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Review Article

Surgical Pathology/Histopathology: Special Article

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ABSTRACT

Surgical pathology is the most significant and time-consuming area of practice for most anatomical pathologists. Surgical pathology involves gross and microscopic examination of surgical specimens, as well as biopsies submitted by surgeons and non-surgeons such as general internists, medical subspecialists, dermatologists, and interventional radiologists. The practice of surgical pathology allows for definitive diagnosis of disease (or lack thereof) in any case where tissue is surgically removed from a patient. This is usually performed by a combination of gross (i.e., macroscopic) and histologic (i.e., microscopic) examination of the tissue, and may involve evaluations of molecular properties of the tissue by immunohistochemistry or other laboratory tests. Surgical pathology has utmost importance in the current era in diagnosis of malignant and nonmalignant lesions to avoid mortality and or morbidity related to disease and treatment plan for the sake of accurate histological diagnosis.

Key Words: Surgical pathology, Pathological diagnosis, Benign & Malignant lesions, treatment plan.

Historical Aspects of Surgical Pathology

Surgical pathology has come a long way since the time that Velpeau, famous professor of clinical surgery at the University of Paris, stated in his work on diseases of the breast published in 1853: The intervention of the microscope is not at all necessary to decide whether such and such a tumor, which has been removed, is or is not of cancerous nature. In the 1870s, Carl Ruge and his associate Johann Veit, of the University of Berlin, introduced the surgical biopsy as an essential diagnostic tool (1,2). Despite the controversies that followed, Friedrich von Esnarch, professor of surgery at Kiel and a leading military surgeon, presented forceful arguments at the German Surgical Congress of 1889 on the need to establish a microscopic diagnosis before operating in suspected cases of malignant tumors requiring extensive mutilating procedures. Shortly thereafter, the freezing microtome was introduced, and the frozen section procedure hastened the acceptance of this recommendation (3). In the United States, the specialty of surgical pathology (defined in its broader sense of the pathology of the living) was conceived and initially developed by surgeons, gynecologists, dermatologists, and other clinical specialists (4-7). It is believed that William S. Halsted was the first American surgeon to create a division of surgical pathology at Hopkins when he made Joseph Colt ('Bloody') Bloodgood the first full-fledged American surgical pathologist (8). These pioneer efforts, which were initially met with indifference and occasionally scorn by the academic pathology establishment, proved to be hugely successful. In the second phase of its development, the specialty came to be performed by pathology-trained individuals, and this was followed by its logical and perhaps inevitable incorporation into pathology departments (9). Because of the differences in background, philosophy, and goals between the ‘surgical’ and the ‘general’ pathologists, the merging of the two schools proved to be a slow, complicated, and sometimes frustrating process, and one that is still evolving. The intellectual, logistic, and financial benefits of this arrangement are, however, too obvious for any alternative scheme to be a realistic consideration, despite some ill-conceived recent attempts from some clinical groups (particularly gastroenterologists) to revert to a variation of the ancient scheme.
INTRODUCTION

A department of pathology in a large medical center should have a division of surgical pathology closely affiliated with the clinical and surgical departments. Surgical pathology implies surgery, but the modern surgical pathologist is closely affiliated with many branches of medicine. This includes all the surgical specialties, internal medicine, dermatology, neurology, diagnostic radiology, radiation therapy, and medical oncology. Although the study of radiology deals with shadows and the study of pathology with substance, the correlation of those shadows with the gross substance strengthens the diagnostic skill of the radiologist, explains errors in radiologic interpretation, and instills humility rather than dogmatism. The radiotherapist and medical oncologist, too, can learn much from the study of surgical pathology, particularly the correlation between sensitivity to therapy and microscopic tumor types and the effects of therapy on normal tissue. Furthermore, explanations for the success or failure of therapy may become apparent by the study of surgical specimens.

The surgical pathologist has the unique opportunity of bridging the gap between the beginning of disease and its end stages, and he should take advantage of this circumstance. He can do this only after a solid foundation of study at the autopsy table, where the ravages of cancer and other diseases are all too clear. With this background, he can then correlate the initial stages of disease seen in specimens from living patients in the surgical pathology laboratory and make fundamental contributions to knowledge. With the integration of clinical findings, pathologic anatomy is still a living science. Surgical pathology has utmost importance in the current era in the diagnosis of malignant and nonmalignant lesions to avoid mortality and or morbidity related to disease and treatment plan for the sake of accurate histological diagnosis. So histopathological evaluation of every surgically removed tissue is mandatory and meticulous histopathological examination by expert surgical pathologist is the mainstay to arrive at the diagnosis.

Types of Various Biopsies

Interpreting biopsies is one of the most important duties of the surgical pathologist. In incisional biopsies, only a portion of the lesion is sampled, and therefore the procedure is strictly of a diagnostic nature. In excisional biopsies, the entire lesion is removed, usually with a rim of normal tissue, and therefore the procedure serves both a diagnostic and a therapeutic function. The decision whether to perform an incisional or an excisional biopsy depends primarily on the size of the lesion; the smaller it is, the more logical to take it out completely when first encountered. For large lesions, particularly those of deep soft tissues, an incisional biopsy is usually preferable because of the fact that the type and extent of excision vary considerably depending on the tumor type. The danger of incisional biopsies promoting metastatic spread, a hotly debated subject in the past, has proved in most cases to be inconsequential.

Biopsies are also classified according to the instrument used to obtain them: cold knife, cautery, needle, or endoscope. Of these, the one usually least suitable for microscopic interpretation is that obtained with a cautery, because this instrument chars and distorts the tissue and prevents proper staining.

Clinical Utility of Biopsy

Surgical pathologists have the definitive role in tumor diagnosis. No matter how high the index of clinical suspicion, the diagnosis of cancer is not conclusively established nor safely assumed in the absence of a tissue diagnosis. With very few exceptions, definitive therapy for cancer should not be undertaken in the absence of a tissue diagnosis. Policies supporting this practice are written into the bylaws of most hospitals and are regularly monitored by hospital tissue committees and by accrediting agencies.

It is the task of the surgical pathologist to provide an accurate, specific, and sufficiently comprehensive diagnosis to enable the clinician to develop an optimal plan of treatment and, to the extent possible, estimate prognosis. There was a time not many years ago when the simple designation “benign” or “malignant” provided the clinician with all of the information necessary to provide appropriate care for the patient. This is no longer the case. Cancer is not a single disease. There are more than 300 distinct varieties of tumors, each with a characteristic biology. Moreover, tumors have a course of historical development and progression; in an individual patient, they may be first recognized at any stage along that course. The tremendous advances in all fields of oncology require a great deal
of additional information, and nearly every case, in fact, requires a fuller understanding of the patient’s particular tumor to allow the most appropriate classification for research, for prognosis, and for therapeutic intervention. Details of the type and origin of the tumor, its differentiation, level of invasion, the numbers of lymph nodes with and without metastatic tumor, the presence or absence of hormone receptors, the activity of specific enzymes, ploidy, frequency of mitosis, and percentage of cells in the S-phase may all be relevant in the pathologic assessment of neoplasia. Molecular pathology, for example, using nucleic acid probes with or without amplification by the polymerase chain reaction to detect expression of specific tumor genes or gene mutations, has not yet reached standard practice.

Surgical pathologists deal primarily with structure. Careful gross examination of excised tissue, first with the naked eye, is followed by a more detailed examination of tissue sections in the compound light microscope. Intraoperative examination may make use of frozen tissue sections, but in most instances, pathologists rely on the better preservation of structure afforded by permanent tissue sections stained with hematoxylin and eosin (H & E) and occasionally other dyes. Histochemistry, immunohistochemistry, and electron microscopy are helpful or necessary supplements for diagnosis in 10% to 15% of solid tumors. In addition, surgical pathologists collaborate closely with cytopathologists in diagnoses involving exfoliated cells or needle aspirates and with clinical pathologists who make use of other techniques, such as culture for microorganisms, flow cytometry, and specialized laboratory tests of a biochemical, immunologic, or molecular nature. In order to perform most of these supplementary studies, the specimen must be specially processed while it is still fresh; that is, prior to tissue fixation. It is a responsibility of the surgical pathologist to coordinate these various activities and to synthesize the information provided by each into a comprehensive diagnosis that is maximally informative to the clinician caring for the patient.

Pitfalls/Drawbacks of Morphological Diagnosis

Pathologists are physicians and human beings. They have as great a capacity for error and susceptibility to subjective distractions as other practitioners of the art of medicine. Because of certain nineteenth cen-
is inadequate in depth or breadth, the pathologist is obliged to append a note stating that he cannot determine from the tissue submitted whether the process is a cancer or a polyp. The normal margin must not be obtained at the expense of representative tumor. Worst of all are expanding soft tissue neoplasms. Junction biopsies may include only a pseudocapsule that can be hard, typically ‘fish flesh’ and grossly more malignant in character than the tumor beneath. Such a barrier found in the retroperitoneum or deep muscle groups of an extremity may achieve a thickness of one centimeter or more.

While it may not always be technically feasible to obtain bigger, better, or multiple biopsies, there are many occasions in which the advantages of a significant increase in the sample of tumor outweigh the risk to the patient. An adequate volume of tissue permits a choice of fixatives, histochemical studies, bioassay or tissue culture. In some instances, one of the specialized examinations may break a morphologic deadlock.

Before a biopsy specimen is delivered to the laboratory, it may be so damaged that the slides prepared from it are worthless. In place of a diagnosis, the pathologist must write, “Tissue unsatisfactory for interpretation.” A more serious consequence of damage is the failure to recognize subtle artefactual changes in cells. False positive, false negative and incorrect histogenetic interpretations have resulted from avoidable mishandling of biopsy fragments.

The complaint of withholding information may also be lodged against the pathologist. The unsophisticated recipient of a pathologist’s written consultation will seek out the usually brief, bald diagnostic statement, accept it as the truth and proceed on his definitive therapeutic way. In the majority of instances, the diagnosis is the ‘truth’, assuming certain minimum standards of professional competence and permitting considerable philosophic license with the word. But the appearance of a sample of tumors and diseases difficult to classify may be thoroughly misleading when considered out of context.

There are ways in which the pathologist can and should indicate doubts and alternative possibilities when he suspects that the tissue submitted to him may tell only part of the story of the patient’s disease or may be a false representation. Retreat to the smug assertion, “I can see only what is in the tissues you gave me”, has been forced on pathologists by colleagues who have sought miracles of extrapolation from inadequate biopsies. Differential diagnoses of tissue have been discouraged by the myth of objectivity, the dogma that pathologists have the final word, and the thundering denunciations of pathologists’ speculations by physicians who want a single, solid answer, right or wrong.

With full knowledge of the relativity of the term, we use (the term) ‘inexperience’ with deliberate intent. Neither pride nor pressure should force a pathologist to make a decision about a disease process that he does not recognize. The nearest approximation or look-alike in his experience may be entirely unrelated. A mismatch may result in mutilation or death of the patient.

Recognition of one’s limitations is as great an asset as the sharpest diagnostic eye. There is a chain of command for handling serious and unfamiliar problems. Colleagues immediately available may offer a rapid solution from past experience or from lack of obsessive preconception. The community may be polled. Among the members may be one, who has perfect and documented recall of an entity not previously encountered. Such a survey may yield only confusion, but from it, one can usually salvage a list of experts with series of entities, ones that may come to the average pathologist only once or twice in his lifetime.

While it is true those world-renowned experts are human and fallible and that there is an almost irreducible percentage of undiagnosable tumors, it is every physician’s obligation to submit his insoluble problems to the highest court of appeal. Such a presentation should be made only after thorough deliberation and must be accompanied by all pertinent clinical data. A complete historical review and serial roentgen studies of a bone tumor may be more important diagnostically than a biopsy. It is sportsman-like and of great educational value to the pathologist (seeking a second opinion) to submit his own report even if it ends with several speculative diagnoses, each preceded by a question mark.

Ancillary Techniques

Immunocytochemistry is the most commonly employed ancillary technique in surgical pathology (see the following section) but special stains also aid in tumor differential diagnosis and classification. Examples include the Van Gieson’s stain or the Masson trichrome method for distinguishing collagen and muscle, the Weigert’s stain for elastic tissue,

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silver stains for reticulin fibers, and special stains for mucins, amyloid, lipids, myelin and glycogen-all substances whose identification may aid in the diagnosis of one or another type of tumor. In other instances, enzyme histochemistry may be essential for defining cell lineage, as in certain types of leukemia; for example, chloroacetate esterase or endogenous peroxidase staining for cells of myelomonocytic lineage, alpha naphthyl butyrate esterase (so-called “nonspecific” esterase) staining for monocytes and macrophages.

Other techniques that may occasionally aid the surgical pathologist in tumor diagnosis are specimen radiography (for localizing and analyzing crystalline material, such as calcium, in breast biopsies) and morphometry.

Utility of Immunohistochemistry with Examples

Immunohistochemistry has become an important adjunct in the evaluation of human neoplasms. The commercial availability of a broad range of reagents (including prediluted reagents in kit form) has made it possible for high-quality immunohistochemistry to be performed in most pathology laboratories. The most commonly employed immunohistochemical techniques are those in which enzymes, such as horseradish peroxidase or alkaline phosphatase, are used in conjunction with specific antibodies to provide color reactions at sites of antigen-antibody interactions. Variations of the avidin-biotin complex (ABC) technique are currently the most widely utilized in current practice. The ABC procedure generally requires three sequential steps: an unlabeled primary antibody, a biotin-labeled anti-immunoglobulin secondary antibody, and, finally, preformed avidin-biotin-peroxidase complexes. One variation of the ABC method employs streptavidin, which has greater sensitivity than avidin and exhibits less nonspecific binding (11). It should be noted that the sensitivity of any immunohistochemical procedure is, in large part, related to the reagents and detailed procedures employed. As a consequence, it is difficult to compare the results of immunohistochemical studies from different institutions that employ different reagents and methods.

Virtually any type of pathologic specimen may be suitable for immunohistochemical staining, including fresh frozen tissue, fixed tissue, and cytologic preparations. Unfortunately, however, not all antigens are equally well preserved after these various treatments, and the approach taken for immunohistochemical staining must depend on the antigen(s) of interest. For example, although a large number of cytoplasmic antigens are detectable in fixed, paraffin-embedded tissue, other antigens, such as many cell surface-associated antigens, are destroyed or masked by common fixatives and may be demonstrable only in the fresh frozen tissue or in cytologic preparations. Antigen retrieval methods, such as pretreatment with proteolytic enzymes or heating (using a microwave oven, steamer, pressure cooker, or autoclave), may permit the identification of otherwise undemonstrable antigens in fixed, paraffin-embedded tissue sections(12,13). Finally, not all fixatives are equivalent with regard to antigen preservation. Although crosslinking fixatives, such as formaldehyde, are often suitable, they are suboptimal for detecting certain antigens of diagnostic importance, such as those located on intermediate filaments, which are best demonstrated in fresh-frozen or alcohol-fixed tissue (14-16).

Common Applications of IHC

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Utility Special Stains in Diagnosing the lesions with Examples

The surgical pathologist will find a relatively small minority to be of real diagnostic utility at present. This is especially true since the advent of immunohistochemistry, which has rendered many of them obsolete. Those most commonly used at present are the following

1. Periodic Acid-Schiff (PAS) Stain.

Substances containing vicinal glycol groups or their amino or alkylamino derivatives are oxidized by
periodic acid to form dialdehydes, which combine with the Schiff reagent to form an insoluble magenta compound. This stain therefore demonstrates glycogen (in a specific fashion, when used with a diastase-digested control) and neutral mucosubstances, outlines basement membranes, and makes evident most types of fungi and parasites. As a trivia, one might add that it is also useful for the demonstration of the intracytoplasmic crystals in alveolar soft part sarcoma.

2. Stains for Microorganisms

These include techniques for gram-positive and gram-negative bacteria, acid-fast mycobacteria, fungi, and parasites. The gram stain allows the separation of bacteria into those that retain the crystal violet-iodine complex (gram positive) and those that are decolorized by alcohol or acetone treatment and counterstained by either safranin or fuchsin (9). Acid fastness depends on the high lipid content (mycolic acids and long-chain fatty acids) in the cell walls of mycobacteria, which confer to the cell the ability to complex basic dyes (such as carbolfuchsin) and to retain them following strong decoloration with acid–alcohol. The techniques in this group most used are Brown and Brenn (B&B; as a modification of the gram stain), Ziehl–Neelsen (for acid-fast organisms), Grocott hexamine–silver (for fungi and Pneumocystis), PAS (for fungi, amebae, and Trichomonas), and Dieterle or one of its modifications (for Helicobacter, Legionella, and the organisms of syphilis and Lyme disease).

3. Argentaffin and Argyrophilic Stain

The argentaffin reaction depends on the presence in the tissue of a substance, often of the phenolic group (such as catecholamines or indolamines), that reduces silver (and other metallic) salts; (18,19). we generally use the Fontana–Masson technique in paraffin-embedded material (20). In the argyrophilic reaction, an extraneous reducing agent such as hydroquinone or formalin is added; we generally employ the unmodified Grimelius technique and prefer to use it in Bouin fixed material whenever available. Others have found the Churukian-Schenk’s modification to give better results (21).

4. Amyloid Stains

The mysteriously named Congo red (21) followed by examination with both standard and polarized light (the notorious apple green birefringence) is regarded as the most reliable and practical technique to detect amyloid. It should be realized, however, that the stain does not have chemical specificity, being dependent upon an arrangement of the molecule in an antiparallel beta-pleated sheet. It should also be noted that nonamyloid-related green birefringence can occur as a result of excess dye retained in the tissue and to other technical factors (22).

5. Reticulin Stains

Reticulin stains demonstrate both ‘reticular fibers’ and basement membrane material. Reticular fibers consist of very thin fibers of mainly type III collagen, which are widespread in connective tissue throughout the body. Basement membranes are largely composed of type IV collagen and laminin. In both instances it appears that the adsorption of silver stains and their PAS positivity are due to a coating of bound proteoglycans. Reticular fibers and reticulin stains should not be equated to reticulum cells, a common misconception. The latter term refers to cells (generally of the accessory immune system, also called dendritic cells) in which the ‘reticulum’ or network is formed not by extracellular material but by thin, complex cytoplasmic prolongations. Traditionally, the main applications of silver-based reticulin stains (such as Gomori, Wilder, and Gordon and Sweet) in tumor pathology have been in distinguishing: (1) epithelial from nonepithelial neoplasms; (2) various mesenchymal neoplasms from each other; and (3) in situ from invasive carcinoma. In general, foci of carcinoma have reticulin around the tumor nests but not between the individual cells, whereas in most sarcomas and large cell lymphomas the silver-positive material separates single cells. The striking contrast between the two patterns can be readily appreciated by comparing the epithelial and mesenchymal components of a synovial sarcoma. In tumors of endothelial cells, the reticulin that identifies the vessel wall (rather than the one which coats the individual tumor cells) is seen on the outside of the neoplastic population, whereas the reverse is true in tumors of pericytes or vascular smooth muscle cells. In typical cases of leiomyosarcoma the reticulin wraps individual cells completely, whereas in typical cases of malignant peripheral nerve sheath tumor it runs in parallel to the spindle tumor cells without surrounding them at the poles. Reticulin stains have also been used to distinguish ovarian granulosa cell tumors (in which the fibers are scanty and surrounding groups of cells) from fibrothecomas (in which they surround individual tumor cells). Unfortunately, these patterns are well in evidence only in classic cases.
of these respective entities, i.e., those which are already easily diagnosable with H&E techniques. In the controversial cases, reticulin stains are likely to provide results that are far from conclusive, to the point that we have found them of very limited utility. We agree wholeheartedly with Azzopardi when he stated that “reticulin silver impregnations are virtually valueless in the differentiation of certain sarcomatoid carcinomas from true sarcomas”, and that “reticulin impregnations may be deceptive and merely serve to ‘confirm’ an erroneous diagnosis of sarcoma” (23,24).

6. Trichrome Stains

In the trichrome methods, such as those devised by Masson (a veritable cornucopia), van Gieson, and Mallory, phosphotungstic or phosphomolybdic acid is used in combination with several anionic dyes. The main value of this group of stains is in the evaluation of the type and amount of extracellular material. The three tissue structures demonstrated by the three component dyes are nuclei, cytoplasm, and extracellular collagen, respectively (24). It is not generally realized that the only component of all trichrome stains having some degree of specificity is that provided by the phosphotungstic or phosphomolybdic acid, which stains the collagen fibers; everything else is background staining, no better from the point of view of specificity than what is obtained with H&E (25). The often-used statement that a trichrome stain ‘proved’ the smooth muscle nature of a tumor is therefore inaccurate.

7. Phosphotungstic Acid-Hematoxylin (PTAH) Stain

This particular variant of trichrome stain has been traditionally used for the demonstration of intracytoplasmic filaments, such as those present in muscle and glial cells. It has been largely superseded by the immunohistochemical reactions for the specific microfilaments being searched for.

8. Stains for Hemosiderin (Perls), Melanin (Fontana–Masson), and Calcium (Von Kossa)

In the Perls technique for hemosiderin, hydrochloric acid splits off the protein bound to the iron, allowing the potassium ferrocyanide to combine specifically with the ferric iron to form ferric ferrocyanide (Prussian blue). In the Fontana–Masson method for melanin (already mentioned in connection with the argentaffin reaction), an ammoniacal silver solution is used without a reducing bath. Only substances capable of reducing directly silver salts (i.e., argentaffin) such as melanin are demonstrated. In the von Kossa method for calcium, silver is substituted for calcium in calcium salts; this silver salt is then reduced to black metallic silver by the use of light or a photographic developer.

9. Stains for Neutral Lipids

Most of these stains are based on the principle that the colored compounds used are more soluble in the tissue lipids than in their own solvent. Actually, these compounds do not qualify as dyes in the conventional sense, in that they contain no auxochromic groups but are chromogens. Oil red O is the one most commonly employed.

A limitation of fat stains is the fact that they cannot be performed in a paraffin-embedded material because of the fat solubilizing properties of xylene and other clearing materials used for processing. In tumor pathology, the utility of fat stains is minimal and largely limited to the inconsequential distinction between fibroma and thecoma in the ovary, support for the diagnosis of renal cell carcinoma and sebaceous gland tumors of the skin, and identification of lipid-rich carcinoma in various organs. Despite ingrained notions to the contrary, fat stains are of little if any use for the diagnosis of liposarcoma; some liposarcomas contain little or no stainable fat, whereas several types of nonadipose tissue neoplasms can contain considerable amounts.

10. Mucin Stains

Mucin is the traditional term used for a large group of macromolecules containing an acidic group, which is divided into two major categories: the epithelial O-glycoproteins (membrane-bound or secreted) composed of a protein core and a sialic acid-containing carbohydrate moiety (whether sulfated or not) and the stromal glycosaminoglycans, which contain hyaluronic acid and which also can be sulfated. Historically, the term ‘mucin’ has been used for the former category, whereas the latter substance has usually been referred to as ‘myxoid’ (hence the term pseudomyxoma for a lesion that may appear myxoid, i.e., stromal, but is really epithelial, i.e., mucinous). The combination of Alcian blue and PAS is probably the best ‘pan-mucin’ stain, since it demonstrates mucosubstances of neutral, slightly acidic, and highly acidic types (26). Enzymatic pretreatment will show whether the acidic groups are made of sialic acid (digestible with sialidase), hyal-
uronic acid (digestible with hyaluronidase), or sulfated groups (digestible with neither). Several stains are available for the specific demonstration of highly acidic mucins. These include Alcian blue performed at pH 1.0, colloidal iron, high iron-diamine, and the classic Mayer mucicarmine. At the risk of sounding old-fashioned, we prefer the latter stain despite its empiric nature (27,28). The abnormalities in mucin secretion sometimes present in carcinomas (usually because of incomplete carbohydrate synthesis) can be surmised from the mucin stains but require more sophisticated techniques for their specific identification (29,30). Hale colloidal iron stain has become the standard for the identification of renal chromophobe carcinoma. Although it should not be necessary, we will mention that Hale stain is a mucin stain in which iron is used as a reagent, not a stain to demonstrate iron.

11. Giemsa Stain

The most spectacular results with Giemsa and other Romanovsky-type stains are obtained with alcohol-fixed smears (30,31). However, reasonably good preparations can also be achieved in paraffin-embedded material, provided one is very scrupulous with the technique and fastidious with the source of the reagents. The technique is most useful for the demonstration of various hematolymphoid elements (including mast cells) and microorganisms.

12. Elastic Fibers

Weigert-type techniques are reasonably specific for elastin and are regarded by many as the method of choice for the demonstration of these extracellular fibers. However, the Verhoeff–van Gieson (VVG) stain is more popular because it is quick and outlines the elastic fibers with a strong black color. Both techniques are usually set against the esthetically pleasant trichrome background provided by the van Gieson stain.

13. Myelin Stains

Luxol fast blue is the nonimmunohistochemical method of choice for the demonstration of myelin. It is based on the strong affinity of the copper phthalo-cyanine dye for the phospholipids and choline bases of myelin(32).

14. Formaldehyde-induced Fluorescence

This is a very special type of technique, remarkably sensitive for the demonstration of catecholamines and indoleamines but requiring rather costly and cumbersome equipment as originally described. A modified version as applied to touch preparations has made it more accessible to the practicing pathologist (12,23). But it is rarely used at present. It is based on the principle that biogenic amines subjected to formaldehyde vapors produced by heating the polymer paraformaldehyde form highly fluorescent derivatives.

Immunofluorescence with Examples:

Immunofluorescence (IF) is a common laboratory technique used in almost all aspects of biology. This technique based on pioneering work by Coons and Kaplan (33,34), and later by Mary Osborne (35), has been widely used both in research and clinical diagnostics. Applications include the evaluation of cells in suspension, cultured cells, tissue, beads and microarrays for the detection of specific proteins. IF techniques can be used on both fresh and fixed samples. In IF techniques, antibodies are chemically conjugated to fluorescent dyes such as fluorescein isothiocyanate (FITC) or tetramethyl rhodamine isothiocyanate (TRITC). These labeled antibodies bind (directly or indirectly) to the antigen of interest which allows for antigen detection through fluorescence techniques. The fluorescence can then be quantified using a flow cytometer, array scanner or automated imaging instrument, or visualized using fluorescence or confocal microscopy.

The two main methods of immunofluorescent labeling are direct and indirect. Less frequently used is direct immunofluorescence whereby the antibody against the molecule of interest is chemically conjugated to a fluorescent dye. In indirect immunofluorescence, the antibody specific for the molecule of interest (called the primary antibody) is unlabeled, and a second anti-immunoglobulin antibody directed toward the constant portion of the first antibody (called the secondary antibody) is tagged with the fluorescent dye.

Advantages of direct immunofluorescence include shorter sample staining times and simpler dual and triple labeling procedures. In cases where one has multiple antibodies raised in the same species, for example, two mouse monoclonals, a direct labeling may be necessary.

Disadvantages of direct immunofluorescence include lower signal, generally higher cost, less flexibility and difficulties with the labeling procedure.
when commercially labeled direct conjugates are unavailable.

Advantages of indirect immunofluorescence include greater sensitivity than direct immunofluorescence. There is an amplification of the signal in indirect immunofluorescence because more than one secondary antibody can attach to each primary. Commercially produced secondary antibodies are relatively inexpensive, available in an array of colors, and quality controlled.

Disadvantages of indirect immunofluorescence include the potential for cross-reactivity and the need to find primary antibodies that are not raised in the same species or of different isotypes when performing multiple-labeling experiments. Samples with endogenous immunoglobulin may exhibit a high background.

Applications of immunofluorescence in diagnostic pathology are:

1. Analysis of antigens in fresh, frozen or fixed tissues; sub-cellular localization of antigens in tissue culture monolayers; observation of bacterial or parasitic specimens;
2. Detection and localization of the presence or absence of specific DNA sequences on chromosomes;
3. Defining the spatial-temporal patterns of gene expression within cells/tissues.

The most widely used method of IF in pathology is indirect IF. However, in some very specialized applications direct IF has been used for localization of IgG in immune complexes along the dermal-epidermal junction of skin biopsies from patients suffering from systemic lupus erythematosus (36).

FISH/PCR principle and utility in nonmalignant and malignant conditions:

Polymerase chain reaction (PCR), a revolutionary technique first introduced in 1985, allows millions of copies of any specific DNA sequence to be generated within a few hours. PCR relies on the ability of DNA polymerases, in the presence of a mixture of deoxynucleotide triphosphates (dATP, dCTP, dGTP, dTTP), to copy a DNA strand using a short complementary DNA fragment as an initiating template (37-41).

The steps involved in the procedure are the following:(42-44)

1. Synthesis of short DNA fragments (oligonucleotide primers) that are complementary to DNA sequences on opposite strands of the DNA flanking the fragment to be amplified
2. Heat denaturation of the DNA
3. Annealing of the primers to their complementary sequences
4. Extension of the annealed primers with DNA polymerase.

Each cycle of the procedure (from step 2 through step 4) is continuously repeated, each successive cycle doubling the amount of DNA synthesized in the previous one, this chain reaction resulting in the exponential accumulation of the specific DNA target fragment to approximately 2n, where n is the number of cycles. The amplified product is analyzed by gel or capillary electrophoresis. The nature of the amplicon can be further confirmed by specific hybridization, restriction endonuclease analysis or DNA sequencing.

The PCR technique can also be used to amplify RNA so that gene expression can be analyzed. For this purpose, extracted RNA is first converted to double-stranded cDNA using the retroviral enzyme reverse transcriptase (RT), and this is followed by PCR on the cDNA copies.

The applications of PCR technology to medicine in general and pathology in particular are too numerous (45). In the specific field of tumor pathology, the uses of PCR are the following:

1. Detection of Ig or TCR gene rearrangements as a means to determine the clonality of B- or T-cell proliferations, respectively, and also to determine whether separate lymphoid neoplasms represent the same clonal process (46,47).
2. Detection of the chimeric transcripts resulting from chromosomal translocations in hematologic and solid malignancies (e.g., t(14;18) in follicular lymphomas). This can be applied for primary diagnosis or for the detection of ‘residual minimal disease’ after treatment (48).
3. Detection of point mutations in oncogenes and tumor-suppressor genes (i.e., activated RAS
oncogenes in pancreatic carcinomas and EGFR mutation in pulmonary adenocarcinoma).

4. Detection of gene amplifications, such as MYCN in neuroblastoma and HER2 in breast carcinoma.

5. Detection of microsatellite instability, which is a key feature of tumors occurring in patients with hereditary nonpolyposis colorectal cancer syndrome (49,50).

6. Demonstration of loss of heterozygosity, which indicates allelic loss of tumor-suppressor genes in tumors (51).

7. Detection of microorganisms, including bacteria such as Mycobacterium tuberculosis (52,53) and viruses, such as HPV in squamous cell carcinomas, EBV in malignant lymphomas, and HHV8 in Kaposi sarcoma (54).

8. Detection of circulating tumor cells in peripheral blood, through the identification of mRNA for thyroglobulin in thyroid carcinoma, for tyrosinase in melanoma, for PSA in prostatic carcinoma, and others (55,56).

9. Distinguishing a de novo second malignancy from a tumor recurrence (57).

10. Identification of mismatched specimens and ‘floaters’ by microsatellite analysis.

11. Detection of gene hypermethylation, a phenomenon which results in transcriptional inactivation.

CONCLUSION

To conclude, histopathological examination is very important for the diagnosis of benign and malignant conditions. Every specimen operated should be sent for histopathological examination before further treatment to avoid untoward complications related to disease and treatment for the sake of accurate pathological diagnosis.

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A Comprehensive Survey on Imaging and Detection Techniques for Diagnosis of Brain Tumor

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ABSTRACT

Computer vision technologies have created a revolutionary development for diagnosis of diseases through biomedical imaging. Accurate detection of the size of the tumor and its location play a crucial role in the diagnosis of brain tumor. There are many techniques to detect brain tumor. Selection of a specific imaging technique is a great challenge in real-time diagnosis of the tumor. Detection of tumor in the earlier stages makes the treatment easier. This paper reviews various imaging techniques, its purpose, limitations in detection of brain tumor, Physicians need both anatomical and functional imaging information at the same time for assessing and also for appropriate treatment planning. Computer-aided precise detection techniques which include, preprocessing, feature extraction, feature selection, and further classification are also reviewed. Recent advances made in multimodal medical image fusion are also reviewed. Effective detection through advanced processing like fusion technique is illustrated.

Key Words: Imaging modalities, Magnetic Resonance Imaging, Denoising, Segmentation, Feature extraction, Feature selection.

INTRODUCTION

Either benign or malignant, brain tumors are serious. It is thus important to develop computer-aided diagnostic techniques for identifying tumor at an early stage. It involves a deep insight into different types of tumor, impact on intensity variation in images and the principle behind the different modalities used for brain tumor detection. Having acquired the above knowledge, image processing techniques can be used for extracting and characterizing the abnormality. Primary and secondary tumors are the two different types of tumors in the brain. As the tumor cells react differently from the normal cells to light, x-rays, magnetic resonance, etc., there is an intensity variation between the tumor and the background regions. Computerized Tomography, Magnetic Resonance Imaging, Functional Magnetic Resonance Imaging, Positron Emission Tomography are different types of diagnostic techniques. Image preprocessing, enhancement, abnormality isolation and feature extraction are the most commonly used techniques for computer-aided interpretation. Also, as these techniques are complimentary to each other, image fusion is also performed by researchers to strengthen the redundant information and to have the complimentary information. This paper deals with different types of brain tumor and modalities used for brain tumor imaging. Also, a comparison is provided between the various diagnostic techniques. A brain tumor is a mass of abnormally growing cells in the brain or skull. It can be benign (noncancerous) or malignant (cancerous). Disparate other cancers, cancer arising from brain tissue very rarely spread. All brain tumors irrespective of whether they are benign or malignant are serious (1). Benign tumors can be removed, and they seldom grow back. Benign brain tumors usually will have an obvious border or edge. Cells from benign tumors rarely invade tissues around them. They do not spread to other parts of the body. Malignant brain tumors grow rapidly and crowd or invade the nearby healthy brain tissue. They rarely spread to other parts of the body. A growing tumor eventually will compress and damage other structures in the brain. There are two types of brain tumors namely primary and secondary. Primary tumors begin in brain tissue, while secondary tumors spread to the brain from other parts of the body. Table 1 shows different types of primary tumors and its origin.

Table 1: Tumor types and its origin

<table>
<thead>
<tr>
<th>Name</th>
<th>Place of origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glioma or Astrocytoma</td>
<td>Glial cells called astrocytes</td>
</tr>
<tr>
<td>Meningioma</td>
<td>Meninges</td>
</tr>
<tr>
<td>Oligodendrogloma</td>
<td>Cerebrum</td>
</tr>
<tr>
<td>Medulloblastoma</td>
<td>Cerebellum</td>
</tr>
<tr>
<td>Ependymoma</td>
<td>Cells that line the ventricles or the central canal of spinal cord</td>
</tr>
</tbody>
</table>
MATERIALS AND METHODS

Brain Imaging Methods
As the individual molecules in the brain cells interact with electromagnetic waves and Radio Frequency waves separately, these waves are used for imaging the brain. Imaging is of two type namely structural imaging (tumor and injury) and functional imaging (Metabolic disease & lesions). Different modalities used for imaging brain for detecting tumor are Magnetic Resonance Imaging (MRI), Computed Tomography (CT), Positron Emission Tomography (PET), Single Photon Emission Computed Tomography (SPECT) are common modalities available for tumor detection (2,3).

Computerized Tomography (CT) involves X-Ray method, and this technique is capable of identifying abnormalities such as Tumors and Lesions. Magnetic Resonance Imaging (MRI) utilizes strong magnetic field which aligns the protons in H atom and can detect an abnormality on cerebral blood volume and flow. With Functional Magnetic Resonance Imaging (fMRI) with the strong magnetic field, part of the brain is active and functions when a patient is performing a given task. With Positron Emission Tomography (PET) injection of radio-active material, identifies the activated area of the brain and also measures the degree of activity. Diffusion Tensor Imaging (DTI) with magnetic field variations observe functions of the brain as they occur.

CT imaging generates multiple images collected from many angles. A new image created shows any abnormalities or tumors with the overlying structures removed. CT scan may also be used if the patient cannot have an MRI, such as if the person has a pacemaker in the heart. Sometimes, a contrast medium is given before the scan to provide better detail on the image. MRIs create more detailed pictures when CT scans and are the preferred way to diagnose a brain tumor. MRI is the commonly used modality for brain tumor growth and location detection due to its high resolution. Magnetic Resonance Imaging provides high contrast images of different anatomical structures. It gives better differentiation of tissues than other medical imaging techniques. Evaluation and analysis of MRI images by radiologists is error-prone (4).

The most common MRI sequences are T1-weighted and T2-weighted scans. T1-weighted images produce by using short TE and TR times. The brightness and contrast of the image are predominately determined by the T1 properties of tissue. Conversely, T2-weighted images are produced by using the longer TE and TR times. In these images, the contrast and brightness are predominately determined by the T2 properties of the tissue. In general, T1 and T2-weighted images can be easily differentiated by looking at CSF. CSF is seen as dark on T1-weighted imaging and bright on T2-weighted imaging.

A third commonly used sequence is Fluid Attenuated Inversion Recovery (Flair). The Flair sequence is similar to T2-weighted image except that the TE and TR times are very long. By doing so, abnormalities remain bright, but normal CSF fluid is attenuated and made dark. This sequence is very sensitive to pathology and makes the differentiation between CSF and an abnormality much easier. T1-weighted imaging can be performed while infusing the Gadolinium (Gad), a non-toxic paramagnetic contrast enhancement agent. When injected during the scan, Gad changes signal intensities by shortening T1. Thus, Gad is very bright on T1-weighted images. Gad enhanced images are especially useful in looking at vascular structures and breakdown in the blood-brain barrier [e.g., tumors, abscesses, inflammation (herpes simplex encephalitis, multiple sclerosis, etc.). MRI Imaging (5,6) leads to

• Noise: There is random noise connected with MR imaging system known as Rician distribution
• Shading Artifact: The non-uniformity in the radio frequency (RF) field during data collection results in shading effect.
• Partial Volume Effect: More than one type of class or tissue occupies one pixel or voxel called mixelsof an image.
Fig 1 shows the steps involved in Brain tumor selection and classification. Pre-processing step is carried out to improve the next step of the segmentation process. Computer-aided brain MRI segmentation requires skull stripping operation along with the global multi-thresholding scheme. Skull-stripping is done to separate the skull portion of the head from soft brain tissues. Initially, the input MRI image is denoised using a filter. Mask is generated by tracing the contour of the brain image. It is multiplied by the input MRI image to get the pre-processed image. To perform denoising operation, different hybrid combinations are adopted to perform the best filtering operation.

1. Mean Filter—Which works on averaging pixels under consideration is capable of reducing Guassian Noise but has the drawback of resulting in distorted edges and boundaries.
2. Median Filter—Obtaining the median value of pixels is effective in reducing salt and pepper noise, speckle noise and preserves boundaries and edges.
3. Wiener filter—Based on inverse filtering in the frequency domain it is efficient in removing blurring effects from images. As it functions in the frequency domain, its speed is slow.
4. Hybrid filter (Combination of median and wiener filter)—Removes speckle noise, impulse noise, and blurring effects but complex and time-consuming.
5. Modified hybrid median filter (Combination of median and hybrid filter)—Efficient in removing speckle, salt and pepper and Gaussian noise but Computation time is more as compared to simple median filter. Compared to hybrid combinations, Morphology Based De-Noising is efficient and produces better results.

**Image Segmentation Methods**

Segmentation can be manual or semiautomatic depending on the degree of human interaction involved. Manual segmentation is a tedious and exhaustive task. Human expert has to mark tumor regions carefully; otherwise, it will generate jaggy images that lead to poor segmentation results. Semi-automatic brain tumor segmentation consists of the computational part, interactive part, and the user interface. Since it involves both computer and humans’ expertise, results depend on expert users. In fully automatic segmentation, there is no intervention of human and segmentation of tumor is determined with the help of a computer. It involves the human intelligence and is developed with soft computing techniques. These methods are likely to be used for a large batch of images in the research environment (7). Different methods of segmentation techniques include (8)

1. Threshold-based (Global/ Local thresholding)
2. Region-based./Region-growing
3. Watershed Segmentation
4. Pixel-based fuzzy c means
5. Artificial neural networks
6. Markov random fields
7. Model-based parametric deformable models
8. Level sets approach
9. Atlas-guided approach

Inspite of availability of a large variety of state-of-art methods for brain MRI segmentation, still research continues to improve the precision and accuracy of segmentation methods.

**Feature Extraction Methods**

Successful pattern recognition methods depend on the extraction of effective features. Features are the representative of the maximum relevant information that the image could offer for a complete characterization. Features are extracted in both spatial and frequency domains to have maximum accuracy. Statistical feature extraction includes Intensity-based features, Symmetry-based features, and texture-based features. In the frequency domain, wavelet transform, curvelet transform based features can be extracted to form a feature vector, which will be used for developing the classification model (9,10).

**Feature Selection Methods**

The curse of dimensionality is addressed by the appropriate feature selection process. It is an important data processing step before recognition and classification. In accordance with pre-designed selection criteria, the most important features of given input data are selected by the optimal operations under the prefixed criterion and the remaining features are removed from the input to reduce the data amount.

1. Feature selection can eliminate the redundancy, interference, noise and less important data in input. According to the definition of Feature selection, this process can choose the data based on some certain criteria to eliminate all the factors which are not relative to classification problems and to effectively fuse the important data and therefore greatly reduce the data amount.
2. Feature selection can improve the accuracy of the classifier. After the Feature selection operation, a large number of non-relevant data which contain many interferential components are removed. Only the most important features remain for training, which makes the obtained classification model much better improve the applicability of the model and its ability for solving the problem, and finally to achieve higher classification accuracy.

3. Feature selection can improve the operational efficiency. After the Feature selection, the training sample data greatly decrease, and the computational complexity is reduced in a relatively lower degree (mainly determined by the algorithm, thus changes on the computational complexity by reducing the amount of data is just in a relative sense) to reduce the computation time (11).

4. Various pruning techniques are reported in the literature for feature selection. However, these techniques are heuristic in nature. The use of a Genetic algorithm to identify the best set of features will improve the classification accuracy.

Image Fusion Methods

As is visible from various Imaging modalities, none of the above techniques can provide complete information about brain tumor. Hence, it is necessary to combine these imagers to provide the complete information about the abnormality. Though the hardware is now available to combine the techniques, the cost of such hybrid techniques is high and is not feasible for a commoner. Hence it necessitates a software technique for combining these images. Image fusion, the process of combining two different images to strengthen the redundant information and to retain the complementary information. Nuclear medicine images are nowadays superimposed either with Computed Tomography (CT) or magnetic resonance imaging (MRI) to produce special views, a practice known as image fusion or co-registration. These special views allow the information from two different exams to be correlated and interpreted on one image, leading to more precise information (high spatial and spectral information) and for accurate diagnoses. Image fusion is widely used to overcome the observational constraints, which account for the disability to build such instruments to provide such information.

Combination of PET and CT imaging is used to conveniently view the tumor activity by visualizing the anatomical and physiological characteristics in oncology. Fusion of CT and MRI is helpful for neuronavigation in skull base tumor surgery. Combination of PET and MRI is helpful for diagnosis of hepatic metastasis.

Image fusion can be performed on the spatial domain or in the transform domain. In the spatial domain, intensities of the two images are manipulated to obtain the desired result. On the other hand, in transform-based technique, the intensity is converted into transform co-efficient, and these co-efficient are combined effectively to form new co-efficient. Then inverse transform is applied on this co-efficient to get back the image. The most commonly used transforms include Discrete Wavelet Transform (DWT), Stockwell transform, Framelet transform and Hilbert transform.

There are three different types of image fusion techniques namely pixel level fusion, region level fusion and feature level fusion (12). In the case of pixel level fusion, the individual pixels are combined using fusion rules namely choose average and choose maximum. In the case of feature level fusion, the features namely edges, points, histogram, etc. are obtained on the individual images, and these features are fused.

Image Classification Methods

Classifiers have been used to classify subjects as normal or abnormal MRI brain images. Many classification techniques are available in the literature (13) for medical images such as an artificial neural network (ANN), fuzzy c-means (FCM), support vector machine (SVM), decision tree and Bayesian classification. Performance of the proposed technique is measured in terms of entropy (a measure of information in the fused image), standard deviation (a measure of contrast in the fused image), correlation (the relationship between original image and the output image), Peak Signal to Noise Ratio (useful information in the image).
dard deviation is 679.2893. Root Mean Square Error between the output image with the first and second input images are 17.2 and 33.5 respectively. Higher standard deviation indicates high contrast of the image. Lesser Root Mean Square with the first image indicates that the output image has more information of the first image than that from the second image. Feature-based image fusion is performed on the same set of input images. Edge is regarded as the feature for it indicates the set of pixels where the intensity changes from one level to another. The edge detected images of the input images are shown. From the subjective analysis, it is evident that the output image in Fig 2j has the edges of both the images.

Figure 2: (a-d) Original and Gray Scale Images of 2 and 4 months Tumor (e-f) Wavelet Decomposed Image (g) – Fused Image (h-i) Edge Detected Image (j) Fused Image

CONCLUSION

In this paper, a detailed study of various brain tumors, its origin, medical imaging techniques, methodologies adopted for computer-aided tumor detection is presented which remains a challenging task. Multimodal image fusion techniques are also reviewed. Several novel hybrid approaches may be developed through the ideas conveyed in this paper for automatic analysis and detection which will help radiologists inaccurate brain tumor diagnosis. Also, in this paper, both pixel level and feature level image fusion techniques were performed, and quantitative characterization was also done.

REFERENCES

Research Papers

**Extract of *Piper Betle* Facilitates Significant Modulation on the Redox Status in Preeclamptic Placental Trophoblast**

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**ABSTRACT**

**Introduction and Aim:** Preeclampsia (PE), a condition associated with incomplete trophoblast invasion leads to redox status alteration. Effective strategies for the management of PE without any side effects are lacking, and the available synthetic drugs seem to cause harmful effects to mother and fetus. Herbal supplements may help to overcome the clinical manifestations of PE, need of the hour. *Piper betle* (PB) leaf contains several biologically active compounds with protective effects. However, the effect of PB for the treatment of hypertensive disorder like preeclampsia is seldom explored. Extracts of betle leaf-BLE (aqueous-ABLE, PBS-PBLE, saliva-SBLE, diastase-DBLE) are utilized to examine its effect on redox changes in placental trophoblast.

**Materials and Methods:** Three different concentrations of BLE were used to evaluate the optimal concentration having potent radical scavenging activity. Following this, incubation time exhibiting maximum efficiency was standardized by analyzing the trophoblast viability upon incubation with BLEs at the optimum concentration of 1mg/mL.

**Results:** All the extracts of BL (1mg/mL/1h) showed variations in their radical scavenging activity in the order as follows SBLE>DBLE> PBLE>ABLE. It inferred that SBLE is more efficient in modulating redox status in preeclamptic placental trophoblast which is followed by DBLE, may be due to the presence of antioxidants in saliva.

**Conclusion:** Reveals that chewing of the betle leaf during pregnancy facilitates a significant change in the oxidants-antioxidants status in the trophoblast. Hence dietary supplementation of BL orally in particular by chewing might effectively render beneficiary effect in the management of oxidative stress and also help to overcome the other related complications of PE.

**Key Words:** Antioxidants, Betle leaf extract (BLE), Preeclampsia, Redox status, Trophoblast.

**INTRODUCTION**

Trophoblasts, the major cell type of the placenta, attach to and invade into the uterine lining, begin the process of pregnancy. Trophoblasts have a potent invasive capacity which is modulated by endometrium. Recent studies have shown that invasion of cytotrophoblast into the uterus is a unique differentiation pathway for proper implantation (1). Researchers revealed that any abnormalities in trophoblast reflect the changes in the placenta leading to pregnancy complications like preeclampsia (2). Preeclampsia is a hypertensive disorder that develops after twenty weeks of pregnancy and associated with the defective manifestation of the placenta. It is the second most frequent cause of death in pregnant women, and 10-15% of maternal deaths are due to preeclampsia and eclampsia (3) leading to severe complications for mother and fetus, especially premature births. In the mother, preeclampsia may cause premature cardiovascular disease, such as chronic hypertension, ischemic heart disease, and stroke, later in life (4). Moreover, failure in proper placental development leads to inadequate fetal nutrition resulting in growth-restricted neonates (5) and increased oxidative product with low antioxidant level.

Subsequently altered redox status is the key factor involved in the development of preeclampsia; sup-
plementing women with antioxidants during pregnancy may help to counteract oxidative stress and thereby prevent or delay the onset of preeclampsia (6) Since many of the synthetic drugs are known to stimulate deleterious effects, natural plant extracts are believed to initiate beneficiary effect on pregnant women. P.betle may be one such plant that may facilitate the placental cell to counter the oxidative stress created during preeclampsia.

Betle leaf is a well-known ethnomedicinal plant consumed in the Asian countries (7). It is a heart shape leaf comes under the family Piperaceae, and its botanical name is *Piper betle* (8). The plant leaf has wide variety of secondary metabolites such as phenolic compounds (chavicol, hydroxyl chavicol), volatile oils like safrole, eugenol, isoeugenol, eugenol methyl ester (9) According to Dorman et al. (10) chavibetol, eugenol, methyl eugenol and carvacrol of BL plays a crucial role in antioxidant effectualness. Rathee (11) also reported on the O2-. radicals scavenging activity betle leaf due to the presence allylpyrocatechol. This was further confirmed by Sripradha (12) that treatment with BL elevates the level of antioxidants. The plant leaf exerts various pharmacological activities like antimicrobial, anti-diabetic, antioxidant, hepato-protective, antitumor, antimutagenic and antihelminthic activities (13). However, the facilitating effect of betle leaf on hypertensive disorder like preeclampsia is seldom explored.

The practice of chewing betle leaf is common after a feast and even after normal food renders complete digestion, and it also acts as prophylaxis for digestion (14). When betle leaves are chewed, it induces salivation. Saliva secretion comprises 99% water, small quantities of various organic and inorganic compounds, bacteria, epithelial cells and gingival fluid. In addition to its other host-protective qualifications, saliva constitutes the first line of defense against free radical-mediated oxidative stress, since the process of mastication and digestion of ingested foods promotes a variety of reaction. The antioxidant defense system of saliva includes various molecules and enzymes (15), and the major enzyme may be diastase.

An imbalance in the level of antioxidant defense systems and the generation of reactive oxygen species (ROS) leads to oxidative stress ultimately resulting in the damage of macromolecules and indicators of this includes lipid peroxidation products such as malondialdehyde (MDA) and lipid hydroperoxides (LHP). To protect the cells from free radicals induced oxidative stress, living systems have developed strong defense mechanisms that include antioxidants and in extreme conditions by signaling molecules (16). The sum of endogenous and food-derived antioxidants is represented by the total antioxidant activity (TAC) of the system which is a measure of both water-soluble such as and fat-soluble antioxidants (17). Such an oxidative imbalance in trophoblast ultimately results in abnormal placentation and placental dysfunction leading to preterm delivery (18) and other clinical complications of PE. Herbal supplements may be the only solution to overcome the manifestations of preeclampsia.

The present study was aimed to investigate the free radical scavenging activity of various extracts (aqueous, PBS, saliva and diastase) of *P. betle* L. Free radical scavenging activities of the extracts were assessed against DPPH and hydroxyl radicals and to evaluate the effective role of various extracts of BL on normotensive and preeclamptic placental trophoblast assessment of viability, oxidant/antioxidant status were performed, which has not been previously reported.

**MATERIALS AND METHODS**

**Collection and Authentication of *P. betle***

*P. betle* plant saplings were purchased from Tamilnadu Horticulture Institute, Chennai and the plants were grown in a well-defined soil and packed in a pot with appropriate water and sunlight. The young leaves were collected from the grown plant and authenticated by the Siddha Central Research Institute, Chennai (Central Council for Research in Ayurveda and Siddha, New Delhi, under the Ministry of Health & Family Welfare, Government of India).

**Preparation of Betle Leaf Extracts**

Aqueous betle leaf extract was prepared by boiling 10% (w/v) leaves the deionised distilled water and allowed to concentrate to about 90% (w/v). The leaves were sieved out and the crude aqueous extract obtained was filtered. 1mL aliquots of the crude extract were dried over night using speed vacuum concentrator. The dried pellets of the crude aqueous extract samples were refrigerated at -40°C until further use. The pellets were then weighed, dissolved and diluted to suitable concentration as mentioned below.
Different concentrations of BLE (1, 2 and 3mg/ml) were prepared with distilled water, PBS of pH 7.4, 0.1% diastase and 1:10 diluted saliva. The isolated trophoblasts of normotensive and preeclamptic placentas were incubated with various concentrations of BLE at different time intervals (1h, 2h, and 3h). Following the incubation, cell viability was assessed and the effective concentration (1mg/ml and 1h) at the time having maximum efficiency was utilized for further studies.

**Selection of Subjects**

The study was carried out for a period of 6 months. The placental samples were obtained from a private hospital at Chennai. Informed consent was obtained from the subjects, and the study was approved by Ethical Committee (IEC/S/BWC/0610/ 2014). Placenta was collected from both normal (n = 10) and preeclamptic (n = 10) pregnant women in the age group of 20-40 years, post delivery. Patients with preeclampsia were defined by the following laboratory criteria: blood pressure greater than 140/90 mmHg but less than 160/110 mmHg, proteinuria > 300 mg/L, and xanthine oxidase activity of approximately 2.6 units/ mg protein. Patients with severe preeclampsia and other severe maternal complications were excluded from the study.

**Isolation of Trophoblast**

Third-trimester villous trophoblast cells, which were used for the comparison, were isolated from term placentas by the method of Douglas (19). Briefly, placental villi were cut and thoroughly washed to remove blood. After that, they were incubated for four times in a digestion medium composed of HBSS, containing trypsin and deoxyribonuclease at 37°C for 30 min in a water bath with continuous shaking. The dispersed cells were layered on the top of a discontinuous 5%–70% percoll gradient and centrifuged at 507 Xg for 25 min. The intermediate layers (density between 1.048 and 1.062) containing cytотrophoblast cells were removed and washed, and the cell viability was determined by trypan blue exclusion. Following trophoblast isolation, cells were seeded at a density of approximately 1.6 x 106 cells per well in 6-well plate. The complete culture medium constituted of M199, 2 mM glutamine, 10% FBS. All the experiments were performed on the same day of trophoblast isolation to overrule the influence of the cultivation process.

**2,2-Diphenyl-1-Picrylhydrazyl (DPPH) Radical Scavenging Activity**

DPPH radical scavenging ability was determined by the method of Viturro *et al*. (20). The scavenging activity was expressed as mg/mL.

**Hydroxyl Radical Scavenging Assay**

Hydroxyl radical scavenging activity of the extracts was determined according to the method reported by Klein *et al*. (21).

**Assessment of Cell Viability**

The viability of the isolated trophoblast was assessed by trypan blue exclusion method (22). Briefly, 10 μL of the isolated cells was mixed with 0.4% trypan blue solution and was allowed to react for 5 min in a moist chamber. The viable unstained cells were then counted using a hemocytometer. The results were expressed as % of viability.

**Estimation of Lipid Peroxide (LPO)**

LPO was determined by thiobarbituric acid (TBA) reaction using the method of Ohkawa *et al*. (23). The LPO content was expressed as nanomoles of MDA/ mg of protein.

**Estimation of Total Antioxidant Capacity (TAC)**

TAC analysis was performed by the method of Prieto *et al*. (24). The total antioxidant activity was expressed as Trolox equivalent in mmol/L.

**Statistical Analysis**

Data were analyzed using statistical software package version 7.0. Student’s t-test was used to ascertain the significance of variations between normotensive and preeclamptic placental trophoblast. All data were presented as mean ± SD. Differences were considered significant at p < 0.05, p < 0.01, and p < 0.001.

**RESULTS**

Figure 1: 2,2-Diphenyl-1-Picrylhydrazyl (DPPH.) radical scavenging activity of different extracts of *Piper betle*. Values are mean ± SD

ABLE: Aqueous extract of betle leaf; PBLE: Phosphate buffered saline extract of betle leaf

DBLE: Diastase extract of betle leaf; SBLE: Salivary extract of betle leaf
From the radical scavenging activities, 1mg/mL concentration of various extracts of \textit{P. betle} was found to be the effective concentration having the significant radical scavenging property, and the SBLE is the most efficient.

**Figure 3:** Outer figure represents the effective duration of incubation of the isolated normotensive and preeclamptic placental trophoblasts with 1 mg/ml of different BLE (ABLE, PBLE, DBLE and SBLE) at different times of incubation (1hr, 2hrs and 3hrs) and the effective time of incubation was standardized. Percentage of significance for the comparative analysis is given in Table 1.

**Figure 3** represents the Viability of normotensive and preeclamptic placental trophoblast. Outer figure shows effect of ABLE, PBLE, SBLE and DBLE on the viability of normotensive and preeclamptic placental trophoblast at different time intervals and the values are expressed as means ± SD (n = 10) and the “p” values are given in Table 1.

NT, normotensive placental trophoblast; PE, preeclamptic placental trophoblast.

ABLE: Aqueous extract of betle leaf; PBLE: Phosphate buffered saline extract of betle leaf

DBLE: Diastase extract of betle leaf; SBLE: Salivary extract of betle leaf

**Figure 3:** Inner figure represents the ability of placental trophoblast to maintain cell viability assessed by Trypan blue staining. Cell viability in preeclamptic placental trophoblast was decreased, which is proved by 80% viable cells when compared to normotensive placental trophoblast, where 95% viable cells were observed. It represents the 16% difference between normotensive and preeclamptic placental trophoblast viability.

**Figure 3:** Inner figure 3 represents the Viability of normotensive and preeclamptic placental trophoblast on the ability of placental trophoblast to maintain cell viability assessed by Trypan blue staining. Cell viability in preeclamptic placental trophoblast was decreased, which is proved by 80% viable cells when compared to normotensive placental trophoblast, where 95% viable cells were observed. It represents the 16% difference between normotensive and preeclamptic placental trophoblast viability.

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Time of Incubation

**Figure 3:** Outer figure represents the effective duration of incubation of the isolated normotensive and preeclamptic placental trophoblasts with 1 mg/ml of different BLE (ABLE, PBLE, DBLE and SBLE) at different times of incubation (1hr, 2hrs and 3hrs) and the effective time of incubation was standardized. Percentage of significance for the comparative analysis is given in Table 1.

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Table 1: Percentage of Significance for the Comparative Analysis for Figure 3

<table>
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<th>SBLE</th>
<th>NTSBLE</th>
<th>NT Vitamin C</th>
<th>PE</th>
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Values represent the percentage of significance for 4 different extracts (ABLE, PBLE, SBLE and DBLE) at a different time interval (1h, 2h and 3h) between normotensive and preeclamptic placental trophoblast. In the table, a column filled with dark grey demonstrates the effective incubation time in preserving the cell viability. DBLE secured the second position in preserving cell viability. Other two extracts ABLE and PBLE were found to be moderately efficient in preserving cell viability.

**Figure 4**: Level of LPO in normotensive and preeclamptic placental trophoblast with and without SBLE. Values are expressed as means ± SD (n = 10).

NT, normotensive placental trophoblast; NTSBLE, normotensive placental trophoblast with SBLE; PE, preeclamptic placental trophoblast; PESBLE, preeclamptic placental trophoblast with SBLE.

#p < 0.001 when compared to normal trophoblast without SBLE.

*p < 0.01 when compared to preeclamptic trophoblast without SBLE.

NS not significant when compared to normotensive trophoblast without SBLE.

The extent of lipid peroxidation was determined by measuring the release of MDA. Figure 4, the level of MDA was significantly higher in preeclamptic placental trophoblast by 60% (p < 0.001) than normotensive placental trophoblast. In preeclamptic placental trophoblast, SBLE significantly decreased the level of MDA by 29% (p < 0.01) when compared to preeclamptic placental trophoblast without SBLE.

**Figure 5**: Level of TAC in normotensive and preeclamptic placental trophoblast with and without SBLE. Values are expressed as means ± SD (n = 10).

NT, normotensive placental trophoblast; NTSBLE, normotensive placental trophoblast with SBLE; NTC, normotensive placental trophoblast with Vitamin C; PE, preeclamptic placental trophoblast; PESBLE, preeclamptic placental trophoblast with SBLE; PEC, preeclamptic placental trophoblast with Vitamin C.

#p < 0.001 when compared to normotensive trophoblast without SBLE.

*p < 0.01 when compared to preeclamptic trophoblast without SBLE.

@p < 0.05 when compared to preeclamptic trophoblast without SBLE.

NS not significant when compared to normotensive trophoblast without SBLE.

Figure 5, shows that the total antioxidant capacity of the SBLE it was determined to assess the antioxidant capacity of SBLE in placental trophoblast and the level of TAC was significantly lower in preeclamptic placental trophoblast by 30% (p < 0.001) than normotensive placental trophoblast. In preeclamptic placental trophoblast, SBLE significantly increased the level of TAC by 21% (p < 0.01) when compared to preeclamptic placental trophoblast without SBLE.

**DISCUSSION**

Preeclampsia, multisystem disease of unknown etiology and its pathophysiology may begin with abnormal placentation resulting in systemic dysfunction, and deficient spiral artery remodeling leads to placental ischemia. In parallel, oxidative stress markers like free radicals, oxidized lipids are released into the maternal circulation which is responsible for the insufficient trophoblast invasion and plugging of maternal vessels in the periphery (25). However, de-
factors in these processes can ultimately lead to severe complications during pregnancy and resulting in hypertensive disorder like PE. Treatment of the disease like preeclampsia with modern medicine is often associated with serious side effects that affect both mother and fetus: herbal remedies having a synergistic effect to overcome the defective trophoblast functions and traditional method of consumption may be a suitable alteration for these patients.

P. betle is such a plant, and the leaf extracts of PB has antioxidant (26), anti-hypercholesterol (27), and vasodilatory actions. PB showed vasorelaxation on isolated perfused mesenteric artery preparation (28), which may be due to the presence of various polyphenols compounds like hydroxy chavicol, chatecol, allylpyrocatecol which inhibits lipid peroxidation. This could be attributed to its ability to scavenge free radicals involved in initiation and propagation steps of lipid peroxidation (29).

DPPH is a stable nitrogen-centered free radical commonly used for testing radical scavenging activity of plant extracts. The DPPH free radical scavenging activity of a compound indicates its hydrogen-donating tendency. A high correlation between DPPH radical scavenging activities and total polyphenolics has been reported by many researchers (30). Similarly, the antioxidant activity of plant extracts is also correlated with their hydroxyl radical scavenging activity (31). Coherent with this our present study also evidences on the antioxidant property for different extracts of BL, SBLE counterbalances the increased utilization of antioxidants to counterbalance the free radical-induced oxidative damage. The diastase extract of the betle leaf was found to have potential antioxidant efficacy in the management of hypertensive mediated OS-AO imbalance.

Antioxidants from natural compounds of medicinal plants are useful in inhibiting or preventing the deleterious consequence of oxidative stress. Badrul et al. (33) earlier demonstrated that P. betle leaves are rich in polyphenolic compounds, such as flavonoids, tannins, and phenolic acids. These are responsible for the multiple biological effects of BL which includes antioxidant activity. When BL is chewed it mixes with saliva which might enhance the bioactive property of the components present in BL. The efficiency of plant extracts in inhibiting lipid peroxidation is an excellent measure of assessment of antioxidant potential. The high scavenging property of P. betle may be due to hydroxyl groups existing in the phenolic compounds, and these are responsible for the antioxidant activity. The antioxidant activity is exhibited by inactivating lipid free radicals or preventing decomposition of hydroperoxides into free radicals (34). Consistent with this study we observed that the various extract of BL could decrease the level of LPO and quench the free radicals along with increasing the total antioxidant capacity. Among the different extracts of BL, SBLE counterbalances the preeclampsia mediated oxidative stress effectively. This may be attributed to its ability to scavenge free radicals which may be due to the presence of secondary metabolites in BL and its interaction with salivary antioxidants. Diastase is also equally potent in counter balancing free radicals and the associated pregnancy induced hypertension.

Sujatha et al. (35) reported that the antioxidant potency of the betle leaf aqueous extract was poor. However, we found that when the aqueous extract was mixed with diastase in vitro or in vivo by the process
of chewing the antioxidant properties increased significantly. Hence, these enzymatic/salivary extracts from leaves could be used as a potential water-soluble antioxidant source which can be effective in the management of hypertensive disorder like PE. BL itself has higher antioxidant potency when leaves are chewed the antioxidant efficacy of BL further enhanced by of saliva. Alternatively, a diastase-BL mixer may be prepared and made available to pregnant women for minimizing the complication that might arise due to pregnancy.

The present study clearly indicates that the salivary extract of *P. betle* was found to be more potential in maintaining the altered redox status of trophoblasts. It may be concluded that the active components present in SBLE and DBLE might be involved in the induction of antioxidant efficacy. Therefore, dietary supplementation of BL orally in particular by chewing might effectively render beneficiary effect in the management of preeclamptic mediated oxidative stress and may help to overcome the other complications of PE.

**Conflict of Interest:** The authors report no conflict of interest.

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The stability and Antifungal Activity of Different Concentrations of Triple Antibiotic Paste against Candida albicans- An In-vitro Study

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ABSTRACT

Introduction and Aim: Biomechanical preparation alone cannot completely eliminate all the potential source of infection, the use of antimicrobial disinfectants is necessary for between appointments to successfully eradicate the colonizers of root canals to prevent reinfection. The aim of this study is to evaluate the antifungal effect of various concentrations of triple antibiotic paste against Candida albicans by antimicrobial susceptibility testing.

Materials and Methods: The stability and antimicrobial effect of triple antibiotic paste were tested by antibiotic susceptibility testing against C. albicans by using Agar disc diffusion method over the period of one day, 3 days, 7 days, 14 days and 21 days. The samples were divided into three groups: Group 1, 1% TAP; Group 2, 2% TAP; Group 3, 3%; Group 4, Chlorhexidine (control). The zone of inhibition was measured after 24 hours and recorded in millimeters, and the same procedure was repeated after 3 days, 7 days, 14 days and 21 days.

Results: Higher concentration of triple antibiotic paste (3%) showed a superior antifungal effect compared to 1%, 2% TAP and chlorhexidine. The antifungal efficacy increased more rapidly after 21 days compared to 24 hours, 3 days, 7 days.

Conclusion: Triple antibiotic paste when used at greater concentration had increased antifungal effect compared to other groups. This study showed that the antifungal effectiveness increases over a period of 21 days.

Key Words: Antibiotics; Antifungal; Candida albicans and Ciprofloxacin and Metronidazole

INTRODUCTION

The successful root canal treatment lies in complete debridement of microbes from the root canals. Chemico-mechanical preparation alone cannot eliminate all the potential source of infection, the use of antimicrobial disinfectants is necessary for between appointments to successfully eradicate the colonizers of root canals to prevent reinfection (1). Primary molars have many lateral and accessory canals which harbour many microbes which become out of reach for normal irrigation solutions (2). So the use of intracanal medicaments is required to disinfect the root canals which are considered important for root canal treatment. Root canal microbiota consists of wide species of aerobic and anaerobic bacteria which persist even after adequate irrigation. Studies have shown that candida is seen predominantly in root canals with persistent apical periodontitis (3, 4, 5). C.albicans is commonly associated with periapical lesions and is also a resistant pathogen to non-surgical endodontic therapy. Recently, it is alarming that the C.albicans is seen both in primary and resistant root canal infections since it has the ability to easily invade the dentinal tubules and enter the root canal system (6, 7). One of the ideal property of intracanal medicament is used to destroy the remaining bacteria from the root canals after canal instrumentation. Various intracanal medicaments have been over many years like formocresol, glutaraldehyde, calcium hydroxide, CMCP etc. Though calcium hydroxide is widely used due to its effect on various endodontic pathogens, it is said that calcium hydroxide has limited effect against certain resistant species such as C.albicans. Candida albicans is predominantly present in the root canals with periapical
lesions because it has many virulence factors which are responsible for its survival after chemo-mechanical preparation. And also *C. albicans* has the ability to penetrate deep into the dentinal tubules and adapts it in the high alkaline environment (8). Antibiotics are essential for inhibition of bacterial infections. A single antibiotic does not provide adequate sterilization and eliminate the microbes present in the root canals. Over the past decades, the resistance of micro-organisms to antibiotics has raised due to regular use of antibiotics for various infections (9). Therefore to eliminate the variety of resistant microbes present in the complex root canal system, a mixture of antibiotics like ciprofloxacin, metronidazole, and doxycycline introduced by Hoshino which is used as an aid to nonsurgical endodontic therapy. A periapical lesion responds to nonsurgical endodontic therapy by proper disinfection of the root canal system with the help of intracanal medicament. The cariology research unit of the niigata university School of Dentistry introduced the concept of “Lesion sterilization and tissue repair therapy”. Nonsurgical endodontic therapy such as local application of antibiotics in the form of “LSTR” has been on the rise over the recent years to avoid the possible side effects of systemic antibiotics (10). Literature has shown that a mixture of Ciprofloxacin, Metronidazole, and Doxycycline produce sterilization of root canal dentin by penetrating and completely eradicating the resistant bacteria. Various studies evaluated the antifungal effectiveness of TAP against *C. albicans* but there is a lacunae of research regarding the stability of triple antibiotic paste at different concentrations against *C. albicans* over a period of 21 days. This study aims in determining the stability of TAP by evaluating the antifungal effect of various concentrations of *C. albicans* by antibiotic susceptibility testing.

**MATERIALS AND METHODS**

The study approval was obtained from the institutional review board, Saveetha Dental College. Triple antibiotic paste made of metronidazole, ciprofloxacin, doxycycline (1:1:1) were prepared by using sterile mortar and pestle with different concentrations of triple antibiotic paste using propylene glycol as a carrier in the ratio of 1:1 and stored under sterile conditions. The antimicrobial effect of triple antibiotic paste was tested by antimicrobial susceptibility testing against *C. albicans* by using Agar disc diffusion method over the period of one day and after 3 days. The samples were divided into three groups: Group 1, 1% TAP; Group 2, 2% TAP; Group 3, 3%; Group 4, Chlorhexidine (control). Mueller- Hinton agar plates were used and inoculated with *C. albicans* and sterile discs with different concentrations of Triple antibiotic paste were placed over it and incubated for 24 hours at 37 °C for 24 hours (11). The zone of inhibition was measured after 24 hours and recorded in millimeters and the same procedure was repeated after 3 days, 7days, 14 days and 21 days. The zone of inhibition was measured after 24 hours and recorded in millimeters.

**RESULTS**

The inhibitory zones at various concentrations of triple antibiotic paste and chlorhexidine against *C. albicans* are shown in [Figure-1] and [Table-1]. From the disc diffusion method, it was observed that the zone of inhibition for [Group 1; 1% TAP] was 30mm, 21 mm and 18mm at 24h, 3 days,7 days, 14 days and 21 days [Group 2; 2% TAP] was 33mm, 29mm and 22mm [Group 3; 3% TAP] was 36mm, 30mm and 28mm at 24h, after 3 days,7 days,14 days and 21 days respectively. [Group 4; Chlorhexidine] showed a zone of inhibition of 14mm, 15mm and 16mm at 24h, after 3 days,7 days,14 days and 21 days respectively. The greater antifungal effect of TAP was seen for higher concentration and consequently decreased over the period of 72 hours [Table/Figure-1] and [Table/Figure-2]. The antifungal effect of triple antibiotic paste decreased after 3, 7 14 and 21days. But chlorhexidine showed a greater zone of inhibition after 3 and 7 days compared to 24 hours of incubation against *C. albicans*.
Table 1: Comparison of Zone of Inhibition of 1%, 2%, 3% Triple antibiotic paste and Chlorhexidine against C.albicans

<table>
<thead>
<tr>
<th>Sample</th>
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<tr>
<td></td>
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<tr>
<td>1% Triple Antibiotic Paste</td>
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<tr>
<td>Chlorhexidine</td>
<td>14mm</td>
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</table>

DISCUSSION

The ultimate success of root canal treatment lies in thorough mechanical and chemical debridement of all the microorganisms harbouring the complex root canal system. In spite of effective root canal irrigation, bacteria have the ability to resist and survive in the anaerobic conditions and cause recurrent infections (12). Endodontic infection is polymicrobial in nature. Hence intracanal medicaments with the appropriate antimicrobial property are required in between appointments to sterilize the root canals. For effective sterilization of this complex root canal system, a combination of antibiotics is necessary, since the single medicament or an antibiotic cannot provide the excellent results and it can increase the spread of resistant microbes (13). An intracanal medicament, when used for a long period of time, decreases the resistant microorganisms by exhibiting synergistic property (14). Over the past many years, numerous intracanal medicaments have been used. Though calcium hydroxide proved to be effective, it has less effective against the microbes present deep in the dentinal tubules which might infect the root canals by penetration (15). So chlorhexidine was used as a control medicament as it has the wide spectrum of antimicrobial action against C.albicans. The triple antibiotic paste which consists of metronidazole, ciprofloxacin, and minocycline was used earlier. But, minocycline binds to calcium ions through chelation process and produces the compound which causes tooth discolorations (15). A study by Jong HK, 2010 has shown that even a short application of minocycline causes discoloration of the teeth (16). Metronidazole is very effective against microbes present deep in the root canals. The results of this present study showed that the antifungal effect of chlorhexidine increased after 7 days compared to the short period of time, which explains its substantivity. This might be due to its property of substantivity which increases over the period of time. Many antibiotic-corticosteroid combinations have been used in the research as a root canal medicament due to its superior anti-inflammatory property (17).

This in vitro study compared various concentrations of triple antibiotic paste made up of metronidazole, ciprofloxacin, and doxycycline to test its antifungal activity against Candida albicans as it the most predominantly seen fungal species in the cultures with secondary root canal infections (3). It harbours in the root canal dentine due to its collagenolytic property and colonizes the root canal (15). From this study, it is clear that higher concentration of triple antibiotic paste showed a superior antifungal effect compared to lower concentration. And also TAP showed a better effect compared to chlorhexidine group. This was in disagreement with a study done by Attia et al, 2015 which reported that 2% Chlorhexidine gel showed a favorable result compared to antibiotic paste and calcium hydroxide (18). The difference may be due to the use of chlorhexidine gel which has a better penetrating property compared to chlorhexidine used in this study (18). This study did not evaluate the antifungal effect of TAP under the clinical scenario. In future, more in vivo and in vitro studies are advocated to determine the stability and antimicrobial effect of TAP under proper oral conditions against C.albicans for a longer period of time to confirm the findings obtained from this study. Within the limitations of this in vitro study, higher concentrations of triple antibiotic paste can effectively eliminate C.albicans from the root canals compared to chlorhexidine. The antifungal effect of chlorhexidine increased over a period of time thereby confirming its substantivity. Further research must be conducted to test the effectiveness of various intracanal medicaments against other common microbes isolated from the root canal system.  

ACKNOWLEDGEMENT

Authors would like to thank all the staffs, colleagues and department of microbiology for their guidance and support.

REFERENCES


Divya, Sujatha: The stability and antifungal activity……………… an in-vitro study
Effect of Extraction on the Phenolic Compounds and Antioxidant Activities of Selected Medicinal Plants used in the Treatment of Dengue Fever

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ABSTRACT

Introduction and Aim: In present study, we investigated the effects of five different solvents, namely Hexane, Ethyl acetate, Acetone, Ethanol (100% V/V) and Aqueous (70% V/V Ethanol) on the extractability of some of its antioxidant compounds, total phenols, and tannins in 9 selected medicinal plants listed in table 1, used for treating Dengue fever.

Materials and Methods: The antioxidant activities of each individual extract were evaluated through DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging activity and reducing power assay using butylated hydroxyl toluene (BHT) and ascorbic acid as a standard; its scavenging concentration (SC50) values were also calculated. The total phenolic content was measured by Foliniocalteau method using Gallic acid as a standard and Tannins were estimated by Folin’s Denis method using Tannic acid as a standard respectively.

Results: Of all the solvents employed ethanol and acetone showed the highest amount of total phenols. Tannins extractability was found to be good with almost all the solvents, but moderate activity is observed in an aqueous solvent. Amongst the tested herbal extracts, extracts of Zingiber officinale and Santalam album showed the highest anti-oxidant activities in both DPPH and reducing power assay. Their anti-oxidative activities were comparable to butylated hydroxyl toluene (BHT) and ascorbic acid.

Conclusion: These herbs are safe and inexpensive sources of natural antioxidants. In addition, a strong correlation between DPPH and reducing power assay implied that anti-oxidants in these herbs were capable of scavenging the free radicals and also in reducing oxidants.

Key Words: Medicinal plants, Anti-oxidant activity- scavenging DPPH radical scavenging capacity/ Reducing power assay, Total phenolic content -Total tannins, SC50.

INTRODUCTION

During the normal metabolic process in aerobic cells, free radicals are generated that readily react with the lipids, nucleic acids, proteins, sugars, and sterols, which might lead to the development of neurodegenerative diseases. To prevent the onset of these diseases, high amount of antioxidants need to be present in the body. The most applicable and healthy way to improve the antioxidant levels in the body is by consuming various naturally available plant resources. Hence studies about commercial exploration and utilization of plant source as antioxidants are of increasing interest.

Oxidative stress has been shown to play an important role in the development of anaemia in Dengue. Oxidative stress arises when the balance between oxidants and antioxidants is tipped in favour of the former. This phenomenon may be influenced by exogenous agents but also by endogenous ones such as viruses. There is now much evidence that oxidants play a complex role in viral diseases, starting from influences on host cell metabolism and viral replication and extending to desirable inactivating effects on viruses and less desired toxic effects on host tissue. Recently, several approaches to study antioxidant consumption and markers of free radical-induced damage have been described. Oxidative damage may affect all biochemical compounds.
including lipids, proteins, nucleic acids, carbohydrates, and macromolecules of connective tissue. This process might cause the loss of fluidity, leads to the destruction of cell membrane because of structural deformity and the production of lipoperoxides and their products, such as malondialdehyde (MDA) and 4-hydroxyalkenals (4-HDA). Indeed, increase in total antioxidant status has been shown to be important in recovery from dengue. Antioxidants that can inhibit or may delay the oxidation of an oxidizable substrate in a chain reaction appear to be very important. Synthetic antioxidants are broadly used, but their use is being restricted nowadays because of their toxic and carcinogenic effects. Thus, interest in finding natural antioxidants, without any undesirable effect, has increased greatly.

A man has been using herbs and plant products for combating diseases since time immemorial. The Indian subcontinent is enriched by a variety of flora-both medicinal and aromatic plants. This is due to the wide diversity of climate conditions in India ranging from deserts to swamplands. Numerous types of herbs have been recognized and cataloged by botanists from the high ranges of the Himalayan tract up to the seashore of Kanyakumari. This extensive flora has been greatly utilized as a source of many drugs in the Indian traditional systems of medicine (1). It is notable that world health organization is showing great interest in drugs from natural sources especially from traditional systems and folklore medicines. Among the natural sources, plants are economic, and they are the source for abundant chemical intermediates to produce new drugs with a fewer side effect. Also, they are also the source of chemical intermediates needed for the production of some drugs.

It has been stated that the antioxidant activity of the plants might be due to their phenolic compounds. The details of the plants considered for present study along with its usefulness was listed in table 1. Studies to date have demonstrated that phytochemicals in common medicinal plants can have complementary and over lapping mechanisms of action, including scavenging of oxidative agents, stimulation of the immune system, hormone metabolism and antibacterial and antiviral effects. Many research have been done for antioxidant activity of medicinal plants, antioxidant activities of A.paniculata (2), S.album (3), Vizianoides (4), C.rotnutus (5), Z.officinale (6), P.nigrum (7), H.corymbosa (8), Pvetiverioodes (9), T.dioica (10) has already been done. However, to our knowledge, no detailed studies on the antioxidant activities of the said medicinal plants with different solvent combinations have been reported.

Generally, for the extraction of polyphenols or other bioactive compounds from plant materials, water and organic solvents (ethanol, methanol, acetone, diethyl ether) are used. Additionally, during the extraction process, the percent recovery depends mainly on the type of solvent and the extraction methods being adapted (11, 12). Depending on the plant materials, the nature of the bioactive compound present also varies. Hence, it is very difficult to recommend a suitable extracting solvent for individual plant materials. The main objective of the present study was to evaluate the effect of various solvents (hexane, ethyl acetate, acetone, ethanol and aqueous) on the extractability of antioxidant compounds such as total phenols, tannins, and anti-oxidant activity (as percent DPPH inhibition activity) of those medicinal plants involved in formulation of Nilavembu Kudineer. We, therefore, hope to provide a suitable base for commercial exploitation as a source of natural antioxidant for medicinal applications.

**MATERIALS AND METHODS**

**Standards and Reagents:** Folin’s reagent (Folin-Ciocalteu reagent), Gallic acid, and 2,2-diphenyl-1-picyrhidrazyl (DPPH) were purchased from Sigma-Aldrich1, USA. Acetone, aluminum chloride, ferrous sulphate, ferric chloride, methanol, potassium chloride, sodium acetate, sodium carbonate and sodium hydroxide were purchased from R & M chemicals (Essex, UK). All the other chemicals used in the present study were of analytical grade quality.

**Sample preparation:** About 100g of each of the powdered plant materials was weighed and macerated with 500 ml of solvents like hexane, ethyl acetate, acetone, ethanol and aqueous sequentially and kept overnight in a shaker. The extract was collected after filtration by using Whatmann No.1 filter paper and then stored. 75 ml of the solvent was added to the residual mixture and then incubated for 24 hrs in a shaker. The extract was again collected using a Whatmann No.1 filter paper. The same procedure was repeated once again, and the solvent was then removed from the extract by reduced pressure below 40 °C, using rotary evaporator which was used for further phytochemical and antioxidant analysis. The residue was refrigerated at 4°C for further use.
Determination of Total Phenolic Content: Amount of phenolic compounds in the extracts was determined by the Folin Ciocalteau colorimetric method and calculated from a calibration curve obtained with Gallic Acid as standard (10mg/10ml). From the standard solution, 0.05 to 0.3 ml was taken and added to different test tubes. Extracts were aliquoted in required concentrations and made up to 1 ml with distilled water and 5ml of folin–ciocalteau (1:10 dilution) was added, and the mixture was incubated for 10 minutes. The absorbance was measured at 765nm in a UV-Visible Spectrophotometer. The results were tabulated.

Determination of Tannin Content: Estimation of extracted tannin concentration in the extract was measured by Folin–Denis method (Schanderi 1970) with minor modifications. The extracts were aliquoted in required concentrations and made up to 1 ml with distilled water and then mixed with 0.5 ml of Folins–Ciocalteau reagent. The reaction mixture was alkalinated by the addition of 1 ml of 15% (w/v) sodium carbonate solution and kept in the dark for 30 min at room temperature. The absorbance of the solution was read at 700 nm using a spectrophotometer, and the concentration of tannin in the extract was determined using pure tannic acid as standard (10mg/100ml).

DPPH Radical Scavenging Activity: The ability of the extracts to annihilate the DPPH radical (1,1-diphenyl-2-picrylhydrazyl) was studied by the method described by (Blois 1958). DPPH is a stable free radical which shows a red-purple color in methanol solution, and it has maximal absorption at 515 nm. This assay is based on the decoloration of DPPH free radical solution due to the free radical scavenging effect of antioxidants. A stock solution of extracts was prepared to the concentration of 1mg/ml.25, 75 and 100 μL of test sample concentrations of dry extracts were dissolved in water, were added to 1.95 ml of the methanolic solution of DPPH (0.1mM) and mixed well. After one-hour incubation, the absorbance value of the sample (A test) was determined by a UV-visible spectrometer (Perkin Elemer Lambda 35). While the absorbance value of a mixture of 1.95 ml of DPPH methanol solution and 50 μl of wa-

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Table 1: Selected medicinal plants for their antioxidant activity with its uses

<table>
<thead>
<tr>
<th>S. No</th>
<th>Botanical Name</th>
<th>Local Name (Tamil)</th>
<th>Family</th>
<th>Parts used for analysis</th>
<th>Traditional uses</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>tonic and vulnerary</td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>Santalum album</td>
<td>Sandanam</td>
<td>Santalaceae</td>
<td>Stem bark</td>
<td>Clear up a dry cough and boosts the digestive system, especially helpfulindiarrea.</td>
<td>Harsha etal, 2014</td>
</tr>
<tr>
<td>4.</td>
<td>Piper nigrum</td>
<td>Milagu</td>
<td>Piperaceae</td>
<td>Fruit</td>
<td>Anticancer, diuretic, laxative,musclerelaxant,antioxidant.</td>
<td>Suchathrachatterjaetal, 2007</td>
</tr>
<tr>
<td>5.</td>
<td>Andrographis paniculata</td>
<td>Nilavembu</td>
<td>Acanthaceae</td>
<td>Aerial part</td>
<td>Anti-microbial, immune stimulant</td>
<td>S.Savithaetal, 2011</td>
</tr>
<tr>
<td>7.</td>
<td>Hedyotis corymbosa</td>
<td>parpadagam</td>
<td>Rubiaceae</td>
<td>Aerial part</td>
<td>Anti-malarial,antiinflammatory and antioxidant</td>
<td>J.M.Sasikumar etal,2010</td>
</tr>
</tbody>
</table>

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mixture was then incubated for 20 min at 50°C. Then 2.5 ml of 10% tri chlor acetic acid was added to the mixture and centrifuged at 3000 rpm for 10 min. Finally, 1.25 ml from the supernatant was mixed with 1.25 ml of distilled water and 0.25 ml FeCl₃ solution (0.1%, w/v). The absorbance was measured at 700 nm using corresponding concentrations of ascorbic acid as the standard. The increased absorbance of the reaction mixture indicates increased reducing power. The percentage of inhibition was measured by the formula.

Radical scavenging activity % = [(Absorbance of Standard – Absorbance of the test sample) / (Absorbance of Standard)] x 100.

Statistical Analysis: The results of the present study (samples run in triplicates, n = 3) are represented as mean values ±S.D. Analysis of variance was performed, and significant differences between mean values were determined by Tukey’s multiple comparison test and post hoc test at a level of significance of p < 0.05. Statistical analyses were conducted using SPSS 17.0 (SPSS Inc., Wacker Drive, Chicago, USA).

RESULTS

Total Phenols: Phenolic compounds in plants are known to act as free radical scavengers, and it has been opined that the antioxidant activity of most of

<table>
<thead>
<tr>
<th>Solvent</th>
<th>C.rotundus</th>
<th>Z.officinale</th>
<th>P.pungens</th>
<th>T.dioica</th>
<th>P.vetiveroides</th>
<th>V.zizanoides</th>
<th>H.corymbosa</th>
<th>A.paniculata</th>
<th>S.album</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hexane</td>
<td>213.17±25.62</td>
<td>206.1±16.02</td>
<td>212.6±26.86</td>
<td>463±15.25</td>
<td>143.67±11.50</td>
<td>35.47±7.606</td>
<td>204.42±5.35</td>
<td>167.8±37.26</td>
<td>6.90±2.325</td>
</tr>
<tr>
<td>Ethylacetate</td>
<td>61.30±3.45</td>
<td>38.33±6.50</td>
<td>397.7±7.6</td>
<td>498.9±29.52</td>
<td>25.17±4.19</td>
<td>106.35±8.67</td>
<td>51.33±3.57</td>
<td>36.58±10.77</td>
<td>5.03±2.86</td>
</tr>
<tr>
<td>Acetone</td>
<td>357.3±13.67</td>
<td>712.6±32.56</td>
<td>690.5±58.5</td>
<td>577.1±38.18</td>
<td>379.05±27.65</td>
<td>216.87±33.29</td>
<td>372.9±51.39</td>
<td>264.4±66.80</td>
<td>626±110.</td>
</tr>
<tr>
<td>Ethanol</td>
<td>429.32±2.42</td>
<td>688.93±23.0</td>
<td>622.6±16</td>
<td>498.6±29.7</td>
<td>560.3±27.75</td>
<td>237.3±10.03</td>
<td>189.9±27.86</td>
<td>403.0±12.39</td>
<td>402.7±12.11</td>
</tr>
<tr>
<td>Aqueous</td>
<td>1.88±2.05</td>
<td>272.17±23.62</td>
<td>37.58±10.41</td>
<td>387.5±24.0</td>
<td>387.5±24.0</td>
<td>190.3±13.47</td>
<td>104.48±34.57</td>
<td>87.05±3.11</td>
<td>403.4±3.884</td>
</tr>
</tbody>
</table>

Table 2: Total phenolics content of 9 medicinal herbal extracts (mean±S.D, n=3)

Vishwarohini, Padmini: Effect of Extraction on the Phenolic….in the Treatment of Dengue Fever
the plant produce is mainly due to the presence of phenolic compounds. Antioxidant mechanism of polyphenolic compounds is based on their hydrogen donating and metal ion chelating abilities (13).

Table 2, shows the extraction yield of total phenols with different solvents. According to the obtained results, it was evident that both solvents namely acetone and ethanol had highest levels of total phenols which is followed by absolute solvents. Overall, considering all the solvent systems, the amount of total phenols recovered by acetone and ethanol was (712.6 ±32.56 mg GAE/100 g) and (688.93±23.0 mg GAE/100g) in Z.officinale which was comparatively higher than other solvents, it is also reported that lower levels of phenol content is expressed in hexane (6.90 mg GAE/100g) and ethyl acetate (5.03 ±2.86 mg GAE/100g) extract of S.album, still lower level is present in aqueous extract of C.rotundus (1.88 ±2.05mg GAE/100g) respectively. The difference was highly significant (p < 0.001) and up to 120 fold. Furthermore, considering the nine selected medicinal plants of interest, the results are represented graphically and summarized below.

The result showed that the extractable potential of total phenolics was high in solvents like acetone and ethanol. Hexane showed poor extractability in V.zizanoides, but moderate activity is observed with other plant extracts. This difference might be attributed to the variations in the sample preparation, extraction methods and extraction time. These results suggest that the extractability of polyphenols is influenced by the polarity and viscosity of the solvents used (23).

**Total Tannins:** In case of tannins, highest amount were present in ethanolic extracts of C.rotundus, and A.paniculata, Z.officinale, P.nigrum, and S.album expressed its maximum tannins content when a solvent like hexane is employed. P.vetiveroides and T.dioica showed its maximum content in acetone extracts. Maximum activity was expressed in V.zizanoides and H.corymbosa when aqueous / water solvents are used. Overall considering all the solvent systems, the tannin levels was recorded maximum with (113.12±2.62mg TAE/100g) and (112.51±11.84 mg TAE/100g)in hexane and acetone extracts of Z.officinale and P.vetiveroides. However, the levels of tannins were low in hexane extracts of A.paniculata (29.89±0.569mg TAE/100g) and H.Corymbosa(37.5±.676mg TAE/100g). This concludes that the levels of tannins among the medicinal plants varies based on solvent’s extractable potential. It is a well-known fact that in addition to the numerous hydroxyl groups, tannins do have some hydrophobic character due to the presence of the benzene rings in the molecule. Therefore, it could be hypothesized that an appropriate extraction solvent for tannins could have hydrophilic character as well as a degree of hydrophobicity (20). Tannins are also reported to contain anti-microbial, anti-oxidant and anti-inflammatory activity (24).
Antioxidant activity (percent inhibition of DPPH assay): DPPH assay is widely used in the determination of free radical scavenging activity of natural antioxidants, mainly due to its simplicity and high sensitivity. In this assay, an antioxidant donates hydrogen or electron which is then accepted by the DPPH radicals (25). Upon the reduction of DPPH radical to a stable diamagnetic molecule, the colour changes from purple to yellow. DPPH radical itself shows a strong absorption maximum at 515 nm which measured by using a spectrophotometer. In the current study, the selected medicinal herbal extracts potential to scavenge stable DPPH radicals was used to measure their general anti-oxidative effects. The more antioxidants occurred in the extract, the more the DPPH reduction will occur. High reduction of DPPH is related to the high scavenging activity performed by particular sample. SC50 was calculated and summarized in Table 6; SC50 is defined as an amount of antioxidant present in the sample necessary to decrease the initial DPPH concentration by 50%. The lower the

Table 3: Total tannins content of 9 medicinal herbal extracts (mean ±S.D, n=3)

<table>
<thead>
<tr>
<th>Solvent</th>
<th>C.rotundus</th>
<th>Z.officinale</th>
<th>P.nigrum</th>
<th>T.dioica</th>
<th>P.vettiveroides</th>
<th>V.zizanoides</th>
<th>H.corymbosa</th>
<th>A.paniculata</th>
<th>S.album</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hexane</td>
<td>99.22±0.181</td>
<td>113.12±2.62</td>
<td>108.66±1.59</td>
<td>39.5±0.593</td>
<td>37.5±0.676</td>
<td>55.02±1.389</td>
<td>37.60±3.411</td>
<td>29.89±0.569</td>
<td>101.54±3.319</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>105.76±0.421</td>
<td>98.42±0.761</td>
<td>64.03±1.314</td>
<td>92.42±1.451</td>
<td>93.54±1.012</td>
<td>73.49±2.664</td>
<td>83.0±2.765</td>
<td>93.52±1.274</td>
<td>72.63±3.214</td>
</tr>
<tr>
<td>Acetone</td>
<td>105.61±1.652</td>
<td>93.20±0.529</td>
<td>99.11±3.912</td>
<td>98.95±1.025</td>
<td>112.51±11.840</td>
<td>93.53±3.414</td>
<td>89.26±3.782</td>
<td>66.15±0.533</td>
<td>73.84±1.626</td>
</tr>
<tr>
<td>Ethanol</td>
<td>112.33±0.416</td>
<td>109.69±5.249</td>
<td>92.75±0.911</td>
<td>83.60±1875</td>
<td>50.94±2.062</td>
<td>103.63±1.014</td>
<td>85.18±3.030</td>
<td>99.20±3.978</td>
<td>72.57±1.695</td>
</tr>
<tr>
<td>Aqueous</td>
<td>50.11±0.456</td>
<td>46.93±1.047</td>
<td>67.87±0.431</td>
<td>84.21±1.233</td>
<td>106.54±3.817</td>
<td>109.69±3.448</td>
<td>91.18±3.629</td>
<td>72.44±2.270</td>
<td>63.36±2.332</td>
</tr>
</tbody>
</table>

Antioxidant activity (percent inhibition of DPPH assay): DPPH assay is widely used in the determination of free radical scavenging activity of natural antioxidants, mainly due to its simplicity and high sensitivity. In this assay, an antioxidant donates hydrogen or electron which is then accepted by the DPPH radicals (25). Upon the reduction of DPPH radical to a stable diamagnetic molecule, the colour changes from purple to yellow. DPPH radical itself shows a strong absorption maximum at 515 nm which measured by using a spectrophotometer. In the current study, the selected medicinal herbal extracts potential to scavenge stable DPPH radicals was used to measure their general anti-oxidative effects. The more antioxidants occurred in the extract, the more the DPPH reduction will occur. High reduction of DPPH is related to the high scavenging activity performed by particular sample. SC50 was calculated and summarized in Table 6; SC50 is defined as an amount of antioxidant present in the sample necessary to decrease the initial DPPH concentration by 50%. The lower the
SC50 value, the higher is the antioxidant activity. SC50 is calculated by linear regression of plot. Among the selected plants *Z.offinicale* and *S.album* showed maximum anti-oxidative activities in all the solvent systems, compared with BHT as standard. However, the highest percentage inhibition (93.61%) was recorded with ethyl acetate extract of *Z.offinicale*, and the lowest (13.58%) is recorded in Hexane extract of *P.vettiveroides*. Based on the results shown in Table 5, it was evident that the percentage inhibition of DPPH was increased with the corresponding increase in sample concentration. Statistical analysis (one-way ANOVA) of the results obtained indicate that the extracts have significantly high anti-oxidant potential (P<0.001) due to high total phenolic content, reducing power and free radical scavenging activity.

Table 4: Standard – BHT – Inhibition of DPPH (%) (Mean ±S.D, n=3)

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Conc. (µg)</th>
<th>Crotundus</th>
<th>Z.offinicale</th>
<th>P.nigrum</th>
<th>T.dioica</th>
<th>P.vettiveroides</th>
<th>V.zizanoides</th>
<th>H.corymbosa</th>
<th>A.paniculata</th>
<th>S.album</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hexane</td>
<td>25</td>
<td>77.6±2.10</td>
<td>73.6±1.25</td>
<td>70.3±1.50</td>
<td>65.4±1.40</td>
<td>60.5±1.75</td>
<td>55.4±1.90</td>
<td>50.4±2.15</td>
<td>45.5±2.30</td>
<td>40.7±2.70</td>
</tr>
<tr>
<td>Ethylacetate</td>
<td>75</td>
<td>73.6±1.25</td>
<td>68.5±1.76</td>
<td>64.3±2.05</td>
<td>59.2±2.45</td>
<td>54.3±2.85</td>
<td>49.3±3.10</td>
<td>44.3±3.45</td>
<td>39.4±3.65</td>
<td>34.5±3.95</td>
</tr>
<tr>
<td>Acetone</td>
<td>100</td>
<td>70.6±1.76</td>
<td>65.5±2.06</td>
<td>60.4±2.45</td>
<td>55.4±2.85</td>
<td>50.4±3.25</td>
<td>45.4±3.65</td>
<td>40.4±3.95</td>
<td>35.4±4.25</td>
<td>30.5±4.55</td>
</tr>
<tr>
<td>Ethanol</td>
<td>25</td>
<td>70.6±1.76</td>
<td>65.5±2.06</td>
<td>60.4±2.45</td>
<td>55.4±2.85</td>
<td>50.4±3.25</td>
<td>45.4±3.65</td>
<td>40.4±3.95</td>
<td>35.4±4.25</td>
<td>30.5±4.55</td>
</tr>
<tr>
<td>Aqueous</td>
<td>75</td>
<td>67.6±2.06</td>
<td>62.5±2.35</td>
<td>57.4±2.75</td>
<td>52.4±3.15</td>
<td>47.4±3.55</td>
<td>42.4±3.95</td>
<td>37.4±4.25</td>
<td>32.4±4.55</td>
<td>27.5±4.85</td>
</tr>
</tbody>
</table>

Earlier, it has been opined that with the change of solvent polarity, vapor pressure and viscosity, the type of antioxidant compound being dissolved in the solvent also varies. Solvents with low viscosity have low density and high diffusivity that allows them to easily diffuse into the pores of the plant materials to leach out the bioactive constituents (22). As a result of this, the antioxidant activity of the extract observed also varies. Hence, the Current study was focused on analyzing the potentiality of solvents towards its extractability of anti-oxidant compounds. Solvents with varied polarity ranges were selected for this purpose. Graphical representation of free radical scavenging activities at three different concentration ranges through DPPH assay is also depicted in fig 1 to 3 and based on the results obtained; high percent inhibition was observed in almost all the solvents extracts of *Z.offinicale*, and *S.album*. Ethyl acetate is found to be a suitable solvent system for *C.rotundus*. In *P.nigrum* maximum percentage inhibition was observed in Hexane extracts. Ethanol and Ethyl acetate solvent systems were effective towards extracting the potentiality of anti-oxidant properties in *T.dioica*. Acetone is found to be a suitable solvent system for *H.corymbosa*, in plants like *A.paniculata* and *V.zizanoides*, ethanol was able to extract its maximum anti-oxidant activities.

Among nine of the selected medicinal plants, 5 plants namely, *Z.offinicale*, *S.album*, *A.paniculata*, *V.zizanoides*, and *T.dioica* showed its maximum free radical scavenging activity with ethanol as a selected solvent. Since the above-listed plants are already available as Nilavembu Kudineer choornam for treating viral fevers like Dengue, and most of the listed plants responded well with ethanol, it is concluded that ethanol could be used as a suitable solvent medium for extracting potential antioxidative compounds and hence recommended for further research.
Figure 1:

**percent inhibition-DPPH assay at 25µg concentration**

- Hexane
- Ethylacetate
- Acetone
- Ethanol
- Aqueous
- BHT

Figure 2:

**percent inhibition-DPPH assay at 75µG concentration**

- Hexane
- Ethylacetate
- Acetone
- Ethanol
- Aqueous
- BHT

Figure 3:

**Percent inhibition-DPPH assay at 100µG concentration**

- Hexane
- Ethylacetate
- Acetone
- Ethanol
- Aqueous
- BHT
BHT – 36.26(SC50)

The scavenging concentration (SC50) of the extracts ranged from 0.335 to 719.14μg/mL (Table 4). Among the tested samples acetone extract of *S.album* showed highest DPPH scavenging activity, This was followed by the ethanolic extract of *H.Corymbosa* and aqueous extract of *S.album*. Ethanolic extracts of hedyotis-corymbosa have been showed to have improving monosodium iodo acetate-induce osteoarthritis in the rat (14). The antioxidative effect of this herb demonstrated in the present study agrees with the previous study (15). Earlier studies opined that the aqueous extract of *Santalam album* possessed anti-microbial and anti-oxidant activities (16), the anti-oxidant effectiveness of this study agrees with the previous study (16). It has been reported that the lowest activity is observed in acetone extract of *V.zizanoides*. However not much literature support is available supporting acetone extract of *V.zizanoides*, But it is proven that crude ethanolic extract of *V.zizanoides* exerted a potent anti-malarial activity against Anaphelesstephensi Liston (17). Ethanolic extracts of *Z.offinciale* and *P.nigrum* also had shown a good scavenging result in our current investigation. Earlier studiess have supported the ethanolic extracts of *Z.offinicale* and *P.nigrum* for its good anti-bacterial activities (18).

A.paniculata, the principal ingredient of Nilavembu Kudineer chornam, possessed its maximum activities in its aqueous and ethanolic extract. Literature evidence also supports *A.paniculata* for its potent anti-viral, anti-microbial and immune stimulant activities (19). Although BHT is considered as a pure compound, its DPPH scavenging activities are less than most of the solvent extracts of the listed medicinal plants taken for the study. The top six highest scavenging efficiency (SE) according to solvent wise exhibited the descending order comparable with standard BHT is summarised below:

<table>
<thead>
<tr>
<th>Solvent</th>
<th><em>S.album</em></th>
<th><em>C.rotundus</em></th>
<th><em>Z.offinciale</em></th>
<th><em>P.nigrum</em></th>
<th><em>T.dioica</em></th>
<th><em>V.zizanoides</em></th>
<th><em>H.corymbosa</em></th>
<th><em>A.paniculata</em></th>
<th><em>P.vettiveroides</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Hexane</td>
<td>21.48</td>
<td>82.32</td>
<td>8.49</td>
<td>18.62</td>
<td>58.44</td>
<td>132.24</td>
<td>117.9</td>
<td>75.28</td>
<td>127.43</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>23.22</td>
<td>44.18</td>
<td>27.29</td>
<td>53.32</td>
<td>56.47</td>
<td>134.46</td>
<td>55.174</td>
<td>76.63</td>
<td>43.36</td>
</tr>
<tr>
<td>Acetone</td>
<td>0.335</td>
<td>39.16</td>
<td>10.94</td>
<td>117.96</td>
<td>64.04</td>
<td>719.14</td>
<td>10.98</td>
<td>31.95</td>
<td>129.26</td>
</tr>
<tr>
<td>Ethanol</td>
<td>12.70</td>
<td>48.21</td>
<td>6.26</td>
<td>21.85</td>
<td>48.27</td>
<td>142.11</td>
<td>1.081</td>
<td>10.83</td>
<td>106.08</td>
</tr>
<tr>
<td>Aqueous</td>
<td>3.05</td>
<td>94.27</td>
<td>12.27</td>
<td>169.39</td>
<td>93.66</td>
<td>169.66</td>
<td>112.62</td>
<td>7.14</td>
<td>47.49</td>
</tr>
</tbody>
</table>

Table 6: Solvent wise summary of SC50 values of the extracts

Comparing the top six highest anti-oxidative samples in the DPPH assay, S.album showed relatively high scavenging activity in the ethyl acetate extract; however total polyphenolic content in the reported plant extract was only 5.03±2.86 mg GAE/100g.Similarly, hexane extract of S.album topped 3rd in the list of top six anti-oxidant scavenging activities, but its total phenolic activity was only 6.90±2.325mg GAE/100g. The results gained from these assays provide simple data that make it possible to classify extracts with respect to their anti-oxidant potential. Because anti-oxidant activity does not always correlate with the presence of large quantities of phenolics, however in our current investigation, both acetone and ethanol extracts of *S.album* is directly correlated with its total polyphenolic contents. Hence, the phenolic content and the anti-oxidant activity data needed to be examined together when screening plant extracts.
Reducing Power Assay: To get a comprehensive evaluation of the anti-oxidative effects of herbal extracts, we decided to further investigate the anti-oxidative effects by using the reducing power assay (21). The reducing power assay is versatile and measures the total reducing capacity of any compounds in the test materials. It can be readily applied to both aqueous and alcohol extracts of plants as well as gives fast and reproducible results. Similar to the results of DPPH scavenging results the extracts of *Z. officinale* and *S. album* exhibited strongest reducing power amongst the tested herbal samples.

All the five organic extracts of the selected medicinal plants showed increasing, reducing power with the increasing concentration. Further, the reducing power and the DPPH free radical scavenging abilities of the extracts show that the extracts could be used as potential free radical inhibitors or scavengers.

### CONCLUSION

The anti-oxidative capacities and total phenolic contents of 9 selected medicinal plants in different solvents were evaluated. This study confirmed that, depending on the type of solvent, the type of anti-oxidant compounds being extracted also vary. Further research is progressing to characterize the anti-oxidant compounds in the selected medicinal plant extracts as well as their mode of action in imparting antiviral activity to combat dengue fever. It is also envisaged that the choornam might possess high antimicrobial activities with the promising potential to be used in drug industries as well.

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Antimicrobial Activity of Marine Endosymbiotic Fungi

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ABSTRACT

Introduction and Aim: Marine endosymbiotic fungi represent an enormous and untapped source of bioactive metabolites. These secondary metabolites help both the endosymbiont and host organism to fight against predators and pathogens. Studies focusing on the antimicrobial activity of marine endosymbiotic fungi are meager, especially from India. Hence the present study was undertaken to evaluate the antimicrobial potential of marine endosymbiotic fungi from southern India.

Materials and Methods: Endosymbiotic fungi associated with various marine organisms were isolated. They were mass cultured. The broth and mycelium were extracted using the organic solvent (EtOAc). The resultant extracts were subjected to antimicrobial assay using 5 clinical isolates of bacteria and fungus.

Results: Of all the twenty-nine endosymbionts tested, twenty-three of them showed antimicrobial activity. The extract derived from the broth of Aspergillus fumigatus and Alternaria alternata showed the highest inhibition zone (7mm) against gram-positive bacteria.

Conclusion: The screening for antibacterial and antifungal activity of the marine-derived endosymbiotic fungi can be pursued for deriving potential antibiotics. At least one of the extracts (either broth or mycelium) of most of the fungi showed antibacterial activity. This is the first report showing the antimicrobial activity of marine endosymbiotic fungi Penicillium javanicum and Aspergillus clavatus.

Key Words: Seaweeds, Sponges, Mangroves, Marine endosymbiotic fungi, Algicolous fungi, Endophyte, Antibacterial activity, Antifungal activity.

INTRODUCTION

The marine environment is unique and holds a rich source of bioactive compounds with interesting chemical diversity (1). Flora and fauna living in this environment have high-stress tolerance capability and are constantly exposed to the high density of bacteria, fungi and viruses. Survival of these organisms depends on how efficient is their antimicrobial defense mechanisms. Most of the marine plants and invertebrates live in close association with the microorganisms. These microorganisms may increase the effectiveness of their host to fight against pathogens by producing secondary metabolites. Of these microbes, the most important is fungal endosymbionts which produce unique compounds which possess high antimicrobial properties.

Only a few reports on antimicrobial activity of marine endosymbiotic fungi exist. Studies concerning the diversity of fungal endosymbionts associated with marine plants, invertebrates and their potential for antimicrobial activities have been performed (2-7). Reports from India coast is scanty (8).

A variety of secondary metabolites showing high antimicrobial activity have been isolated from terrestrial endophytic fungi but studies on the antimicrobial activity of marine endophytic fungi are scarce. Hence the present study has been undertaken to evaluate the antimicrobial potential of marine endosymbiotic fungi from South West coast of India.

MATERIALS AND METHODS

Fresh samples of seaweeds, sponges and mangroves without any signs and symptoms of disease were collected from Kerala (Thirumullavaram, Thangassery,
Kovalam, Vizhinjam, Tamil Nadu (Kanyakumari, Muttom, Rameswaram) and Andaman and Nicobar Islands by handpicking, snorkeling and scuba diving during the period from May 2013 to September 2016. The collected specimens were washed in seawater to remove extraneous matter, followed by sterile seawater and immediately transferred into the sterile conical flask. Then the samples were transported to the laboratory with minimum possible time and processed within 12 hours.

Then the alga was surface sterilized with 70% ethanol for 1 min and shaken in 1.2 % Sodium Hypochlorite (NaClO) solution for 5 min. Samples were then washed three times with sterile distilled water by shaking. Various segments of the surface sterilized alga were cut aseptically and plated on to PDA medium which was supplemented with Chloramphenicol (150 mg/L) to prevent the growth of bacteria. Control of algal thalli before and after sterilization was also plated to check the contamination. All plates were incubated at 28ºC for 3 days or until fungal growth was observed. Using aseptic technique, emergent hyphae were transferred and purified on sterile PDA medium. The pure culture was stored in slants under 4ºC for further studies. The fungal strains thus obtained were identified by classical taxonomic techniques and confirmed by molecular taxonomy (Fig.1 A-F).

The fungal isolates were mass cultured in Sabouraud dextrose broth. They were incubated for 5-7 days at 26ºC. This flask was shaken in periodic intervals to get uniform fungal growth. After 5-7 days the broth was filtered to remove all the mycelial remnants. By fractionation, the Ethyl acetate (EtOAc) soluble extract was obtained. The separated mycelium was macerated and stirred with EtOAc in order to derive the EtOAc soluble fraction. The fractions of mycelia and broth were evaporated to dryness using Rotavapor (BUCHI, Switzerland) at 40ºC under reduced pressure. The dried crude extracts were kept frozen at -20ºC for further analysis.

Antimicrobial assay of the crude extracts of endophytic fungi was carried out against four clinical bacterial (Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Bacillus subtilis) and one fungal isolate(Candida albicans) obtained from Govt. Medical College, Thiruvananthapuram. Disc diffusion assay of the extracts in duplicate was carried out at 200µg/disc. Sterile filter paper disc of 5 mm diameter was used. Positive (Streptomycin and Nistatin) and negative controls (solvent) were run parallely. The zone radius (from the edge of the disc to the edge of inhibition zone) was noted after 24 hours for clinical bacteria and 48 hours for fungi.

RESULTS

A total of 29 endosymbiotic fungi belonging to three classes viz., Eurotiomycetes, Dothideomycetes and Sordariomycetes were collected from the host organisms (Table 1). Both the broth and mycelial crude extracts of endosymbionts were subjected to antimicrobial assay. Among this, only twenty-three species/strains showed antimicrobial activity, of which 11 of them belong to the class Eurotiomycetes, 3 to class Dothideomycetes and 9 of them to the class Sordariomycetes (Table 2).
Table 1: List of Endophytic fungi derived from marine organisms.

<table>
<thead>
<tr>
<th>No</th>
<th>Fungi</th>
<th>Place of Collection</th>
<th>Date of Collection</th>
<th>Source/organism</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Aspergillus clavatus</em></td>
<td>Vizhinjam</td>
<td>09/11/2012</td>
<td>Dermonema virens</td>
</tr>
<tr>
<td>2</td>
<td><em>Aspergillus Sclerotiorum</em></td>
<td>Kanyakumari</td>
<td>27/07/2013</td>
<td>Jania sp.</td>
</tr>
<tr>
<td>3</td>
<td><em>Aspergillus sclerotiorum</em></td>
<td>Vizhinjam</td>
<td>12/10/2012</td>
<td>Gratelouphia lithophila</td>
</tr>
<tr>
<td>4</td>
<td><em>Aspergillus fumigatus</em></td>
<td>Rameshwaram</td>
<td>30/03/2012</td>
<td>Oceanapia sp.</td>
</tr>
<tr>
<td>5</td>
<td><em>Aspergillus flavus</em></td>
<td>Rameswaram</td>
<td>31/03/2012</td>
<td>Sponge B5B</td>
</tr>
<tr>
<td>6</td>
<td><em>Aspergillus fumigatus</em></td>
<td>Kanyakumari</td>
<td>27/07/2013</td>
<td>Jania sp.</td>
</tr>
<tr>
<td>7</td>
<td><em>Alternaria alternata</em></td>
<td>Kovalam</td>
<td>12/10/2012</td>
<td>Gratelouphia lithophila</td>
</tr>
<tr>
<td>8</td>
<td><em>Alternaria alternata</em></td>
<td>Rameswaram</td>
<td>14/04/2017</td>
<td>Prostlyssa foetida</td>
</tr>
<tr>
<td>9</td>
<td><em>Acremonium</em> sp. 1</td>
<td>Kovalam</td>
<td>21/07/2015</td>
<td>Tedania anhelans</td>
</tr>
<tr>
<td>10</td>
<td><em>Acremonium</em> sp. 2</td>
<td>Kovalam</td>
<td>02/09/2016</td>
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</tr>
<tr>
<td>11</td>
<td><em>Cheatomium globosum</em></td>
<td>Kanyakumari</td>
<td>02/09/2016</td>
<td>Sponge B5A</td>
</tr>
<tr>
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<td><em>Cheatomium globosum</em></td>
<td>Kovalam</td>
<td>02/02/2015</td>
<td>Sargassum wightii</td>
</tr>
<tr>
<td>13</td>
<td><em>Cheatomium globosum</em></td>
<td>Kovalam</td>
<td>31/03/2016</td>
<td>Sargassum wightii</td>
</tr>
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<td>Kovalam</td>
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</tr>
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<td>22</td>
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<td>Kovalam</td>
<td>31/03/2014</td>
<td>Sargassum wightii</td>
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</tr>
<tr>
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<td><em>Fusarium</em> sp. 2</td>
<td>Kovalam</td>
<td>31/03/2016</td>
<td>Sigmadocia carnosa</td>
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<td>30/04/2013</td>
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<tr>
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<td>Kovalam</td>
<td>24/02/2015</td>
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<td>02/02/2015</td>
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<td>31/03/2016</td>
<td>Sargassum wightii</td>
</tr>
<tr>
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<td><em>Penicillium</em> citrinum</td>
<td>Vizhinjam</td>
<td>25/11/2014</td>
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<tr>
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<td>Endosymbiotic fungi</td>
<td>Source</td>
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<td>Staphylococcus aureus</td>
</tr>
<tr>
<td>-------</td>
<td>---------------------</td>
<td>--------</td>
<td>------------------</td>
<td>----------------------</td>
</tr>
<tr>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Aspergillus penicilloides</td>
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<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
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<td>Tr</td>
<td>Tr</td>
</tr>
<tr>
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<td>Broth</td>
<td>3*</td>
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<td></td>
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<td>Mycelium</td>
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<td>2</td>
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<tr>
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<td>Aspergillus flavus</td>
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<td></td>
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<td>tr</td>
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<td>2</td>
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<td></td>
<td>Mycelium</td>
<td>Tr</td>
<td>Tr</td>
</tr>
<tr>
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<td>Broth</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mycelium</td>
<td>1</td>
<td>Tr</td>
</tr>
<tr>
<td>8</td>
<td>Pencillium javanicum</td>
<td>Broth</td>
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<td>1</td>
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<tr>
<td></td>
<td></td>
<td>Mycelium</td>
<td>5</td>
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<tr>
<td>9</td>
<td>Pencillium sp.</td>
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<td>-</td>
<td>Tr</td>
</tr>
<tr>
<td></td>
<td></td>
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<td>4</td>
<td>4</td>
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<tr>
<td>10</td>
<td>Sagenomella sp.</td>
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<td>Mycelium</td>
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</tr>
<tr>
<td>11</td>
<td>Talaromyces islandicus</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>Mycelium</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Class Dothideomycetes</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>Alternaria alternata</td>
<td>Broth</td>
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<td>7</td>
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<td></td>
<td></td>
<td>Mycelium</td>
<td>4</td>
<td>5</td>
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<tr>
<td>13</td>
<td>Curvularia lunata</td>
<td>Broth</td>
<td>2</td>
<td>2</td>
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<td></td>
<td></td>
<td>Mycelium</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>14</td>
<td>Cladosporium sp.1</td>
<td>Broth</td>
<td>-</td>
<td>-</td>
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<td></td>
<td></td>
<td>Mycelium</td>
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<td>-</td>
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<tr>
<td>15</td>
<td>Cladosporium sp.2</td>
<td>broth</td>
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<tr>
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<td>16</td>
<td>Corynespora cassicola</td>
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<tr>
<td>17</td>
<td>Lasiodiplodia theobromae</td>
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<td>mycelium</td>
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<tr>
<td>18</td>
<td>Letendraea helminthicola</td>
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<td>-</td>
<td>-</td>
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<tr>
<td>19</td>
<td>Acremonium sp.1</td>
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<td>4</td>
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<tr>
<td></td>
<td></td>
<td>Mycelium</td>
<td>1</td>
<td>Tr</td>
</tr>
<tr>
<td>20</td>
<td>Acremonium sp.2</td>
<td>broth</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>mycelium</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
From among the 11 species of fungi belonging to class Eurotiomycetes, 2 of them viz., Eurotium am-
stelodami and Penicillium citrinum inhibited all the five test microorganisms. All the nine fungi belonging to this class, showed inhibitory activity towards both the gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*). The highest activity was shown by the mycelial extract of Aspergillus fumigatus against the gram-positive bacteria *Bacil-
-lis subtilis* (7mm zone). Three of them (*Aspergillus fumigatus*, *Aspergillus flavus*, *Talaromyces islandicus*) inhibited gram-negative bacteria. *Talaromyces islandicus* inhibited the gram-negative bacterium *Pseudomonas aeruginosa*. *Sagenomella* sp. and *As-
-pergillus penicillioides* showed the least activity.

The broth and mycelial extracts of seven fungal strains belonging to class Dothideomycetes were tested for antimicrobial activity, of which only three of them (*Alternaria alternata*, *Curvularia lunata*, and *Lasiodiplodia theobromae*) exhibited inhibitory activity. Extracts of all the three species inhibited both the gram-positive bacteria. Mycelial extract of *Alternaria alternata* showed a zone of inhibition of about 3mm against E.coli and its broth extract showed 2mm inhibition zone against *Pseudomonas aeruginosa*. *Alternaria alternata* was found to be the highly active fungi (7mm) towards *Staphylococcus aureus*. This is the only fungi belonging to this class showing antifungal activity.

From the class Sordariomycetes, extracts of both the broth and mycelial extracts of *Chaetomium globo-
sum* showed broad-spectrum antimicrobial activity inhibiting all the test bacteria and fungi. *Acremoni-
um sp. 1* was the other species with its broth exhibiting broad-spectrum inhibitory activity.

### DISCUSSION

A large number of bioactive natural products that have been isolated from endophytic fungi and these bioactive natural products have been reported to possess broad range of biological activities viz., antibacterial, antifungal, antiviral, antioxidant, anticancer, immunomodulatory, antitubercular, antiparasitic and insecticidal activities. They are also chemically diverse and are of benzopyranones, cytchalasins, enniatins, isocoumarins, phenols, quinones, steroids, terpenoids, xanthones, etc., (9). The unique nature of association with host tissue results in the production of such diverse secondary metabolites and many of them are reported with antimicrobial activities (10, 11). It is known that fungal metabolites have primarily served as lead molecules for the development of antibacterial and antifungal drugs.

Previous studies on the terrestrial *Aspergillus* sp. have revealed its high antimicrobial microbial po-
tential, but similar investigations on its marine-derived endophytic fungi is scarce. Based on the studies of Zhang et al. (12, 13), Qiao et al. (14), Miao et al. (15) and Sun et al. (16) the antimicrobial secondary metabolites of marine-derived Aspergillus sp. have been well established. The elaborate review of Flewelling et al. (7) established the antimicrobial potential of Aspergillus fumigatus as observed in the present investigation. The present study on the observation of antimicrobial activity of Aspergillus sclerotiorum agrees with report of Zheng et al. (17) Antimicrobial activity of Eurotium sp. is already known (6). The moderate activity of Eurotium amstelodami agrees with the earlier study.

As observed in the present investigation, Christophersen et al. (18) also reported on the antibacterial activity of marine-derived Penicillium citrinum against the gram-positive bacteria Staphylococcus aureus. The antibacterial activity exhibited by Talaromyces islandicus in the present study may be due to the presence of new diphenylketones and xanthone (19).

Zhang et al. (13) and Jadulco et al. (20) have also reported the antibacterial activity of marine-derived Alternaria alternata and Curvularia lunata. The endophytic fungi Chaetomium globosum belonging to the class Sordariomycetes showed conspicuous antibacterial activity. This agrees with the report of Wang et al. (21). Yang et al. (22) have reported the antibacterial properties of Nigrospora sp. against the gram-positive bacteria Bacillus sp. which is due to the antibacterial metabolite anthraquinone. The antibacterial activity of extracts of Nigrospora sp. against Staphylococcus aureus may also be due to anthraquinone.

**CONCLUSION**

The antimicrobial activity of endosymbiotic fungi isolated from marine organisms showed potential for further isolation and characterization. At least one of the extracts (either broth or mycelium) of most of the fungi showed antibacterial activity. The extracts are comparatively more active against gram-positive bacteria than gram-negative and fungal stains. Hence the nature of metabolite production, i.e., exogenous or endogenous could not be ascertained. The antimicrobial activity of marine endophytic fungi Aspergillus clavatus and Penicillium javanicum has not been reported before.

**ACKNOWLEDGEMENTS**

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**Conflict of Interest:** Nil.

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Biochemical and Genetic Profile of Women with Early and Late Onset Severe Preeclampsia: A Prospective Observational Study

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ABSTRACT

Introduction and Aim: To study endothelial nitric oxide synthase (eNOS) and plasminogen activator inhibitor-1 (PAI-1) gene polymorphisms and their biochemical markers in women with early and late onset severe preeclampsia (PE)

Materials and Methods: Fifty pregnant women each of early and late onset severe PE as study population and 50 normotensive pregnant women were chosen as controls. Maternal blood was obtained and genotyping for eNOS and PAI-1 polymorphism was done. Biochemical analysis of plasma PAI-1 and plasma nitric oxide level was done.

Results: The eNOS T allele (Glu 298 Asp) is found to be posing a significant 6.71 fold increased the risk for early onset PE and 4.95 fold increased the risk for late-onset PE as compared to the respective controls. Both early (EO) and late onset (LO) preeclamptic (PE) cases were found to be characterized by significantly higher PAI levels and significantly lower NO levels.

Conclusion: Plasma nitric oxide levels were significantly decreased and plasminogen activator inhibitor levels were significantly increased among PE (EO and LO) cases as compared to controls.

Key Words: Preeclampsia, eNOS, PAI-1, Pregnancy, Polymorphism; Endothelial dysfunction

INTRODUCTION

Preeclampsia is a multifactorial gestational vascular complication, affecting about seven to ten percent of all pregnancies (1). It is the third leading cause of maternal mortality, responsible for over seven percent of maternal deaths and ten percent of perinatal deaths (2). Women who suffer early-onset preeclampsia have a significantly higher maternal and perinatal morbidity and mortality as compared to women with the onset of preeclampsia at or near term. Although there has been extensive research on preeclampsia during the last decade, the exact etiology of preeclampsia is yet not known. The identification of susceptibility genes is one of the strategies designed to elucidate underlying pathogenetic mechanism. Normal pregnancy is associated with a fall in blood pressure in the first half of pregnancy due to systemic arteriolar vasodilatation before returning to pre-pregnancy levels towards term (3). Elevated synthesis of the endothelium-derived mediator nitric oxide (NO) from L-arginine by endothelial nitric oxide synthase, plays an important role in this vasodilatation (4, 5). Many studies support the association of reduced NO availability in hypertensive disorders of pregnancy (6). The gene that encodes endothelial NO synthase (eNOS), the enzyme that regulates endothelial NO availability is one of the candidate genes for preeclampsia (7-9). One of the potential causes of venous thrombosis is decreased fibrinolysis, which may be due to high plasma plasminogen activator inhibitor type 1 (PAI-1) concentrations (10). PAI-1 inhibits the central step of fibrinolysis i.e. conversion of plasminogen to plasmin, causing thrombosis of spiral arteries and inadequate placental perfusion ultimately resulting in preeclampsia. There is paucity of data available to support the risk determinants related to the time of
onset of severe preeclampsia. The present study was an attempt to understand the role played by the genetic polymorphisms of eNOS and PAI-1 in women with severe preeclampsia (both early and late onset) and normal pregnancies. Further, the effect of polymorphisms on the biochemical markers i.e. plasma nitric oxide and PAI-1 levels is also evaluated.

MATERIALS AND METHODS

Subjects: A total of 150 pregnant women were enrolled in the study. Of these, 50 healthy pregnant women with uncomplicated pregnancies served as controls. Fifty women with early onset severe preeclampsia i.e. onset before 34 weeks of pregnancy and, another 50 women with late onset severe preeclampsia that is onset at ≥ 34 wks pregnancy were included in the study group. Ethical approval was obtained from the institutional ethical committee of Lady Hardinge Medical College, New Delhi and an informed consent was taken from all the participants.

Severe preeclampsia was defined in accordance with the guidelines of the National High Blood Pressure Education Program Working Group on High Blood Pressure in Pregnancy (11). Preeclampsia was defined as systolic and/or diastolic blood pressure of ≥ 140 and 90 mmHg, respectively, on two occasions, at least 6 hours apart along with proteinuria of ≥ 300 mg in a 24hr urine collection developing after 20 weeks of gestation in previously normotensive women. Severe preeclampsia was defined as a diastolic blood pressure ≥110 mmHg or systolic blood pressure ≥ 170 mmHg on two occasions along with significant proteinuria, or preeclampsia with symptoms of severe headache or visual disturbances, platelet count should be below 1 lac per microlitre, epigastric pain and/or vomiting, abnormal liver enzymes (ALT or AST rising to above 70 IU/l) or HELLP syndrome (haemolysis, elevated liver enzymes and low platelet count).

Pregnant women with chronic hypertension, chronic renal disease, and history of thromboembolism, repeated miscarriages or pre-existing liver disease were excluded from the study population.

All recruited women were subjected to a detailed history and examination. The routine investigations of all the cases and controls were done as per the hospital protocol including complete haemogram with peripheral smear, coagulation profile, liver function tests {serum bilirubin, alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP)}, kidney function tests (blood urea, serum creatinine, serum uric acid), urine routine and microscopic examination, 24 hrs urinary protein and ultrasonography.

Maternal venous blood samples were collected in standard vacutainer tubes containing EDTA. The tubes were immediately centrifuged at 3100 rpm for five minutes at room temperature, and plasma samples were stored at -80°C till biochemical analysis was done. Rest of the blood sample with nucleated cells was then subjected to DNA isolation using salting out precipitation method given by Miller et al. (12) for genetic profiling.

For eNOS (Glu298Asp) polymorphism, the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) protocol described by Serrano et al. (13) 2004 was standardized in the laboratory of biochemical and molecular anthropology, Department of Anthropology, University of Delhi. The primer sequences used for the PCR amplifications were: Forward Primer 5’ CCC CTC CAT CCC ACC CAG TCA AC 3’
Reverse Primer 3’ GTC GTG CAG CTT CGC TGG CAA AGG A 5’

For PAI-1 (4G/5G), the PCR-RFLP protocol described by Hasan NS et al. (14) 2004 was standardized in the laboratory. Primer sequences used for the PCR amplifications were:

Forward Primer 5’ CACAGAGAGATCTGGCCACGT 3’
Reverse Primer 5’ CAGCCACGTGTGTGTAGGT 3’

The PCR products obtained after allele specific PCR and digested product obtained after PCR-RFLP method were genotyped using agarose gel electrophoresis technique.

The biochemical analysis was done using stored plasma samples. Plasma nitric oxide was measured by spectrophotometry. The levels of Plasma PAI-1 were measured using a commercially available ELISA method.

Statistical Analysis

For assessing the difference between means of two groups: unpaired t- test was used. All the groups were compared for distribution difference using Pearson’s chi square test. All tests were two tailed. P value <
0.05 was considered statistically significant. For predicting the dependent variable (severe preeclampsia) from the independent variable (biochemical markers and genetic markers), multiple regression analysis was employed. SPSS (Statistical Packages for Social Sciences) version 20 software was used for statistical analysis.

RESULTS

Table 1 summarizes the clinical characteristics of the study population. Fifty-two percent of women among early onset preeclampsia and 36% of women among late onset preeclampsia were primigravidae as compared to only 22% of women in the control group. The age was found to be similar in all the three groups of early (26.2 years) and late onset (25.8 years) preeclampsia with their respective controls (24.9 years). Of 100 cases, 20 (20%) women had a family history of hypertension and nine women (9%) had a history of hypertension in previous pregnancy.

The nitric oxide levels were found to be significantly low among the early onset (12.94µmol/l) and late onset (13µmol/l) preeclamptic cases as compared to controls (19.79µmol/l) (Table 2). The mean nitric oxide levels were found to be comparable (p=0.99) in early onset and late onset severe preeclampsia cases.

The plasma plasminogen activator inhibitor-1 (PAI-1) levels were found to be significantly high among the early onset (71.47ng/ml) and late onset (74.13ng/ml) preeclamptic cases as compared to controls (54.49ng/ml) (Table 2). The mean PAI-1 levels were found to be similar (p=0.29) in early onset and late onset severe preeclampsia cases.

The distribution of eNOS polymorphism was analysed among the EO, LO PE cases and respective controls. The distribution was found to be different with respect to GG, GT and TT genotypes between EO, LO PE cases and controls, wherein significantly higher number of EO and LO PE cases was found to be having GT genotype as compared to controls. Moreover, over representation of GT and TT has been observed among EO and LO PE cases as compared to absence of TT genotype individuals among the controls (Table 3).

To understand the relation between eNOS polymorphism and NO levels, the distribution of NO levels with respect to eNOS polymorphism was analysed. It was observed that the preeclamptic women (EO and LO) carrying GG, GT and TT genotypes were found to have significantly low levels of nitric oxide as compared to that of controls (Table 5). Further,
women carrying T allele were found to have significantly low levels of Nitric oxide as compared to that of women carrying GG genotype in all the EO, LO preeclamptic and control group (Table 5).

Table 3: Genotypic distribution of eNOS Glu298Asp polymorphism in study and control group

<table>
<thead>
<tr>
<th>eNOS genotype</th>
<th>EO+LO cases</th>
<th>EO cases</th>
<th>LO cases</th>
<th>Controls</th>
<th>P value Chi square test</th>
</tr>
</thead>
<tbody>
<tr>
<td>GG</td>
<td>73 (73%)</td>
<td>35 (70%)</td>
<td>38 (76%)</td>
<td>47 (94%)</td>
<td>P1=0.004</td>
</tr>
<tr>
<td>GT</td>
<td>24 (24%)</td>
<td>14 (28%)</td>
<td>10 (20%)</td>
<td>3 (6%)</td>
<td>P2=0.03</td>
</tr>
<tr>
<td>TT</td>
<td>3 (3%)</td>
<td>1 (2%)</td>
<td>2 (4%)</td>
<td>0</td>
<td>P3=0.004</td>
</tr>
</tbody>
</table>

p<0.05 is considered as significant; P1: p value between EO cases and controls with Yates correction; P2: p value between LO cases and controls with Yates correction; P3: p value between severe EO and LO cases and controls (Yates correction)

Table 4: Genotypic distribution of PAI-1 polymorphism among Early and Late onset PE cases and control group

<table>
<thead>
<tr>
<th>PAI-1 genotype</th>
<th>EO+LO cases</th>
<th>EO cases</th>
<th>LO cases</th>
<th>Controls</th>
<th>P value Chi square test</th>
</tr>
</thead>
<tbody>
<tr>
<td>4G-4G</td>
<td>43 (43%)</td>
<td>20 (40%)</td>
<td>23 (46%)</td>
<td>18 (36%)</td>
<td>P1=0.81</td>
</tr>
<tr>
<td>4G-5G</td>
<td>33 (33%)</td>
<td>15 (30%)</td>
<td>18 (36%)</td>
<td>14 (28%)</td>
<td>P2=0.5</td>
</tr>
<tr>
<td>5G-5G</td>
<td>29 (29%)</td>
<td>15 (30%)</td>
<td>14 (28%)</td>
<td>18 (36%)</td>
<td>P3=0.57</td>
</tr>
</tbody>
</table>

p<0.05 is considered as significant; P1: p value between EO cases and controls; P2: p value between LO cases and controls; P3: p value between severe EO and LO cases and controls

Table 5: Correlation of genetic polymorphism with their biochemical markers

<table>
<thead>
<tr>
<th>PAI genotype</th>
<th>Early Onset PE</th>
<th>Late onset PE</th>
<th>Controls</th>
<th>T test p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>4G-4G</td>
<td>69 ± 7.75</td>
<td>74.45 ± 8.7</td>
<td>54.11 ± 5.73</td>
<td>P1**=&lt;0.0001, P2**=0.0001, P3**=0.0001</td>
</tr>
<tr>
<td>4G-5G/5G-5G</td>
<td>73.11 ± 11</td>
<td>73.81 ± 10.33</td>
<td>54.7 ± 7.43</td>
<td>P1**=0.0001, P2**=0.0001, P3**=0.0001</td>
</tr>
<tr>
<td>eNOS genotype</td>
<td>Early Onset PE</td>
<td>Late onset PE</td>
<td>Controls</td>
<td>T test p value</td>
</tr>
<tr>
<td>GG</td>
<td>13.97 ± 2.56</td>
<td>13.68 ± 3.37</td>
<td>20.29 ± 3.81</td>
<td>P1α=0.0001, P2α=0.0001, P3α=0.0001</td>
</tr>
<tr>
<td>GT/TT</td>
<td>10.53 ± 2.46</td>
<td>10.85 ± 2.48</td>
<td>11.73 ± 1</td>
<td>P1β=0.002, P2β=0.02, P3β=0.007</td>
</tr>
</tbody>
</table>

p<0.05 is considered as significant; P1: p value between EO cases and controls; P2: p value between LO cases and controls; P3: p value between severe EO and LO cases and controls;odule 6: Binary Logistic Regression Analysis of genetic markers in severe preeclampsia (both Early onset and Late onset) and respective controls

<table>
<thead>
<tr>
<th>Risk Genotype</th>
<th>Odds Ratio for Early onset PE cases</th>
<th>Odds Ratio for Late onset PE cases</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>eNOS (GT+TT)</td>
<td>6.71 (1.8-24.99)</td>
<td>4.95 (1.3-18.8)</td>
<td>P**=0.003; P*=0.02</td>
</tr>
<tr>
<td>PAI-1 (4G-5G+5G-5G)</td>
<td>0.84 (0.37-1.89)</td>
<td>0.78 (0.35-1.72)</td>
<td>P*=0.83; P**=0.55s</td>
</tr>
</tbody>
</table>

p<0.05 is considered as significant; P*: p value between EO cases and controls; P**: p value between LO cases and controls

The distribution of PAI-1 polymorphism was analysed among the EO, LO PE cases and controls (Table 4). All the three groups i.e. EO, LO and controls do not seem to vary with respect to PAI-1 polymorphism. On analysing the cases (early and late onset) were found to be following Hardy Weinberg Equilibrium with respect to PAI-1 and eNOS mutation (p>0.05); suggestive of the fact that no evolutionary forces are acting on the particular set of participants or the cases. Although, this is not a real situation as the cases were selected based on a certain criteria. Further, the controls were also found to be following Hardy Weinberg Equilibrium with respect to PAI-1 mutation (p<0.05) and not with respect to eNOS polymorphism (p>0.05).

On correlating the plasma PAI-1 levels with PAI-1 polymorphism in all the groups; it was observed that the plasma PAI levels were found to be significantly increased among early and late onset preeclamptic women as compared to the controls irrespective of the genotype.

Logistic regression analysis for eNOS polymorphism revealed that mutant T allele is posing a significant increased risk of 6.71 and 4.95 fold to the early onset and late onset preeclampsia respectively (Table 6).

DISCUSSION

Main Findings

The presence of higher number of primigravida women among early onset and late onset preeclamptic cases as compared to that of controls is in concordance with previous cohort studies (15).

In the present study, both early onset and late onset preeclamptic case groups were found to be having significantly low nitric oxide levels. These results
are in concordance with other studies wherein significant association of PE with reduced levels of nitric oxide is reported (16). Increased blood volume and systemic vasodilation are reported to be a common phenomenon in normal pregnancy (17) whereas the levels of circulating nitric oxide are both expected and also reported to be low in abnormal pregnancies with special reference to preeclampsia. All the studies which attempted to understand the role of NO on PE have restricted only to levels and gestational matched cases and controls. A few studies reported conflicting results and showed higher and unaltered production of nitric oxide among preeclamptic women (18, 19) as compared to healthy pregnant women. These contradictory results reported in few studies could be attributed to their study designs, the difference in sample sizes and ethnic differences between the cases and controls. Nitric oxide acts as a vasodilator and hence reduced NO levels would result in endothelial dysfunction, contributing to PE. The results obtained in the present study are further comparable to that reported by Sandrim VC et al. (20) in which plasma nitrite levels were found to be lowered by 37% in preeclamptic women as compared to healthy pregnant women.

The merit of the present study is that PE is being understood through genotype-phenotype interactions as any disease is an outcome of interactions between the genetic variation specific to a population and its environment. Apart from genotype, there can be various reasons which lead to disturbances in NO levels. One such thing is the use of antihypertensive drugs to improve endothelial dysfunction via restoring vasodilation through an increase in NO levels (21).

In the present study PE cases are not only associated with decrease in NO levels but also significantly reduced levels of plasma nitric oxide among women carrying mutant T allele (GT/TT) (eNOS Glu298Asp) as compared to women with normal genotype (GG) both in the cases as well as in control group (Table 5) which clearly indicates that Glu298Asp polymorphism of eNOS is associated with eNOS activity and lowers the amount of endogenous NO formation in carriers of Asp allele whether it is a case or a control thus indicating the role played by the mutant allele in the predisposition of the pregnant women to preeclampsia.

These results are in concordance with a study by Yaghmaei et al. (22) done on Iranian population (N= 240) which showed that the frequencies of Glu298Asp mutant genotypes were significantly higher in preeclamptic women as compared to controls. However, they reported the risk of PE to be 2.4 fold in pregnant women with Asp allele in their study as compared to 6.71 fold for early onset and 4.95 fold increased risk for late onset preeclampsia in the present study.

The anti hypertensive drugs administered to PE women (present study) may be targeting other pathways which could be directing increase in NO levels but as the mutant T allele frequency is high among both early and late onset PE cases; the T allele is found to reduce the gene expression and hence accounting for reduced levels of nitric oxide among PE cases. This indicates that variable distribution of eNOS genetic variants among different ethnic groups could possibly bring about population specific differences in nitric oxide mediated pathways which can account for inter-ethnic/ inter-population differences in drug response to various complex adverse phenotypes in general and preeclampsia in particular.

The plasma levels of Plasminogen activator Inhibitor-1 (PAI-1) were found to be significantly high among early and late onset PE cases as compared to the controls. The results are supported by many studies that have observed the association of elevated plasma levels of PAI-1 in women with PE compared with gestation matched normotensive pregnant women. However, whether the raised plasma PAI-1 levels and reduced NO levels are causal to PE or are epiphemomena of the disease process is not yet understood.

The PE cases both early and late onset and controls were not found to differ with respect to PAI polymorphism in the present study. The same has been reported by DeMaat et al. (23), wherein they found no significant association of normal allele 4G in women with preeclampsia and controls.

On reviewing literature, it was found that there is no unanimous consensus as yet regarding genotypic distribution of PAI-1 in preeclampsia although some authors suggested association of normal genotype 4G/4G with preeclampsia while others reported a role of mutant genotype 5G/5G in its causation. A study in agreement with the present study by Kobashi G et al. (24) has found no association between 4G/4G genotype and severe PIH among Japanese population. However Gerhardt et al. (25) reported that preeclamptic women who are carriers of the PAI-1 5G/5G genotype are at risk for early onset of the disease. However no significant difference in
distribution of 4G/4G, 4G/5G or 5G/5G genotype in EO and LO preeclampsia was observed. They emphasized that the pathogenetic mechanism of PE is not induced by 5G/5G genotype but it might accelerates the course of preeclampsia. Further, as per the present study, women with intact eNOS T allele are likely to be prone to preeclampsia whereas no such association was found with PAI-1 polymorphism.

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REFERENCES


Why Consumption Pattern of Sugar-Sweetened Beverage is Potential to Increase the Risk of Overweight in School Age Children?

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ABSTRACT

Introduction and Aim: Sugar-sweetened beverages (SSBs) were strongly related to the upward trend of childhood obesity in a few decades. This study was aimed to 1) describe the sugar-sweetened beverages (SSBs) consumption and the risk of obesity among students, 2) analyze the sugar content of homemade-SSB and the nutrient contents of commercial-SSBs.

Materials and Methods: This study was cross-sectional and consisted of three parts. First, we have conducted SSBs consumption survey in the school. A total of 168 students were selected by proportional-stratified random sampling. The second part was laboratory-based in which analyzed 25 samples of homemade-SSB by using the Luff-Schoorl method. The third part, we have observed 91 of commercial-SSBs selected randomly from the minimarket. The nutrients content has been observed through nutrition label.

Results: Conditional logistic, ANOVA, and linear regression were used for data analyses. Students who always consume SSB were 2 times likely to be obese (95%CI: 1.12-3.60). The amount of sugar in homemade-SSB was 68.96g per 100ml. All nutrients, except fiber, are significantly different among the commercial-SSBs type (p<0.001). The nutrients content of commercial-SSBs: 10.23g carbohydrate, 30.55mg sodium, 85.18mg potassium, and 1.47mg fiber.

Conclusion: SSB sold in school is potentially increasing the obesity risk among children because of high sugar. Further study regarding sugar type and artificial sweetener in SSBs should be conducted to provide detail information.

Key Words: Sugar-sweetened beverage, Childhood obesity, School age children, Sugar, Fat.

INTRODUCTION

In a few decades, the number of overweight and obesity has rapidly increased toward to epidemic proportion. In addition, childhood obesity prevalence has elevated in both developed and developing areas. In the developed country such as the United States, some studies show that the number of childhood obesity has dramatically increased (1) whereas, Indonesia, one of developing countries, has a high prevalence of obesity in school-age children which has been recorded clearly over the time. It has been increased from 8.0% in 2007 to 10.8% in 2013 (2). Negative consequences in health and well-being will be addressed to childhood obesity in the future and also it can increase the risk of obesity in later life.

It is important to take action earlier for childhood obesity prevention due to the impact widely in the future is very detrimental. One of the risk factors increases the risk of obesity in children is the western diet including fast food consumption and sugar-sweetened beverages (3). Sugar-sweetened beverages (SSBs) are liquid drinks containing high-fructose corn syrup (55% fructose and 45% glucose) (4). Some studies have revealed the uniqueness of fructose metabolism in human body. Fructose metabolism occurs in the liver entirely and it has metabolic effects if being consumed excessively such as insulin resistance, obesity, and hyperlipidemia (5). An expert defines the SSB as sugary drinks such as carbonated and non-carbonated drink, energy drink and other sugared water (6). Other studies have categorized sports...
drink, fruit drink, soda, tea, milk, coffee, yogurt, supplement drink, and other non-alcoholic drinks as SSBs (7).

Increasing SSB consumption is in line with the elevation of childhood obesity prevalence and even among adults in the US (8). In Indonesia, there is no national survey has been conducted yet which figure out the number of sugary drink consumption. Moreover, the sugar content of SSB is very high so therefore it is possible to increase the risk of weight gain leading to obesity. A study showed the average sugar content of SSB per-serving was 23 grams (7). SSB consumption also has an impact on elevated of waist circumference and inversely associated with HDL cholesterol levels (8).

In Indonesia, there is no regulation to encourage the reducing of SSB consumption especially among children and young adults otherwise only a recommendation of sugar consumption daily. It has been confirmed that the high consumption of sugar-sweetened can cause the risk of obesity in children (9). The objectives of the study were to:

1. Describe the pattern of sugar-sweetened beverage consumption and estimate the risk of obesity among students
2. Analyze the sugar content (sucrose) homemade sugar-sweetened beverage
3. Analyze the nutrients in the commercial sugar-sweetened beverage

MATERIALS AND METHODS

This study was cross-sectional consisting of 3 parts of data collection. The study was conducted in Makassar city. The first part was to describe SSB consumption of students. This part has been conducted from April to June 2017 in IKIP elementary school of Makassar. A total of 168 students were selected through proportional stratified random sampling. The variables included in this study were SSB consumption and nutritional status. 5 underweight students were not included in the analysis because irrelevant to the consumption of SSB. Therefore, the number of samples analyzed was 163 students. The second part was assessing sugar content in homemade sugar-sweetened beverages (HSSBs) through luff schoorl method. A total 25 HSSBs products have been selected and analyzed.

The last part is to observe the nutrients in the product including sugar content from commercial sugar-sweetened beverages (CSSBs). The part of this study has been published previously (Haning, Arundhana, and Muqni, 2016). Total 91 of CSSBs products were selected randomly from the popular minimarket in Makassar city. The nutrient contents have been observed through nutrition label of the products. The nutrient content, the price of the product and type of the product have been defined as variables of the current study. The relationship between the price and nutrients content was analyzed by linear regression. Data resulted were analyzed by using SPSS version 18 (SPSS Inc.).

RESULTS

The characteristic in table 1 showed that the average age of students is 10-years-old. The meal allowance is Rp 11,964 and, almost 80% has been spent out for buying snacks. However, less than a half of students consumed SSB at school. Based on socio-economic aspect, 52.4% of respondents have TVs in the bedroom and 42.3% having game consoles. Most of the students own hand phone, car, and motorcycle (88.1%, 88.7%, 88.1%, respectively). SSB consumption pattern potentially increases the risk of obesity among children. In this study, as shown in Table 2, more than one-third of obese students always consume SSB. It was clearly showed that students who consumed SSB always (more than once per day) were 2 times likely to be obese than those consumed less than once a day.

<table>
<thead>
<tr>
<th>Variables (n=168)</th>
<th>mean±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>10.33 ± 0.96</td>
</tr>
<tr>
<td>Meal allowance</td>
<td>11,964.29 ± 5,692.62</td>
</tr>
<tr>
<td>Allowance proportion for snacks</td>
<td>9,416.67 ± 4,309.04</td>
</tr>
<tr>
<td>Weight</td>
<td>35.48 ± 11.20</td>
</tr>
<tr>
<td>Height</td>
<td>135.35 ± 8.12</td>
</tr>
<tr>
<td>Body Mass Index</td>
<td>19.07 ± 4.53</td>
</tr>
<tr>
<td>SSB consumption (always), n (%)</td>
<td>76 (45.2)</td>
</tr>
<tr>
<td>Having TV in the bedroom, n (%)</td>
<td>88 (52.4)</td>
</tr>
<tr>
<td>Having game console, n (%)</td>
<td>71 (42.3)</td>
</tr>
<tr>
<td>Having handphone, n (%)</td>
<td>148 (88.1)</td>
</tr>
<tr>
<td>Having car, n (%)</td>
<td>149 (88.7)</td>
</tr>
<tr>
<td>Having motorcycle, n (%)</td>
<td>148 (88.1)</td>
</tr>
</tbody>
</table>
Table 2: Obesity risk from SSB consumption

<table>
<thead>
<tr>
<th>Consumption frequency</th>
<th>Obesity (n (%))</th>
<th>Normal (n (%))</th>
<th>p</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;1 per day</td>
<td>24 (32.0)</td>
<td>51 (68.0)</td>
<td>0.025</td>
<td>2.011</td>
</tr>
<tr>
<td>≤1 per day</td>
<td>14 (15.9)</td>
<td>74 (84.1)</td>
<td></td>
<td>(1.123-3.602)</td>
</tr>
</tbody>
</table>

This study also shows the sugar content in the HSSB products sold in IKIP Elementary School (shown in Table 3). On average, the amount of sugar contained in the 100-mL HSSB was 68.96 gram. The type of SSBs that contain the highest sugar is other types (sports drink, jelly drink, etc) while the lowest is shake drink (78.45±15.89 and 61.72±7.14, respectively). Conversely, the high sugar content is negatively related to the price of the product. For the CSSB, nutrient content is varied considerably (Table 4). All of the nutrients, except fiber, are significantly different among the beverages types. The carbonated drink has the highest number of carbohydrate compared to the other types. This result is three-times higher compared to the sugar content in the CSSB as published previously (Haning, Arundhana, and Muqni, 2016). The nutrients content of CSSB respectively, 10.23g for carbohydrate, 30.55mg for sodium, 85.18mg for potassium, and 1.47 for fiber.

Table 3: Sugar content in 100 ml of homemade SSB products

<table>
<thead>
<tr>
<th>Type</th>
<th>n</th>
<th>Sugar (g) mean±SD</th>
<th>Price in IDR mean±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk</td>
<td>5</td>
<td>70.48±25.93</td>
<td>4,800±837</td>
</tr>
<tr>
<td>Fruit juice</td>
<td>3</td>
<td>73.08±10.65</td>
<td>3,000±0</td>
</tr>
<tr>
<td>Coffee/tea</td>
<td>8</td>
<td>66.24±15.52</td>
<td>2,188±843</td>
</tr>
<tr>
<td>Shake drink</td>
<td>5</td>
<td>61.72±7.14</td>
<td>4,800±447</td>
</tr>
<tr>
<td>Other</td>
<td>4</td>
<td>78.45±15.89</td>
<td>4,250±2,217</td>
</tr>
<tr>
<td>TOTAL</td>
<td>25</td>
<td>68.96±16.21</td>
<td>3,660±1,532.43</td>
</tr>
</tbody>
</table>

#ANOVA test

Table 4: Nutrients content in 100 ml of commercial SSB products

<table>
<thead>
<tr>
<th>Type</th>
<th>Carbohydrate mean±SD (N=91)</th>
<th>Sodium mean±SD (N=91)</th>
<th>Potassium mean±SD (N=31)</th>
<th>Fibers mean±SD (N=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbonated drink</td>
<td>12.06±1.43</td>
<td>18.65±10.05</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Coffee</td>
<td>9.94±1.83</td>
<td>44.01±10.21</td>
<td>100.46±14.80</td>
<td>1.08±0.72</td>
</tr>
<tr>
<td>Fruit Juice</td>
<td>10.92±2.27</td>
<td>21.34±17.97</td>
<td>66.00±39.04</td>
<td>1.60±2.32</td>
</tr>
<tr>
<td>Milk</td>
<td>11.36±2.66</td>
<td>46.78±21.26</td>
<td>140.61±45.39</td>
<td>1.56±1.11</td>
</tr>
<tr>
<td>Sport drink</td>
<td>5.33±1.80</td>
<td>33.33±15.47</td>
<td>14.67±1.15</td>
<td>N/A</td>
</tr>
<tr>
<td>Supplement drink</td>
<td>10.44±2.33</td>
<td>25.71±21.89</td>
<td>54.43±42.65</td>
<td>0.80±1.13</td>
</tr>
<tr>
<td>Tea</td>
<td>8.70±2.35</td>
<td>13.69±12.17</td>
<td>9.31±2.55</td>
<td>N/A</td>
</tr>
<tr>
<td>Yogurt drink</td>
<td>11.56±2.31</td>
<td>38.25±10.07</td>
<td>60.67±56.58</td>
<td>2.25±1.06</td>
</tr>
<tr>
<td>Energy drink</td>
<td>11.56±3.05</td>
<td>66.88±44.58</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>TOTAL</td>
<td>10.23±2.77</td>
<td>30.55±23.60</td>
<td>85.18±59.82</td>
<td>1.47±1.52</td>
</tr>
</tbody>
</table>

#Significant (p<0.001)

Linear regression between the price of product and sugar content has been examined (Fig. 1). It was clearly showed that the higher the sugar content the lower the product price. Both homemade SSB and commercial SSB, the product price variable was not significantly correlated with the sugar content variable. However, there is a negative relationship between price and sugar content. The regression linear equations for HSSB (1) and CSSB (2), are as the following:

Price = 4,959.17 – 18.84 sugar ... (1)
Price = 7,085.20 – 104.74 sugar ... (2)
DISCUSSION

Sugar-sweetened beverage (SSB), a large contributor of calories and the source of added sugar, is associated with the problem of obesity. In America, the SSB consumption of adolescent reached 9.1% of total energy. The high consumption of sweet drinks is affected by the environment in both through the beverage advertisements and availability of SSB which increases the accessibility to SSB. In addition, the consumption pattern of parents for SSB and fast food also desired children to consume SSB (10). The present study shows that the opportunity of children exposing by the commercial of “unhealthy” drinks has been increased due to existing of TV in the bedroom. The exposure to SSB advertisements more than 2 hours per day has been associated with an increased risk of overweight in children and adolescents. The content in food and beverage product advertisements is mostly inconsistent with dietary recommendations (11). In this study, we found that those who frequently consumed sugary drinks have associated with obesity. In addition, many beverages packed with “healthy” labels influence consumers, including children and adolescents, to buy the products (10).

The amount of sugar in HSSB is approximately more than 6 tbsp. However, the daily sugar requirement, according to Indonesian dietary guidelines, is only 4 tbsp or 40g. If the children consume HSSBs in the school then their sugar intake will be high. This amount is extremely higher than sugar content in CSSBs that reaches around 22.8g per serving. Nevertheless, it is possible that in the commercial SSB has been added artificial sweetener which also acts as the preservative of these drinks. In the long term effect, it was associated with doubled risk of obesity (12). Excessive consumption of SSB impacts not only to weight gain but also the loss of essential nutrients for children and adolescents (13). The current study also shows the other nutrients such as sodium, potassium, and fiber. However, the number of these nutrients are very low and it indicates no risk of diseases that may arise. The mechanism of this problems emerges through the sugar content associated with increased blood pressure, mainly CSSBs containing high fructose corn syrup. Fructose affects the increase in uric acid that has a correlation with blood pressure elevation. In addition, sugar consumption correlates negatively with sodium retention (14). In the other studies, reducing consumption of SSBs is associated with reducing BP (15). Furthermore, elevated BP is associated with high body mass index.

In the other hands, people who were obese, possibly due to SSB consumption, will have a high risk to be hypertension (16).

In summary, the possible mechanisms that underlie SSBs cause obesity in children are probably through two logical explanations. The first explanation is that the SSB products, both HSSB and CSSB, have cheap price (only ±30% from the meal allowance of the children in IKIP Elementary School). The affordable price of SBB is associated with high consumption and otherwise increases the SSB price could raise the demand for other drinks (17). Furthermore, the high sugar content in SSB leads to causing excessive energy intake. The second explanation is the reinforcement mechanism that may arise from the exposure of SSB advertisement through the presence of TV in the bedroom. The children who always watch television would increase snacking behavior, including SSB consumption, and reduce the diet quality of the children (18). Therefore, it is clear that elementary school children of IKIP probably will be exposing to obesogenic environments.

CONCLUSION

The findings of this study show that sugar-sweetened beverages sold in school have potential to increase the risk of obesity in children due to high sugar content. The students could easily get varied SSBs with affordable price. Further study in terms of sugar type and artificial sweetener used in SSBs should be conducted to provide detail information. In addition, school-based intervention could be designed to overcome the childhood obesity problems.

REFERENCES


Assessment of Acceptance Rate of Various Behavioural Management Techniques among Undergraduate Dental Students, Post Graduates and Pediatric Dentists

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ABSTRACT

Introduction and Aim: The knowledge and training of Behaviour management techniques for the management of Pediatric dental patient is an integral component of the Pediatric dental practice. It is imperative that any approach to behavioural management for the dental child must ensure well-being of the child. The aim of this study is to assess and compare the acceptance of various behavioural management techniques in pediatric dentists among the Undergraduates, Postgraduates of Pedodontics and Pediatric dentists.

Materials and Methods: The study included 300 undergraduate dental students (104 third years, 98 final years and 98 interns), 65 postgraduates in Pedodontics (20 first years, 31 second years and 17 third years), and 18 pediatric practitioners. A questionnaire was designed with 25 questions that depict various behavioural management techniques. The scores were marked on an ordinal scale. Data were tabulated and statistically analysed using SPSS 2.0.

Results: Comparing the acceptance of behavioural management techniques among the three groups, Tell show do (72.95%), using child’s imagination, (69.82%), General anesthesia (52.22%) were found to be having higher acceptance among undergraduates, while providing an explanation of the treatment (68.56%) and modeling (79.16%) and voice control (75.33%) were found to be having higher acceptability rate among postgraduates in Pedodontics and pediatric dentists and are found to be statistically significant.

Conclusion: The Reinforcement techniques are mostly preferred by undergraduates and the postgraduates and Pediatric dentists supported pharmacological and aversive techniques along with desensitisation techniques. Incorporating practical knowledge and awareness of various Behavioural management among undergraduates is necessary.

Key Words: Behavioural Management technique, Pediatric Dentist, Dental graduates

INTRODUCTION

Knowledge and training of Behaviour management techniques for the management of the Pediatric dental patient is an integral component of the Pediatric dental practice. The goal of behavior management techniques practiced by the Pediatric dentists is to establish communication and an element of trust with the child patient (1,2). A friendly relationship between the Pediatric dentists and the child is important for effective and efficient treatment (3).

A wide variety of behavioral management techniques are available to pediatric dentists. In today’s scenario Dentists are expected to treat the child efficiently with the knowledge and skills in Behavioural management techniques acquired during dental education (4). The previous study suggested, suggested that the utilization pattern of behavioral management techniques has changed over past 10 years. These changes are predicted to continue as several factors impact on the Pediatric dentist’s selection and usage of techniques. It is imperative that any approach to behavioral management for the dental child must ensure well being of the child. The aim of the study is to evaluate the acceptance of different behavioral management techniques among undergraduates, postgraduates, and Pediatric Dentists.

MATERIALS AND METHODS

Procedure

This study was approved by the institutional scientific review board at Saveetha University, Chennai. (Ethical Committee clearance number: STP/SDBD-SIV/29). A questionnaire used in the previous study (2) was used in the present study to obtain students’ acceptability scores for various dental behavior guidance techniques in clinical situations. The questionnaire included questions and clinical situations to depict various Behavioural management techniques which is elaborated in table 1.

- Traditional Non pharmacological management
(Tell show do, modeling, distraction, positive reinforcement),

- Aversive behavioral management techniques (Voice control, HOM, immobilization) and

- Pharmacological (Nitrous oxide sedation, General Anesthesia) behavioral management techniques.

Scores for twenty five questions were marked on an ordinal scale.

**Participants**

The study included three groups. Group A consists of 300 undergraduate dental students of which 104 third years, 98 final years, and 98 interns, Group B consists of 65 postgraduates in Pedodontics (20 first years, 31 second years, and 17 third years), and Group C consists of 18 pediatric practitioners. The filled questionnaires were collected and tabulated.

**Analysis**

Comparison of acceptability rate of behavioral management techniques between Group A - undergraduate dental students (3rd years, 4th years, and interns), Group B - postgraduate students (1st year, 2nd year, 3rd year) and Group C pediatric practitioners were done by using one way ANOVA test in IBM SPSS statistics software 23.0 Version.

**RESULTS**

Sex distribution among the undergraduates, postgraduates, and Pediatric dentists were 75% females and 25% males, among the postgraduates, 68.5% females and 38.5% males, and 44.4% females and 55.6% males among the Pediatric dentists respectively. Based on the statistical analysis, comparison of acceptability among the three groups are shown in Table 2.

Among the Desensitizing behavioral management techniques, increased acceptability was suggested to tell show do. 75.60% of Undergraduates preferred this technique compared to PG’s (58.55%), whereas Pediatric dentists (68.3%). The techniques which showed significance on the comparison of three groups are as follows using child’s imagination, providing an explanation of the treatment and modeling. Most of them responded that they sometimes use the following Pharmacological management technique like General anesthesia with a total acceptance rate of 52.22%, which also shows statistical significance on comparing the three groups. Not using LA as per child’s request which is categorized under communicative techniques is reported with

| Reinforcement techniques | • Positive verbal reinforcement  
| | • Promising a toy  
| | • Using the word “coward”  |
| Aversive techniques | • Voice control  
| | • Hand over mouth (HOM)  
| | • Immobilization by staff or parent  
| | • Papoose board  |
| Desensitization techniques | • Tell-show-do  
| | • Providing exact explanation  
| | • Using music or video distraction  
| | • Using the child’s imagination  
| | • Use of euphemisms  
| | • Modeling  |
| Pharmacological techniques | • Nitrous oxide sedation  
| | • General anesthesia  |
| Communicative techniques | • Disallowing child speaking during treatment  
| | • Mentioning the possibility of pain  
| | • Treatment without local anesthetic when it is refused by child  
| | • Allowing child to stop the treatment  
| | • Dentist and/or assistant talking with child during treatment Dentist and/or assistant remains quiet during treatment  
| | • Dentist talks with parent during treatment  
| | • Allowing parent in the clinic during treatment  
| | • Forbidding parent in the clinic during treatment  |
the significant shift in acceptance from Undergraduates to postgraduates. The major number of survey participants reported no change in the utilization pattern for the use of physical restraints, Hand over Mouth technique, etc.

**Comparison between Undergraduates**

Comparing the acceptance of different behavioral management techniques within the Undergraduates (3rd year, 4th year and Interns), the following showed significant results.

Providing appreciation to the child who is a positive reinforcement technique has the maximum acceptability of 81.37% and followed by modeling 79.65%. Pharmacological management techniques also showed significant results which includes Nitrous oxide sedation is reported with an acceptance rate of 59.03% and General anesthesia with 71.8%. Among communicative techniques, parents in the operatory showed increased acceptance rate of 76.04%. Significant results were obtained for desensitizing techniques like using euphemisms (62.8%), giving appreciation (84.58%) and Active immobilization (18%).

**Comparison between the Postgraduates**

The results showed significant results: using euphemisms (62.8%), giving appreciation (84.58%) and active immobilization (73.18%) comparing the acceptance of behavioral management among the 1st, 2nd and 3rd yr postgraduates. Most respondents are pursuing postgraduate on reported they allowed the parent in the operatory (78.65%).

### Table 2: Comparison analysis of Behavior guidance acceptability scores of Undergraduates, Postgraduates and Pediatric dentists.

<table>
<thead>
<tr>
<th>Question number</th>
<th>Behavioural Management Technique or situation</th>
<th>UG acceptance rate</th>
<th>PG acceptance rate</th>
<th>Paediatric Dentists acceptance rate</th>
<th>ANOVA significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Q1</td>
<td>Tell show do</td>
<td>75.60</td>
<td>58.15</td>
<td>82.22</td>
<td>0.000</td>
</tr>
<tr>
<td>Q2</td>
<td>Child not allowed to speak during treatment</td>
<td>60.60</td>
<td>58.77</td>
<td>60.00</td>
<td>0.894</td>
</tr>
<tr>
<td>Q3</td>
<td>Voice control</td>
<td>73.60</td>
<td>73.23</td>
<td>86.67</td>
<td>0.066</td>
</tr>
<tr>
<td>Q4</td>
<td>Hand over mouth</td>
<td>77.27</td>
<td>74.15</td>
<td>83.33</td>
<td>0.290</td>
</tr>
<tr>
<td>Q5</td>
<td>Nitrous oxide</td>
<td>83.13</td>
<td>82.15</td>
<td>85.56</td>
<td>0.710</td>
</tr>
<tr>
<td>Q6</td>
<td>Encouraging not to be a coward</td>
<td>86.47</td>
<td>86.46</td>
<td>87.78</td>
<td>0.935</td>
</tr>
<tr>
<td>Q7</td>
<td>Active immobilisation</td>
<td>78.60</td>
<td>80.31</td>
<td>80.00</td>
<td>0.789</td>
</tr>
<tr>
<td>Q8</td>
<td>Papoose board</td>
<td>84.20</td>
<td>87.08</td>
<td>84.44</td>
<td>0.448</td>
</tr>
<tr>
<td>Q9</td>
<td>Music or video distraction</td>
<td>78.20</td>
<td>77.23</td>
<td>80.00</td>
<td>0.883</td>
</tr>
<tr>
<td>Q10</td>
<td>Positive reinforcement</td>
<td>78.27</td>
<td>78.15</td>
<td>73.33</td>
<td>0.605</td>
</tr>
<tr>
<td>Q11</td>
<td>Taking advantage of child's imagination</td>
<td>71.20</td>
<td>62.77</td>
<td>72.22</td>
<td>0.051</td>
</tr>
<tr>
<td>Q12</td>
<td>Mentioning the possibility of pain</td>
<td>76.07</td>
<td>76.92</td>
<td>87.78</td>
<td>0.116</td>
</tr>
<tr>
<td>Q13</td>
<td>Providing an exact explanation</td>
<td>68.27</td>
<td>76.00</td>
<td>46.67</td>
<td>0.000</td>
</tr>
<tr>
<td>Q14</td>
<td>Pharmacological conscious sedation</td>
<td>78.00</td>
<td>74.46</td>
<td>82.22</td>
<td>0.333</td>
</tr>
<tr>
<td>Q15</td>
<td>General anaesthesia</td>
<td>67.87</td>
<td>49.72</td>
<td>52.22</td>
<td>0.000</td>
</tr>
<tr>
<td>Q16</td>
<td>Giving appreciation</td>
<td>62.07</td>
<td>58.46</td>
<td>60.00</td>
<td>0.637</td>
</tr>
<tr>
<td>Q17</td>
<td>Using Euphemisms</td>
<td>78.73</td>
<td>79.05</td>
<td>84.44</td>
<td>0.476</td>
</tr>
<tr>
<td>Q18</td>
<td>Not using LA as per the child's wish</td>
<td>18.00</td>
<td>20.00</td>
<td>4.44</td>
<td>0.010</td>
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<td>Q19</td>
<td>Dentists being quite during treatment</td>
<td>50.33</td>
<td>50.46</td>
<td>50.00</td>
<td>0.994</td>
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<tr>
<td>Q20</td>
<td>Child allowed to stop the treatment</td>
<td>36.87</td>
<td>34.15</td>
<td>35.56</td>
<td>0.576</td>
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<td>Q21</td>
<td>modelling</td>
<td>50.10</td>
<td>44.31</td>
<td>51.11</td>
<td>0.022</td>
</tr>
<tr>
<td>Q22</td>
<td>Parent in the operatory during treatment</td>
<td>39.27</td>
<td>41.54</td>
<td>32.22</td>
<td>0.046</td>
</tr>
<tr>
<td>Q23</td>
<td>Parent talking with the child during treatment</td>
<td>7.00</td>
<td>4.92</td>
<td>11.11</td>
<td>0.288</td>
</tr>
<tr>
<td>Q24</td>
<td>Parent talking with the dentist during treatment</td>
<td>3.47</td>
<td>4.31</td>
<td>2.22</td>
<td>0.761</td>
</tr>
<tr>
<td>Q25</td>
<td>Showing the needle to the Child</td>
<td>30.80</td>
<td>28.92</td>
<td>22.22</td>
<td>0.104</td>
</tr>
<tr>
<td>Question number</td>
<td>Behavioural Management Technique or situation</td>
<td>PG 1st year</td>
<td>PG 2nd year</td>
<td>PG 3rd year</td>
<td>ANOVA significance</td>
</tr>
<tr>
<td>-----------------</td>
<td>---------------------------------------------</td>
<td>-------------</td>
<td>-------------</td>
<td>-------------</td>
<td>--------------------</td>
</tr>
<tr>
<td>Q1</td>
<td>Tell show do</td>
<td>73.47</td>
<td>52.13</td>
<td>84.65</td>
<td>0.068</td>
</tr>
<tr>
<td>Q2</td>
<td>Child not allowed to speak during treatment</td>
<td>76.47</td>
<td>73.60</td>
<td>62.45</td>
<td>0.936</td>
</tr>
<tr>
<td>Q3</td>
<td>Voice control</td>
<td>84.57</td>
<td>77.27</td>
<td>84.56</td>
<td>0.586</td>
</tr>
<tr>
<td>Q4</td>
<td>Hand over mouth</td>
<td>82.32</td>
<td>83.13</td>
<td>78.65</td>
<td>0.290</td>
</tr>
<tr>
<td>Q5</td>
<td>Nitrous oxide</td>
<td>88.35</td>
<td>86.47</td>
<td>77.58</td>
<td>0.546</td>
</tr>
<tr>
<td>Q6</td>
<td>Encouraging not to be a coward</td>
<td>71.85</td>
<td>76.89</td>
<td>65.58</td>
<td>0.842</td>
</tr>
<tr>
<td>Q7</td>
<td>Active immobilisation</td>
<td>75.54</td>
<td>83.85</td>
<td>60.16</td>
<td>0.049</td>
</tr>
<tr>
<td>Q8</td>
<td>Papoose board</td>
<td>85.57</td>
<td>78.85</td>
<td>81.00</td>
<td>0.956</td>
</tr>
<tr>
<td>Q9</td>
<td>Music or video distraction</td>
<td>67.78</td>
<td>84.56</td>
<td>58.54</td>
<td>0.457</td>
</tr>
<tr>
<td>Q10</td>
<td>Positive reinforcement</td>
<td>84.55</td>
<td>78.58</td>
<td>85.45</td>
<td>0.658</td>
</tr>
<tr>
<td>Q11</td>
<td>Taking advantage of child's imagination</td>
<td>82.15</td>
<td>78.65</td>
<td>71.15</td>
<td>0.649</td>
</tr>
<tr>
<td>Q12</td>
<td>Mentioning the possibility of pain</td>
<td>85.15</td>
<td>69.78</td>
<td>88.66</td>
<td>0.334</td>
</tr>
<tr>
<td>Q13</td>
<td>Providing an exact explanation</td>
<td>78.15</td>
<td>66.54</td>
<td>55.49</td>
<td>0.078</td>
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<tr>
<td>Q14</td>
<td>Pharmacological conscious sedation</td>
<td>59.12</td>
<td>58.68</td>
<td>88.59</td>
<td>0.567</td>
</tr>
<tr>
<td>Q15</td>
<td>General anesthesia</td>
<td>64.67</td>
<td>58.77</td>
<td>59.85</td>
<td>0.983</td>
</tr>
<tr>
<td>Q16</td>
<td>Giving appreciation</td>
<td>85.45</td>
<td>79.55</td>
<td>88.74</td>
<td>0.054</td>
</tr>
<tr>
<td>Q17</td>
<td>Using Euphemisms</td>
<td>64.78</td>
<td>54.85</td>
<td>68.77</td>
<td>0.035</td>
</tr>
<tr>
<td>Q18</td>
<td>Not using LA as per the child's wish</td>
<td>66.87</td>
<td>51.44</td>
<td>20.77</td>
<td>0.985</td>
</tr>
<tr>
<td>Q19</td>
<td>Dentists being quite during treatment</td>
<td>46.22</td>
<td>39.55</td>
<td>48.67</td>
<td>0.245</td>
</tr>
<tr>
<td>Q20</td>
<td>Child allowed to stop the treatment</td>
<td>87.56</td>
<td>56.88</td>
<td>64.46</td>
<td>0.468</td>
</tr>
<tr>
<td>Q21</td>
<td>modeling</td>
<td>80.55</td>
<td>64.82</td>
<td>56.67</td>
<td>0.024</td>
</tr>
<tr>
<td>Q22</td>
<td>Parent in the operatory during treatment</td>
<td>46.58</td>
<td>54.77</td>
<td>78.65</td>
<td>0.051</td>
</tr>
<tr>
<td>Q23</td>
<td>Parent talking with the child during treatment</td>
<td>15.64</td>
<td>20.77</td>
<td>16.85</td>
<td>0.288</td>
</tr>
<tr>
<td>Q24</td>
<td>Parent talking with the dentist during treatment</td>
<td>16.89</td>
<td>54.87</td>
<td>20.64</td>
<td>0.568</td>
</tr>
<tr>
<td>Q25</td>
<td>Showing the needle to the Child</td>
<td>13.55</td>
<td>29.00</td>
<td>34.69</td>
<td>0.801</td>
</tr>
</tbody>
</table>

Table 4: Comparison of acceptability of various behavioural management techniques within the Undergraduates (3rd years, 4th years, Interns).

<table>
<thead>
<tr>
<th>Question number</th>
<th>Behavioural Management Technique or situation</th>
<th>UG 3 rd year</th>
<th>UG 4 th year</th>
<th>UG intern</th>
<th>ANOVA significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Q1</td>
<td>Tell show do</td>
<td>82.65</td>
<td>60.15</td>
<td>67.48</td>
<td>0.589</td>
</tr>
<tr>
<td>Q2</td>
<td>Child not allowed to speak during treatment</td>
<td>78.54</td>
<td>58.65</td>
<td>60.00</td>
<td>0.498</td>
</tr>
<tr>
<td>Q3</td>
<td>Voice control</td>
<td>84.66</td>
<td>56.55</td>
<td>80.35</td>
<td>0.092</td>
</tr>
<tr>
<td>Q4</td>
<td>Hand over mouth</td>
<td>53.44</td>
<td>68.66</td>
<td>55.45</td>
<td>0.659</td>
</tr>
<tr>
<td>Q5</td>
<td>Nitrous oxide</td>
<td>54.66</td>
<td>67.55</td>
<td>54.88</td>
<td>0.037</td>
</tr>
<tr>
<td>Q6</td>
<td>Encouraging not to be a coward</td>
<td>45.87</td>
<td>66.78</td>
<td>54.92</td>
<td>0.053</td>
</tr>
<tr>
<td>Q7</td>
<td>Active immobilisation</td>
<td>45.66</td>
<td>54.60</td>
<td>55.30</td>
<td>0.687</td>
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<tr>
<td>Q8</td>
<td>Papoose board</td>
<td>40.55</td>
<td>55.98</td>
<td>65.78</td>
<td>0.645</td>
</tr>
<tr>
<td>Q9</td>
<td>Music or video distraction</td>
<td>52.66</td>
<td>78.65</td>
<td>45.78</td>
<td>0.338</td>
</tr>
<tr>
<td>Q10</td>
<td>Positive reinforcement</td>
<td>51.66</td>
<td>47.65</td>
<td>87.65</td>
<td>0.067</td>
</tr>
</tbody>
</table>
DISCUSSION

Earlier studies regarding acceptance of various behavioral management techniques by dental students before and after clinical education indicated a preference for the less restrictive pediatric dental behavior guidance techniques (6). Among the reinforcement methods, in agreement with the previous studies, high acceptability scores were given by the Undergraduate students for positive reinforcement, tell-show-do, distraction with words/video, and use of euphemisms. These show that cognitive-behavioral techniques, such as the use of operant conditioning (rewards systems), distraction, graded exposure, and relaxation, have become a consistent preference and are included in the dental curriculum (6-9,11).

Also, the increase in acceptability of passive and active immobilization and Hand over mouth technique (HOM) was suggested by postgraduates and pediatric dentists which is in contrary to the studies done earlier. Thus it shows that increase in education and clinical experience can change the perception of even controversial behavior guidance techniques. Lowest scores were given for showing the needle to the child, treatment without local anesthetic despite it being required, or the child not being allowed to speak during the treatment students and pediatric dentists. These findings indicate that dental education has adapted to the changing needs and expectations of today’s patients, parents, and families, with increasing preference for empathic techniques over aversive techniques (5-7,10,12).

Increased acceptance of Pharmacological, behavioral management methods such as nitrous oxide sedation/conscious sedation, General anesthesia show that there is increased perception to avoid unpleasant experience to the patient. Newton et al. reported that undergraduate dental students from a five-year curriculum perceived sedation as less acceptable than simple behavioral management (13). But this study shows increased acceptance of using nitrous oxide sedation, though the acceptability of performing the procedure under General anesthesia is quite less among the UGs as the undergraduate curriculum does not expose to clinical training on treating a patient under general anesthesia. Parent – patient, Parent – doctor interaction is less preferred by most of the UG and PG students and Pediatric dentists, as they consider the parent’s interaction with the operator or the child can cause interference in the treatment.

In this study, we have also compared the acceptability of various behavioral management techniques among the Undergraduates and postgraduates based on the year of their study. There is the significant increase in the perception of encouraging the child not to be a coward and providing an exact explanation to the child, modeling with an increase in years of education (14,15). This represents that the undergraduate education gives more importance to nonaversive/positive reinforcement techniques whereas the postgraduate education focuses on all the Behavioural management techniques.

Comparing the level of perception among postgraduates, increased acceptability was demonstrated in active immobilization, HOM, and nitrous oxide sedation as they are exposed to clinical training which
provides them the confidence to use aversive behavioral management techniques.

CONCLUSION

We conclude from our study that the Reinforcement techniques are mostly preferred by undergraduates, and the postgraduates and Pediatric dentists supported pharmacological and aversive techniques along with desensitization techniques. The dental education components have the potential to shape student perceptions of pediatric dental behavior guidance techniques during their career. Hence incorporating practical knowledge in the undergraduate curriculum is necessary to impart the knowledge and awareness of various behavioral management among undergraduates.

REFERENCES


Knowledge Attitude and Practice of General Dentist towards Pediatric Dentistry

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ABSTRACT

Introduction and Aim: Every general dental practitioner is expected to diagnose and treat dental diseases of pediatric patients effectively with the acquired knowledge and skills during their professional education. Among all the services that are being performed by a Dental surgeon, Pediatric dentistry is considered to be the most neglected yet an important area to be considered. So, the aim of the present study is to assess the knowledge, attitude, and practice of general dentist towards pediatric dentistry.

Material and Methods: 452 general Dentist, both practitioners and academicians in Chennai and Coimbatore, were included in the questionnaire survey. The questions were based on the knowledge, attitude, and practice of pediatric dentistry among general dentist.

Results: Among the 452 dentists, 373 dentists (82.5%) feel that they are trained to treat children between the age group of 3-12 years, 161 dentists (35.8%) have a clinical practice set up with play area, pictures, and toys.

Conclusion: This study showed that a greater percentage of the general dental surgeons are not prepared to treat child patients, and they find it difficult to treat very young children.

Key Words: General dentist, Pediatric dentistry, Treating children.

INTRODUCTION

Dentistry is defined as the evaluation, diagnosis, prevention and/or treatment (nonsurgical, surgical or related procedures) of diseases, disorders and/or conditions of the oral cavity, maxillofacial area and/or the adjacent and associated structures and their impact on the human body; provided by a dentist, within the scope of his/her education, training and experience, in accordance with the ethics of the profession and applicable law. (As adopted by the 1997 ADA House of Delegates) (1). Children whose dental health care needs were unmet, are the ones who are more likely to experience physical development problems (2,3) a missing of school days, and having a restricted activity (4-6) and the ability to learn may shrink (7-10). It is the responsibility of the practitioner to make sure that every child receives the proper oral health care services that they need and also to ensure that they have good general health and a positive quality of life and can live up to their academic potential.

In Pediatric Dentistry, it is really important to consider the feelings of the child, which helps to gain confidence in the child, and in turn, receive a better co-operation from the child and perform the desired treatment. Pediatric dentist (PD) also plays a major role in contributing child’s dental health in the future by modifying the attitude of the child and encourage the child for his behavior regarding dental care (11).

Dental surgeons may simply be reluctant to see children so young because they perceive them to be difficult to examine. Further, they may not know what to do if, during the examination, it is discovered that the child will require further treatment (12). Every general dental practitioners are expected to diagnose and treat dental diseases of pediatric patients effectively with the acquired knowledge and skills during their professional education. Modifying any child’s behavior can be helpful in providing a safe and effective treatment that is required (13). Among all the services that are being performed by a Dental surgeon, Pediatric dentistry is considered to be the
most neglected an important area yet to be considered (14). So, the aim of this study was to assess the knowledge, attitude, and practice of general dentist towards pediatric dentistry.

MATERIALS AND METHODS

The study protocol was approved and received ethical approval (STP/SDMDS15PED2d) from an institutional review board of Saveetha Dental College.

A questionnaire in English was framed and given to 20 dental academic practitioners within the institution. Face validation and content validation were done. The questionnaire was then modified based on the responses. The questionnaire was then finalized with 16 close-ended questions. This survey was undertaken among the general dentists both in Chennai and Coimbatore district, Tamil Nadu. The study was conducted March 2016 to May 2016. The questionnaires were personally handed over to 452 general Dentist both practitioners and academicians in both the districts by a postgraduate student from Saveetha Dental College and collected within a day or after a day. The questionnaire comprised a series of questions pertaining demographic details and years of practice; knowledge regarding treating pediatric patients; the attitude of the practitioner towards the managing pediatric patients; and practicing pediatric dentistry. The framed questions were obtained from the study done by Rich JP 2016.

The data was entered and analyzed for frequency and percentages by using Statistical Package for the Social Sciences (SPSS) version 19.0. Simple descriptive statistics were done using Chi-square (χ²) test. The chosen level of significance was set at P < 0.05. Unanswered questions were treated as missing values.

RESULTS

The demographic details are described in table 1. Out of 452 dentists, 230 were postgraduate practitioners, and 222 were undergraduate practitioners. 180 and 272 dentists were from Coimbatore and Chennai district respectively. Of the 452 questionnaires that was sent, the response was at a rate of 100%.

The series of questions based on knowledge regarding treating pediatric patients were 373 dentists (82.5%) feel that they are trained to treat children between the age group of 3-12 years. 291 dentists (64.4%) think children cannot tolerate treatment in a general dental office while 161 dentists (35.6%) think children can tolerate treatment in a general dental office. 161 dentists (35.6%) have a clinical practice set up with play area, pictures and toys whereas 290 dentists (64.2%) of them don’t have a play area for children. 336 dentists (74.3%) sure that their clinical staff is not comfortable treating children and only 116 dentists (25.7%) think that their clinical staff is comfortable in treating children. 213 dentists (47.1%) think that their clinical staffs are knowledgeable in treating children and 239 dentists (52.9%) are sure that their clinical staffs are not knowledgeable in treating children. 218 dentists (48.2%) like treating children <6 years and 374 dentists (82.7%) like to treat children >6 years.

Among general dentist who practices pediatric dentistry, 13 dentists (2.9%) use fluoride, 124 dentists (27.4%) gives oral hygiene instruction, 133 dentists (29.4%) administer pit and fissure sealants, and 182 dentists (40.3%) use all the above mentioned as a part of preventive dentistry. 357 dentists (79%) use communication, 25 dentists (5.5%) use oral sedation, 13 dentists (2.9%) use physical restraints and 57 dentists (12.6%) use all the above behavior management techniques. 49 dentists (10.8%) use amalgam, 37 dentists (8.2%) use composite resin restoration, 342 dentists (75.7%) glass ionomer cement, 24 dentists (5.3%) uses all the above for restoration of primary teeth. Only 126 dentists (27.9%) use rubber dam while 326 dentists (72.1%) do not use a rubber dam. 309 dentists (68.4%) use local anesthesia for dental treatment in children while 143 dentists (31.6%) do not use. 279 dentists (61.7%) perform extraction for most of the pediatric patients over pulp therapy, which was carried out in private practice by 135 dentists (29.9%). 166 dentists (36.7%) do pulpectomy, pulpotomy, indirect pulp capping in clinical practice.

The questions based on the attitude of the practitioner towards pediatric dentistry include, 287 dentists (63.5%) think treating children are financially inadequate while 165 dentists (36.5%) think treating children is financially adequate. 353 dentists (78.1%) think treating children are time-consuming where 99 dentists (21.9%) think it is not time-consuming.
DISCUSSION

The study was carried out to assess the knowledge of the dentist about treating child patients, closed-ended questionnaire was considered more effective. 452 general dentists were included in the study.

373 dentists (82.5%) feel that they are trained to treat children between the age group of 3-12 years. Seale and Casamassimo, 2003 reported that more than 90% of dental surgeons provide treatment for children younger than 4 years of age while Rich et al., 2006 reported that only 33.4% of general dentists indicated that their clinical experiences during dental school had prepared them well to treat children under the age of 6 years (15).

357 dentists (79%) use communication as the behavior management techniques while treating children which was similar to a study reported by McKnight-Hanes C, 1993 where 213(93%) dental surgeons stated that the most popular technique for managing children was tell-show-do (17).

In the present study, 116 dentists (25.7%) and 213 dentists (47.1%) think that their clinical staff are comfortable and knowledgeable in treating children which was reported by Rich et al., 2006 that dentists with more positive educational experiences in pediatric dentistry were more likely to report that their staff was comfortable and knowledgeable about treating children (15).

In the present study, 279 dentists (61.7%) perform extraction for most of the pediatric patients over pulp therapy. Hussain et al. reported 40% of dentists perform extraction in primary teeth (18).

In the present study, 287 dentists (63.5%) think treating children is financially inadequate. Rich et al., 2006 stated that financial compensation for treating children was judged as more inadequate by dentists who felt less well prepared by their dental school programs than by those respondents who felt better prepared (15).

CONCLUSION

This study showed that a greater percentage of the general dental surgeons are not prepared to treat child patients, and they find it difficult to treat very young children. All the dental professionals must be aware of treating child patients to meet children’s needs. The dental curriculum should include guidelines and techniques to train the upcoming dentists for excellent practice in pediatric dentistry.

REFERENCES


Prevalance and Associated Factors of Bruxism in Children in Chennai-A Pilot Study

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ABSTRACT

Introduction and Aim: Bruxism, the habitual grinding, gnashing or clenching of teeth at times other than during mastication of food. Bruxism usually causes tooth wear that precipitates wear facets that can range from mild to severe and can be localized or found throughout the dentition. To evaluate the dental wear in school going children and correlate the associated factors causing bruxism.

Materials and Methods: The study comprises of 255 school going children in the age groups of 7-15yrs who reside at Velapanchavdi, Tamil Nadu and also includes their respective parents.

Results: In a total of 255 children, 48 (18%) children exhibited bruxism based on parental observation. Out of 58 children with a positive or possible habit of bruxism, Sleep bruxism was reported in 26 children. Out of 48 children who exhibited bruxism, 6 children had relatives exhibiting the same habit. Based on Johansson criteria of dental wear, moderate bruxism was seen in 24 (9.4%) children. Out of 26 children who brux at night, wear facets indicating moderate bruxism is seen in 23 (88.5%) children. This could be attributed to agents like stress and anxiety.

Conclusion: The evaluated data suggest the prevalence of bruxism being 18% with the dental wear supporting the signs of moderate bruxism in 9.4% of the children. There lies an association between the positive responses and the wear facets indicating parental awareness of this habit. The need for recognizing this habit in children is very much important due to the etiologic agents, most common being stress, anxiety.

Key Words: Bruxism, Children, Teeth wear, Stress, Temporomandibular disorders.

INTRODUCTION

Bruxism, the habitual grinding, gnashing or clenching of teeth at times other than during mastication of food, was originally described by Marie and Pietkiewicz in1907 (1). In 1931, Frohman first coined the term bruxism (2). In the 1960s, a periodontist named Sigurd Peder Ramfjord championed the theory that occlusal factors were responsible for bruxism (3). Bruxism can occur during the day or night (4). Generally, individuals clench their teeth throughout the day and gnash and clench them during sleep (5). Bruxism usually causes tooth wear that precipitates wear facets that can range from mild to severe and can be localized or found throughout the dentition (6). This condition is very common in children (7). The consequences of bruxism in children are the fracture of teeth, increased teeth sensitivity, erosion of dental enamel, hypermobility of the teeth, periodontal ligament injury, fractured cusps, or pulp necrosis (7). Teeth grinding is an activity of significance to the dentist because of breakage of dental restorations, damage to the teeth, induction of temporal headache and temporomandibular disorders (8). Sleep bruxism is a non-functional behavior characterized by the grinding or clenching of teeth; it occurs during non-rapid eye movement sleep stages N1 and N2 and is associated with arousals from sleep (9). Sleep bruxism in children is most often identified by a family member, parents who observe the stereotypic tooth grinding sound, or by a dentist who recognizes abnormal occlusal wear (10). This parental recognition of bruxism habit may or may not show clinical signs of dental wear. The etiology of sleep-bruxism is considered multifactorial (11-13) consisting primarily of pathophysiologic factors like arousal response during sleep and immature masticatory neuromuscular system, psychologic factors like stress and personality and possibly morphologic factors like the anatomy of the orofacial skeleton and occlusal align-
ment(14). In this present study, a community-based prevalence rate of bruxism is assessed using parental observation of the child and clinical evidence supporting the same. Also, a brief description of the correlating factors of bruxism is discussed.

MATERIALS AND METHODS

The study comprises of 255 school going children in the age group of 7-17yrs and their respective parents. The study is concerning the participants who all reside in the same locality, velapanchavdi, Tamil Nadu. Ethical approval was obtained from the saveetha university, Chennai (SRB/STUG15/70).

The survey consisted of a parental questionnaire that was distributed to the parents of the 255 children in their respective residential locations and alongside, the intra oral examination was done. The parental questionnaire consisted of questions regarding their observation of bruxism in their ward and the time of occurrence. Also, questions regarding the occurrence of the same habit in other members belonging to the same family as that of the child were questioned. The evaluation was done by identifying the presence of any wear facets on the teeth based on Johansson’s criteria (15). According to which, the sum of the wear facets in the teeth was calculated to give the composite score. A range of 6 or more indicated moderate bruxism whereas a score of less than 6 accounted for no significance. It was identified using a mouth mirror under daylight. The values for the wear facets were recorded, and the overall score (15) was generated for each child. Evaluation of tooth hypermobility and TMJ disorders were also done clinically. The whole survey took place in the respective residential homes of the participants and prior informed written consent was obtained from the parents. Statistical analysis was applied to the set of questions in the questionnaire, comparing them against each other. Table 2 depicts the parental response of bruxism in their child and dental evaluation of wear facets. Table 3 depicts the relation between the children exhibiting bruxism and the same habit among any other family members. Table 4 depicts the relation between the time of bruxism in children with the positive parental response and the dental wear evaluation.

RESULTS

The overall frequency of all the questionnaire responses and clinical examination are shown in table 1.

Table 1: Frequency for Questions under Parental Questionnaire

<table>
<thead>
<tr>
<th>QUESTIONS</th>
<th>RESPONSES</th>
<th>NUMBER OF PARENTS WHO RESPONDED</th>
<th>PERCENT-AGE (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Does your child currently grind his/her teeth? Other than when eating?</td>
<td>Yes</td>
<td>48</td>
<td>18.8</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>197</td>
<td>77.3</td>
</tr>
<tr>
<td></td>
<td>May be</td>
<td>10</td>
<td>3.9</td>
</tr>
<tr>
<td>2. In the past have you ever noticed your child grinding his/her teeth?</td>
<td>Yes</td>
<td>48</td>
<td>18.8</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>174</td>
<td>68.2</td>
</tr>
<tr>
<td></td>
<td>May be</td>
<td>10</td>
<td>3.9</td>
</tr>
<tr>
<td></td>
<td>NA</td>
<td>23</td>
<td>9.0</td>
</tr>
<tr>
<td>3. If your child currently grinds his/her teeth, when does grinding occur?</td>
<td>Day</td>
<td>19</td>
<td>7.5</td>
</tr>
<tr>
<td></td>
<td>Night</td>
<td>26</td>
<td>10.2</td>
</tr>
<tr>
<td></td>
<td>Both day &amp; night</td>
<td>13</td>
<td>5.1</td>
</tr>
<tr>
<td></td>
<td>NA</td>
<td>197</td>
<td>77.3</td>
</tr>
<tr>
<td>4. Does any member of the child's immediate family grind his/her teeth?</td>
<td>Yes</td>
<td>40</td>
<td>15.7</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>125</td>
<td>49.0</td>
</tr>
<tr>
<td></td>
<td>May be</td>
<td>88</td>
<td>34.5</td>
</tr>
<tr>
<td></td>
<td>NA</td>
<td>2</td>
<td>0.8</td>
</tr>
<tr>
<td>5. If yes, when does this family member grind his/her teeth?</td>
<td>Day</td>
<td>4</td>
<td>1.6</td>
</tr>
<tr>
<td></td>
<td>Night</td>
<td>85</td>
<td>33.3</td>
</tr>
<tr>
<td></td>
<td>Both day &amp; night</td>
<td>1</td>
<td>.4</td>
</tr>
<tr>
<td></td>
<td>NA</td>
<td>165</td>
<td>64.7</td>
</tr>
</tbody>
</table>
6. What is this person’s relationship to this child?

<table>
<thead>
<tr>
<th>Relationship</th>
<th>No.</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Close relatives</td>
<td>38</td>
<td>14.9</td>
</tr>
<tr>
<td>Distant relatives</td>
<td>50</td>
<td>19.6</td>
</tr>
<tr>
<td>NA</td>
<td>166</td>
<td>65.1</td>
</tr>
</tbody>
</table>

7. Dental wear evaluation (Johannson criteria)

<table>
<thead>
<tr>
<th>Criteria</th>
<th>No.</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>No Sig.</td>
<td>231</td>
<td>90.6</td>
</tr>
<tr>
<td>Moderate bruxism</td>
<td>24</td>
<td>9.4</td>
</tr>
</tbody>
</table>

8. Tooth hypermobility

<table>
<thead>
<tr>
<th>Status</th>
<th>No.</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>255</td>
<td>100.0</td>
</tr>
</tbody>
</table>

9. Tooth fractures secondary to bruxism

<table>
<thead>
<tr>
<th>Status</th>
<th>No.</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>255</td>
<td>100.0</td>
</tr>
</tbody>
</table>

10. Pulpal involvement

<table>
<thead>
<tr>
<th>Status</th>
<th>No.</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>255</td>
<td>100.0</td>
</tr>
</tbody>
</table>

11. Morning fatigue or pain of masticatory muscles

<table>
<thead>
<tr>
<th>Status</th>
<th>No.</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>33</td>
<td>12.9</td>
</tr>
<tr>
<td>No</td>
<td>222</td>
<td>87.1</td>
</tr>
</tbody>
</table>

12. Pain to palpation to masseter/temporal muscles

<table>
<thead>
<tr>
<th>Status</th>
<th>No.</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>14</td>
<td>5.5</td>
</tr>
<tr>
<td>No</td>
<td>241</td>
<td>94.5</td>
</tr>
</tbody>
</table>

13. Masseter hypertrophy

<table>
<thead>
<tr>
<th>Status</th>
<th>No.</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>255</td>
<td>100.0</td>
</tr>
</tbody>
</table>

14. Parent reports click when opening TMJ or are positive at examination.

<table>
<thead>
<tr>
<th>Status</th>
<th>No.</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>255</td>
<td>100.0</td>
</tr>
</tbody>
</table>

Parental observation of bruxism

Questionnaire responses were obtained for 255 (100%) children. Of these, parental response indicated that 48 (18%) had a positive history of the habit, 10 (3.9%) had a possible history and 174 (68%) did not present with any history of bruxism. Of the 58 children with either a positive or a possible history of the habit, 26 (10.2%) children exhibited sleep bruxism, 19 (7.5%) children, awakeness bruxism and 13 (5.9%) children who presented with the habit occurring both day and night.

Bruxism among Relatives

A total of 128 (16.5%) responses indicated that members of the child’s family exhibit bruxism and a further 125 (49%) responses indicated no such reports of bruxism among the relatives. In a total of 48 children who were bruxers, had relatives, 6 (12.5%) of them who were positive for the same condition and 25 (52.1%) who weren’t positive for bruxism.

Clinical Detection of Bruxism

On clinical examination, out of 255 children, wear facets in the teeth were observed and evaluated based on Johannson criteria (15). The results reveal 24 (9.4%) children showing signs of moderate bruxism based on evaluating the dental wear. Out of 48 children who exhibited bruxism based on the positive parental response, wear facets indicating moderate bruxism were seen in 24 (50%) children and 24 children exhibited no such significant signs of wear facets. Out of 26 children who brux at night, wear facets indicating moderate bruxism is seen in 23 (88.5%) children. This provides a statistical inference that the positive parental response of bruxism in their children, being complimentary to the wear facets seen most often, secondary to the habit is of significance. On the other hand, there is no such significance in dental wear in children who brux during the daytime. Out of 13 children who brux both day and night wear facets correlating bruxism is seen in only 1 (7.7%) of the total cases. According to table 2, out of 58 children who show bruxism, wear facets secondary to...

Table 2: Correlation between Parental Observation of Bruxism and Clinical Diagnosis of Wear Facets

<table>
<thead>
<tr>
<th>Parental observation of bruxism among children examined (no.)</th>
<th>Clinical diagnosis of wear facets</th>
<th>Wear facets showing moderate bruxism</th>
<th>Wear facets of no significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive history (48)</td>
<td>24 (50%)</td>
<td>24 (50%)</td>
<td></td>
</tr>
<tr>
<td>Negative history (197)</td>
<td>0 (0%)</td>
<td>197 (100%)</td>
<td></td>
</tr>
<tr>
<td>Possible history (10)</td>
<td>0 (0%)</td>
<td>10 (100%)</td>
<td></td>
</tr>
<tr>
<td>Total (255)</td>
<td>24 (9.4%)</td>
<td>231 (90.6%)</td>
<td></td>
</tr>
</tbody>
</table>
DISCUSSION

Bruxism, the habitual grinding, gnashing or clenching of teeth at times other than for the mastication of food, was originally described by Marie and Pietkiewicz in 1907 (1). Since then, the condition has been variously attributed to dental, systemic or psychological factors. Wear facets are most commonly seen secondary or as a result of this habit. The onset of bruxism in children can occur after the first year of life, with the eruption of the deciduous incisors. A study among five- and six-year-old children attending kindergarten in the geographic and socio-economic area of Minneapolis, Minnesota has shown a prevalence of bruxism of 15% (16). The figure of 15% was close to that of 14.4% reported in 1966 by Reding, Rubright and Zimmerman4 in a study of 1157 three- to seven-year-old children attending the University of Chicago Laboratory Schools. Studies on older persons by the same authors have indicated the decrease of the habit of bruxism with age; a prevalence of 5.1% was reported among a population of 2290, 16- to 36-year-old undergraduate and graduate students from the University of Chicago (17). In our present community based study stated that the prevalence of parent-reported sleep-bruxism that occurred at least once a week among preschool and first grade children and was 36.8% and 49.6%, respectively (19). In the present study, out of children whose parents presented a positive or a possible history of bruxism, sleep bruxism was prevalent in 10.2% of those children. Clinical assessment of wear facets as a result of bruxism were also reported with an incidence of 9.4% in relation to those presenting with this habit. This clinical evaluation presented as a limitation due to the different exfoliative stages in our patients. Few studies remarked a hereditary association of bruxism. A study on identical twins showed that there was a greater chance of the presence of grinding teeth compared to individuals heterozygous; a child with bruxer parent has a Chance of bruxism 1.8 increased to submit this dysfunction (20). Whereas in our study, the statistical significance of this comparison shows no such familial predisposition of bruxism in children. In another study (16) 24.6% of the children presented with positive history of the habit.15.4% of the total sample showed clinical evidence of wear facets but did not have a history of the habit at home. Since the majority of bruxing children exhibited the habit during the night, the timing may have contributed in part to the lack of parental awareness. Although bruxism is frequently audible, a sleeping parent could be unaware of the child’s habit (16). However,

<table>
<thead>
<tr>
<th>Parental observation of bruxism among children examined (no.)</th>
<th>Parental observation of bruxism in any member of the same family %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive history (48)</td>
<td>Positive response 25 (52.1%)  Negative response 33 (67.9%)  Possible response 2 (4.2%)</td>
</tr>
<tr>
<td>Negative history (172)</td>
<td>Positive response 61 (35.7%)  Negative response 104 (60.5%)  Possible response 17 (9.8%)</td>
</tr>
<tr>
<td>Possible history (10)</td>
<td>Positive response 1 (10.0%)  Negative response 9 (90.0%)  Possible response 0 (0%)</td>
</tr>
<tr>
<td>Total (230)</td>
<td>Positive response 92 (39.6%)  Negative response 226 (97.8%)  Possible response 7 (3.0%)</td>
</tr>
</tbody>
</table>

Table 3: Correlation between Parental Response of Bruxism Habit in Children and any other Member of the Same Family

<table>
<thead>
<tr>
<th>Parental observation of time of bruxism among children examined (no.)</th>
<th>Clinical diagnosis of wear facets %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bruxism during daytime (19)</td>
<td>Wear facets showing moderate bruxism 23 (88.5%)  Wear facets of no significance 6 (22.2%)</td>
</tr>
<tr>
<td>Bruxism during night time (26)</td>
<td>Wear facets showing moderate bruxism 23 (88.5%)  Wear facets of no significance 3 (11.5%)</td>
</tr>
<tr>
<td>Bruxism during both day and night (13)</td>
<td>Wear facets showing moderate bruxism 1 (7.7%)  Wear facets of no significance 12 (92.3%)</td>
</tr>
<tr>
<td>Total (58)</td>
<td>Wear facets showing moderate bruxism 24 (41.4%)  Wear facets of no significance 34 (58.6%)</td>
</tr>
</tbody>
</table>

Table 4: Correlation between Time of Bruxism with Dental Wear Evaluation
in our present study there is an equal statistical significance in the association between the parental observation of the habit and presence of wear facets as a sign of bruxism. Statistical significance was seen equally in children who had positive response and showed wear facets on examination and those who did not show any significant wear facets. The reason for this may be the awareness of the parent in this community based study. This can vary depending upon the culture, the parental upbringing of the child and other associated factors. Renner et al (21) conducted a sound and informative investigation onto the relationship between bruxism and mental health in children. They demonstrated that bruxism is associated with emotional difficulties and socio-economic status. Sleep bruxism may serve as a behavioural indicator and may be a signal of early health care intervention need. A recent report indicated that children who brux are 2.4 more likely to experience migraines than children who do not brux (22). Another study (23) was aimed to determine the prevalence of childhood bruxism and associated correlates in children that were a cross-sectional survey of parents that was conducted at 4 private paediatric dental offices in Boston. In which, 5% of parents reported that their children had at least one TMD symptom; no TMD symptoms were associated with reported bruxism (23). No significant signs of severe bruxism were observed in our current study and thereby no significance is seen regarding TMD symptoms. This may be attributed to the developing TMJ in the age group of children included in our study.

CONCLUSION
A prevalence rate of 18% was reported in the present study. The dental wear with the positive parental history of bruxism exhibited that the habit of bruxism was recognized by the parents. This showed that the parents were quite aware and recognized this habit in their children. No significant familial predisposition was seen concerning bruxism in our study. Sleep bruxism is the most common type of bruxism and is of major concern due to its psychological etiology in bruxism. Therefore, an early recognition and a cognitive approach in treating bruxism in children required in addition to dental therapy.

Limitation
Certain factors served as a limitation of our studies such as the smaller sample size and the limited area that was chosen.

Recommendations
The relationships between the habit of bruxism and the associated factors are complex, further longitudinal studies over a longer period are required regarding the same.

REFERENCES


Influence of Diet Score and Feeding Practices on the Severity of Caries Occurrence in Pre-School Children-A Cross Sectional Study

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ABSTRACT

Introduction and Aim: Children and adolescent are giving more preference for sweetened food and soft drinks that are rich in carbohydrates and thus are at a risk of increased caries development. The frequency of rinsing habit practised among the children as well as the effect of rinsing after meals has effect on caries occurrence. So, the aim of the study is to evaluate the effect of diet, rinsing habit after meals and feeding practices on dental caries.

Materials and Methods: A 3 day diet chart given to the parents of 50 pre-school children evaluating the dietary and snacking habits, rinsing habit after meals and feeding practices and their dmft score was noted. The comparison was based as per the severity of dmfs score being caries free, ECC, s-ECC.

Results: Positive correlation is found between the dietary score and dmfs score. (p<0.001). Lower dietary score is seen in caries free children as compared to children having caries. No significant difference is found in between the two severities of dmfs and the age of stoppage of feeding on dmfs.

Conclusion: Diet and oral hygiene practice is an important determinant of caries severity in childhood. As a preventive means, measures have to be taken to control these factors individually and in combination.

Key Words: Cariogenicity, Dietary score, dmfs, Feeding habit, Oral hygiene.

INTRODUCTION

The prevalence of dental caries in primary teeth, early childhood caries (ECC), increased from approximately 40 percent in children aged 2-11 years in the 1988-1994 National Health and Nutrition Examination Survey (NHANES) to 42 percent in the year 1999-2004 survey (1). For the children 2-5 years of age, the rate increased greater, rising from 24 percent to 28 percent (2). In 1940, the prevalence of dental caries in 5 and 12-year-old school children in India were 55.5%, and it jumped to 68% in the 1960 and climbed to 89% in subsequent years (3,4,5).

According to WHO, the multifactorial nature of dental caries is attributed to the colonization of microorganisms, fermentable carbohydrates in the diet, particularly when consumed frequently, susceptible teeth, such as early stage of development, after injury or in malnutrition or chronic disease, genetic factors, time of the teeth eruption in the oral cavity. However, dietary factors and food choices play a major role in the causation of various chronic disease including dental caries (3,4). The association of dental caries to excessive sugar intake has been affirmed by an Expert Panel of World Health Organisation, whose members reviewed the strength of evidence linking dietary factors to caries in 2003. The panel reported a decrease in the risk of caries post the consumption of hard cheeses and use of sugar-free chewing gum. Studies have also shown that mouth rinsing after cariogenic food consumption has a protective effect against dental caries (5). In the causation of caries, there is a dynamic balance between the caries risk factors and protective factors (Figure 1).

Children and adolescent are giving more preference for sweetened food and soft drinks that are rich in carbohydrates and thus are at risk of increased caries development (6). Studies have shown that pre-schoolers do not tend to rinse the mouth after snacking (7). With the known cultural difference, a wide variation exists in the Indian and western diet as well as within the various states in India. Not many studies assess the frequency of rinsing habit practiced among the children as well as the effect of rinsing after meals on caries occurrence.
Thus, in this regard a study was conducted in the metropolitan city of Chennai, assessing the effect of a 3-day diet chat score on dmft. This study also assessed the effect of rinsing after the meals on the caries occurrence in pre-schoolers. Caries being a multifactorial disease, isolating an individual factor as the causation of caries is difficult; however, the risk of caries due to the presence or absence of the risk as well as protective factors in combination can be determined. The objective of the study includes recording the diet and the oral hygiene practices practiced after meals and recording the corresponding dmf(t) and dmf(s) (8).

**MATERIALS AND METHODS**

A cross-sectional study was done in preschool children. The study design protocol and informed consent were approved by the ethical committee board of the institution with the ethical number STP/SD-MDS16PED3. The inclusion criteria were that children should be between 2-6 years of age, they were medically healthy, had full primary dentition with or without the eruption of first permanent molar and the parent or guardian provided informed consent for the child’s participation. These children were enrolled from the preschools in Chennai as well as randomly selected patients visiting Saveetha Dental College and Hospital for dental treatment. A total of 50 children were included in the study out of which 28 were caries free and others having carious dentition. The sample population was divided into caries free (severity 0), ECC (severity 1) and S-ECC (severity 2) for the comparison with the diet score (9).

**Survey Information and Clinical Examination**

Parents and caregivers completed the survey information given to them. The basic clinical examination of carious teeth was carried out by the visual method, and the corresponding carious teeth were noted as DMF(T) and DMF(S) (10-12). Parents were asked to enter the demographic data and feeding habits of the child stating the age at which the feeding was stopped. Also, if the feeding practice continued they were asked if night feeding was practiced. Secondly, the parents were asked to record a 3-day diet chart for the child stating the time of food consumed and type of food consumed. Also, the parents were asked to note if rinsing practice or any other oral hygiene measure was practiced within 20 minutes after the consumption of the meal.

**Data Analysis**

The study hypothesized that dietary factor and oral hygiene practices followed after that would differentiate carious and caries-free children. The cariogenicity of the food consumed by each child was calculated as the weighted cumulative mean of the individual cariogenicity of food (13). The individual cariogenicity of the food consumed is divided as caries protective (cario00), non-cariogenic (cario0), low cariogenicity (cario1), liquids (cario2), solid retentive (cario3) (13). If a rinsing practice was mentioned as done in the survey after the individual food consumed, the corresponding score for that food was considered as zero. The total cumulative score calculated was compared with the dmfs score.

**Statistical Analysis**

The data obtained were analyzed using SPSS software version 22. The Normality tests Kolmogorov-Smirnov, and Shapiro-Wilks tests results reveal that variables do not follow a normal distribution. Therefore to analyze the data Nonparametric methods were applied. To examine whether a 3 day diet score and the frequency of feeding habit varied with the severity OF DMFS, a Kruskal-Wallis Test was performed. Bonferroni adjusted Mann – Whitney’s test was used for multiple pair wise comparison between the severities of DMFS and the dietary score. Spearman rank correlation is used to calculate the relationship between variables which is DMFS score, dietary score and, feeding habit. Significance level is fixed as 5% (α = 0.05).

**Ethical Aspects**

This study was approved by the Ethical Research Committee of the Saveetha University. Informed consent was obtained from the parents on behalf of their children, authorizing participation and data collection in the study.

**RESULTS**

Of the children recruited for the study, 53.1% were boys and 46.9% were girls with all the children being below 6 years of age (mean-3.81, SD-1.86). The 3-day diet score ranged from 0-1.87(Table3) with the mean (SD) of 1.09(0.52) and median (interquartile range) of 1.21 (0.98-1.46). The age of stoppage of feeding habit varied between 11 months to 42 months with the mean (SD) of 3.81 (1.86) and
median (interquartile range) of 1.30 (1.0-2.0). The dmfs score had 57.1% children as caries free, 10.2% having a severity of 1, and 32.7% having a severity of 2.

Spearman’s Nonparametric Correlations (Table 1), (figure2) reveal that there is a positive co-relation between the 3-day diet score and DMFS score (p<0.001). Children having higher dietary score had more decayed teeth as compared to those who had less dietary score being caries-free. There was, however, no significant co-relation between the age of stoppage of feeding with DMFS score (p<0.305) and 3-day dietary score (p<0.954).

Table 1: Spearman’s Nonparametric Correlations (original)

<table>
<thead>
<tr>
<th>Variables</th>
<th>SEVERITY</th>
<th>Mean Rank</th>
<th>Chi-Square value</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 DAY SCORE</td>
<td>0</td>
<td>18.07</td>
<td>15.847</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>38.10</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>33.03</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 3: Comparison of 3 day diet score with severity

The mean (SD) 3 day diet score was 0.89(0.493), 1.50(0.204) and 1.31(0.499) for severity 0, 1 and 2 respectively. The median (interquartile range) for the severities were 1.05(0.50-1.24), 1.54(1.46-1.67), 1.43(1.27-1.59) respectively.

Comparative analysis of Kruskal-Wallis Test (Table2), (figure3) show that higher 3 day diet score exists in the children having severities 1 and 2 as compared to lower dietary score n caries-free children (P<0.001). Multiple pairwise comparisons revealed a significant difference of the 3-day diet score between the caries-free children and severity 1 and caries-free children and severity 2. However, no significant difference exists between the dietary score in between the two severities. Also, no significant difference was found between the age of stoppage of feeding and severities of DMFS (Table4), (figure 4).

Figure 4: Comparative evaluation of 3 day diet score and DMFS

Table 3: Descriptive Statistics (original)

<table>
<thead>
<tr>
<th>SEVERITY</th>
<th>0</th>
<th>1</th>
<th>2</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 DAY SCORE</td>
<td>N</td>
<td>Mean</td>
<td>Std. Dev</td>
</tr>
<tr>
<td></td>
<td>28</td>
<td>.89</td>
<td>.493</td>
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<tr>
<td></td>
<td>6</td>
<td>1.50</td>
<td>.204</td>
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<tr>
<td></td>
<td>16</td>
<td>1.31</td>
<td>.499</td>
</tr>
<tr>
<td>AGE OF STOPPAGE OF FEEDING</td>
<td>N</td>
<td>Mean</td>
<td>Std. Dev</td>
</tr>
<tr>
<td></td>
<td>28</td>
<td>1.49</td>
<td>.601</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>1.10</td>
<td>.579</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>1.74</td>
<td>.705</td>
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</tbody>
</table>
DISCUSSION
The principal finding of this study was that diet as a primary influence on caries. The higher dietary score is positively associated with higher DMFS score. Also, the study also included the rinsing habit and the timing of rinsing in the dietary score, which can also be co-related to the caries score in combination with the cariogenicity of food. This study shows a significant difference between caries-free and children with high severity of caries.

Increase in snacking is associated with increased decay. Snacking on three to four times a day is associated to have a lower dmft score as compared to children snacking more than 5 times a day. Snacking at least 3 times a day was associated with three times higher dmft score as compared to kids who snacked only once a day (11). Studies have also shown an increase in food with high cariogenicity is associated with the high dmft score and increased s. mutans level (10,13). The dietary score obtained in the present study is the combination of the food has increased cariogenicity and the amount of snacking done during the day. This co-relates with the increased frequency of snacking and food having high cariogenicity to be positively associated with caries score. This finding is consistent with other studies(14-18).

Oral hygiene plays one of the main roles in caries prevalence (12). A decrease in the dietary score is present if rinsing practice was done within 20 mins after food consumption. Thus a lower dietary score in caries-free individual can be co-related to consumption of low cariogenic food or practice of oral hygiene measure after the consumption of food or both. Studies have shown a direct influence of oral hygiene practice on caries experience (19,20).

Studies have shown that feeding practice more than 12 months of age showed a higher incidence of caries as those whose breast feeding is stopped before 12 months (21-23). The present study, however, does not show any significant difference between the age of stoppage of feeding and dmft severity. This may be due to less sample size. A larger sample size may provide a significant co-relation between the stoppage of feeding and caries score.

CONCLUSION
Diet, oral hygiene practice, and feeding habits influence the prevalence of dental caries in children individually and in combination which works in favor of the present study. A wide variety of factors are considered for evaluation as the determinant of dental caries influencing each other. Further studies need to be carried out with a larger sample size to achieve more significant results. Also, the present study being a cross-sectional study measures the outcome at a given point of time which cannot be co-related as causation. Longitudinal studies need to be carried out to provide the correct causation.

REFERENCES


Insilico Studies of Thymoquinone in *Nigella sativa* as Potential Antitumor Agent

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**ABSTRACT**

**Introduction and Aim:** The objective of this study was to study the binding energy of *N. sativa* biological active compounds, and drug likeliness by *insilico* techniques for anticancer activity. Thymoquinone inhibited vascular endothelial growth factor–induced extracellular signal-regulated kinase (ERK) activation. The proteins were retrieved from PDB bank and plant data compounds are taken from literature survey and chosen 4 compounds such as thymoquinone, alpha-hederin, dithymoquinone and thymohydroquinone for the study.

**Materials and Methods:** Auto dock 1.2.6 software is a suite of automated docking tools. It is designed to predict how small molecules, such a substrate or drug candidates, bind to receptors of the known 3d structure. 2XIR, 1NME, 1Q0R and IR9O protein preparation and optimization, ligand preparation and optimization and docking simulations were carried out by using biological databases like PubChem, Drug Bank, Protein Data Bank. These compounds are visualized by using Discovery studio 4.1 Visualizer.

**Results:** From the docking results, thymoquinone was showing satisfactory dock score values and it also satisfied the Lipinski’s rule of five for drug likeness.

**Conclusion:** Present study indicates that thymoquinone inhibits tumor angiogenesis and tumor growth and could be used as a potential drug candidate for cancer therapy.

**Key Words:** *Nigella sativa*, thymoquinone, ERK, AKT pathway, AutoDock 4.2, Discovery Studio Visualizer 4.1.

**INTRODUCTION**

Spices and medicinal herbs are known to have anti-cancer characteristics which can be used to target the tumor growth and further damage (1). One such spice is *Nigella sativa*, also called as black cumin. *Nigella sativa* (*N. sativa*) is the annual plant of the family Ranunculaceae is a widely used medicinal plant throughout the world. It is very popular in various traditional systems of medicine like Unani, Ayurveda and Siddha. It has been widely used as antihypertensive, liver tonics, diuretics, antidiarrheal, apetite stimulant, analgesics, antibacterial and skin disorders (2). It is listed in the “Medicine of the Prophet” as a natural remedy to cure all pathological conditions. The main active component of black cumin volatile oil is Thymoquinone (TQ), of about 54% and others include monoterpenes like p-cymene, dithymoquinone (TQ2), thymohydroquinone (THQ) and alpha hederin (3). Extensive studies on *N. sativa* have been carried out by various researchers and a wide spectrum of its pharmacological actions have been explored which may include antidiabetic, anticancer, immunomodulator, antimicrobial, anti-inflammatory, spasmylytic, bronchodilator, hepato-protective, renal protective, gastro-protective, antioxidant properties (4). The seeds

**Figure 1:** *Nigella sativa* flower

**Figure 2:** Seeds
are thought to contain active ingredients having antitumor characteristics.

Thymoquinone is a principle component in Black cumin inhibits the DNA synthesis process leading to apoptosis and thereby hindering the cell proliferation. 5-fluorouracil inhibits thymidine synthesis and is seen to cure many forms of cancer. A number of studies have established that the pro-apoptotic mechanism of thymoquinone is mediated by both p53 dependent pathways and p53 independent pathways (5). The Anti-tumor effects of Black cumin is because of its potent anti-inflammatory properties. This was established by Chehl N et al (6), a research study on thymoquinone on pancreatic cells, in which it was observed that this compound inhibits NF-κβ pathway leading to reduction in the growth of pancreatic cancer. And also, the anticancer effect of Black cumin is because of its anti-angiogenic mechanism. In this content, it was observed that thymoquinone inhibits its AKT and EKT signalling pathways and depicts inhibitory action on key cancer regulatory proteins (7).

MATERIALS AND METHODS

The protein and ligand interactions takes an important part in protein function. Both ligand and its binding site are essential components for understanding how the protein-ligand complex functions. Molecular docking is a key tool in structural molecular biology and computer assisted drug design (8).

Black cumin has number of chemically diverse agents contributing to its anti-cancer functionality. Literature review revealed the principle bioactive components of black cumin i.e. Thymoquinone, alpha hederin, dithymoquinone and thymohydroquinone and their structures are given in Table No.1. Lipinski’s properties such as molecular weight, log P, molar refractivity, number of hydrogen bond acceptors and donors taken from SCFBio software for thymoquinone and it is satisfied the Lipinski’s rule of five for drug likeness. The values of the Lipinski’s properties are highlighted in Table: 2.

Protein Preparation

The three-dimensional coordinates of the crystal structure of the proteins (PDB-ID: 2XIR, 1NME, 1Q0R and 1R9O). were downloaded from the RCSB protein data bank archive and used for docking studies. The protein structures were prepared in order to obtain the correct ionization and tautomeric state of amino acids residues. Further, water molecules were removed and polar hydrogen atoms were added. Then, the kollman united atom partial charges and salvation parameters were assigned. The protein preparation process resulted in a PDBQT file that contained Autogrid and Autodock.

Ligand Preparation

The three-dimensional structures of small molecules were prepared by identifying the root and its expansion, as required by the docking programs.

Ligand Docking

AutoDock is a tool used for predicting the interactions between the receptor (macromolecule) and the ligand molecule. Autodock 4.2 suite was used for molecular docking analysis and the docking logs were analyzed using the graphical user interface of ADT. Initially, the grid box was generated for the entire protein molecule, because the protein structure was not complexed with a small molecule. Further, at the end of the docking process (for each of the four protein), a possible ligand binding site was identified and another grid box was generated around that area. Then the final docking results in order to confirm the accuracy of the predicted binding sites. The results were clustered into similar conformations based on the cluster root mean square deviation and orientation. The dock score values are tabulated in Table: 3.

Visualization

Discovery studio 4.1 visualizer is a free, molecular modelling environment, for both small and macromolecule applications. It is developed by accelrys which specializes in scientific software products. It is used regularly in a range of academic and commercial entities, but is most relevant to pharmaceutical and biotechnology industries. The visualization of the docked compounds is tabulated in Table: 4.

RESULTS

The targets identified for thymoquinone from insilico techniques are depicted in Table: 3

The Pharmacological activities of the Nigella sativa

*Nigella sativa* are antidiabetic, anticancer, immunomodulator, antimicrobial, anti-inflammatory, spasmyloytic, bronchodilator, hepatoprotective, renal protective, gastro-protective, antioxidant properties. The anti-cancer potency of *N.sativa* is predominantly contributed by its principle constituent thymoquin-
none. The targets were identified for thymoquinone by *Insilico* reverse screening process, it was observed that many of these identified targets (PDB-ID: 2XIR, 1NME, 1Q0R and IR9O), are significant contributor to the process of cell proliferation and apoptosis in cancer. Amino acid residues such as arginine, asparagine, lysine and leucine have bound to the thymoquinone compound. The compound showed good binding energy thus these compounds can be effectively used for the treating anti-cancer activity.

Table 1: Compounds and their Structure

<table>
<thead>
<tr>
<th>COMPOUNDS</th>
<th>STRUCTURES</th>
</tr>
</thead>
<tbody>
<tr>
<td>THYMOQUINONE</td>
<td><img src="image1" alt="Thymoquinone" /></td>
</tr>
<tr>
<td>DIHYDRO THYMOQUINONE</td>
<td><img src="image2" alt="Dihydro Thymoquinone" /></td>
</tr>
<tr>
<td>DITHYMOQUINONE</td>
<td><img src="image3" alt="Dithymoquinone" /></td>
</tr>
<tr>
<td>ALPHA HEDERIN</td>
<td><img src="image4" alt="Alpha Hederin" /></td>
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Table 2: Lipinski’s Rule of Five

<table>
<thead>
<tr>
<th>COMPOUND</th>
<th>MOL. WT</th>
<th>LOG P</th>
<th>HYDROGEN BOND DONOR</th>
<th>HYDROGEN BOND ACCEPTOR</th>
<th>MOLAR REFRACTIVITY</th>
</tr>
</thead>
<tbody>
<tr>
<td>THYMOQUINONE</td>
<td>312.000000</td>
<td>-0.053101</td>
<td>5</td>
<td>6</td>
<td>77.145782</td>
</tr>
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</table>

Table 3: Chemical Compound Thymoquinone and its Dock Score Values

<table>
<thead>
<tr>
<th>COMPOUND</th>
<th>PROTIENS</th>
<th>BINDING ENERGY</th>
<th>HYDROGEN BOND CONTACT</th>
</tr>
</thead>
<tbody>
<tr>
<td>THYMOQUINONE</td>
<td>2XLR</td>
<td>-5.78</td>
<td>Asn (900) H…D21</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Leu (901) H…N</td>
</tr>
<tr>
<td>1NME</td>
<td>-5.02</td>
<td></td>
<td>Asn (80) H…D21</td>
</tr>
<tr>
<td>1Q0R</td>
<td>-6.25</td>
<td></td>
<td>Arg (179) H…H22</td>
</tr>
<tr>
<td>IR9O</td>
<td>-6.2</td>
<td></td>
<td>Lys (48) H…N</td>
</tr>
</tbody>
</table>
CONCLUSION

The biologically active compounds of *N. sativa* are thymoquinone, alpha hederin, dithymoquinone, dihydrothymoquinone. From this study, TQ could be considered as an efficient phytochemical in regulating the protein responsible for cancer and thereby preventing the cancer initiation and development. This study will help to understand how the target protein is regulated by the ligands and inhibiting the carcinogenic pathway. In future studies, the identified targets have to be further validated in *in-vivo* and *in-vitro* bioassays to support the findings of this study.

ACKNOWLEDGEMENT

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REFERENCES

A Study of Hematological and Biochemical Parameters in Seropositive Cases of Dengue

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ABSTRACT

Introduction and Aim: Dengue Fever is the most common arboviral disease in the world and it is considered a major global health threat by the World Health Organization. Dengue infection presents with varied clinical conditions ranging from simple fever to shock. Dengue infection can lead to severe bleeding, shock, and death. Hence early and rapid laboratory diagnosis of dengue is crucial. The present study aims to investigate the age and sex prevalence in the spectrum of dengue and to correlate the hematological, serological and biochemical findings in relation to antigens and antibodies in dengue infection.

Materials and Methods: The present study was conducted in Department of Pathology, in a south Indian tertiary hospital between April 2015-September 2016. All the patients with immunochromatographic positive (NS1, IgM, and IgG) for dengue infection were included in the study. Their clinical history, laboratory data, and outcome were recorded.

Results: A total of 137 serologically positive patients were included in the present study. Of them, 67.2% (92) had dengue fever, 31.4% (43) had dengue hemorrhagic fever and 1.4% (2) presented with dengue shock syndrome. Males 53.3% (73) were more commonly affected than females (46.7% (64) with the M:F ratio of 1.1:1. NS1 positive cases constituted 48.1% (66), IgG positive cases accounted for 25.8% (35) cases, IgM, NS1+IgM and NS1+IgG were observed in 13.1% (18), 10.2% (14) and 2.9% (4) cases respectively. Thrombocytopenia was observed in 67.8% (93) cases and its association with NS1 antigen was significant. Hemoconcentration was seen in 28.4% (39) of the cases.

Conclusion: We conclude that early diagnosis of the infection with the help of complete blood examination and serological dengue tests can prevent the fatality of the disease.

Key Words: NS1 antigen, Dengue shock syndrome, thrombocytopenia, DEN V, Hemoconcentration

INTRODUCTION

According to the World Health Organization (WHO), there are about 390 million new cases per year of dengue infection worldwide. Dengue infection presents with varied clinical conditions ranging from simple fever to shock. Dengue infection can lead to severe bleeding, shock, and death. Hence early and rapid laboratory diagnosis of dengue is crucial. Hematological, serological and biochemical parameters are important entities which indicate and assess the severity of the disease. The aim of present study is to study the age and sex prevalence in the spectrum of dengue infection and to correlate the hematological, serological and biochemical findings in relation to antigens and antibodies in dengue infection.

MATERIALS AND METHODS

The present study was conducted in Department of Pathology, in a tertiary Hospital for a period of 15 months between April 2015-September 2016. All the patients with immunochromatographic positive (NS1, IgM and IgG) for dengue infection were included in the study. The serological test for dengue was done using a rapid solid phase immunochromatographic test (SD dengue duo) for the qualitative detection of NS1 antigen and differential detection of IgM and IgG antibodies to dengue virus in human serum and confirmed by ELISA. Haematological and biochemical parameters were investigated...
and studied in dengue infection confirmed cases. 4ml of blood was drawn, 2 ml of blood in EDTA vacutainers for complete hemogram, 2ml of blood in plain vacutainers for biochemical investigations. The blood counts were performed on fully automated hematology analyzer with a five part differential (Mindray BC-5380). The peripheral smears were stained with Leishman stain and studied by a pathologist. Complete blood count includes peripheral smear, RBC, WBC, hemoglobin, platelet counts, red cell indices and hematocrit. The biochemical investigations like hepatic enzymes-ALT, AST and ALP, blood urea and serum creatinine were performed on automated biochemistry analyzer (Mindray BS-390). The results were recorded and analyzed.

RESULTS

A total of 137 cases were studied, based on positive dengue test (Rapid card test), and were divided into 3 groups based on WHO classification. Out of 137 cases 92 (67.2%) patients were of Dengue fever, 43 (31.4%) were of Dengue haemorrhagic fever and 2 (1.4%) were Dengue shock syndrome. There was no fatality. Dengue infection was observed commonly in the age group between 11 to 30 years, with the mean age of 26.02 years [Table -1]. Seventy three (53.30%) were male and 64 (46.7%) were female out of 137 cases. The male and female ratio was about 1:1:1. In the serological investigations, the most common antigen observed was NS1 presenting in 66 (48.1%) cases followed by IgG in 35 (25.5%) cases. The positivity of NS1 among DF cases were 45 (48.9%) out of 91 cases and IgG was positive in 24(26.1%) cases. Among 43 cases of DHSF, NS1 was positive in 21(48.8%) cases whereas IgG positivity was observed in 10 (23.2%) cases [Table -2]. Thrombocytopenia (<1 lakh/ml) was observed in 93 (67.8%) out of 137 cases. Fifteen (10.9%) cases were below 20000/ml. There were 50 (75.5%) cases of thrombocytopenia among 92 NS1 positive cases, which is followed by 20 (57.2%) cases of thrombocytopenia among 35 IgG positive cases [Table-3]. We have calculated the hemoconcentration by hematocrit hemoglobin ratio, where the value of <3.5 is counted as normal and > 3.5 as increased in hemoconcentration1. Out of 137 patients studied 39 (28.4%) cases show raise in hemoconcentration. Raise in hemoconcentration was observed in 24 cases among DF, 13 cases among DHSF. Both the cases of DSS exhibit Hemoconcentration [Table-4]. There was raise in hepatic enzyme concentration in about 50 (36.4%) cases. Serum ALT was raised in 18(13.1%) cases and AST in 25 (18.2%) cases [Table-5]. Serum urea was raised in 1 case among DFH and 2 cases among DSS. Serum creatinine was raised in 3 cases among DFH and 2 cases among DSS.

Table 1: Age Distribution of Dengue Infection

<table>
<thead>
<tr>
<th>Age Group</th>
<th>Birth -10</th>
<th>11-20</th>
<th>21-30</th>
<th>31-40</th>
<th>41-50</th>
<th>51-60</th>
<th>61-70</th>
<th>&gt;70</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>6</td>
<td>42</td>
<td>42</td>
<td>13</td>
<td>7</td>
<td>4</td>
<td>4</td>
<td>2</td>
<td>137</td>
</tr>
</tbody>
</table>

Table 2: Serological Distribution of Antigen and Antibodies in spectrum of dengue infection

<table>
<thead>
<tr>
<th>Spectrum of Dengue</th>
<th>NS1</th>
<th>IgM</th>
<th>IgG</th>
<th>NS1 + IgM</th>
<th>NS1 + IgG</th>
</tr>
</thead>
<tbody>
<tr>
<td>DF (n=92)</td>
<td>45</td>
<td>14</td>
<td>24</td>
<td>8</td>
<td>2</td>
</tr>
<tr>
<td>DHSF (n=43)</td>
<td>21</td>
<td>4</td>
<td>10</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>DSS (n=2)</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>66</td>
<td>18</td>
<td>35</td>
<td>14</td>
<td>4</td>
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</tbody>
</table>

Table 3: Platelet Counts Distribution

<table>
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<tr>
<th>Platelet count</th>
<th>NS1 (n=66)</th>
<th>IgM (n=18)</th>
<th>IgG (n=35)</th>
<th>NS1+IgG (n=14)</th>
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</tr>
<tr>
<td>50000 -1 lakh</td>
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</tr>
<tr>
<td>Total</td>
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<td>12</td>
<td>20</td>
<td>9</td>
<td>1</td>
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</table>

Table 4: Hemoconcentration Distribution by Hematocrit Hemoglobin Ratio

<table>
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<tr>
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<th>DF (n=92)</th>
<th>DHSF (n=43)</th>
<th>DSS (n=2)</th>
<th>Total (n=137)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;/= 3.5</td>
<td>13</td>
<td>24</td>
<td>2</td>
<td>39</td>
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</table>

Table 5: Hepatic Enzymes Distribution

<table>
<thead>
<tr>
<th>Total Patients</th>
<th>Alanine Aminotransferase</th>
<th>Aspartate Aminotransferase</th>
<th>Alkaline Phosphatase</th>
</tr>
</thead>
<tbody>
<tr>
<td>DF</td>
<td>2</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>DHSF</td>
<td>14</td>
<td>22</td>
<td>5</td>
</tr>
<tr>
<td>DSS</td>
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<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>17</td>
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<td>7</td>
</tr>
</tbody>
</table>

DISCUSSION

Dengue is caused by Dengue virus (DENV), a mosquito-borne flavivirus which is a single-stranded RNA positive-strand virus. There are 4 distinct but closely related serotypes of the virus that cause dengue (DEN-1, DEN-2, DEN-3 and DEN-4) with a wide range of diseases in humans, from a self-limited dengue fever to life-threatening syndromes namely Dengue hemorrhagic fever and Dengue shock syndrome. In 2014 a total of 40571 cases of dengue infection were reported in India out of which 137 cases
were fatal and in 2015, 99913 cases were reported with 220 fatalities which is more than two-fold increase in the occurrence of the disease. After the bite of an infected mosquito, the dengue virus enters the body and replicates within cells of the mononuclear phagocyte lineage (macrophages, monocytes, and B cells). Additionally, infection of mast cells, dendritic cells, and endothelial cells are known to occur (2). The incubation period of dengue infections is 7–10 days. A viraemic phase follows where the patient becomes febrile and infective. Thereafter, the patient may either recover or progress to the leakage phase, leading to DHF and/or dengue shock syndrome. Peak plasma viraemia correlates with the severity of dengue infections (3). Some of the postulated hypotheses on dengue immunopathogenesis are the antibody enhancement theory, cross-reactive memory T cells activation and the original antigenic sin (4), where all in a way cause an overproduction of cytokine release and it is termed as a cytokine storm. The mean age of dengue infection in the present study was 26.2 years with the range of 4 -83 years and male to female ratio was about1.1:1. The present study correlated with the study done by Malavige et al., (5) where the mean age was 26.6 years. The present study has shown av male preponderance (53.3%) as compared to the females (46.7%) with the male: female ratio being 1.1:1. Similar results were observed by Su DH et al., (6) who gave they male: female ratio as being 1.5:1. The commonest antigen detected in the present study was NS1 66 (48.1%) cases followed by IgG 35 (25.5%) cases out of 137 cases. The present study has similar findings with the study done by Gargi Ghosh et al., (7) where NS1 antigen accounted for 51.5% of the cases. But cases showing IgG positivity (25.5%) were more in the present study and this may be explained by the presence of more number of secondary infections in the present study population. In the present study thrombocytopenia (< 1 lakh) was observed in 93 (67.8%) cases out of 137 cases, among 93 cases 15 (10.9%) cases were presented with platelet count < 20000. The present study correlated with the research done by RD Kulkarni et al., (8) and Parameswarrappa Jyothi et al., (9) where they have observed thrombocytopenia in 68.8% and 61.6% of cases respectively. There were 48 (72.2%) cases of thrombocytopenia among 92 NS1 positive cases in the present study. The similar study of the association of thrombocytopenia with NS1 antigen was observed by RD Kulkarni et al., (8) wherein 130 cases positive for NS1, thrombocytopenia was evident in 103 (79.2%) cases, which correlated with the present study. Out of 137 patients studied we have observed 28.4% (39) cases with raise in hemoconcentration. A study, conducted by Malavige et al., (5) has evidence that out of 108 serologically positive cases 26% show increase in hemoconcentration. In the present study, maximum levels of hepatic enzymes recorded were 190 U/L, 205 U/L and 194 U/L in ALT, AST and ALP respectively. Increase in serum ALT and AST concentration was observed in about 30 (21.9%) and 29 (21.2%) cases respectively. There was an increase in liver enzymes especially ALT and AST in females with age less than 20 years of age in the present study. The similar finding was evident by Luiz Jose de Souza et al., (10). Among DHF cases there was raise in ALT, AST, and ALP concentration in about 14(32.5%), 22 (51.1%) and 5 (11.6%) cases respectively, which was similar to the study conducted by Wahid SF et al., (11). In the present study out of 137 patients 95.6% cases presented with normal concentration of serum urea and creatinine. A study, conducted by Futrakul et al., (12) has reported that there is a mild elevation in serum creatinine in 43% out of 24 DHF cases.

CONCLUSION

The present study observed that dengue infection is common among young adults and a significant association of thrombocytopenia with NS1 antigen positivity has been found. Hence we conclude that early diagnosis of the patient with the help of complete blood examination and serological dengue tests along with prompt clinical care can prevent the fatality of the disease.

Compliance with Ethical Standards

This study was not funded by organization/agency

An informed consent was obtained from participants of the study

REFERENCES


A cross sectional study to evaluate the prevalence of TMD in relation to Gender, Occlusion and Psychological factors in Under Graduate Students

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ABSTRACT

Introduction and Aim: The purpose of the study was to assess that the prevalence of TMD among the group of undergraduate dental students. The association of gender, occlusion and psychological factors to the symptoms of TMD were also evaluated in this study.

Materials and Methods: A sample of 234 subjects aged 18-25 years participated in this study. A questionnaire-based survey was carried out among the students and results were scored, and severity of the TMD was assessed. TMD degree was evaluated using Anamnestic Questionnaire; the morphologic occlusion was assessed according to Angel’s classification. The hospital anxiety and depression scale with 14 subscales self-administered questionnaire was used. Chi-square test was used to compare the incidence of TMD level and its association gender, malocclusion and anxiety and depression levels.

Results: Among 234 subjects study group 51.3% of the students were found have some form of TMD. Subjects with class II malocclusion have a higher prevalence of mild TMD (70.8%). TMD had an association with anxiety and depression levels in this study even though the values are statistically not significant.

Conclusion: There is a high prevalence of TMD in student population however it is classified as mild. Occlusion, anxiety and depression levels play an important role in the occurrence of TMD.

Key Words: TMD, Occlusion, Anxiety, Depression

INTRODUCTION

Tempromandibular disorders (TMD) represents a heterogeneous group of patho-anatomical dysfunctions that affect TMJ and its surrounding tissues. TMD is a term that is used to describe a number of disorders resulting in pain and dysfunction of TMJ and related orofacial structures. It is one of the most common non-infectious painful conditions in the orofacial region.

Etiology of TMD is multifactorial and controversial in nature. Occlusal discrepancies were once considered as one of the major etiological factors in TMD, but later research revealed other factors such as somato form disorders such as emotional stress, personality disorders such as OCD (Obsessive Compulsive Disorder), loss of tooth, interferences in occlusion, spasm of muscles, parafunctional habits such as bruxism, internal and external changes in the TMJ apparatus (1). The multidimensional etiology includes neurobiological, biomechanical, neuromuscular and biopsychosocial factors.

The most common signs and symptoms of TMD are restricted and painful mandibular movement; pain in TMJ, preauricular area and masticatory muscles; muscle fatigue; clicking sounds in the TMJ region (2). TMD because of its multifactorial etiology and variability in the presentation of signs and symptoms in clinical and subclinical degrees are prevalent even among the non-patient population (those who do not seek for health care attention) (3).

Prevalence of TMD is difficult to determine because of different diagnostic qualifications and investigative designs. Prevalence of TMD ranges from 5-60% as given by Magnusson et al. (4). Estimated man-
agement of TMD in adults is about 16% (5). The TMD evaluation and screening is a task challenging to the researchers due to the variation in designing the samples, criteria for evaluation and methods of collecting the information. Recently Schiffman and colleagues in 2014 claimed a comprehensive version of RDC/ TMD known as diagnostic criteria for TMD (DC/ TMD) a valid and reliable screening questionnaire for the diagnosis of pain-related TMD’s. In spite of their advantages, they are cumbersome and require individual to be present at the time of diagnosis and difficult to use on large samples (6). Since there are no criteria to attain numeric value for assessment of severity of TMD indices, play an important role to determine the prevalence of this disorder. Helkimo was a pioneer to establish an index to measure the severity and pain in TMD patients. Helkimo’s index was further broken down into anamnestic and clinical dysfunction and occlusal state (7). Fonseca in 1992 developed anamnestic question based on Helkimo’ s index that classifies the sign and symptoms of TMD as mild, moderate, severe or free of TMD.

Fonesca’s Anamnestic Index (FAI) is a self-administered questionnaire that serves as a preliminary TMD assessment tool for the non-patient population. This questionnaire offers a multidimensional evaluation with less influence from examiners and less variability in measures (8). An occlusal examination was also carried out to assess the correlation between morphologic occlusion and TMD.

Hospital Anxiety and Depression Scale(HADS) is used in this study since the somatoform disorders such as emotional stress may play a role in TMD occurrence among the non-patient population. HADS scale was first used by Zigmond and Snaith that has seven subscales for depression called HADSD and seven subscales for anxiety called HADSa. It is a psychometric tool that gives a clear indication of the severity of mental disorder. This tool takes less time and gives a correlation between stress-related disorder namely anxiety and depression with the severity of TMD. The rationale of this study was to assess the signs and symptoms of TMD that may worsen with time in a Non-patient population of dental students. The association between TMJ and psychological factors such as emotional stress among students as they are away from their home or academic stress are made aware through this study that helps in early diagnosis and management of TMD.

**MATERIALS AND METHODS**

This cross-sectional study was conducted on a sample of 234 students including both genders were randomly selected with age group ranging from 18-25 years. The consent was obtained from the students for conducting the research.

**Data Collection and Analysis**

- An anamnestic self-administered questionnaire was utilized in evaluating TMD degree that comprised of 10 questions. It was a modified version of Helkimo’s anamnestic index that provided substantial information in a short period of time and was easy to use. The number and frequency of positive responses were used to categorize the subjects into mild, moderate, severe and free of TMD.

- An occlusal examination was evaluated according to Angle’s classification (Class I, II and III)

- 14 Subscale of self-administered questionnaire for classifying anxiety and depression level in non-patient population called as HADS scale given by Zigmond and Snaith was utilized. The advantage of this scale was that the somatic symptoms were eliminated. HADSa and HADSD subscales give an indication of the severity of the mental disorder.

The scales were given as follows:

- 0-7: Normal
- 8-10: Mild
- 11-14: Moderate
- 15-21: Severe

The scores that were obtained through the questionnaire was subjected to statistical analysis and the results were obtained.

**Statistical Analysis**

To compare proportions between groups Chi-Square test was applied, if any expected cell frequency is less than five, then Fisher’ s exact test was used. To analyze the data SPSS statistical software (IBM PSS Statistics for Windows, Version 22.0, Armonk, NY: IBM Corp. Released 2013) was used. The significance level was fixed as 5% (α= 0.05).
RESULTS

The result from the anamnestic index of this study shows the marginally higher number of subjects affected by a mild form of TMD (51.3%) compared to normal subjects (44.9%) (Table 1). The percentage of women with TMD (52.7%) was also more compared to men (45.8%) (Table 1). The majority of the subjects with class II malocclusion have a slightly higher percentage of mild form of TMD (70.8%) when compared to class I malocclusion (49.0%) (Table 2), but the values were not statistically significant.

This study does not find any correlation between prevalence of TMD with anxiety and depression levels of the subjects, in fact the percentage of prevalence of TMD was higher in the subjects with normal anxiety and depression levels (70.8% for mild and 77.8% for moderate form of TMD) (Table 3) and depression levels (58.3% for mild and 88.9% for moderate form of TMD (Table 4).

Chi-Square test to compare proportions

Table 1: Severity of TMD in relation to gender (n=234)

<table>
<thead>
<tr>
<th>TMD Degree</th>
<th>Gender</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
<td>Total</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Free of TMD</td>
<td>25 52.1</td>
<td>80 43.0</td>
<td>105 44.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mild</td>
<td>22 45.8</td>
<td>98 52.7</td>
<td>120 51.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moderate</td>
<td>1 2.1</td>
<td>8 4.3</td>
<td>9 3.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>48 100.0</td>
<td>186 100.0</td>
<td>234 100.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Chi-Square Tests Value P-Value
Fisher’s Exact Test 1.292 0.567

Table 2: Severity of TMD in relation to Angle’s classification (n=234)

<table>
<thead>
<tr>
<th>Molar Class</th>
<th>TMD Degree</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Free of TMD</td>
<td>Mild</td>
<td>Moderate</td>
<td>Total</td>
<td></td>
</tr>
<tr>
<td></td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
<td></td>
</tr>
<tr>
<td>CL -1</td>
<td>94 47.0</td>
<td>98 49.0</td>
<td>8 4.0</td>
<td>200 100.0</td>
<td></td>
</tr>
<tr>
<td>CL -2</td>
<td>7 29.2</td>
<td>17 70.8</td>
<td>0 0.0</td>
<td>24 100.0</td>
<td></td>
</tr>
<tr>
<td>CL -3</td>
<td>4 40.0</td>
<td>5 50.0</td>
<td>1 10.0</td>
<td>10 100.0</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>105 44.9</td>
<td>120 51.3</td>
<td>9 3.8</td>
<td>234 100.0</td>
<td></td>
</tr>
</tbody>
</table>

Chi-Square Tests Value P-Value
Fisher’s Exact Test 5.346 0.213

DISCUSSION

Gary R Rollman stated that there was a very strong predilection for TMD (9). TMD is almost 2-3 times more common in women than in men. Krogstad et al. (10) observed more gender differences. While other studies (11, 12) showed that TMD was more prevalent in women of older age groups due to female reproductive hormones which contribute to etiology of TMD. In this study young adolescent females were examined which eliminated etiology due to hormonal disturbance and psychological causes. The apparent increase in the female group could be due to the greater number examined.

According to the HADS scale results of this study 19.2% of the subjects with mild TMD had mild anxiety and with moderate TMD had mild anxiety level of 22.2% These results were consistent with the previous study done by Bonjardim et al (13, 14). Mingheli et al (15) in his studies showed that students in health care schools showed anxiety or depression levels. However, results were consistent with Bonjardim et al (2009) which found an association with TMD degree and HADSa but not with HADSd. In the current study none of the correlations were sta-

Table 3: Severity of TMD in relation to Anxiety levels(n=234)

<table>
<thead>
<tr>
<th>TMD Degree</th>
<th>Anxiety level</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal</td>
<td>Mild</td>
<td>Moderate</td>
<td>Total</td>
<td></td>
</tr>
<tr>
<td></td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
<td></td>
</tr>
<tr>
<td>Free of TMD</td>
<td>80 76.2</td>
<td>19 18.1</td>
<td>6 5.7</td>
<td>105 100.0</td>
<td></td>
</tr>
<tr>
<td>Mild</td>
<td>85 70.8</td>
<td>23 19.2</td>
<td>12 10.0</td>
<td>120 100.0</td>
<td></td>
</tr>
<tr>
<td>Moderate</td>
<td>7 77.8</td>
<td>2 22.2</td>
<td>0 0.0</td>
<td>9 100.0</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>172 73.5</td>
<td>44 18.8</td>
<td>18 7.7</td>
<td>234 100.0</td>
<td></td>
</tr>
</tbody>
</table>

Chi-Square Tests Value P-Value
Fisher’s Exact Test 1.893 0.741

Table 4: Severity of TMD in relation to Depression levels(n=234)

<table>
<thead>
<tr>
<th>TMD Degree</th>
<th>Depression level</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal</td>
<td>Mild</td>
<td>Moderate</td>
<td>Severe</td>
<td>Total</td>
</tr>
<tr>
<td></td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
</tr>
<tr>
<td>Free of TMD</td>
<td>68 64.8</td>
<td>17 16.2</td>
<td>11 10.5</td>
<td>9 8.6</td>
<td>105 100.0</td>
</tr>
<tr>
<td>Mild</td>
<td>70 58.3</td>
<td>20 16.7</td>
<td>15 12.5</td>
<td>15 12.5</td>
<td>120 100.0</td>
</tr>
<tr>
<td>Moderate</td>
<td>8 88.9</td>
<td>1 11.1</td>
<td>0 0.0</td>
<td>0 0.0</td>
<td>9 100.0</td>
</tr>
<tr>
<td>Total</td>
<td>146 62.4</td>
<td>38 16.2</td>
<td>26 11.1</td>
<td>24 10.3</td>
<td>234 100.0</td>
</tr>
</tbody>
</table>

Chi-Square Tests Value P-Value
Fisher’s Exact Test 3.103 0.792
tistically significant compared to other studies (13-16) which showed a definitive association.

There was a long contention as to psychological causes of TMD. The current study shows a weak correlation. The reason could be that a definitive group undergoing dental curriculum was examined with a greater number of females. The reason then students showed mild anxiety level could be stress due to curriculum, peer pressure, deprivation of family surrounding and reduced counseling from family members. This small group could be called for students counselling.

According to the study, 70.8% subjects with class II malocclusion were reported with mild TMD while 50% reported for the class I and class III. Our results agree with other studies (17-21). This study suggests that malocclusion could play a role in TMD. However, none of the subjects showed severe TMD, and the statistical difference between groups was not established. Definitive, conclusive evidence cannot be drawn as the symptoms were mild and an adolescent group was examined (22).

**Limitations**

Compared to the general population the examined population was small. A smaller group of undergraduate students undergoing dental curriculum were examined. A larger population of students belonging to different course groups could give better authenticity to the results.

**CONCLUSION**

The conclusion within the limits of this study there seems to be the prevalence of mild TMD in the undergraduate dental students. Class II malocclusion may have a role to play. Anxiety levels were mild in patients with mild TMD. However definitive conclusions cannot be drawn based on this study. The etiology of TMD has always been an enigma as shown by this study. More detailed and extensive research has to be taken up in this area to make definitive conclusions.

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Drinking Water Stored in Copper Vessel - Reveals Antibacterial Activity

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ABSTRACT

Introduction and Aim: Recently About 2.2 million diarrheal deaths per year especially in developing countries, in which 1.4 million deaths among children under age five. Safe drinking water, especially in developing countries, is still a major problem. The present study was designed to explore the antibacterial effect of copper vessel stored with drinking water inoculated with diarrhea-causing bacterial strains enterotoxigenic Escherichia coli (ETEC), Vibrio cholera and Shigella flexneri.

Materials and Methods: The bacterial strains isolated from fecal sample, identified by colony morphology and biochemical test. About 500 Colony Forming Unit (CFU/mL) of the bacterial strains of E. coli, V. cholera and S. flexneri were inoculated separately in drinking water stored in the copper vessel and non-copper vessel for 12 hrs. After incubation 100 μL of samples was taken from each copper vessel and non-copper vessel container and spread on nutrient agar for the enumeration of bacteria colonies. After 24hrs of incubation at 37°C, dishes were observed for visible bacterial colonies using colony counter.

Results: The bacterial strains E. coli (ETEC) (532 CFU), V. cholera (502 CFU) and S. flexneri (512 CFU) was inoculated in copper vessel observed no growth on the selective media and when the bacterial strains inoculated in the non-copper vessel observed growth upon inoculated in selective media E. coli (ETEC) (624 CFU), V. cholera (328 CFU) and S. flexneri (483 CFU).

Conclusion: The antibacterial activity may be due to the oligodynamic effect of copper which destroy the cell wall and cell membrane results in membrane damage. The present study recommends the use of cost-effective copper vessels to store drinking water especially in rural areas for protection from water-borne infection.

Key Words: Diarrheal, Copper vessel, Drinking water

INTRODUCTION

The 70% of the World is covered by water of which only 2.5% is of fresh water, which 1% of the freshwater is accessible easily, remaining trapped in glaciers and snowfields and from the 1% of fresh water only 0.3-0.5% is available for drinking purpose. As the human population enhances, day by day and daily consumption of fresh water also increased several folds.

Water plays an essential dual role - one is vital for life, as more than 60% of human body is composed of water and sustains the natural environment, contributes to the development of economic, health, social, recreational and cultural activities. On the other hand, it plays a vital role in the transmission of water-borne infectious diseases. It is estimated to cause about 2.2 million diarrhea deaths per year especially in developing countries in which 1.4 million deaths among children under age five (1). Worldwide, an economic burden for a water-borne disease is about 12 billion US dollars per year (2). Waterborne infections are caused by ingestion, airborne or contact with contaminated water by a variety of infectious agents which includes bacteria, viruses, protozoa and helminths (3). In addition, flood accelerates the risks of outbreaks of waterborne diseases. About 1415 species of microorganisms have been reported to be pathogenic, among which approximately 348 are water-borne, causing 115 infectious diseases (4).
From 1991 to 2008, about 1,428 water-borne outbreaks were reported (5).

The major bacterial agents that account for millions of diarrhoeal deaths particularly in developing countries, are enterotoxigenic Escherichia coli (ETEC), Vibrio cholerae, and species of Shigella, which spread through contaminated water, food or from person to person. Shigella flexneri known as acute bacillary dysentery causes approximately 10% of all diarrhoeal episodes among children aged less than five years (6). Infection with ETEC is associated with traveler’s diarrhea, and the rate of infection is higher in India compared to other developing countries (7).

Currently, about 780 million people do not have access to a purified drinking water, and 2.5 billion people lack access to improve sanitation worldwide especially in the developing countries (8). It is estimated that about 3.2% of deaths globally are attributable to consume unsafe water caused by poor sanitation and hygiene (9). The WHO recommends that improving water quality may reduce the global diseases burden by approximately 4%. Thus, there is an urgent need to move all possible efforts to minimize the water-borne infection. Though detection methods play an essential role in monitoring water quality, surveillance, and quantitative microbial risk assessment but providing safe drinking water globally especially in developing countries like India is still a challenging one.

The household boiling water for disinfection is in danger of leaching harmful chemicals from the plastic bottles and container, also prone to recontamination during handling and storage. At present three main water treatment methods are available includes distillation, reverse Osmosis and solid block activated carbon, apart UV, Ozone, activated alumina, sediment filters, ion exchange, granular activated carbon, and boiling are also used but combined with other methods for effective outcomes. Many of the currently available water purification systems are more expensive, required regular maintenance, electricity and beyond the reach of the rural population in countries such as India is questionable. Even inexpensive Candle filters (with diatomaceous earth) require regular cleaning and replacement are usually ignored by users.

In most of the rural places at India, people usually collect drinking water from the lake, ponds, wells, running streams, municipal pipes, from stored water tanks and water, may become contaminated at any point between collection, carrying and storage before use. The use of copper by human civilizations dates behind to between the 5th and 6th millennia B.C. It was the 1st metal used, presumably because it could be found in a native, metallic form which did not demand to smelt. The Indian Ayurveda describes storing water in a copper vessel overnight and drinking it in the morning had many health benefits. The present study was designed to explore the antibacterial effect of copper vessel container by storing the drinking water inoculated with diarrhea causing bacterial strains enterotoxigenic E. coli (ETEC), V. cholera and S. flexneri.

**MATERIALS AND METHODS**

**Isolation and Identification of Bacterial Strains**

Bacterial strains enterotoxigenic E. coli (ETEC), V. cholera and S. flexneri were isolated in the faecal sample. Bacterial strains enterotoxigenic E. coli (ETEC), V.cholera and S. flexneri were isolated in the stool samples by culturing on Mac conkey agar, thiosulphate-citrate-bile salt sucrose agar (TCBS) and Salmonella shigella agar respectively. Initially colony morphological identification was Mac conkey plates showed pure lactose fermenting, translucent, glossy, smooth colonies and were identified as E. coli. On TCBS agar V. cholera produce large, flattened yellow colour colonies with opaque centers due to fermentation of sucrose in the medium. S. flexneri is non lactose fermenter appear as a transparent colorless colonies. Biochemical test showed Indole and Methyl Red (MR) showed Positive and negative for Voges-Proskauer (VP) and citrate for E. coli. For identification of V. cholera Catalase and oxidase test showed positive, urease test showed negative, indole, citrate and MR test showed positive and Negative for VP test. For identification of S. flexneri showed Catalase, MR and nitrate reduction showed positive and negative for Citrate, Oxidase, and VP test. After morphological and biochemical identification single colonies of E. coli (ETEC), V. cholera and S. flexneri were isolated, inoculated in sterile Luria Bertani broth as a starter culture and incubated at 370C for 12-16 hrs, followed by serial dilution with normal physiological saline.

**Serial Dilution Method**

A pure culture may be obtained by serially diluting (tenfold-1 in 10 dilutions-1:10, 1:100 or 1/10, 1/100, 1/1000 or 10-1, 10-2, 10-3) the sample with sterile...
water or saline to the point of extinction in numbers of cells and transferred on an agar plate. In serial dilution technique sample has been diluted serially. It is assumed that each viable bacterium in the original sample can produce one discrete colony (colony forming unit or CFU) and thus, the number of colonies represents the number of bacteria that can grow under the conditions. Petridish with between 30 and 300 colonies are ideal for completing a standard plate count.

**Spread Plate Method**

An aliquot of the serially diluted sample (0.1ml) is placed onto the agar surface in a petri dish and is spread uniformly with a sterile, bent ‘L’ shaped glass rod until the surface becomes dry and the spreader begins to stick to the surface. Petri dishes are turned upside down to avoid condense on the agar and incubated at 37°C for 24 hours and after 24 hours of incubation the Petri dishes are examined for visible colony formation. Above said procedure repeated in triplicate and average mean of the three was calculated.

**Inoculation of bacterial culture in sterilized water stored in copper and non-copper vessel**

Drinking water (2 liters) was sterilization at 120°C for 15-20 minutes and transferred to sterile copper vessel and non-copper vessel. Then sterilized water was inoculated with 500 CFU/mL of test bacterial strain. The CFU was carried by serially dilution and spread plate method (for each bacterial culture separate copper / non-copper containers were used). Before and after inoculation bacterial population was enumerated by spread plate method. Then the copper vessel and non-copper vessel’s with bacterial cultures were incubate at room temperature (28±2°C) for 12hours. After incubation 100 μL of samples was taken from each copper vessel and non-copper vessel container and spread on nutrient agar for the enumeration of bacteria colonies. After 24hrs of incubation at 37°C, dishes were observed for bacterial colonies using colony counter.

**RESULTS**

The bacterial strains E. coli (ETEC) (532 CFU), V. cholera (502 CFU) and S. flexneri (512 CFU) was inoculated in copper vessel showed no growth on the selective media and when the bacterial strains inoculated in the non-copper vessel showed growth upon inoculated in selective media E. coli (ETEC) (624 CFU), V. cholera (328 CFU) and S. flexneri (483 CFU) shown in Table No. 1.

<table>
<thead>
<tr>
<th>SL. No</th>
<th>Bacterial sample</th>
<th>Before incubation number of CFU</th>
<th>After incubation number of CFU for sample taken copper vessels container</th>
<th>After incubation number of CFU for sample taken non-copper vessels container</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Escherichia coli (ETEC)</td>
<td>532±24</td>
<td>0</td>
<td>624±35</td>
</tr>
<tr>
<td>2</td>
<td>Vibrio cholera</td>
<td>502±12</td>
<td>0</td>
<td>328±24</td>
</tr>
<tr>
<td>3</td>
<td>Shigella flexneri</td>
<td>512±11</td>
<td>0</td>
<td>483±56</td>
</tr>
</tbody>
</table>

* Number of colony forming unit (CFU)

**DISCUSSION**

Water is essential for maintaining life on Earth, but can also serve as a media for many pathogenic organisms. The present study was designed to provide safe water to people especially at the rural places where the water purifier cannot reach since more expensive, required regular maintenance which is beyond for the rural population. The use of copper by human civilizations dates back to between the 5th and 6th millennium B.C. Copper vessel is merely passive storage of water, and it will be a one-time investment with no recurring costs for further maintenance. It is suitable for developing countries like India where there is a frequent intermittent supply of drinking water, necessitating storage of drinking water for days. In such conditions, copper vessels can be introduced during storage of drinking water. In the present study, the result showed, no growth was observed after 12hrs of incubation of bacterial strains stored in a copper vessel and when the water stored in non-copper vessel showed the presence of colony forming unit indicates its viability (Table No. 1).

When the water is stored in a copper vessel overnight a detectable amount of copper ions gets dissolved into the water called Oligodynamic effect. The dissolved copper result in structural and membrane damage, by observing to inactivate bacteria by destroying their cell wall and cell membrane (10,11). Damage to the membrane leads to leakage of potassium or glutamate through the outer membrane of
bacteria results in osmotic shock. In addition, copper ions binding to proteins that cause oxidative stress by generating hydrogen peroxide and damaging DNA may result in genotoxicity (11).

As copper vessels are not very common today since the costly and easy availability of plastic and stainless steel containers with less cost. The present study recommends to use a copper vessel to store drinking water since Copper vessel is easy to use, requires no electricity/battery and does not need any maintenance and replacements like other commercial water purifiers, making it ideal for situation prevailing in developing countries like India especially in rural areas. This study also recommends that canned drinking water supply companies can use copper coated containers for processing the water and also to use copper coated pipelines.

**CONCLUSION**

The antibacterial properties of water stored in copper vessel inoculated with bacterial strains were firmly established. The result showed no bacterial colony counts were enumerated which implies that storage of water in the copper vessel is a promising additional tool alongside other hygienic measures to curb the number and severity of waterborne diarrhoeal infections. At this point, additional studies would be recommended to help in determining the most cost-effective way to give maximal protection for other water-borne diseases.

**Conflicts of Interest**

Author’s declared that no Conflict of Interest

**REFERENCES**

Extraction and Identification of Phytoconstituents from Leaves of *Phyllanthus gardnerianus* by GC-MS

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**ABSTRACT**

**Introduction and Aim:** Medicinal plants are well known for their therapeutic properties and they are highly esteemed all over the world due to its prevention capacity of several diseases and ailments. A large number of secondary metabolites such as tannins, alkaloids, phenolics, and terpenoids responsible for the pharmacological properties of medicinal plants. The present study was extraction and identification of phytoconstituents from Leaves of *Phyllanthus gardnerianus* by GC-MS.

**Materials and Methods:** The shade dried (200 g) of *Phyllanthus gardnerianus* leaves were ground to powder and soaked in 2 Litre of methanol solvent. Periodically, the container was shaken well and filtered. The extract was removed using the distillation method. Again fresh methanol was added to the material. The same procedure was followed until to get disappearance of colour. On evaporation of the solvent yielded the crude extracts. The crude extract methanol was thoroughly washed with hexane, chloroform and ethyl acetate. This material was then subjected to column chromatography. GC–MS analysis of ethanol extract of *Phyllanthus gardnerianus* leaves was carried out using a GC Perkin–Elmer system. The spectrum of the unknown components was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained.

**Results:** Eighteen compounds were detected in the methanol extract of *Phyllanthus gardnerianus* (Table 1). Some of the major compounds were found from the GC-MS analysis are 3-t-Butyl-4-methoxyphenol methyl derivative, 1,2-Benzenedicarboxylic acid, Guaiol, Patchouli alcohol, Gibberellin, Hexadecanoic acid methyl ester, 1,3-dimethyl-4-azaphenanthrene and 2-(6,7-Dimethoxy-1-isquinolinyl)phenol and the remaining compounds are listed in Table 1.

**Conclusion:** In the present study, GC-MS analysis of methanol extract of leaves of *Phyllanthus gardnerianus* showed eighteen biomolecules according to the analysis. In recent years, the traditional uses of *Phyllanthus* spp. had been partly confirmed and more evidence such as pharmacological research and clinical studies are urgently needed to be taken to extend the use of *Phyllanthus* and to develop leading compounds from the species studied.

**Key Words:** GC-Ms analysis, *Phyllanthus gardnerianus*, Distillation method, NIST library

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**INTRODUCTION**

Medicinal plants are an expensive gift from nature to human. The approval of traditional medicine as an alternative form of health care and the improvement of microbial resistance to the existing antibiotics has lead researchers to scrutinize the antimicrobial compounds(1). Medicinal plants have been used allover the world for the treatment and prevention of various ailments, particularly in developing countries, where, infectious diseases are endemic and modern health facilities and services are inadequate(2). The medicinal actions of plants unique to particular plant species or groups are consistent with the concept that the combination of secondary products in a particular plant is taxonomically distinct(3). Screening active compounds from plants has to lead to the invention of new medicinal drugs which have efficient protection and treatment roles against various diseases including cancer and alzheimer’s disease (4,5).
The plants of genus *Phyllanthus* have been reported to possess a variety of curative effects. *Phyllanthus emblica* has been reported to possess antiviral and antimicrobial properties (6). *P. amarus* is a rich source of phytochemicals such as alkaloids, astragalin, brevifolin, carboxylic acids, corilagin, cymene, ellagic acid, ellagitanins, galloatechins, geraniin, hypophyllanthin, phyllanthin, lignans, lintetralins, lupeols, methyl salicylate, phyllanthine, phyllanthenol, phyllochrysine, phyltetralin, repandusinic acids, quercetin, quercetol, quercitrin, rutin, saponins, triacontanal and tricontanol (7). *Phyllanthus muelleri*ans extracts are reported to possess antimicrobial (8) properties. *Phyllanthus niruri* may possibly help prevent stone formation (9). *Phyllanthus amarus* root and leaf extract have been reported to show significant hepatitis C antiviral activity (10). *Phyllanthus acidus* (leaf) showed antiplasmodial activity against *Plasmodium falciparum* (11). Thus literature survey reveals that the *Phyllanthus* plants have varied medicinal properties hence it is worth experimenting to isolate, characterize the study the antioxidant properties of their phytoconstituents. With this background, the present study was aimed to identify the phytoconstituents present in *P. amarus* using GC-MS analysis. GC-MS is the best technique to identify the bioactive constituents of long chain hydrocarbons, alcohols, acids esters, alkaloids, steroids, amino acid and nitro compounds (12).

**MATERIALS AND METHODS**

**Materials**

The plant material for the present investigation was collected from Kalakad (*Phyllanthus gardnerianus* (Wight) Baill.) The plant material was washed and rinsed with tap water to remove all the dirt and unwanted particles prior to the drying process. The collected sample was kept under shade and dried for 3–4 weeks at room temperature (27–32°C) till a constant weight was obtained. The dried plant material was granulated or powdered by using a blender and sieved to get uniform particles by using sieve No. 60. The dried powder was used for Phytochemical studies.

**Preparation of crude extracts**

The shade dried (200 g) of *Phyllanthus gardnerianus* leaves were ground to powder and soaked in 2 Litre of methanol solvent. Periodically, the container was shaken well and filtered. The extract was removed using the distillation method. Again fresh methanol was added into the material. The same procedure was followed until to get disappearance of colour. On evaporation of the solvent yielded the crude extracts. The crude extract methanol was thoroughly washed with hexane, chloroform and ethyl acetate. This material was then subjected to column chromatography.

All the chemicals used to carry out the research work were of AR grade and used as such without further purification. All solutions were prepared using double distilled water.

**GC–MS (Gas Chromatography-Mass Spectroscopy) Analysis**

GC–MS analysis of ethanol extract of *Phyllanthus- gardnerianus* leaves was carried out using a GC Perkin–Elmer system comprising an Autosampler and gas chromatograph interfaced to a mass spectrometer (GC–MS) equipped with a Elite–1, fused silica capillary column (330 mm x 0.25 mm ID x 1 mm df, composed of 100% Dimethyl polysiloxane). For GC–MS detection, an electron ionization system with the ionizing energy of 70 eV was used. Helium gas was used as a carrier gas at the flow rate of 1ml/min, and an injection volume of 0.5 ml was employed. Injector and Ion source temperature was 250–280°C. The oven temperature was started at 110°C with an increase of 10°C /minutes. Mass spectrum was recorded at 70 eV. Total GC running time was 30 min. The relative percentage of each compound was calculated by comparing its retention time. Through the library search, the name of the compounds was obtained.

Interpretation of mass spectra of GC-MS was performed using the database of the National Institute of Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the unknown components was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained.

**RESULTS**

The chemical composition of methanolic extract of leaves of *Phyllanthus gardnerianus* was analyzed by using GC-MS. The chromatogram of the leaf powder of *Phyllanthus gardnerianus* was shown in Figure 1. Mass spectra were used to identify the structure of the compounds found by comparing with those in
Figure 1: GC-MS analysis of the methanol extract of leaves of *Phyllanthus gardnerianus*.

<table>
<thead>
<tr>
<th>S. No</th>
<th>RT</th>
<th>Name of the Compounds</th>
<th>Molecular formula</th>
<th>Molecular weight</th>
<th>Nature of Compound</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>15.616</td>
<td>3-t-Butyl-4-methoxyphenol methyl derivative</td>
<td>C_{12}H_{18}O_{2}</td>
<td>194</td>
<td>Phenolic compound</td>
</tr>
<tr>
<td>2</td>
<td>15.194</td>
<td>1,2-Benzenedicarboxylic acid</td>
<td>C_{8}H_{6}O_{4}</td>
<td>166</td>
<td>Carboxylic acid</td>
</tr>
<tr>
<td>3</td>
<td>17.303</td>
<td>Guaiol</td>
<td>C_{15}H_{26}O</td>
<td>222</td>
<td>Phenolic compound</td>
</tr>
<tr>
<td>4</td>
<td>17.641</td>
<td>Patchouli alcohol</td>
<td>C_{15}H_{26}O</td>
<td>222</td>
<td>Alcohol</td>
</tr>
<tr>
<td>5</td>
<td>18.158</td>
<td>1-Methoxy-2-methyl-4-(methylthio)benzene</td>
<td>CH_{3}OC_{6}H_{5}(CH_{3})SCH_{3}</td>
<td>168</td>
<td>Benzene derivative</td>
</tr>
<tr>
<td>6</td>
<td>18.417</td>
<td>1,5-dimethylbicyclo[3.3.1]non-3-en-2,9-dione</td>
<td>C_{10}H_{14}O_{2}</td>
<td>166</td>
<td>Ester</td>
</tr>
<tr>
<td>7</td>
<td>18.532</td>
<td>3,6-Dimethyl-5-hepten-1-ol acetate</td>
<td>C_{9}H_{18}O</td>
<td>142</td>
<td>Ester</td>
</tr>
</tbody>
</table>
DISCUSSION

The GC-MS analysis of Phyllanthus gardnerianus indicated the presence of eighteen phytocompounds which are presented in Table 1. Various types of compounds were found in the methanolic extract of leaves of Phyllanthus gardnerianus including methyl derivatives, carboxylic acid, ester, gibberellin, indole, and phenol. Among the phytochemicals identified from the methanolic extract of leaves of Phyllanthus gardnerianus, 1,2-Benzenedicarboxylic acid, diethyl ester possesses antimicrobial, antioxidant and anti-inflammatory, as reported by earlier works. It is also reported that Guaiol from Cymbopogan parkari has the perfumery, pesticide and termitifuge activity(13). In the present investigation, Guaiol is present in the leaves of Phyllanthus gardnerianus. Recently, it is confirmed that hexadecane-2-one, oleyl alcohol and heptfluorobutyrate from Phyllanthus niruri using GC-MS method. 2H-pyran-2, 6 (3H)–dione, 5-hydroxy methyl-2-furan carboxyaldehyde and γ-sitosterol and stigmastan-3, 5-diene have been reported from the leaves of Phyllanthus emblica and Phyllanthus madarspatensis by GC-MS (15,16). They have also investigated in the leaves of P. amarus using GC-MS analysis many compounds such as 3, 5-di-t-butylphenol (1.2%), methyl 14-methyl pentadecanoate (1.4%), hexadecanoic acid, (11.8%), 10-octadecanoate (5.5%), 9-hexadecenal (9.0%), glycerol 1, 3-dipalmitate (5.7%), 2, 13-octadecadiene-1-ol (8.2%), dioctytl ester (10.1%) and heptanoic acid (9-dece-1-yl ester), (4.6%)(17). Thus, GC-MS analysis showed active and important compounds from both plant spp. and it can be utilized for further development of new phytomedicines for various therapeutic purposes.

CONCLUSION

In the present study, GC-MS analysis of methanol extract of leaves of Phyllanthus gardnerianus showed eighteen biomolecules according to the analysis. In recent years, the traditional uses of Phyllanthus spp. had been partly confirmed and more evidence such as pharmacological research and clinical studies are urgently needed to be taken to extend the use of Phyllanthus and to develop leading compounds from the species studied.

<table>
<thead>
<tr>
<th>No.</th>
<th>M.S. No.</th>
<th>Molecular Weight</th>
<th>Molecular Formula</th>
<th>Retention Time</th>
<th>Compound Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>18.950</td>
<td>270</td>
<td>C17H34O2</td>
<td>8</td>
<td>Isopropyl Myristate</td>
</tr>
<tr>
<td>9</td>
<td>19.783</td>
<td>270</td>
<td>C17H34O2</td>
<td>9</td>
<td>Hexadecanoic acid methyl ester</td>
</tr>
<tr>
<td>10</td>
<td>20.414</td>
<td>282</td>
<td>C17H18N2O2</td>
<td>10</td>
<td>2,2'-[1,2-Propanediylbis(nitrilomethylidylen)]diphenol</td>
</tr>
<tr>
<td>11</td>
<td>22.346</td>
<td>371</td>
<td>C21H25NO3</td>
<td>11</td>
<td>Demecolcine</td>
</tr>
<tr>
<td>12</td>
<td>23.181</td>
<td>495</td>
<td>C29H27NO7</td>
<td>12</td>
<td>1,3-dimethyl-4-azaphenan-threne</td>
</tr>
<tr>
<td>13</td>
<td>23.479</td>
<td>346</td>
<td>C19H22O6</td>
<td>13</td>
<td>Gibberellin</td>
</tr>
<tr>
<td>14</td>
<td>24.394</td>
<td>85</td>
<td>C25H11N</td>
<td>14</td>
<td>Hexahydroxyridine</td>
</tr>
<tr>
<td>15</td>
<td>26.057</td>
<td>341</td>
<td>C30H23NO4</td>
<td>15</td>
<td>Dibenzoazabicycloundecane</td>
</tr>
<tr>
<td>16</td>
<td>32.193</td>
<td>210</td>
<td>C9H6O6</td>
<td>16</td>
<td>1,2,4-Benzendentrioxylic acid</td>
</tr>
<tr>
<td>17</td>
<td>33.889</td>
<td>207</td>
<td>C15H13N</td>
<td>17</td>
<td>4’ Methyl-2 Phenylindole</td>
</tr>
<tr>
<td>18</td>
<td>33.946</td>
<td>285</td>
<td>C17H19NO3</td>
<td>18</td>
<td>2-(6,7-Dimethoxy-1-isquinolyl)phenol</td>
</tr>
</tbody>
</table>

Phyllanthus spp. are well known for the phytochemicals. Many researchers are working on Phyllanthus spp. for the exploration of biomolecules. They have reported alkaloids, terpenoids, tannins, flavonoids, saponins 9, 12-octadecadienoic acid, bicycle (13, 1, 0) hexadecane-2-one, oleyl alcohol and heptfluorobutyrate from Phyllanthus niruri using GC-MS method. 2H-pyran-2, 6 (3H)–dione, 5-hydroxy methyl-2-furan carboxyaldehyde and γ-sitosterol and stigmastan-3, 5-diene have been reported from the leaves of Phyllanthus emblica and Phyllanthus madarspatensis by GC-MS (15,16). They have also investigated in the leaves of P. amarus using GC-MS analysis many compounds such as 3, 5-di-t-butylphenol (1.2%), methyl 14-methyl pentadecanoate (1.4%), hexadecanoic acid, (11.8%), 10-octadecanoate (5.5%), 9-hexadecenal (9.0%), glycerol 1, 3-dipalmitate (5.7%), 2, 13-octadecadiene-1-ol (8.2%), dioctytl ester (10.1%) and heptanoic acid (9-dece-1-yl ester), (4.6%)(17). Thus, GC-MS analysis showed active and important compounds from both plant spp. and it can be utilized for further development of new phytomedicines for various therapeutic purposes.
<table>
<thead>
<tr>
<th>S.No</th>
<th>Name of the Compounds</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>3-t-Butyl-4-methoxyphenol methyl derivative</td>
<td>Antioxidant activity</td>
</tr>
<tr>
<td>2.</td>
<td>1,2-Benzenedicarboxylic acid</td>
<td>Antimicrobial, antioxidant and antiinflammatory</td>
</tr>
<tr>
<td>3.</td>
<td>Guaiol</td>
<td>Perfumary, pesticide and termitifuge</td>
</tr>
<tr>
<td>4.</td>
<td>Patchouli alcohol</td>
<td>Antibacterial, antiplaque, fungicide, antidepressants and pesticide</td>
</tr>
<tr>
<td>5.</td>
<td>1-Methoxy-2-methyl-4-(methylthio)benzene</td>
<td>Antifungal activity</td>
</tr>
<tr>
<td>6.</td>
<td>1,5-dimethylbicyclo[3.3.1]non-3-en-2,9-dione</td>
<td>Anticancer activity</td>
</tr>
<tr>
<td>7.</td>
<td>3,6-Dimethyl-5-hepten-1-ol acetate</td>
<td>Cytotoxicity</td>
</tr>
<tr>
<td>8.</td>
<td>Isopropyl Myristate</td>
<td>Antimicrobial &amp; antioxidant activity</td>
</tr>
<tr>
<td>9.</td>
<td>Hexadecanoic acid methyl ester</td>
<td>Antiinflammatory, cytotoxicity, Antioxidant, Hypocholesterolemic, Antimicrobial activity, Nematicide, Insecticide, Lubricant, Antiandrogenic Flavor and Hemolytic</td>
</tr>
<tr>
<td>10.</td>
<td>2,2’-[1,2-Propanediylbis(nitrilo-methylidylen)]diphenol</td>
<td>Antileukemic, antimitotic, antipodagric, antitubulin, antitumor, marrow-suppressant and MDR-inhibitor</td>
</tr>
<tr>
<td>11.</td>
<td>Demecolcine</td>
<td>Antibacterial and antifungal activity</td>
</tr>
<tr>
<td>12.</td>
<td>1,3-dimethyl-4-azaphenan-threne</td>
<td>Cicatrizant and Plant growth inhibition</td>
</tr>
<tr>
<td>13.</td>
<td>Gibbrellin</td>
<td>Anticancer activity</td>
</tr>
<tr>
<td>14.</td>
<td>Hexahydropyridine</td>
<td>Anticancer activity</td>
</tr>
<tr>
<td>15.</td>
<td>Dibenzoazabicycloundecane</td>
<td>Not reported</td>
</tr>
<tr>
<td>16.</td>
<td>1,2,4-Benzeneetricarboxylic acid</td>
<td>Anticancer and antifungal activity</td>
</tr>
<tr>
<td>17.</td>
<td>4’ Methyl-2 Phenylindole</td>
<td>Antioxidant and anticancer activity</td>
</tr>
<tr>
<td>18.</td>
<td>2-(6,7-Dimethoxy-1-isquinolinyl)phenol</td>
<td>2-(6,7-Dimethoxy-1-isquinolinyl)phenol</td>
</tr>
</tbody>
</table>

Source: D. Duke’s: Phytochemical and Ethnobotanical Databases

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Comparison of Abdominal Crunch with and without Tongue in Physiological Position in Improving Deep Cervical Flexors Endurance

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ABSTRACT

Introduction and Aim: To Compare the Effect of Abdominal crunch with and without Tongue in Physiological position in Improving Deep Cervical Flexor Endurance. The objective of the study is (a) To find the effectiveness of abdominal crunch with tongue in resting position in improving deep cervical flexor endurance. (b) To find the effectiveness of abdominal crunch without tongue in physiological position in improving deep cervical flexor endurance. (c) To compare the effectiveness abdominal crunch with and without tongue in physiological position in improving deep cervical flexor endurance.

Materials and Methods: Stop watch Examination couch. 60 subjects (students) from Physiotherapy College were selected based on the Inclusion and Exclusion Criteria. Subjects were divided into 2 groups: Group A-Abdominal crunch without tongue in Physiological position, Group B- Abdominal crunch with tongue in resting position. All participants were made to exercise for 10 repetitions for 4 sets with one session per day for 5 days a week for totally 8 weeks. Endurance of Deep cervical flexors measured by Deep Cervical Flexor Endurance Test. The collected data was tabulated & analyzed using descriptive & inferential statistics. Mean and Standard deviation (SD) were used for all parameters. Paired t-test was used to analyze significant changes between pre-test and post-test measurements. Unpaired t-test was used to analyze significant changes between two groups.

Results: From the statistical analysis made with quantitative data revealed statistically significant difference between the Group A and Group B, and also within the group. The post-test mean value of Deep cervical Flexor Endurance test in Group A is 28.27 and in Group B is 30. This shows Group B comparatively having mean value more than Group A. Statistical Analysis of the post-test, Deep cervical flexor Endurance test revealed that there is statistically significant difference seen between group A and group B.

Conclusion: From the result, it has been concluded that Abdominal crunch tongue in physiological position (Group B) is more effective than Abdominal crunch without Tongue in Physiological position (Group A) to improve deep cervical flexor endurance.

Key Words: Abdominal Exercise, Training, Endurance, Deep Cervical Flexors.

INTRODUCTION

The Suprhyoid muscles are four muscles located above the hyoid bone in the neck. They are the Digastric, Stylohyoid, Geniohyoid, and Mylohyoid muscles. They are all pharyngeal muscles. The Infrahyoid muscles (strap muscles) are a group of four pairs of muscles in the anterior (frontal) part of the neck. The four Infrahyoid muscles are; the Sternohyoid, Sternothyroid, Thyrohyoid and Omohyoid Muscles(1). The Infrahyoid muscles function to depress the Hyoid bone and larynx during Swallowing and Speech. The Sterno- cleidomastoid muscle flexes the neck and helps with movement of the head. The muscle originates at the temporal bone’s mastoid process near the ear and the base of the skull, and it stretches the entire length of the neck. This muscle helps the neck to turn to the side, flex to the side, and bend forward(1). Proper tongue position is essential during repeated trunk flexion exercise against gravity. The physiological rest position of the tongue is the roof of the mouth, just behind the front teeth. If the tongue is not held in this position, as the head is lifted during a Crunch or Sit-ups, the only way to lift the head is with the sternocleidomastoid (SCM) muscle and deep cervical flexors. If the SCM muscles are used for this purpose, they will hypertrophy just like any other muscles being exercised. The SCM muscles are extensors of the upper cervical spine and flexors of
the lower cervical spine, and therefore, hypertrophy and hypertonicity can cause a forward head posture. Another problem with incorrect tongue position is that the deep cervical flexors must play a major role in lifting the head. These muscles are long, thin and slight. They fatigue easily, and excessive fatigue encourages spasm and hypertonicity. If shortened because of poor technique, these muscles flex the cervical spine, straightening it, thus reversing is natural curvature. This creates many unwanted problems. The supra and Infrahyoid muscles have a tremendous mechanical advantage over the deep cervical flexors. Also, the SCM are extensors of the upper cervical spine and are counter balanced by the supra and Infrahyoid muscles. This is a primary mechanism for maintaining a normal posture of the head. The cervical flexors should be allowed to help stabilize the head during abdominal exercises. This is a natural activity and is taught in this course. If you pull on your head during abdominal exercise, the cervical flexors don’t contract. Such inactivity makes them weak and encourages a forward head posture. First of all, we must establish that the neck, or cervical spine, tends to work with the lumbar spine during human motion. This relationship is well established via reflexes such as the ‘pelvo-ocular’ reflex, which ties together the eye motion with pelvic motion, and the vestibulo-spinal reflex, which relates the vestibular system with spinal muscular reactions. One key point of these reflexes is that looking down is associated with activation of the abdominals and front neck muscles, looking up with activation of the spinal muscles, which antagonize the abdominals (work in the opposite direction). Secondly, the muscles on the front of the neck have a tendency to lengthen and get weak. This allows the head to migrate forward into the ubiquitous ‘forward head posture.’ This posture allows the increased probability of neck pain, headaches, and shoulder dysfunction. Crunches, then, can also help train these muscles by forcing the neck to lift one’s own head while during a crunch. In effect, we should think about ‘nodding’ as we crunch upwards, then reversing the nod on the way back down. This will help promote neck stabilization and good posture. All of this being said, what about the tongue? Well, it is interesting to note that some of these front neck muscles, which help us ‘nod’ our head, have an attachment at the tongue, specifically the supra and Infrahyoid muscles. With that in mind, a ‘loose’ tongue, or a tongue that is hanging loose in the mouth, provides a poor foundation for this muscle attachment. This can be illustrated by protesters who go limp at demonstrations to make the police exert more force in moving them. A stable, or tense, foundation would be much easier to move! Similarly, we want to have a solid base of motion for these all-important anterior neck muscles, making it easier for them to move the head. Research supports this notion as well.

Having said all that, there is something called flexor chain component like deep cervical flexors, abdominals flexors and hip flexors. If we do crunches without concentrating on deep cervical flexors we do over strengthen the abdominals relative to deep cervical flexor’s strength, and this will lead to forward head posture, reduced subcostal angle, thoracic kyphosis and other biomechanical dysfunctions. Though it may not have greater influence in apparently normal and sedentary individuals, it does have a greater role in sports personnel for their high performances.

Crunches

The exercise lying flat on your back as soon as you lift your head from the floor, the abdominal muscles begin to work. This is because the muscles lifting the neck pull on the rib cage. To stop the ribs from moving and hold them firm, the abdominal muscles must tighten. As the exercise continue, you begin to lift your trunk from the floor to do this, your legs are lighter than your trunk, the tendency is always for the legs to lift unless the trunk is bent. Bending the trunk reduces the effect of leverage, and makes the trunk lighter (2).

MATERIALS AND METHODS

A total 60 male subjects Aged between 18-23 years. Samples will be selected from students of Saveetha college of Physiotherapy according to inclusion and exclusion criteria using Simple Random Sampling. Approved by the scientific review board and Institutional Human Ethical Committee at the Saveetha University before participating in the study.

Selection criteria for participants: Both genders aged between 18 and 23 years, apparently, normal individuals.

Procedure

Samples of 60 individuals were recruited from physiotherapy college student population. Both genders aged between 18 and 25 were included and people who are already into structured exercise program and people with cervical referring and or radiating were excluded from the study. For this study totally 82 subjects initially showed willingness. Among them to 60 subjects are finally chosen. The principal researcher has explained the procedures to all the subjects. Pre and post functional outcome were obtained by Deep flexor endurance test for all subjects.
Subjects were divided into 2 groups: Group A-Abdominal crunch without tongue in Physiological position, Group B- Abdominal crunch with tongue in resting position.

Participants in Group A were taught to do abdominal crunches in crook lying. The exercise begins lying on floor on the back, Lifting head from the floor. Here participants were not taught about the tongue position, they were just asked to do regular Abdominal Crunches in right technique. Participants were instructed to do crunch till their shoulder blade comes off the floor or simply by keeping their arm on floor and drag or slide the arm till it reaches both the heels. And they were taught not to hold the breath during crunches instead they were asked to blow the air out though mouth during crunch and inhale through nose when they back to starting position, and keep their eyes facing ceiling. All participants were made to exercise for 10 repetitions for 4 sets with one session per day for 5 days a week for totally 8 weeks.

Where Participants in Group B were thought to do abdominal crunches as like the participants in Group A, with additional instruction on placing the tip of tongue on base on roof of the mouth, they were asked to maintain the tongue position throughout the exercise session, Same exercise parameters followed as for Group A.

Outcome Measures

Participants were supine positioned with tuck chin in and lift off table 1 inch. Then they were asked to hold as long as they can and time duration is noted. The pre-test and post-test values were taken and the average was considered for statistical analysis.

Data Analysis

The collected data were tabulated and analyzed using descriptive and inferential statistics. To all parameters mean and standard deviation (SD) will be used. A paired t-test will be used to analyze significant changes between pre-test and post-test measurements. Unpaired t-test will be used to analyze significant changes between two groups.

Table 1: Comparison of Deep cervical flexor endurance test between Group A&B

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group A</th>
<th>Group B</th>
<th>T test</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Standard deviation</td>
<td>Mean</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>Deep cervical flexor endurance test</td>
<td>28.27</td>
<td>3.67</td>
<td>30.00</td>
<td>2.62</td>
</tr>
</tbody>
</table>

The data from the above table shows the post-test values of Deep cervical endurance test in Group –A and Group-B subjects. The post-test mean value of Deep cervical flexor endurance test in Group A is 28.27 (SD3.67) and post-test mean value of deep cervical flexor endurance test is Group-B is 30.00 (SD 2.62). This shows that Group-B is greater than Group-B with the P value (0.1479).

Figure 1: Comparison of the Deep Cervical Flexor Endurance Test Post-test values

RESULTS

From the statistical analysis made with quantitative data revealed statistically significant difference deep cervical Flexor Endurance between the Group A and Group B, and also within the group. The post-test mean value of deep cervical Flexor Endurance test Group A is 28.27 and in Group B is 30. This show Group B was comparatively more than Group A. Statistical Analysis of the post-test, shows that the Deep cervical flexor Endurance is more significantly increased in Group B than Group A.

DISCUSSION

This study was conducted with the aim of improving cervical endurance among normal individuals by comparing abdominal crunch with and without tongue in physiological position. The study was conducted for a period of 4 weeks and results showed significant result in Group A (i.e.) Abdominal crunch without tongue in resting position Group B i.e. Abdominal crunch tongue in physiological position. Major muscles are the neck flexor muscles-Suprahyoid, Infrahyoid and Sternocleidomastoid. Minor muscles Rectus Abdominis and Obliques. The supra and infrahyoid muscles have a tremendous mechanical advantage over the deep cervical flexors. Also, the SCM are extensors of the upper cervical spine and are counter balanced by the supra and infrahyoid muscles. This is a primary mechanism for maintaining normal posture of the head. Winnberg A  Suggested that during upright head posture, the hyoid bone
exhibited on average an elliptical movement pattern and reached its most elevated position at the start of opening and its most depressed position at the start of closing. A small movement before opening an upward and forward direction and before closing in a further downward and backward direction was found. Suprahyoid EMG activity was registered before start of opening, during opening and during the first half of the open phase (4). The cervical flexors should be allowed to help stabilize the head during abdominal exercises. This is a natural activity and is taught in this course. If you pull on your head during abdominal exercise, the cervical flexors do not contract. Such inactivity makes them weak and encourages a forward head posture. Muscle endurance is the ability of a muscle or group of muscles to sustain repeated contraction against a resistance for an extended period of time. Muscular endurance is one of the components of muscular fitness, along with muscular strength and power. Proper tongue position is essential during repeated trunk flexion exercise against gravity. The physiological rest position of the tongue is the roof of the mouth, just behind the front teeth. Anders Winnberg, Hans Pancherz, et al 1983: Suggested that no absolute reciprocity was found between the Suprahyoid and Masseter muscle activity during chewing. Suprahyoid overlap at the end of the masseter, chewing phase was reduced when the head was flexed forward. The amount of Masseter overlap at the end of the Suprahyoid chewing phase was unaffected by variation in head position (5). Domenech MA, Sizer PS, Dedrick GS, McGalliard MK, Brismee JM. Suggest that “The Deep Neck Flexor Endurance Test: normative data scores in healthy adults.” (9) Deep Neck Flexor Endurance Test To assess the endurance of the deep neck flexors (Rectus Capitus Anterior, Rectus Capitus Lateralis, Longus Capitus, Longus Colli - “Muscle specificity in tests of cervical flexor muscle performance”). Importance of the Test: Those with neck pain were found to have significantly decreased deep neck flexor endurance, average of 21.4 seconds (“Reliability of a measurement of neck flexor muscle endurance”). They tend to over-utilize other muscles (Platysma, Hyoid muscles, and especially the sternocleidomastoid) for postural maintenance, which leads to the commonly seen position of forward head postures—a position we commonly see in those who use computers frequently or engage in sedentary activity on a regular basis. This may lead you to think of some impairment that is contributing to the patient’s pain. Be sure to assess a patient’s posture and segmental mobility in the cervical spine. This study shows significant difference between Abdominal crunch without tongue in Physiological position and Abdominal crunch tongue in with resting position and reveals Abdominal crunch tongue in with resting position have superior effect than Abdominal crunch without tongue in Physiological position in improving deep cervical flexor endurance. We do exercise to keep our self fit and healthy but doing so should not open the door for other health problems which can easily be prevented by adapting right techniques of exercises. Poor posture will affect the functions of joints it involved and leads to even poor digestion and visceroptosis. Common cause for poor posture is doing a monotonous activity and or adapting a common posture for longer durations. Even among sports personnel, poor posture is quiet common because of their regular pattern of sporting activity and conditioning in sports and they usually use to come up with cross training to avoid the poor adaptations of body structure and function towards similar activity. Though it is common that most individuals do abdominal crunches unfortunately they do it with poor techniques.

CONCLUSION

From the result, it has been concluded that Abdominal Crunch with Tongue in resting position (Group B) is more effective than abdominal crunch without tongue in Physiological position (Group A) in improving deep cervical flexor endurance.

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GC-MS analysis of Bioactive Components of *Andrographis Alata* (Vahl) Nees (Acanthaceae)

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ABSTRACT

Introduction and Aim: The purpose of the present investigation was carried out to determine the various bioactive components by the ethanol extract of whole plant of *Andrographis alata* using GC-MS analysis.

Materials and Methods: The chemical composition of the ethanol extract of whole plant of *A. alata* was investigated using GC-MS (GC Clarius 500 perklin Elmer) analyser equipped with mass selective detector TQ Quadrupole mass spectrometer with national institute of standard and technology (NIST) library.

Results: Nineteen compounds were identified. The prevailing compounds were Alpha-1-rhamnopyranose, 3,7,11,15-Tetramethyl-2-hexadecen-1-ol, n-Hexadecanoic acid, 9,12-Octadecadienoic acid (Z,Z)-, Oleic acid, Octadecanoic acid, n-Tetracosanol-1, Phytonadione, Ergosta-4,6,8(14),22-tetraen-3-one, Lupeol, 4H-1-Benzopyran-4-one,5-hydroxy-2-(4-hydroxyphenyl)-7-methoxy-, 4H-1-Benzopyran-4-one,5-(acetoxy)-2-[3-(acetoxy)-4-methoxyphenyl]-7-methoxy-, Anthroquinone,7-methoxy-2-methyl-1,4,5-trihydroxy-, Butylphosphonic acid, butyl 4-(2-phenylprop-2-yl)phenyl ester, Stigmasterol.

Conclusion: From the result it could be concluded that the whole plant of *A. alata* contains various bioactive compounds which have various medicinal properties.

Key Words: GC-MS, A.alata, Ethanol extract, Bioactive compounds.

INTRODUCTION

Medicinal plants and herbs have various bioactive compounds with therapeutic potential and the properties found in the traditional medicinal plants and their constituents offer exciting opportunity to develop them into novel plants. India is blessed with different medicinal practices like Ayurveda, Siddha and Unani. Plants have use in folk medicine and traditional system of medicine. Traditional medicine makes use of theories, beliefs and experience that helps to maintain health and also to protect from various ailments. Plants consist of various phyto pharmaceuticals which have led to its use in medicinal fields (1). Worldwide, India is a richest biodiversity country and it has a 45,000 plant species. In India, around 20,000 medicinal plants have been recorded recently. 500 traditional communities cure the different disease using medicinal herbs. According to the World Health Organization (WHO) in 2008, more than 80% of the world’s population relies on traditional medicine for their primary healthcare needs (2).

Higher plants as sources of bioactive compounds continue to play a dominant role in the maintenance of human health. Reports available on green plants represent a reservoir of effective chemotherapeutics, these are non-phytotoxic, more systemic and easily biodegradable (3-5)

GC-MS is normally used for direct analysis of components existing in traditional medicines and medicinal plants. In recent years GC-MS studies have been increasingly applied for the analysis of medicinal plants as this technique has proved to be a valuable method for the analysis of non polar components and
volatile essential oil, fatty acids, lipids and alkaloids

The aim of the present study is to identify the phy- 
tocomponents of this plant and subjecting the etha-
nol extract of the whole plant to Gas chromatogra-
phy-Mass Spectrum analysis. This work will help to
identify the compounds, which may be used to cure
many diseases.

About 21 species of Andrographis are reported to
occur in India (6-8) Several other species of Androg-
raphis are used as substitutes and adulterants of A.
paniculata the most important among these being A.
alata and A.lineata.(9).

Andrographis Wallish ex Nees belongs to the fami-
ly Acanthaceae. The genus Andrographis wallish ex
Nees is comprised of annual herbs or small shrubs,
including about 40 species, distributed in the tropi-
cal Asia (10). A. alata (Vahl) Nees commonly known
as“Periyanangai” is distributed in south-west India,
Sri Lanka, Tamilnadu, Kerala, AndhraPradesh at alti-
itudes ranging from 100m-3000m(11).

The plant is used as stomachic, intermittent fevers,
malaria, alternative, snake bite, antisnake venom,
appetizer, activating taste, indigestion, skin diseases,
worms, poisonous bites, giving strength fever, diar-
rhoea, childhood diseases, ascitis and impurities to
blood. (12,13,7&9).It is used in snake-bites, consti-
pation,skin diseases and lung diseases. These plants
are also claimed to possess snake- bites.Leaf paste is
locally applied for skin diseases in cattle (9)

MATERIALS AND METHODS

Plant Material

The plant specimens for the present study were
collected from Kalakad foot hills. The plants were
identified with the help of “Flora of the presidency
of Madras” (14) and “Flora of Tamil Nadu Carna-
tic” (15) and the binomials of the plants were as
given in the “Flora of Tamilnadu” (16). The mate-
rials were also identified and authenticated by Dr. P.
Jayaraman, Director, PARC, Chennai-45 and Dr. V.
Chelladurai (Rtd.), Research officer, Medicinal plant
Survey Unit, Government Siddha Medical College,
Palayamkottai.

Preparation of Extract

The powders of the plant (30 gm) were extracted
with ethanol at room temperature for 48h. The ex-
tract was filtered and concentrated under reduced
pressure in a rotary evaporator. The extract was sub-
jected to GC-MS analysis.

GC-MS Analysis

GC MS analysis of ethanol extract was performed
with GC Claritus 500 Perkin Elmer System and Gas
Chromatograph interfaced to a Mass Spectrometer
(GC-MS) equipped with a BR-5MS column (30
mmx0.25 mm ID x 0.25 µm df, composed of 5%
Diphenyl/ 95% Dimethyl poly siloxane). For GC-
MS detection and electron ionization system with
an ionizing energy of 70eV was used. Helium gas
(99.999%) was used as the carrier gas at constant
flow rate 1 ml/min and an injection volume of 2 µl
was employed (split ratio of 50:1). Injection tem-
perature 280ºC; ion source temperature 200ºC.
GC oven temperature started at 80ºC and holding
for 2min and it was raised to 160ºC at the rate of
20ºC/min, without holding. Holding was allowed at
285ºC for 5 min with program rate of 5ºC/min. The
injector and detector temperatures were set at 250ºC
and 280ºC respectively. The mass spectrum of com-
pounds in samples was obtained by electron ioniza-
tion at 70 eV and the detector was operated in scan
mode from 50-500amu (atomic mass units). A scan
interval of 0.5seconds and fragments from 45 to 450
Da was maintained. The total running time of GC
programme and MS programme were 36minutes.

Identification of Components

Identification was based on the molecular structure,
molecular mass and calculated fragments. Interpre-
tation on mass spectrum GC-MS was conducted us-
ing the database of National Institute Standard and
Technology (NIST) having more than 62,000 pat-
terns and mass spectra of WILEY . The relative per-
centage amount of each component was calculated
by comparing its average peak area to the total areas.
The spectrum of the unknown component was com-
pared with the spectrum of the component stored in
the NIST library .The name, molecular weight and
structure of the components of the test materials
were ascertained.

RESULTS

GC-MS is one of the best techniques to identify the
constituents of volatile matter, long chain, branched
chain hydrocarbons, alcohols acids, esters etc. The
GC-MS analysis of Andrographis alata whole plants
revealed the presence of nineteen compounds. The
identification of the phytochemical compounds was
confirmed based on the peak area, retention time and molecular formula. The active principles with their Retention time (RT), Molecular formula, Molecular weight (MW) biological activities, the structure of the compound and peak area in percentage are presented in Table (1&2) and Fig 1. The first compound identified with less retention time in *Andrographis alata* (10.03 min) was Alpha-1-rhamnopyranose whereas Stigmasterol was the last compound which took longest retention time (34.29 min) to identify. The other compounds with intermediate retention time like 3,7,11,15-Tetramethyl-2-hexadecen-1-ol(12.53 min), n-Hexadecanoic acid(14.12 min), 9,12-Octadecadienoic acid (Z,Z)-(16.51 min), Oleic acid(16.59 min),Octadecanoic acid(16.94 min), n-Tetracosanol-1(17.59 min), Phytonadione(19.93 min), Ergosta-4,6,8(14),22-tetraen-3-one(21.56 min), Lupeol(23.34 min), 4H-1-Benzopyran-4-one,5-hydroxy-2-(4-hydroxyphenyl)-7-methoxy-(24.96 min), 4H-1-Benzopyran-4-one,5-hydroxy-6,7-dimethyl-2-phenyl-(26.03 min), Squalene(26.34 min), Cholestan-3-ol,2-methylen, (3β,5α)-(26.55min), 4H-1-Benzopyran-4-one,5-(acetoxy)-2-[3-(acetoxy)-4-methoxyphenyl]-7-methoxy-(27.84min),4H-1-Benzopyran-4-one,5,7-dihydroxy-2-(3,4,5-trimethoxyphenyl) (28.58min), Anthroquinone,7-methoxy-2-methyl-1,4,5-trihydroxy-(29.26 min), Butylphosphonic acid, butyl 4-(2-phenylprop-2-yl)phenyl ester(33.67 min)

### Table 1: Phytocompounds identified in the *Andrographis alata* (Vahl) Nees by GC-MS

<table>
<thead>
<tr>
<th>S.No</th>
<th>RT</th>
<th>Name of the compound</th>
<th>Molecular Formulae</th>
<th>Molecular Weight</th>
<th>Peak Area %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10.03</td>
<td>Alpha-1-rhamnopyranose</td>
<td>C6H12O5</td>
<td>164</td>
<td>0.65</td>
</tr>
<tr>
<td>2</td>
<td>12.53</td>
<td>3,7,11,15-Tetramethyl-2-hexadecen-1-ol</td>
<td>C20H40O</td>
<td>296</td>
<td>1.87</td>
</tr>
<tr>
<td>3</td>
<td>16.51</td>
<td>n-Hexadecanoic acid</td>
<td>C16H32O2</td>
<td>256</td>
<td>5.94</td>
</tr>
<tr>
<td>4</td>
<td>16.59</td>
<td>Oleic acid</td>
<td>C18H34O2</td>
<td>282</td>
<td>4.14</td>
</tr>
<tr>
<td>5</td>
<td>16.94</td>
<td>Octadecanoic acid</td>
<td>C18H36O2</td>
<td>284</td>
<td>0.93</td>
</tr>
<tr>
<td>6</td>
<td>17.59</td>
<td>n-Tetracosanol-1</td>
<td>C24H50O</td>
<td>354</td>
<td>3.87</td>
</tr>
<tr>
<td>7</td>
<td>19.93</td>
<td>Phytonadione</td>
<td>C31H46O2</td>
<td>450</td>
<td>3.90</td>
</tr>
<tr>
<td>8</td>
<td>21.56</td>
<td>Ergosta-4,6,8(14),22-tetraen-3-one</td>
<td>C28H40O</td>
<td>392</td>
<td>0.75</td>
</tr>
<tr>
<td>9</td>
<td>23.34</td>
<td>Lupeol</td>
<td>C30H50O</td>
<td>426</td>
<td>4.20</td>
</tr>
<tr>
<td>10</td>
<td>24.96</td>
<td>4H-1-Benzopyran-4-one,5-hydroxy-2-(4-hydroxyphenyl)-7-methoxy-</td>
<td>C16H12O5</td>
<td>284</td>
<td>17.40</td>
</tr>
<tr>
<td>11</td>
<td>26.03</td>
<td>4H-1-Benzopyran-4-one,5-hydroxy-6,7-dimethyl-2-phenyl-</td>
<td>C17H14O5</td>
<td>298</td>
<td>7.93</td>
</tr>
<tr>
<td>12</td>
<td>26.34</td>
<td>Squalene</td>
<td>C30H50</td>
<td>410</td>
<td>0.47</td>
</tr>
<tr>
<td>13</td>
<td>26.55</td>
<td>Cholestan-3-ol,2-methylen,(3β,5α)-</td>
<td>C28H48O</td>
<td>400</td>
<td>4.93</td>
</tr>
<tr>
<td>14</td>
<td>27.84</td>
<td>4H-1-Benzopyran-4-one,5-(acetoxy)-2-[3-(acetoxy)-4-methoxyphenyl]-7-methoxy-</td>
<td>C21H18O8</td>
<td>398</td>
<td>11.51</td>
</tr>
<tr>
<td>15</td>
<td>28.58</td>
<td>4H-1-Benzopyran-4-one,5,7-dihydroxy-2-(3,4,5-trimethoxyphenyl)-</td>
<td>C18H16O7</td>
<td>344</td>
<td>1.74</td>
</tr>
<tr>
<td>16</td>
<td>29.26</td>
<td>Anthroquinone,7-methoxy-2-methyl-1,4,5-trihydroxy-</td>
<td>C16H12O6</td>
<td>300</td>
<td>10.83</td>
</tr>
<tr>
<td>17</td>
<td>33.67</td>
<td>Butylphosphonic acid, butyl 4-(2-phenylprop-2-yl)phenyl ester</td>
<td>C23H33O3P</td>
<td>388</td>
<td>13.87</td>
</tr>
<tr>
<td>18</td>
<td>34.29</td>
<td>Stigmasterol</td>
<td>C29H48O</td>
<td>412</td>
<td>3.26</td>
</tr>
</tbody>
</table>
Figure 1: GC-MS Chromatogram of *Andrographis alata* (Vahl) Nees

Table 2: Structure of the compounds identified in the *Andrographis alata* (Vahl) Nees by GC-MS

<table>
<thead>
<tr>
<th>S.NO</th>
<th>Name of the compound</th>
<th>Structure of the compound</th>
<th>Nature of the compound</th>
<th>Therapeutic activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Alpha-1-rhamnopyranose</td>
<td><img src="image" alt="Structure of Alpha-1-rhamnopyranose" /></td>
<td>Sugar moiety</td>
<td>Antiviral activity, Analgesic activity, Anti-leishmanial activity, Anti-inflammatory, Anti-Hypoglycemic</td>
</tr>
<tr>
<td>3.</td>
<td>n-Hexadecanoic acid</td>
<td><img src="image" alt="Structure of n-Hexadecanoic acid" /></td>
<td>Palmitic acid</td>
<td>Analgesic, Anti-inflammatory, Ulcerogenic, Antihyperglycemic, antihyperlipidemic, Antioxidant activity</td>
</tr>
<tr>
<td>4.</td>
<td>9,12-Octadecadienoic acid (Z,Z)</td>
<td><img src="image" alt="Structure of 9,12-Octadecadienoic acid" /></td>
<td>Linoleic acid</td>
<td>Analgesic, Anti-inflammatory, Ulcerogenic, Antioxidant</td>
</tr>
<tr>
<td>5.</td>
<td>Oleic acid</td>
<td><img src="image" alt="Structure of Oleic acid" /></td>
<td>Fatty acids</td>
<td>Antioxidant, Bacteriocidal activity, Anticancer activity, Antimicrobial, Immunotherapeutic activity</td>
</tr>
<tr>
<td>6.</td>
<td>Octadecanoic acid</td>
<td><img src="image" alt="Structure of Octadecanoic acid" /></td>
<td>Stearic acid</td>
<td>Analgesic, Anti-inflammatory, Ulcerogenic, Antiviral activity, Antioxidant activity, Antihyperglycemic, Antihyperlipidemic activities</td>
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<td>7.</td>
<td>n-Tetracosanol-1</td>
<td><img src="image" alt="Structure of n-Tetracosanol-1" /></td>
<td>Fatty alcohol</td>
<td>Antibacterial activity, Anti-breast cancer activity, Antioxidant activity</td>
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<td>8.</td>
<td>Phytonadione</td>
<td><img src="image" alt="Structure of Phytonadione" /></td>
<td>Vitamin K</td>
<td>Anticoagulation therapy, Antioxidant activity</td>
</tr>
<tr>
<td>9.</td>
<td>Ergosta-4,6,8(14),22-tetraen-3-one</td>
<td><img src="image" alt="Structure of Ergosta-4,6,8(14),22-tetraen-3-one" /></td>
<td></td>
<td>Anti proliferative activity, Hepatocellular/drug</td>
</tr>
<tr>
<td>No.</td>
<td>Compound</td>
<td>Type</td>
<td>Activity</td>
<td></td>
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<tr>
<td>-----</td>
<td>--------------------------------------------</td>
<td>-----------------------</td>
<td>-----------------------------------------------</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Lupeol</td>
<td>Triterpenoid</td>
<td>therapy, Diuretic activity</td>
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</tr>
<tr>
<td>11</td>
<td>4H-1-Benzopyran-4-one,5-hydroxy-2-(4-hydroxy-phenyl)-7-methoxy-</td>
<td>Flavonoid</td>
<td>Anti inflammatory, Anticancer activity, Anti proliferative activity</td>
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<td>12</td>
<td>4H-1-Benzopyran-4-one,5-hydroxy-6,7-dimethyl-2-phenyl-</td>
<td>Nil</td>
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<td>13</td>
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<td>Triterpene</td>
<td>Anticancer activity, antimicrobial activity</td>
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<td>Cholestane-3-ol,2-methylene,(3β,5α)-</td>
<td>Nil</td>
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<td>4H-1-Benzopyran-4-one,5-(acetyloxy)-2-[3-(acetyloxy)-4-methoxyphenyl]-7-methoxy-</td>
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<td>16</td>
<td>4H-1-Benzopyran-4-one,5,7-dihydroxy-2-(3,4,5-trimethoxyphenyl)-</td>
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<td>17</td>
<td>Anthroquinone,7-methoxy-2-methyl-1,4,5-trihydroxy-</td>
<td>Nil</td>
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<tr>
<td>18</td>
<td>Butylphosphonic acid, butyl 4-(2-phenyl-prop-2-yl)phenyl ester</td>
<td>Glyceride esters of polyethylene glycols</td>
<td>Anti-inflammatory, Termiticidal activities,</td>
<td></td>
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<tr>
<td>19</td>
<td>Stigmasterol</td>
<td>Unsaturated plant sterol</td>
<td>Immunomodulatory activity, Antiproliferative, Apoptotic, Antimutagenic, Antiulcerogenic, Antitumor activities</td>
<td></td>
</tr>
</tbody>
</table>
DISCUSSION

One of the ongoing problems scientist and medical workers face in the fight against infectious diseases is the development of resistance to the agents used to control them. There has been a remarkable progress in the prevention, control and even eradication of infectious diseases with improved hygiene and development of antimicrobials and vaccines. In recent times, in addition morphological markers, anatomical, cytological, biochemical, and molecular markers are also being used to categorize the organisms (17). GC-MS is a valuable tool for reliable identification for phytocompounds (18&19). In this study 19 compounds have been identified from the ethanolic extract of the whole plant of *Andrographis alata* by GC-MS analysis. The therapeutically important active principle andrographolide was observed in the aerial parts of *A.paniculata* (20-22). Among the identified phytochemicals, 4H-1-Benzopyran-4-one,5-hydroxy-6,7-dimethyl-2-phenyl-4H-1-Benzopyran-4-one,5-(acetyloxy)-2-[3-(acetyloxy)-4-methoxyphenyl]-7-methoxy-and 4H-1-Benzopyran-4-one,5,7-dihydroxy-2(3,4,5-trimethoxyphenyl) and it may be acts as an Anticancer, Antimicrobial, Antioxidant, Anti inflammatory, Antitumor and Antiproliferative, Apoptotic, Antimutagenic, Antiulcerogenic, Anticoagulant therapy, Anxiolytic and it may be acts as an Immunomodulatory activity, Antidiabetic activity, Anticancer, Antimicrobial, Antioxidant, Anti inflammatory, Antiinvasive activities, Antimitotic activity and Anti-TB activity. 3,7,11,15-Tetramethyl-2-hexadec-1-ol, Lupeol and Squalene are the terpenes and it may be acts as an Anticancer, Antimicrobial, Antioxidant, Anti inflammatory, Antioxidant, Antibacterial, Anthelmintic activity, Anticancer activity and *In vitro* cytoprotective activity, Vitamin K is suggested to be a vitamin compound and it may be acts as Anticoagulation therapy, Antioxidant activity. n-Hexadecanoic acid (Palmitic acid) and 9,12- Octadecadienoic acid (Z.Z)- (Linoleic acid) have the pharmacological activities like Analgesic, Anti-inflammatory, Ulcerogenic, Antioxidant, Antibacterial, Anthelmintic activity, Anticancer activity and *In vitro* cytoprotective activity, Vitamin K is suggested to be a vitamin compound and it may be acts as Anticoagulation therapy, Antioxidant activity. n-Hexadecanoic acid (Palmitic acid) and 9,12- Octadecadienoic acid (Z.Z)- (Linoleic acid) have the pharmacological activities like Analgesic, Anti-inflammatory, Ulcerogenic, Antioxidant, Antibacterial, Anthelmintic activity, Anticancer activity and *In vitro* cytoprotective activity, Vitamin K is suggested to be a vitamin compound and it may be acts as Anticoagulation therapy, Antioxidant activity. 

CONCLUSION

Phytochemical investigation and GC-MS analysis of ethanolic extract of whole plant of *Andrographis alata* showed the presence of carbohydrates, steroids, alkaloids, phenols, flavonoids, fatty acids, vitamins, palmitic acid, linoleic acid, and esters. The presence of various bioactive compounds confirms the application of *Andrographis alata* for various ailments by traditional practitioners. The isolation of individual phytochemical constituents may proceed to find a novel drug. It can be concluded that in future *in vitro* and *in vivo* studies on biological systems can find a new way for drugs that can be employed in clinical trials.

ACKNOWLEDGEMENT

The authors are grateful to the Indian Institute of Crop Processing Technology (IICT), Thanjavur, for providing the laboratory facilities and also wish to gratefully acknowledge to Dr. P. Jayaraman, Director, PARC, Chennai-45 and Dr. V. Chelladurai (Rtd.), Research officer, Medicinal plant Survey Unit, Government Siddha Medical College, Palayankotai for identification of this plant.

REFERENCES


Effects of Resistance Exercise combined with Balance Exercise on Static and Dynamic Balance among Community Dwelling Older Adults

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ABSTRACT

Introduction and Aim: Falls in elderly is a significant cause of morbidity and mortality and are an important class of preventable injuries due to the reduction in the muscle strength and balance among older adults. To determine the effects of resistance exercise combined with balance exercise on the static and dynamic balance among community-dwelling older adults.

Materials and Methods: Forty subjects with or without previous history of falls are selected based on the selection criteria and divided into two groups. Group-A subjects received resistance exercise and balance exercises. Group-B subjects received balance exercise alone. Both groups were treated for 3 days a week for 4 weeks. The outcome measures were Berg Balance Scale (BBS) and Timed Up and Go test (TUG).

Results: All the values were tabulated and statically analyzed using paired and unpaired t-test.

Conclusion: Resistance exercise combined with balance exercise is effective than balance exercise alone in improving the static and dynamic balance thereby reducing falls among the older adults.

Key Words: Falls, Balance, Resistance exercise, Balance exercise

INTRODUCTION

The number of people over the age of 60 years is vastly increasing, especially in India. India is the secondly ranked for having people at or over 60 years, constituting about 7.7% of the entire population (2). The major reasons for this substantial increase are because of higher life expectancy and decreased birth rates (8). Aging is associated with increased risk of disability and dependency. With increasing age, the risk of falls can increase up to 60% (16).

Evaluation of the risk factors is essential for tailoring effective fall prevention strategies (11). Some risk factors are modifiable while others can be modified with appropriate intervention (15,19). Despite rehabilitation, several individuals do not regain the level of functional independence they had before fracture which is why prevention of falls are important (9).

The causes of falls in elderly are multifactorial. The causes can be classified into intrinsic and extrinsic factors. The intrinsic risk factors are muscle weakness (lower extremity weakness), balance deficits and gait instabilities while extrinsic factors include polypharmacy (use of 4 or more medications) and environmental hazards such as loose carpets, poor lighting and lack of safety equipment such as handrails in bathroom (AGS Panel on Falls Prevention, 2001).

Some of the factors that are associated with greater probability of falls are problems such as gait and balance impairment, sensory impairment such as the peripheral neuropathy, vestibular dysfunction, vision impairment, and orthostatic hypertension. Balance is important for maintaining postural equilibrium and thus avoiding falls. Aging may affect the central nervous system and neuromuscular system leading to a reduction in balance and gait performance (7). Studies reveal that for prevention of falls, resistance exercises and balance exercises can be prescribed for the elderly population.

Lower extremity resistance exercise is important for prevention of falls in elderly (4). Resistance exercise using the Thera-band is possible to improve the static and dynamic balance of elderly adults. The pre-
ferred resistance used for the elderly population is a yellow band as they are of lower resistance and can be used as beginner resistance training in the elderly population.

Balance exercises with the help of Swiss ball are proven at improving the static and dynamic balance ability of the elderly women. It is very important to have a physically active life in the satisfying independence of the elderly, preserving balance and mobility is essential.

This study is done to find out the effectiveness of the resistance exercises and balance exercises Vs balance exercises alone in improving the balance in elderly population by the administration of resistance exercise using thera band and balance exercise using swiss ball and then to suggest an appropriate exercise protocol with the interpretation of the results done in the study.

MATERIALS AND METHODS

A total of 40 subjects with or without previous history of falls between the age 60-80 year participated in the study. Subjects were selected from Anandam Old Age Home. All the subjects were signed an informed consent form approved by the Scientific review board and Institutional Human Ethical committee at Saveetha University before participating in the study.

Selection criteria for subjects with or without previous history of falls

Subjects of both the sexes, with or without previous history of falls, a score of 41-56 in the Berg Balance Scale were selected. Subjects were excluded if they had the Vestibular or visual disorder, Cardiorespiratory disorders, Neurophysiological diseases, Vertigo, any recent injury or previous trauma within 6 months.

Procedure

Group A received a combination of resistance exercise by Thera band, and balance exercise using Swiss ball and Group B received only balance exercise using Swiss ball. Dependent variables measured in the study were the Berg balance scale and Timed Up and Go test. The Berg Balance Scale is used to measure balance. It is a 14-item balance assessment tool that is scored on a 5-point scale (0-4) measuring levels of ability in performing each task (4-safe and independent, 0-incapable). The BBS includes task such as standing with eyes closed, reaching, standing on one foot and picking objects from the floor. The highest possible score is 56, indicating excellent balance.

The Timed Up and Go test is used to measure functional mobility. The time taken to complete rising from a chair, walking 10ft, and turning, walking back to the chair and sitting was recorded in seconds. The starting position is standardized so that the subjects commenced the test with feet flat on the floor and the arm resting on armrests. No physical assistance was provided. The subject was asked to perform three trails, and the mean score was recorded.

Group A received resistance exercise using Thera band and balance exercise using Swiss ball for improving the balance. Thera band of yellow colour is given as it provides the least and beginner’s resistance. Balance exercises are provided using Swiss ball. The resistance exercises and the balance exercises were performed for about 2 sets per day, 3 days a week for 4 weeks. Resistance exercise using Thera band and balance exercise using Swiss ball are performed for all the lower extremity joint movements in the hip, knee and ankle joints. The entire duration is about 30 minutes per session for 3 days per week for 4 weeks.

Group B received the balance exercises alone using Swiss ball. The balance exercises were performed for about 2 sets per day, 3 days a week for 4 weeks. Balance exercise using Swiss ball is performed for all the lower extremity joint movements in the hip, knee and ankle joints. The entire duration is about 30 minutes per session for 3 days per week for 4 weeks.

RESULTS

The collected data were tabulated and analyzed using descriptive and inferential statistics. To all parameters mean and standard deviation (SD) was used. A paired t-test will be used to analyze significant changes between pre-test and post-test measurements. Unpaired t-test was used to analyze significant changes between two groups.

The post-test mean value of BBS in Group A is 52 (SD is 2.99), and post-test mean value in Group B is 48 (SD is 3.70). This shows that BBS scores in Group A are gradually increased, with P value (<0.0001) extremely statistically significant.

The post-test mean value of TUG is 9.4 (SD is 1.57) in Group A, and post-test mean value in Group B is 8.4 (SD is 0.91). This shows that TUG scores
in Group A are gradually decreased, with P value (<.0001) extremely statistically significant.

From the statistical analysis made with the quantitative data, it is concluded that there is a significant difference between pre-test and post-test values within Group A and Group B.

**Within the Group Analysis**

**Graph 1:** Graph showing Pre and post test BBS values of Group A

**Graph 2:** Graph showing Pre and post test TUG values of Group A

**Graph 3:** Graph showing Pre and post test BBS values of Group-B

**Graph 4:** Graph showing pre and post test TUG values of Group B

**Graph 5:** Graph showing Post test-post test BBS values of Group A and Group B

**Graph 6:** Graph showing Post test-post test TUG values of Group A and Group B

From the above statistical analysis with the quantitative data, it is concluded that there is a significant difference between pre and post-test values within Group A and Group B, signifying that Group A (Resistance exercise and balance exercise) is an effective treatment protocol than Group B.
DISCUSSION

This study investigates the effect of resistance exercise combined with balance exercise on the static and dynamic balance among community-dwelling older adults, proving that resistance exercise combined with balance exercise is better than balance exercise alone. There were 40 older adults who participated in the study. They were divided into two groups, Group A receiving resistance and balance exercise and the latter group (Group B) received balance exercises alone and further showed a statistical significance better than BBS, TUG for 4 weeks duration in Group A more than Group B. Handling geriatric population was a challenge. They were apprehensive at the beginning and later got comfortable with the exercise schedule.

According to WHO, falls are prominent among the external causes of unintentional injury. They are coded as E880-E888 in International Classification of Disease-9 (ICD-9), and as W00-W19 in ICD-10, which include a wide range of falls including those on the same level, upper level, and other unspecified falls. Falls are commonly defined as “inadvertently coming to rest on the ground, floor or other lower level, excluding intentional change in position to rest in furniture, wall or other objects.

Falls exponentially increase with age-related biological change. Therefore, a pronounced number of persons over the age of 80 years will trigger a substantial increase in falls and fall injury at an alarming rate. In fact, the incidence of some fall injuries, such as fractures and spinal cord injury, have markedly increased by 131% during the last three decades (14).

According to Yves J Gschwind et al.: A best practice fall prevention exercise program to improve balance, strength/power, and psychosocial health in the older adults-Study protocol for Randomised controlled trial (2013), he stated that balance is important for maintaining postural equilibrium and thus for the avoidance of falls (1).

Besides balance, muscle strength also is required for the proper performance of ADL. There are many conventional procedures that are being practiced for improving the balance such as wobble board, swiss ball, balance exercises. But the additional training of the muscles was not focussed along with balance training in most of the clinical practices. Exercises such as resistance exercises can be used to improve the muscle power of the individual. Resistance exercise and balance exercise improves the balance, unlike any other treatment.

Granacher U et al. (2010), Howley ET et al. (2003) and several other authors have proved that combined balance and resistance exercise may positively improve physical (i.e., balance and strength) and also functional performance.

Granacher U et al.: Balance training and multi-task performance in seniors (2010) stated that age-related impairment in gait patterns when simultaneously performing cognitive (CI) and/or motor (MI) interference tasks are associated with an increased risk of falling in seniors. The objective of his study was to investigate the impact of balance training (BT) on walking performance with and without concurrently performing a CI and/or MI task in seniors. The intervention group conducted a six week BT (3/week). Pre and post-tests included the assessment of stride-to-stride variability during single (walking), dual (CI or MI+ walking), and triple (CI+ MI+ walking) task walking on an instrumented walkway. BT resulted in statistically significant reductions in stride time variability under a single (p=0.02, Delta34.8%). His findings showed that performance during single task did not transfer while walking, suggesting BT as an alternative training modality for MI and CI (10).

Wonjong Yu et al.: Effects of Resistance exercise using Thera band on balance of elderly adults-A randomized control trial (2013) decreased balance causes disturbances while standing and during gait, through a decrease in the ADL and an increase in the incidence of falls. The objective of this study is to investigate the effects of resistance exercise using theraband on balance of elderly adults. The intervention group conducted a five-week, and the participants were evaluated using Berg balance scale and Timed Up and Go test. The findings of the study concluded that resistance exercise using Thera band is possible to improve the static and dynamic balance of the elderly adults (4).

According to Yves J Gschwind et al: A best practice fall prevention exercise program to improve balance, strength/power, and psychosocial health in older adults-Study protocol for Randomised controlled trial (2013), mentioned that Podsiadlo D et al, “The “Timed Up and Go” a test of basic functional mobility for frail elderly persons stated that the TUG showed excellent test-retest reliability (ICC=0.99) in older adults. The Berg Balance Scale is also considered to be one of the most commonly used scales to measure balance in the elderly.

This study proves that combined resistance and balance exercise are useful in improving the balance of an older adult, thereby improving their functional independence. It is also proven that combined exercise strategies are better in improving the balance ability of the older adults.

The post-test mean value of BBS in Group A is 52 (SD is 2.99) the post-test mean value of TUG is 9.4 (SD is 1.57), and the graphical representation proves the same.
In future, easy to administer balance and muscle strength exercises can be developed for prescribing for the geriatric population.

To conclude, this trial will provide an insight into the effect of fall prevention on older adults and also the importance of leading a physically healthy lifestyle, especially to the older adults.

REFERENCES


23. www.wikipedia.com (Balance)
Study the Association of Plasma Homocysteine, Retinol Binding Protein, Pre Albumin, and Albumin In Partial Hydatidiform Mole

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ABSTRACT

Introduction and Aim: The Hydatidiform mole is one of a group of diseases referred to as gestational trophoblastic disease (GTD), and the commonest type of GTD. Classification of a hydatidiform mole into complete and partial depends on histopathological and cytogenetic features. An H mole is considered to be a premalignant condition, with approximately 15% of complete and 1% of partial moles progress to a malignant gestational trophoblastic neoplasia (GTN). The objectives of the study were to assess nutritional status as a possible cause of Hydatidiform mole. In addition, to investigate the changes in the homocysteine, Retinol binding protein, Pre albumin, and albumin in the first-trimester pregnant women compared to non-pregnant.

Materials and Methods: This was a case-control study which included (75) subjects, (25) of them were patients group, the other (25) were pregnant women in the first trimester (first control group), and the remainder (25) were nonpregnant women were (second control group). The sera obtained from the fasting blood of subjects were used to measure the level of homocysteine, Retinol binding protein, pre-albumin, and albumin.

Results: The results of the present study showed that The mean age of patients group was (27.44 ± 5.36) years, ranging from 18-39 years and the peak incidence of PHM between 22-29 years. The majority (76%) of a patient with hydatidiform mole came from the rural area. Accident finding at ultrasonography was the commonest way of presentation (44%) followed by vaginal bleeding (40%) while (16%) hyperemesis gravidarum. The Pre albumin level was significantly lower in the Pregnant women with mole compared with healthy pregnant women control group (P < 0.001), but the homocysteine level was not significantly different (P = 0.146). The levels of homocysteine and albumin were found significantly different between first-trimester pregnant women and non-pregnant (P < 0.001).

Conclusion: The highest incidence of PHM was found in the age group between 22-29 years, belonging to the rural city. PHM was more common in nulliparous and low parous patients having 1-2 children. The accidental finding of ultrasonography was the commonest way of presentation followed by vaginal bleeding. This is the first report to suggest that there may be an association between pre-albumin and PHM. But it is also suggesting that there may be no association between homocysteine and PHM.

Key Words: Hydatidiform mole, Homocysteine, Retinol binding protein, Pre albumin, Albumin

INTRODUCTION

A variation Hydatidiform mole or molar pregnancy is one of a group of diseases referred to as gestational trophoblastic disease (GTD), H mole is the commonest type of GTD (1). Most of the molar pregnancies are benign but on rare occasions, they have the tendency for local and distant metastasis. Classification of the hydatidiform mole into complete and partial depends on histopathological and cytogenetic features (2). An H mole is considered to be a premalignant condition, with approximately 15% of complete and 1% of partial moles progress to a malignant gestational trophoblastic neoplasia (GTN) (3). Several potential etiologic risk factors have been evaluated for the development of H mole. These include the extreme of maternal age, prior history of H mole, deficiency of Beta-carotene and animal fat intake (4). Vaginal bleeding is the most common presenting symptom.
During the second trimester, the diagnosis of molar pregnancy is usually made, and the classical signs and symptoms include toxemia, large uterine size, hyperemesis, anemia, hyperthyroidism, and respiratory distress (3). The clinical presentation of molar pregnancy has changed largely in recent years; the diagnosis of H mole occurs at earlier gestational age owing to the ultrasonographic examination made at early pregnancy (3).

MATERIALS AND METHODS

This study was conducted in Babylon Maternity and Pediatrics Teaching Hospital in Babylon Province and Al Zahraa teaching hospital in Al Najaf Province from the first of September 2016 to the end of March 2017.

This was a case control study which included 75 subjects, twenty-five were patients diagnosed with partial H mole, the other twenty-five pregnant women in the first trimester were healthy subject (first control group), and the remainder twenty-five non pregnant women were healthy subject (second control group). Patients who suffered from metabolic or endocrine disease, renal dysfunction, and BMI > 30 were excluded. The sera obtained from the fasting blood of subjects were used to measure the level of homocysteine and Retinol binding protein by (ELISA), pre albumin by Nephlometry, and albumin by Spectrophotometry).

Statistical Analysis

All Statistical analysis had been done by using (SPSS) version 20th for social sciences. Categorical variables had been presented as percentages, frequencies. Contentious variables had been presented as mean and standard deviation (SD). The t-test had been used to determine the mean statistically significant difference between two groups. P value less than (0.05) had been considered to be significant.

RESULTS

Table (3.1) showed that the mean age of patients group was (27.44 ± 5.36) years, ranging from 18-39 years and the mean BMI of patients group was (23.58 ± 2.27), ranging from 18.2-26.5 Kg/m2 and also showed that the mean level of B HCG was (15815.6 ± 14442.12), ranging from (2160-54354.0) (mIU/ml).

<table>
<thead>
<tr>
<th>Study variable</th>
<th>(Means ± SD)</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>(27.44 ± 5.36)</td>
<td>(18-39)</td>
</tr>
<tr>
<td>BMI (Kg/m2)</td>
<td>(24.58 ± 2.27)</td>
<td>(20.2-27.5)</td>
</tr>
<tr>
<td>β- HCG (mIU/ml)</td>
<td>(15815.6 ± 14442.12)</td>
<td>(2160-54354.0)</td>
</tr>
</tbody>
</table>

Figure 3.1 showed that the majority (76%) of patient with hydatidiform mole came from rural area. While only (24%) of patient with hydatidiform mole came from urban area.

| Figure 3.1: The distribution of patients according to residence |

Figure 3.2 showed that (34%) of patients presented with blood group A+ve and (31%) of patients presented with blood group O+ve and (20%) of patients presented with blood group AB+ve, the reminder Only (15%) of patients presented with blood group B+ve.

| Figure 3.2: Distribution of patients according to blood group |

Table 3.2 showed the distribution of patients according to study variables including (gestational age, history of mole, family history of mole, clinical presentation, number of miscarriage and parity).
Table 3.2: The Distribution of patients according to clinical history

<table>
<thead>
<tr>
<th>Study variables</th>
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<th>%</th>
</tr>
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<tbody>
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<td>Gestational age</td>
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<td></td>
</tr>
<tr>
<td>≤ 9 weeks</td>
<td>14</td>
<td>66%</td>
</tr>
<tr>
<td>&gt; 9-13 weeks</td>
<td>11</td>
<td>44%</td>
</tr>
<tr>
<td>Total</td>
<td>25</td>
<td>100%</td>
</tr>
<tr>
<td>History of mole</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Present</td>
<td>2</td>
<td>8%</td>
</tr>
<tr>
<td>Absent</td>
<td>23</td>
<td>92%</td>
</tr>
<tr>
<td>Total</td>
<td>25</td>
<td>100%</td>
</tr>
<tr>
<td>Family history of mole</td>
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</tr>
<tr>
<td>Present</td>
<td>1</td>
<td>4%</td>
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<tr>
<td>Absent</td>
<td>24</td>
<td>96%</td>
</tr>
<tr>
<td>Total</td>
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</tr>
<tr>
<td>Way of presentation</td>
<td></td>
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</tr>
<tr>
<td>Hyperemesis gravidram</td>
<td>4</td>
<td>16%</td>
</tr>
<tr>
<td>Ultrasound</td>
<td>11</td>
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</tr>
<tr>
<td>Vaginal bleeding</td>
<td>10</td>
<td>40%</td>
</tr>
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<td>Total</td>
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<td>100%</td>
</tr>
<tr>
<td>Number of miscarriage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nill</td>
<td>20</td>
<td>80%</td>
</tr>
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<td>8%</td>
</tr>
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<td>0</td>
<td>8</td>
<td>32%</td>
</tr>
<tr>
<td>1-2</td>
<td>9</td>
<td>36%</td>
</tr>
<tr>
<td>3-4</td>
<td>5</td>
<td>20%</td>
</tr>
<tr>
<td>5-6</td>
<td>2</td>
<td>8%</td>
</tr>
<tr>
<td>7 or more</td>
<td>1</td>
<td>4%</td>
</tr>
<tr>
<td>Total</td>
<td>25</td>
<td>100%</td>
</tr>
</tbody>
</table>

Table 3.3 showed that there was no significant difference in the mean of serum Homocysteine level (mmol/l) between patient’s pregnant women with mole and non-pregnant women (p<0.05). 

Table 3.3: The mean differences of study markers between patients and control pregnant women

<table>
<thead>
<tr>
<th>Study markers</th>
<th>Group</th>
<th>N</th>
<th>Mean ± SD</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homocysteine</td>
<td>Pregnant women with mole</td>
<td>25</td>
<td>7.3 ± 2.03</td>
<td>0.146</td>
</tr>
<tr>
<td></td>
<td>healthy pregnant women</td>
<td>25</td>
<td>6.5 ± 1.92</td>
<td></td>
</tr>
<tr>
<td>Retinol binding</td>
<td>Pregnant women with mole</td>
<td>25</td>
<td>326.5 ± 109.4</td>
<td>0.222</td>
</tr>
<tr>
<td>protein (ng/ml)</td>
<td>healthy pregnant women</td>
<td>25</td>
<td>366.4 ± 118.2</td>
<td></td>
</tr>
<tr>
<td>Pre albumin</td>
<td>Pregnant women with mole</td>
<td>25</td>
<td>18.71 ± 5.08</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>(mg/dl)</td>
<td>healthy pregnant women</td>
<td>25</td>
<td>23.40 ± 2.39</td>
<td></td>
</tr>
</tbody>
</table>

Figure 3.3 showed that there was a significant difference in the mean of serum homocysteine level (mmol/l) between patient’s pregnant women with mole and non-pregnant women (p<0.05),(P= <0.001*).

Figure 3.3 Mean differences of homocysteine level between patients and control non-pregnant group

Figure 3.4 showed that there was no significant difference in the mean of serum Retinol binding protein level (ng/ml) between patient’s pregnant women with mole and non-pregnant women (p<0.05). (P= 0.07).

Figure 3.4 Mean differences of retinol binding protein level between patients and control non pregnant group

Figure 3.5 showed that there was a significant difference in the mean of serum pre albumin level (mg/dl) between patient’s pregnant women with mole and non-pregnant women (p<0.05). (P= <0.001*).
Figure 3.5 Mean differences of pre albumin level between patients and control non pregnant group

Figure 3.6 showed that there was a significant difference in the mean of serum albumin level (g/dl) between patient's pregnant women with mole and non-pregnant women (p<0.05), (P= <0.001*).

Table 3.4 showed that there was significant decrease in the mean of serum Homocysteine and Albumin between healthy pregnant women and non-pregnant women (p>0.05), while there was no significant decrease in the mean of serum Retinol binding protein, and Pre albumin level.

<table>
<thead>
<tr>
<th>Study markers</th>
<th>Group</th>
<th>N</th>
<th>Mean ± SD</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homocysteine (mmol/l)</td>
<td>healthy pregnant women</td>
<td>25</td>
<td>6.5 ± 1.92</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td></td>
<td>Non pregnant women</td>
<td>25</td>
<td>10.02 ± 2.48</td>
<td></td>
</tr>
<tr>
<td>Retinol binding protein (ng/ml)</td>
<td>healthy pregnant women</td>
<td>25</td>
<td>366.4 ± 118.2</td>
<td>0.639</td>
</tr>
<tr>
<td></td>
<td>Non pregnant women</td>
<td>25</td>
<td>381.45 ±106.7</td>
<td></td>
</tr>
<tr>
<td>Pre albumin (mg/dl)</td>
<td>healthy pregnant women</td>
<td>25</td>
<td>23.40 ± 2.39</td>
<td>0.149</td>
</tr>
<tr>
<td></td>
<td>Non pregnant women</td>
<td>25</td>
<td>24.49 ± 2.85</td>
<td></td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>healthy pregnant women</td>
<td>25</td>
<td>4.10 ± 0.25</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td></td>
<td>Non pregnant women</td>
<td>25</td>
<td>4.58 ± 0.44</td>
<td></td>
</tr>
</tbody>
</table>

*P value ≤ 0.05 was significant.

DISCUSSION

In the present study, the highest incidence was found in the age group 22–29 years and similar results were reported from Sadiq S et al. (5). The overall mean age of patients was 27.44±5.3 years, nearly similar results have been reported by Moodley M et al (6). The result of the pre-evacuation β-HCG in this study was nearly similar with study conducted by Agrawal N et al. in 2015 (7). The highest percentage of patients belonging to the rural city, these results may be attributed to low socioeconomic status and malnutrition (8). Nutritional and socioeconomic factors appear to be important risk factors for molar pregnancy in some populations (9). Another study conducted by Taboo ZA et al. (10) concluded that sixty-five percent of cases were of low socioeconomic state, while 35% of the cases were of high socioeconomic status (10). Our finding in this study that blood group A and O were commonly associated with HM, are compatible with other study Abdelrub AS et al. (11). However, the data regarding such association are conflicting. Nevertheless, the number of studies that have found a positive association with blood group makes this issue worthy of further studies. All of the patients in this study were diagnosed at first trimester with gestational age ranging from (7-12) weeks. In a study done in Malaysia by Aye and Karate (2009) revealed that 75% of cases of GTD were diagnosed at first trimester (12), which nearly coincide with the result of the current study. Both CM and PM are now mainly diagnosed in the first trimester. A first-trimester fetal ultrasound scan is now recommended and commonly performed; this may have contributed to the earlier diagnosis of PM (13).

Two of the patients (8%) had a previous history of molar pregnancy. Similar results have been reported from Ocheke AN et al. (1). A previous history of molar pregnancy has consistently been shown to influence the risk of HM (4). Also in this study, we found only one of the patients (4%) had a family history of molar pregnancy. Very rarely, several women in the same family have one or more molar pregnancies. It is a rare autosomal recessive condition in which the affected women have a predisposition to pregnancy losses, but most of which are CM (14). In the current study, we found that accidental finding of ultrasonography was the commonest way of presentation in PHM followed by vaginal bleeding. The increased use of Ultrasound scan has significantly improved the diagnosis and promoted prompt uterine evacuation of molar pregnancy (15). Ultrasonography aided the diagnosis of hydatidiform mole in most cases. Ultrasonography plays a critical role in the diagnosis of both complete and partial mole, it has virtually replaced all other means of preop-
erative diagnosis (15). It can be said that patients with molar pregnancy are increasingly being diagnosed earlier in pregnancy and treated before they develop the classic clinical signs and symptoms as a result of widespread use of Ultrasonography (4). In this study the most frequency was seen in the women who were experiencing their second pregnancy 36% and nulliparous 32%. In Ocheke AN et al. a large proportion of the hydatidiform mole patients (64%) were of low parity (0-2) (1). In Moodley M et al. study, hydatidiform mole pregnancy was seen more among nulliparous women (6). Although this is at variance with reports from some authors where most patients were of high parity (16). But also, other authors have reported that hydatidiform mole has no significant association with parity (17). The present study showed that there was no statistically significant mean difference of plasma homocysteine between partial moles and healthy pregnant women. These result disagree with Kokanali MK et al. study that reported statistically significant mean difference in the levels of plasma Hcy was found between partial moles and healthy pregnant women (18).

Regarding Retinol binding protein in the present study, the mean level was little bit higher in the control group but the difference was not statistically significant. This may be explained by the relationship between the Retinol binding protein and pre albumin. The retinol-RBP compound is secreted into the blood flow, where it is bound to PA (19). Therefore, the observed correlations between PA and RBP levels (20). Interestingly, in this study Pre albumin levels were significantly lower in the Pregnant women with mole compared with healthy pregnant women control group This may be explained by the fact that the pre albumin has short half-life (2-3) days, this make it showed a much higher degree of sensitivity to the change in nutritional status (21). In the present study pregnant women have been found to have lower levels of plasma Hcy, the mean plasma homocysteine levels between first trimester pregnant women and non-pregnant was found statistically difference. This result was nearly similar to study conducted by Muhammad K et al. (22). It is interesting to note that Hcy concentration in plasma actually decreases in normal pregnancy. Various hypotheses have been proposed to explaining the decrease in Hcy concentration during pregnancy. Among these are hormonal influences on Hcy metabolism, maternal dietary protein intake during pregnancy, pregnancy-associated hemodilution, and fetal utilization (23). The mean Serum albumin levels between first trimester pregnant women and non-pregnant was found statistically significant difference. Serum albumin was decreased in first and third trimester of pregnancy than in non-pregnant women and maximum decrease seen in third trimester. This study affirms with that of the study done by Gohel et al. in 2013 and Zannat MR et al. in 2016 (24, 25). They found that serum albumin level is significantly lower in all three trimester compared to non-pregnant women.

CONCLUSION

• This study demonstrates that the highest incidence of PHM is found in the age group between 22-29 years.
• The highest percentage of Patients with PHM belonging to the rural city, nutritional and socioeconomic factors appear to be important risk factors for molar pregnancy.
• Accidental finding of ultrasonography was the commonest way of presentation followed by vaginal bleeding, routine first trimester ultrasound has made diagnosis of Molar pregnancy easier and earlier before any complications arise.
• PHM was more common in nulliparous and low parous patients having 1-2 children.
• This is the first report to suggest that there may be an association between pre albumin and PHM, pre albumin is sensitive to change in nutritional status due to short half-life (2-3) days.
• It is also suggesting that there may be no association between homocysteine and PHM.

Recommendations

• Future studies should focus on the association between Retinol binding protein, and Pre albumin in PHM.
• The main weakness of this study is small numbers of the patients, other studies should focus not only on the association between homocysteine and PHM but also on the association between homocysteine and CHM.
• Providing health education of community with regards to the importance of nutrition may decrease the incidence of HM.

REFERENCES

according to hydatidiform mole type. Gynecology Oncology. 2013; 130: 86-89.


Anthropometric Correlates of Anaerobic Power

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ABSTRACT

Introduction and Aim: The present paper intends to correlate the results of vertical jump test with different anthropometric parameters.

Materials and Methods: Eastern Indian schoolboys from the similar socio-economic background were selected. Vertical jump height (VJH) score and anaerobic power (AP) were two performance attributes considered which are correlated with Height (Ht), Weight (Wt), Body Mass Index (BMI) and Ponderal Index (PI).

Results: Mean VJH (26.5 ± 9.6 cm) was found to be lower than earlier studies. AP was 44.6 ± 21.8 kg-m/sec. Inter-correlation coefficients among all anthropometric parameters except PI with VJH and AP scores ranged between 0.22 – 0.93 (p < 0.01). The increasing trend of VJH and AP scores with age is clearly noted. A similar trend was also observed for height and weight. Multiple regression of AP with height and weight in three different age groups viz., 7 – 10, 11-14 and 15 – 18 years and as well as for the whole group yields an $R^2$ value ranging between 0.42 – 0.88.

Conclusion: Performance is greatly influenced by factors like age, height, and weight. Association of Wt and BMI with AP and VJH score can be correlated with their already established relation with muscularity, which in turn can directly affect anaerobic power. Since muscularity, leanness and body fat percentage are not included in the present study, their relative contributions in determining AP have been indicated as a further scope of research.

Key Words: Anaerobic power, Vertical jump test, Height, Weight, Anthropometry.

INTRODUCTION

Anaerobic power is the maximum ability of the anaerobic system to produce energy per unit of time. Anaerobic energy production is primarily a function of the Adenosine Triphosphate, phosphocreatine system (ATP – PC) system present in the skeletal muscles, which causes the instant release of energy from ATP breakdown with subsequent rapid regeneration of PC. This energy generates maximal muscle power in short bursts of activities. However, due to limited CP stores in muscles, this system can produce energy for a limited period lasting maximally for 8 – 10 seconds (1, 2).

Direct measurement of anaerobic power by measuring the ATP - PC stores is complicated and uses the invasive technique of muscle biopsy. However, this procedure has been successfully replaced by performance measure of anaerobic power. These alternative procedures are presented as field test methods. As a principle, the test employs very short bursts of high-intensity exercise which momentarily mobilize and expend maximum amount of energy that releases instantaneously from the muscle pool of high energy phosphates (3). In accordance, various test protocols have been developed and successfully administered for years (4-6).

Vertical Jump test (VJT) originally described by Sargent (4) has been widely accepted as a valid measure to estimate the anaerobic power generating the capacity of an individual. Recent studies have indicated that power output in the vertical jump is strongly associated with weightlifting ability and can be a valuable tool in assessing weightlifting performance (7). This test has been used to assess the maximum power of volleyball players (8, 9) and has also been introduced as a standardized test to evaluate the anaerobic fitness of basketball player (10) and to assess athletic performance (11, 12). The vertical jump test has also been advised as one of the major anaerobic training items in the physical fitness programs (10).

Recent data on vertical jump test in the Indian context is still scanty. Some earlier studies have reported vertical jump scores for school going boys and girls (13) and junior badminton players (14) from www.biomedicineonline.org
Eastern India. However, no attempt so far has been documented to correlate these values with anthropometric measures. In the present study, attempt has been made to report vertical jump scores for children and pre-adolescent boys and to correlate the anaerobic power generating capacity with fundamental anthropometric measures like height, weight and some other derived anthropometric indices.

**MATERIALS AND METHODS**

**Subjects**

A total of 143 boys within the age group of 7 – 18 years were selected from different schools of North 24 Pargana district of West Bengal, India to volunteer in the study. All of them were selected from a preselected population with matched daily habits, similar engagement in recreational sports activity for 2 hours per day and 3-4 days per week and similar socio-economic background and dietary patterns. All the subjects were from middle- class families.

Prior to subject election, a letter explaining the brief plan of the study was presented to the school management as well as to the parents for consent. Both the parents and children were explained about the study and the extent of their involvement.

**Investigation**

Height and weight of the subjects were obtained using an anthropometric rod and human weighing machine. Body Mass Index (BMI) was obtained from weight in kg divided by the square root of the height in meters. The height and weight of the subjects were also used to obtain the Ponderal Index (PI), calculated as the cube root of the weight (kg) divided by the body height (cm).

All subjects performed three trials of vertical jump as per protocol (4). Before the test, the subjects were explained and demonstrated about the procedure. In accordance, the subjects stood erect by a wall with both heels touching the floor. In this position they were asked to extend their arms vertically as far as possible and make a mark with their fingers dipped in chalk dust. This was designated as the standing reach. Next they were asked to jump and touch a height at their maximal possible extent and make a mark again. This height was termed as jump reach. The difference of jump and standing reach – the vertical jump height (VJH) was measured in cm. The best of the three trials were recorded and before each trial the subjects were verbally encouraged to beat their previous score. Finally the VJH is converted to anaerobic power (AP) expressed in kg – meter/ sec from the Lewis nomogram (6) by the following formula:

$$\text{Anaerobic power (kg – m/sec)} = \sqrt{4.9 \times \text{Wt (kg)} \times \sqrt{\text{VJH (m)}}}.$$ 

**Statistical Analysis**

Descriptive statistics including mean and standard deviations (SD) were generated from the data. Pearson product moment correlation coefficient was determined to judge the relationship between different anthropometric parameters and power scores. One way ANOVA was used to compare means of several parameters studied among different age groups and linear multiple regressions models were computed to assess anaerobic power from anthropometric parameters.

**RESULTS**

**Physical characteristics and anaerobic power for the whole group**

Descriptive statistics for different anthropometric parameters and anaerobic power scores are summarised in table 1. A wide dispersal of the data is apparent from the range of different parameter studied. The matrix of correlation between different parameters is presented in table 2. It is conspicuous from the table that PI showed least association with all parameters except BMI. In contrast, all the other anthropometric parameters showed significant positive association among themselves and also with the VJH score and anaerobic power. Furthermore, the interrelation between the VJH score and anaerobic power appeared insignificant.

**Table 1: Anthropometric characteristics and power scores of the subjects (n= 143)**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Mean ± SD (Range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>12.2 ± 3.1 (7 – 19)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>147.1 ± 15.2 (113 – 175)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>40 ± 13.1 (19.5 – 77.0)</td>
</tr>
<tr>
<td>BMI (kg/m2)</td>
<td>18.0 ± 3.2 (12.9 – 27.6)</td>
</tr>
<tr>
<td>Ponderal Index x103</td>
<td>23.03 ± 1.25 (20.6 – 27.4)</td>
</tr>
<tr>
<td>Vertical Jump Height (VJH) (cm)</td>
<td>26.5 ± 9.6 (9.3 – 51.4)</td>
</tr>
<tr>
<td>Anaerobic Power (kg – m/sec)</td>
<td>44.6 ± 21.8 (10.4 -106.8)</td>
</tr>
</tbody>
</table>
Relation between anthropometric and anaerobic power profile with age

For a comprehensive assessment of the anaerobic power the entire group of subject was subdivided into three age group classes, viz; 7 – 10 (mean age 8.8 ± 1.11) years, 11-14 (mean age 12.4 ± 1.14) years, and 15 – 18 (mean age 16.6 ± 1.37) years. Descriptive statistics of different parameters of the age groups is summarised in table 3. It appeared that the subjects of different age groups differ significantly in terms of all the parameters studied. All the parameters except PI were lowest in 7 – 10 years and highest among the 15 -18 years age group while 11-14 age group demonstrated intermediate value. However, this pattern was not followed for the PI. The increasing trend of all the parameters with age except PI is also reflected in positive significant correlation coefficient as presented in table 2.

Table 3: Anthropometric profiles and anaerobic power scores in different age groups

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Age group (year) Classes</th>
<th>Results of ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>7 – 10 (n= 47)</td>
<td>11 – 14 (n= 63)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>131.8 ± 7.3</td>
<td>149.4 ± 11.0</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>29.2 ± 5.9</td>
<td>39.8 ± 9.6</td>
</tr>
<tr>
<td>BMI (kg/m2)</td>
<td>16.7 ± 2.7</td>
<td>17.7 ± 3.0</td>
</tr>
<tr>
<td>Ponderal Index x 103</td>
<td>23.3 ± 1.2</td>
<td>22.7 ± 1.3</td>
</tr>
<tr>
<td>VJH (cm)</td>
<td>18.2 ± 5.1</td>
<td>27.8 ± 7.7</td>
</tr>
<tr>
<td>Anaerobic Power (kg-m/sec)</td>
<td>22.3 ± 8.6</td>
<td>46.3 ± 14.4</td>
</tr>
</tbody>
</table>

Regression equations generated for predicting anaerobic power from anthropometric parameters viz, height and weight for different age groups are summarised in table 4. The models were derived using height and weight as variables and AP (kg –m) as criterion variable (Y).
### Table 4: Relationship among height weight and anaerobic power in different age groups

<table>
<thead>
<tr>
<th>Age group (years)</th>
<th>Relation</th>
<th>( R )</th>
<th>( R^2 )</th>
<th>SE</th>
<th>( p )</th>
<th>Regression</th>
</tr>
</thead>
<tbody>
<tr>
<td>7 – 10</td>
<td>Age vs Height</td>
<td>0.47</td>
<td>0.22</td>
<td>7.7</td>
<td>&lt; 0.001</td>
<td>( Y = Ht \times 0.547 - 49.81 )</td>
</tr>
<tr>
<td></td>
<td>Age vs Weight</td>
<td>0.64</td>
<td>0.41</td>
<td>6.6</td>
<td>&lt; 0.001</td>
<td>( Y = Wt \times 0.928 - 4.83 )</td>
</tr>
<tr>
<td></td>
<td>Age vs Ht and Wt</td>
<td>0.65</td>
<td>0.42</td>
<td>6.7</td>
<td>&lt; 0.001</td>
<td>( Y = Ht \times 0.135 + Wt \times 0.825 -19.61 )</td>
</tr>
<tr>
<td>11 – 14</td>
<td>Age vs Height</td>
<td>0.71</td>
<td>0.50</td>
<td>10.2</td>
<td>&lt; 0.001</td>
<td>( Y = Ht \times 0.927 - 92.10 )</td>
</tr>
<tr>
<td></td>
<td>Age vs Weight</td>
<td>0.88</td>
<td>0.77</td>
<td>6.9</td>
<td>&lt; 0.001</td>
<td>( Y = Wt \times 1.31 - 5.92 )</td>
</tr>
<tr>
<td></td>
<td>Age vs Ht and Wt</td>
<td>0.89</td>
<td>0.79</td>
<td>6.7</td>
<td>&lt; 0.001</td>
<td>( Y = Ht \times 0.223 + Wt \times 1.131 -32.0 )</td>
</tr>
<tr>
<td>15 – 18</td>
<td>Age vs Height</td>
<td>0.63</td>
<td>0.40</td>
<td>11.8</td>
<td>&lt; 0.001</td>
<td>( Y = Ht \times 1.274 - 136.11 )</td>
</tr>
<tr>
<td></td>
<td>Age vs Weight</td>
<td>0.83</td>
<td>0.70</td>
<td>8.3</td>
<td>&lt; 0.001</td>
<td>( Y = Wt \times 1.24 +3.75 )</td>
</tr>
<tr>
<td></td>
<td>Age vs Ht and Wt</td>
<td>0.85</td>
<td>0.72</td>
<td>8.1</td>
<td>&lt; 0.001</td>
<td>( Y = Ht \times 0.395 + Wt \times 1.066 - 51.26 )</td>
</tr>
<tr>
<td>Whole group</td>
<td>Age vs Height</td>
<td>0.87</td>
<td>0.76</td>
<td>11.1</td>
<td>&lt; 0.001</td>
<td>( Y = Ht \times 1.310 - 148.10 )</td>
</tr>
<tr>
<td></td>
<td>Age vs Weight</td>
<td>0.93</td>
<td>0.86</td>
<td>8.6</td>
<td>&lt; 0.001</td>
<td>( Y = Wt \times 1.615 - 20.00 )</td>
</tr>
<tr>
<td></td>
<td>Age vs Ht and Wt</td>
<td>0.94</td>
<td>0.88</td>
<td>7.8</td>
<td>&lt; 0.001</td>
<td>( Y = Ht \times 0.468+Wt \times 1.151 -70.29 )</td>
</tr>
</tbody>
</table>

### DISCUSSION

Average VJH score for all the subjects as obtained in this study is lower than Eastern Indian school going boys and junior badminton players with a mean of 34.9 ± 6.3 cm and 33.0 ± 9.0 cm respectively but comparable to that of school going girls (26.5 ± 4.4 cm) of 10 – 16 years of age (13, 14). Considering the VJH score in similar age groups as obtained in previous studies, the current study yields an average VJH score of 27.6 ± 8.2 cm which was still lower.

It has been acknowledged that performance is greatly influenced by factors like age, height, and weight. In the present study performance, scores are well correlated with age, height, weight, and BMI except for PI. The ponderal index used in the present study was constructed considering the fact that weight is the result of a three-dimensional expansion of the body, and thereby represents a valid indicator of body stoutness (15).

Although the relationship of PI with muscle strength is not well documented, an apparent low negative correlation between PI both with VJH and AP scores in the present study may be attributed to the fact that ponderal index implies the body position in relation to ectomorphy which reflects thinness. This is opposed to mesomorphy which is characterized by muscularity (16) that positively influences the anaerobic power output of an individual.

It is easily assumed that muscular strength and relative muscularity have got direct influence on anaerobic power and the relation between muscular strength, performance and anthropometric profile have been well established in different kinanthropometric studies (17-19). This may be further established by the fact that vertical jump score is a well predictor of muscle strength and has been found significant in predicting leg strength (20). Again correlation obtained by knee extensor muscular strength with vertical jumping height and peak power yield strong to the moderate relationship among women volleyball players (21). The choice of body mass index in the present study is influenced by the fact that it represents one of the best indices for assessment of nutrition, growth status and body composition for school-age children and adolescent (22) and has also been used to evaluate the leanness among Indian children (23). It is also highly correlated with body weight (24) and reported to have a strong positive association with muscularity (r = 0.66, p< 0.001) among Eastern Indian ‘Bengalee’ boys (25). Considering the effect of muscularity on the jump height and AP scores, the relationship between muscularity and BMI can, therefore, be assumed as an explanation for the positive association of BMI with AP and VJH score as obtained in the present study. It can be noted that the coefficient of correlation obtained between BMI and VJH score is almost similar to that obtained in a previous study conducted among young adults (26).

The weight in the present study is more associated with AP as compared to VJH. This can be partly attributed to the fact that the Lewis method directly includes body weight as a factor in determining anaerobic power. Moreover, since weight has a high relationship with developmental level and influenc-
es muscularity, it, therefore, acts as a determining factor for strength and anaerobic power measures. This has been reflected in strength test performances relying on anaerobic power (27).

A conspicuous increasing trend was observed in the anaerobic power score with increasing age that parallels with a similar trend for height and weight of the subjects. The observed growth pattern of the subjects in relation to height and weight is in well conformation with the results obtained for similar eastern Indian population (28).

The higher values of VJH and AP scores of the subjects in different age groups in ascending order may be attributed to their increased height and weight with age which could have influenced the anaerobic power scores. A very early study conducted by Dawson (29) reported a rising trend in AP scores with age advancement up to middle age.

Based on the protocol of the experiment it can be stated that height has a positive influence on the VJH as taller subjects will likely to have more reach scores. It is also evident that this relationship is subjected to variation by individual muscularity. The subjects selected in the present study are not trained for a particular sports activity that may influence the muscle build. Moreover, as per the selection criteria, they can be considered homogenous on the source of socio-economic background which is observed to have an influence on the dietary pattern thereby influencing growth status (30). The average BMI of the subjects in this study was obtained to be 18 which can be classified as thin (31) indicative of a lower musculature. Now, based on an assumed equality of nutritional status of the subjects it can be stated that VJH and following AP score might have been more influenced by the stature than muscularity in this case. The ‘thinness’ of the boys under present investigation may be responsible for a lower value of VJH score as compared to the previous findings mentioned earlier (13,14). From this could be humbly acknowledged that assessment of muscularity along with leanness and fat percentage, which was not considered in the present study, can be proved to be a better estimator of AP and evaluation of their relative contributions in VJH and AP thus represent a potential scope of the future study.

**CONCLUSION**

As like other methods of classifying anaerobic power in relation to age, the present study has generated regression equations to predict anaerobic power from height and weight for different age group categories, each with high R² values. These equations may be useful for assessment of anaerobic power in large-scale studies and primary evaluation of one's capability to different field events requiring short bursts of muscle activity.

**ACKNOWLEDGEMENT**

The author expresses his deep respect and heartfelt gratitude to late Arghya Biswas for his immense enthusiasm in organizing necessary arrangement for the study.

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A Single Blind, Single Center, Two Group Pretest-posttest Randomized Controlled Trial to Determine the Efficacy of Vestibular Rehabilitation and Conventional Balance Training Programmes in the Management of Diabetic Peripheral Neuropathy

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ABSTRACT

Introduction and Aim: Diabetic peripheral neuropathy (DPN) is defined as the presence of signs and symptoms of peripheral nerve dysfunction in subjects with diabetes. Vestibular dysfunction accounted to be greater than 70% in people with diabetes which eventually increases the risk of fall. Conservative treatment methods like vestibular rehabilitation (VR) and conventional balance training (CBT) programmes are available to treat them. More efficient among them is not known. The aim of the study was to compare the efficacy of VR and CBT programmes in the management of individuals with DPN.

Materials and Methods: A total of 60 individuals with DPN were recruited by the simple random sampling to participate in this two group pretest-posttest, single blind, single center randomized clinical study. Recruited individuals with DPN were randomly allocated into two groups, Group A and Group B. Group A were provided with VR programme for 60 seconds duration x 5 repetition /session / day x 3 days / week x 12 weeks. While group B received CBT programme. Both the groups received the treatment for 12 weeks, totaling 36 sessions (three sessions per week). Level of confidence [Activities specific Balance Confidence (ABC) Scale] and functional mobility [Timed up and go test (TUG)] were documented at baseline and 12th week after intervention and analyzed.

Results: Group A demonstrated significance difference (p<0.05) in ABC scale and TUG when compared to group B.

Conclusion: Twelve week VR programme has the sufficient potential to increase the level of confidence and functional mobility among individuals with DPN.

Key Words: Diabetic neuropathy, Falls, Physical therapy techniques, Single-blind study.

INTRODUCTION

Diabetes is a serious and chronic metabolic disorder that occurs when the body cannot effectively use the insulin that it produces, or when the pancreas does not produce enough insulin (1). Diabetes and its complications, especially retinopathy and peripheral neuropathy, is steadily increasing over the past few decades and The increased risk of complications of diabetes has led to 1.6 million (2.8%) deaths in 2015 across the world (2). Diabetic peripheral neuropathy (DPN) is defined as the presence of signs and symptoms of peripheral nerve dysfunction in subjects with diabetes after the exclusion of other cause (3). Peripheral neuropathies, commonly seen in subjects with diabetes mellitus are due to the chronic hyperglycemia (1). In type 1 diabetes mellitus, distal polyneuropathy typically becomes symptomatic after many years of chronic prolonged hyperglycemia, but in cases of type 2 diabetes mellitus, the subjects may present with distal polyneuropathy only after a few years of known poor glycaemic control (1). It is always necessary to take into account the following changes in the subjects like Touch sensitivity symptoms which include pain, numbness, and altered pain sensation in hands, legs, and feet leading to skin damage (1).

The complication of diabetes which is least understood in its effect on the vestibular system (4). Due to diabetes, pathophysiological changes in central and peripheral vestibular structures have been noted (3). If the vestibular system is adversely affected by diabetes, it is necessary to consider its impact of
diabetes on the risk of falls in older adults and other population (5). Although vestibular system dysfunction is not commonly recognized as a microvascular complication of diabetes, a recent epidemiological study reported that vestibular dysfunction was 70% higher in people with diabetes than in people matched for age and serving as controls (6). In addition, decreased vibration sense and loss of pressure sensitivity have been shown to be associated with recurrent falls (7). Because of decreased proprioceptive feedback during walking, older adults with diabetes walk slower and have greater stride variability; these factors increase the risk of falls (8).

Physiotherapists play a vital role in improving the general condition and fall prevention in subjects with diabetic peripheral neuropathy. The need to consider vestibular rehabilitation (VR) exercise becomes essential due to the involvement of vestibular system in people with DPN. VR exercises aim at training the eye and head movements and thereby improving general coordination. The comprehensive evaluation of the vestibular system may be necessary for people who have diabetes along with balance impairment. Hence the purpose of this study was to compare the effects of vestibular rehabilitation (VR) exercises and conventional balance training (CBT) program in subjects with DPN.

MATERIALS AND METHODS

Recruitment and Allocation

The study protocol was approved by the university research and ethics committee (ACS/2016/68) and the study was done strictly in accordance with the guidelines of Helsinki declaration, revised 2013 (9). A total of 30 individual with DPN were recruited by the simple random sampling (random number tables from standard statistics book) to participate in this two group pretest-posttest, single blinded randomized clinical study. After the demographics, 60 recruited individual with DPN were randomly divided into two groups, group A and group B with, 30 in each by block randomization. There were ten blocks, with the matrix design of 6 x 10, where 6 being rows. Each block contained 6 chits (3 chits for each group), totaling 60. The subjects were allotted to the group based on the randomly chosen chit. Once the block was allotted, next row block was opened. Thus, equal number of subjects was assigned to each group over time. Group A received vestibular rehabilitation (VR) exercises. While Group B received conventional balance training (CBT) programme. Both the group received the interventions for 12 week period, totaling 36 sessions (3 sessions / week). First session was performed under supervision, and other at their home without supervision. The Consolidated Standards of Reporting Trials (CONSORT) (10) flow chart describing the details of the study is displayed in Figure 1.

Vestibular Rehabilitation Exercise in Group A

Group A (30 subjects) received vestibular rehabilitation exercises. The vestibular rehabilitation exercises consist of the Cawthorne Cooksey exercises and the gaze stabilization exercises. All the exercises were performed daily with 8 repetitions for each exercise / session. They performed single session / day x 3 days / week x 12 weeks. The first session of VR exercises were demonstrated and supervised by qualified person who have more than five years’ experience in training VR exercises.

Conventional balance training (CBT) programme in Group B

Group B received CBT program, which includes the exercises like single limb stance (30s x 3 repetitions / limb), staggered stance (30s x 3 repetitions / limb), side stepping (3m/side x 3 repetition) and crossed stepping (3m/side x 3 repetition) exercises for single session aimed at improving the balance of the subjects. These exercises were performed single session / day x 3 days / week x 12 weeks.

Outcome Measures

Both the group were measured for their level of confidence [Activities specific Balance Confidence (ABC) Scale] (11) and functional mobility [Timed up and go test (TUG)]12 between Group- A and Group- B at baseline and 12th week post intervention.

Data Analysis

The collected demographic and outcome measures were assessed for their normality using Kolmogorov–Smirnov test. As the data follow normal distribution, all the descriptive were expressed in mean ± standard deviation. Paired t test was adopted to find out the differences within Group- A and group-B for pre-post intervention changes. While independent t-test was used to compare the changes in mean values of ABC scale and TUG test between Group- A and Group- B at baseline and end of 12 week intervention. The data was analysed using statistical software, statistical package for social science (SPSS),
IBM SPSS version 20.0 (Armonk, NY: IBM Corp.). The p-value ≤0.05 was considered to be statistically significant.

RESULTS

Sixty individuals with DPN were recruited for the study. The demographic characteristic of the individuals with DPN recruited were displayed in Table 1. The demographic characteristics were elaborated in Table 1. There exists no significance difference between the two groups. Between the session and group comparison at baseline and end of 12 weeks exercise intervention for the outcome measures ABC scale (Figure 4) and TUG test (Figure 5) were displayed. In all the outcome measures, group A shows significant (p<0.05) improvement when compared to group B.

Table 1: Demographic characteristic of the individuals with diabetic peripheral neuropathy recruited in group A and group B.
Table 1: Demographic characteristic of the individuals with diabetic peripheral neuropathy recruited in group A and group B

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group A</th>
<th>Group B</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>30</td>
<td>30</td>
<td>-</td>
</tr>
<tr>
<td>Age (years)</td>
<td>53.2±3.8</td>
<td>51.7±3.1</td>
<td>0.71</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>75.4±7.5</td>
<td>79.2±5.6</td>
<td>0.23</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>165.2±9.2</td>
<td>167.5±6.9</td>
<td>0.13</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27.7±2.1</td>
<td>28.4±2.9</td>
<td>0.09</td>
</tr>
</tbody>
</table>

Figure 2: Mean Activities specific Balance Confidence (ABC) Scale score at baseline and end of 12th week intervention between Group A and Group B

Figure 3: Mean Timed up and go test (TUG) at baseline and end of 12th week intervention between group A and group B

DISCUSSION

The individual with DPN has increased chances of developing an unsteady movement pattern that is due to the decline in the balance and proprioception. Lack of proprioception in the subjects with diabetic peripheral neuropathy may affect the quality of life of the patients and their dynamic balance. Studies suggest that the complications of the diabetics are more severe when the disease is for the prolonged period. Balance problems are one among the common problems faced by the subjects with DPN in which all the three somatosensory systems are affected.

The conventional balance training program was the treatment strategies for the subjects with balance impairment in the diabetics, Allet et al. (8) reported that the balance training program had an excellent outcome in improving the dynamic balance of the subjects (8). It is mandatory that the subjects with balance impairment should follow the balance training because it was the older population who were more prone to the fall risk. The conventional balance training program aims at improving the balance of the patients and thereby prevents the risk of fall. The balance training programme includes gait and balance exercises combined with strengthening that improves gait speed and balance, and increase both muscle strength and joint mobility of diabetic patients (6,8,13). This supports the findings of our study that CBT improves confidence level and functional mobility in individuals with DPN.

Recent studies suggested that the balance impairment in the diabetics is also due to vestibular dysfunction. D’Silva et al (14) reported the relationship among diabetes, vestibular function, and the fall risk are complex (14). People with diabetes have many deficits, including neuropathy, retinopathy, all of which would compromise the activity and their functional status. Vestibular dysfunction is another possible complication of diabetes and may increase the risk of falls. Understanding this relationship, identifying and treating, and working toward integrating all systems visual, vestibular, and somatosensory to improve balance are the ways in which physical therapists can prevent falls. Hence, treating vestibular dysfunction by the vestibular rehabilitation exercises may also have an impact on improving both the static and dynamic balance of the subjects.

The present study was conducted in 60 subjects with Diabetic Peripheral Neuropathy. 30 subjects were included in each Group in which Group A received the Vestibular rehabilitation exercises, and Group B received the Conventional balance training program. The study intended to compare the effectiveness of vestibular rehabilitation exercises and conventional balance training program in DPN subjects with balance impairment. This study showed that both the treatment program worked well on both the groups, in which vestibular rehabilitation exercises were considered to be more effective than the conventional balance training program in order to maintain the
balance while performing different activities.

The possible reasons behind the effectiveness of treatment with vestibular rehabilitation exercises are due to the involvement of the visual, vestibular and somatosensory system by the process of vestibular compensation (14). It is the process which allows the brain to regain the balance control when there is an imbalance between the right and left vestibular organs in the ear. It is achieved when the brain copes with disorienting signals from the inner ear by learning to rely on the alternative signals coming from eye, neck, and legs to maintain balance (14-16). Hence incorporating vestibular rehabilitation exercises in the general treatment protocol for treating subjects with DPN is considered to be beneficial in improving balance and reducing the risk of falls.

**CONCLUSION**

Twelve-week VR programme has the potential to increase the level of confidence and functional mobility among individual with DPN when compared with CBT programme.

**REFERENCES**


Predisposing Haplotypes of TGF-ß1 among Malay Women with Breast Density in Kuala Terengganu, Malaysia: A Pilot Study

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ABSTRACT

Introduction and Aim: Breast density has been used as a potential cofounder factor in breast carcinoma risk. The breast density is determined by several genes that involved in cell proliferation, particularly Transforming Growth Factor-Beta 1 (TGF-ß1). A defect in TGF-ß1 molecular pathway may lead to abnormal cell proliferation which is seen in human cancers, particularly breast carcinoma. This preliminary study was aimed to predict the predisposing haplotypes of TGF-ß1 among Malay women in Kuala Terengganu with breast density.

Materials and Methods: The participants were recruited among women who underwent mammography check-up at Department of Radiography, Hospital Sultanah Nur Zahirah of Kuala Terengganu. The case group (n=44) was consisted of individuals with BIRADS 1 and BIRADS 2, while the control group (n=48) was taken among individuals with BIRADS 3 and BIRADS 4. SNP genotyping was conducted for three main TGF-ß1 variants, assigned as rs1800469, rs1800470 and rs4803455 using PCR-RFLP technique.

Results: Two SNPs (rs1800469 and rs180047) did reveal a significant association with breast density with a p value of 0.008 and 0.006, respectively. However, rs4803455 did not yield association in this small cohort. In a further investigation, haplotype GAA conferred a susceptible haplotype (p value=0.027, OR=2.28 [1.09-4.76]) towards breast density whilst haplotype AAC projected a significant association with protective effect (p value=0.018, OR=0.45[0.23-0.88]) in breast density development.

Conclusion: This pilot study might provide baseline information on the role of TGF-ß1 haplotypes in determining the density of breast that projected a genetic determinant as a risk factor for breast carcinoma among Malay women.

Key Words: Breast density, TGF-ß1, Haplotypes, Association.

INTRODUCTION

A mammogram is a screening tool to detect the mass of breast by assessing the ratio of the epithelial and stromal tissue towards fat mass in which can determine the density of the breast. According to the Breast Imaging-Reporting And Data System (BIRADS) classification, there are four main classes, namely BIRADS 1, BIRADS 2, BIRADS 3 and BIRADS 4 that is based on the level of density. Breast density has been recognized as a breast cancer predictor in diagnosis alongside with other factors such as family history, environmental factor and genetic determinants (1). Individuals with higher breast density assigned as BIRADS 3 and BIRADS 4 are prone to develop breast cancer with 1.8- to 6-fold increase risk compared to individuals with lesser breast density (BIRADS 1 and BIRADS 2) (2). The mammographic density is determined by gene regulation in mammary cell development that includes Transforming growth factor-beta (TGF-ß) gene. The gene is a cytokine that involves cell proliferation, differentiation and cell signaling in which consists of three isoforms assigned as TGF-ß1, TGF-ß2, and TGF-ß3. TGF-ß have been shown to promote in ductal branching in mammary gland
which leads to alveolar hyperplasia of breast and premature functional differentiation (3).

Transforming Growth Factor-Beta 1 (TGF-ß1) is the most isof orm that was observed in upregulating in tumourogenesis(4) and has been extensively studied in association with its role in cell divisions and proliferation especially in mammary cell development (5). The gene was observed to associate in breast carcinogenesis, and thus, this study was conducted to determine the role of TGF-ß1 variants in breast density development among Malay women in our population in which to the best our knowledge no study is carried out yet in Malaysia for this purpose.

MATERIALS AND METHODS

Subject Recruitment

This case-control study was conducted among Malay women (n=92) who underwent mammography screening either for diagnosis or follow up examination at Hospital Sultanah Nur Zahirah, Kuala Terengganu. They were given informed consent before participating in this study. The human ethical approval was obtained from UniSZA Human Research Ethical Committee (No:UniSZA.C/1/UHREC/628-1(38)) and National Medical Research Registry (No:NMRR 15-1021-26292 (IIR). For those who developed with other cancer than breast carcinoma was excluded in this study. The mammography parenchymal pattern was determined using BIRADS (breast imaging-reporting and data system) classification. BIRADS 1 and 2 with lesser breast density were identified/categorized as a control group (n=44), whilst BIRADS 3 and 4 were grouped into case cohort (n=48) with denser breast density. BIRADS 1 consists of the mostly fatty breast with 0-24% density and BIRADS 2 is a scattered fibroglandular breast with 25-50% density. Meanwhile, BIRADS 3 is categorized as a heterogeneously dense breast with 51-75% density, and BIRADS 4 is classified as an extremely dense breast with 76-100% density. The diagnosis on BIRADS classification was conducted by radiologists.

SNP Genotyping

DNAs were extracted from 3 ml of peripheral blood using the commercialized extraction kit according to the manufacturer’s protocol (Qiagen, Germany). Three SNPs of TGF-ß1 were genotyped using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique, assigned as rs1800469 (G/A), rs1800470 (A/G) and rs4803455 (C/A). The primers and restriction enzymes used in this technique are listed in the table below (refer Table 1). PCR technique was performed in a total of 25µl that consisted of 5X PCR buffer, 2.0mM MgCl2, 200M dNTP, 0.2M of forward and reverse primer and 0.08 U/µl of Taq polymerase (Promega, Madison, WI) before restriction enzyme digestion procedure. The PCR and PCR-RFLP product was preceded with 2% agarose gel electrophoresis and stained with ethidium bromide before viewed with the Alpha Imager Analyzer.

Statistical Analysis

Allelic and genotypic frequencies were calculated according to Hardy Weinberg equilibrium using SHEsis online software providing odd ratios (ORs) and 95% confidence interval (95%CI) and a significant p value at 0.05 (Chi square). The statistical analysis is used for single association and haplotype association analyses in determining the association of TGF-ß1 with breast density.

RESULTS

Single association revealed a significant difference for rs1800469 and rs1800470 with breast density among Malay women who attended for mammography screening with p value at 0.008, OR=2.23, 95%CI [1.22-4.08] and p value at 0.006, respectively. However, this pilot study did not find any association of rs4803455 (p value=0.130, OR=0.62, 95%CI [0.34-1.15]. The odd ratio for rs1800470 was not obtained due to no heterogeneity observed in case group (refer Table 2). In an attempt to provide evidence of predisposing effect with a combination of an allele from each of the TGF-ß1 variants, haplotype analysis was conducted. It is noteworthy that haplotype GAA conferred susceptible effect in breast density development with p value at 0.027, OR=2.28, 95%CI [1.09-4.76]. In a contrarily, haplotype AAC yielded a significant association with breast density in a protective manner, projected p value at 0.018, OR=0.45, 95%CI [0.23-0.88] (refer Table 3).
DISCUSSION

In this preliminary study, the data showed a significant role of TGF-ß1 polymorphisms with breast density development projected by two variants assigned as rs1800469 and rs1800470. The association study may support the function of Transforming Growth Factor (TGF) gene in the mammary parenchymal cell formation, differentiation, and proliferation with the role of single nucleotide polymorphisms within the gene region. The mammographic density is determined by the ratio of the amount of epithelial and stromal tissue to the amount of fat tissue. The importance of breast density determination and classification is useful as a strong predictor of breast cancer risk. Individuals with higher breast density such as BIRADS 3 which contains 51-75% of density and BIRADS 4 comprises of 76-100% of density may prone to predispose them to breast carcinogenesis. Therefore this study presented the minor allele of rs1800469 (a promoter variant with a substitution of guanosine to adenosine at position -800 bp) to in-

Table 1: Primer sequences and restriction enzymes used with PCR product sizes

<table>
<thead>
<tr>
<th>SNP</th>
<th>Primer sequences</th>
<th>Restriction enzyme</th>
<th>Product sizes</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs1800469</td>
<td>Forward:5'-GGGAGGAGGGGCAACAGGAGCAGCTGC Reverse: 5'-TGGGGGAGGATGGGCAGCTG</td>
<td>HpyCH4V</td>
<td>289bp, 30 bp, 259 bp</td>
</tr>
<tr>
<td>rs1800470</td>
<td>Forward:5'-ACCTAGTCACCCAGCAGCGGTAGCAGCTGC Reverse: 5'-AGGACCTCAGCCTTCCCTGC</td>
<td>Bst1</td>
<td>191bp, 31 bp, 160 bp</td>
</tr>
<tr>
<td>rs4803455</td>
<td>Forward: 5’-TTTGACACCCCTGAATTCTCAA Reverse: 5’-TTAGTAGAGACGAGGTTTCAC</td>
<td>MluCI</td>
<td>172 bp, 34 bp, 138 bp</td>
</tr>
</tbody>
</table>

Table 2: Single association analysis of TGF beta polymorphisms with breast density

<table>
<thead>
<tr>
<th>SNP</th>
<th>Genotype data</th>
<th>Case Control analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Homozygous wild type n (%)</td>
<td>Heterozygous, n (%)</td>
</tr>
<tr>
<td>Rs1800469</td>
<td>25(0.57)</td>
<td>10(0.23)</td>
</tr>
<tr>
<td></td>
<td>16(0.33)</td>
<td>15(0.31)</td>
</tr>
<tr>
<td>Rs1800470</td>
<td>44(1.00)</td>
<td>0(0.00)</td>
</tr>
<tr>
<td></td>
<td>43(0.90)</td>
<td>2(0.04)</td>
</tr>
<tr>
<td>Rs4803455</td>
<td>18(0.41)</td>
<td>17(0.39)</td>
</tr>
<tr>
<td></td>
<td>27(0.56)</td>
<td>14(0.30)</td>
</tr>
</tbody>
</table>

*Case data is at the top line while control data is at the bottom line. MAF= Minor allele frequency. P value < 0.05 is considered significant in Pearson Chi Square.

Table 3: Haplotype association analysis of TGF-ß1 polymorphisms with breast density

<table>
<thead>
<tr>
<th>Allele combination</th>
<th>Lesser breast density group, n (freq)</th>
<th>Denser breast density group, n (freq)</th>
<th>P value</th>
<th>OR [95%CI]</th>
</tr>
</thead>
<tbody>
<tr>
<td>GAC</td>
<td>28.29(0.295)</td>
<td>35.38(0.402)</td>
<td>0.153</td>
<td>1.56[0.85-2.88]</td>
</tr>
<tr>
<td>GAA</td>
<td>13.71(0.143)</td>
<td>24.62(0.280)</td>
<td>0.027</td>
<td>2.28[1.09-4.76]</td>
</tr>
<tr>
<td>GCC</td>
<td>3.00(0.031)</td>
<td>0.00(0.000)</td>
<td>0.091</td>
<td>-</td>
</tr>
<tr>
<td>AAC</td>
<td>33.71(0.351)</td>
<td>17.62(0.200)</td>
<td>0.018</td>
<td>0.45[0.23-0.88]</td>
</tr>
<tr>
<td>AAA</td>
<td>12.29(0.128)</td>
<td>10.38(0.118)</td>
<td>0.793</td>
<td>0.89[0.37-2.14]</td>
</tr>
<tr>
<td>AGC</td>
<td>3.00(0.031)</td>
<td>0.00(0.000)</td>
<td>0.091</td>
<td>-</td>
</tr>
</tbody>
</table>

*Alleles from left to right are rs1800469, rs1800470 and rs4803455, respectively. P value < 0.05 is considered significant in Pearson Chi Square.
crease risk in breast density development by giving the odd ratio of 2.23 in a single association analysis. Even though the odd ratio for rs1800470 (a non-synonymous variant with a substitution of leucine to proline at position -509 bp) could not be calculated due to no heterogeneity in the case group, the variant showed association with breast density. The data is consistent with other studies in different populations (6,7). The minor allele from these two variants was also implicated in higher TGF-ß1 serum level and suggesting the genetic factor controls and regulates the biochemical in which this study did not perform (8). Meanwhile, rs4803455 did not yield a significant association in this study with the minor allele conferred protective effect based on OR=0.61. The data was contradicted by a study conducted among Singapore Chinese women particularly among nulliparous women (9) with a fair association (p=0.034). The contradictory factor might be due to different ethnicity in which the women in this study was among 98% Malays.

In a further investigation to evaluate predisposing haplotype in breast density, haplotype GAA with a combination of wild alleles from rs1800469 and rs1800470 with the mutant allele from rs4803455 revealed an association in susceptibility to higher breast density as projected by the odd ratio. A change allele from haplotype GAC to GAA provided an evidence of allele A from rs4803455 with a susceptible effect significantly in haplotype GAA. Thus, individuals with this haplotype GAA are prone to have denser breast density as categorized as BIRADS 3 and BIRADS 4. Contradictory, a protective haplotype assigned as haplotype AAC with a combination of a mutant allele from rs1800469 with wild alleles from rs1800470 and rs4803455 might reduce the risk to denser breast density in which less potential to develop breast cancer among Malay women in our population. This evidence can be observed when an allele change from haplotype GAC with a combination of all wild alleles was changed to haplotype AAC. The role of the protective effect was projected from allele A from rs1800469. It is noteworthy that mutant alleles from rs4803455 and rs1800470 play a major role in giving stronger effect to these predisposing haplotypes either a susceptible or protective manners.

Malaysia is a multiracial country that accounts Malays with 54.7%, followed by Chinese 24.3%, Indian 7.3%, other Bumiputera 12.8% and others 0.9%. The incidence of breast cancer in Malaysia was highest among Chinese, followed by Indian and Malays. However, the incidence of breast cancer among Malay women in Malaysia was 27.2% based on Malaysian National Cancer Registry Report 2007-2011(10). Since this study was conducted in Terengganu where the Malays contribute the majority ethnic group with 96.6%, the data may provide evidence of breast density among Malay women with predicting the breast cancer incidence rate in Terengganu, particularly. Therefore, SNP profile in the different ethnic group is worth to be taken into account in a future direction in a well-characterized population-based study.

This current study revealed that the variations of TGF-ß1 provide molecular information on potential diagnostic biomarkers for a majority of patients-based ethnic in breast cancer risk. The data may facilitate the personalized medicine based on targeted SNP profile and may provide baseline information to other researchers in exploring more evidence in the role of TGF-ß1 variants in breast density.

ACKNOWLEDGEMENT

We would like to thanks to all participants in this study who underwent the mammogram and all the staffs at Radiology Department of Sultanah Nur Zahirah of Kuala Terengganu. This study was supported by the Dana Penyelidikan Universiti (DPU) (No grant: UniSZA/2015/DPU/83) and approved by National Medical Research Registry (No grant: NMRR 15-1021-26292 (IIR)).

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To Compare the Effectiveness between LASER and Neuromuscular Electrical Stimulation in Knee Osteoarthritis

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ABSTRACT

Introduction and Aim: The aim of the study is to compare the efficacy of LASER, and conventional Exercise versus Neuromuscular Electrical Stimulation and conventional Exercise in the treatment of subjects with osteoarthritis of the knee joint in terms of pain relief and improving the functional activities. Osteoarthritis is the most common form of Arthritis. It is a disease caused by the breakdown of cartilage in the joints. The layers of the cartilage become damaged, and with time they lose the function of smoothing the contact between the bone surface and the joints. The pain is a result of the attrition of one bone against the other in the absence or decreased cartilage in the joints. The knee joint supports nearly the whole weight of our body. This study helps to increase the Range of motion, Muscle strength and Recovery of Muscle Mass.

Materials and Methods: The study was done at Outpatient Physiotherapy Department ACS Medical College and Hospital. This experimental study design had a sample size of 30, between the age group of 40 to 60 years. The patients were allocated to Group A and Group B by simple random sampling, duration of study 6 Sessions in a week, for 4 weeks. The material used a LASER, NMES, Bicycle Erygometer, Treatment couch and Assessment sheet and outcome measures Visual analogue scale [VAS] and Western Ontario and Mc Master Osteoarthritis Index [WOMAC]

Results: A Total number of 30 patients with osteoarthritis of the knee joint were taken between 40-60 years of age, both males, and females. Group A -was treated with LASER and conventional exercise, Group B - received NMES with Conventional Exercise for 4 weeks. VAS and WOMAC scale was recorded before and after treatment session as pre and post-test score after 4 weeks of treatment. Group -B proved better improvement than Group A. Hence Null hypothesis is rejected.

Conclusion: The present study concluded that 4-weeks treatment program using LASER and NMES in subjects with osteoarthritis of the knee joint showed that both the interventions were effective in reducing pain intensity and improves the functional activities.

Key Words: Osteoarthritis, LASER, NMES, WOMAC

INTRODUCTION

Osteoarthritis is a musculoskeletal condition characterized by the loss of cartilage (1). It is a chronic degenerative disorder of multifactorial etiology associated with the loss of articular cartilage, hypertrophy of the bone at the margins, subchondral sclerosis, and range of biochemical and morphological alteration of the joint capsule and the synovial membrane (2). Osteoarthritis is the 2nd most common Rheumatologic problem. In India, osteoarthritis is the most frequent joint disease with the prevalence of 22% to 39% (3). Osteoarthritis is more common in women than men but the prevalence of the OA increases dramatically with age (4,5).

The cartilage provides impact resistance, low friction and bearing that contributes to efficient pain-free stability in the posture and locomotion (6). The cartilage like any other material or tissue is damaged by the high pressure or repetitive loading.

Knee OA is associated with the symptoms of pain, instability, stiffness, swelling and decreased the range of motion (8). These symptoms result in functional impairment, increasing the risk of morbidity and mortality (9). The wear and tear of cartilage can be prevented by lowering the stress across the joint so as to allow the functional healing of the bone and articular surface to take place (10). Stress in the joint can be diminished by reducing the overall load on
the joint and by increasing the surface over which the load acts.

The LASER therapy has promoted for effects on tissue healing and for the pain relief. It refers to the production of a beam of radiation which differs from the ordinary light. The laser exerts a positive influence on fibroblast and osteoblast proliferation, collagen synthesis, bone metabolism and cartilage damage. The laser has an effect on prostaglandin synthesis and thus it reduces inflammation (12). It is found to be effective in the healing of connective tissues in the knee OA. Laser therapy has bactericidal effects because of increased phagocytosis by the leukocytes (13).

Neuromuscular Electrical Simulation is defined as the application of electrical current to the neuromuscular junction and the surrounding muscle fibers to produce a visible muscle contraction due to the activation of intramuscular nerve branches (14). It is used for the preservation of muscle mass and the function during prolonged periods of disuse or immobilization, improvement of the muscle function in different healthy populations, the recovery of muscle mass and preoperative strengthening (15). It is an effective therapy for quadriceps strengthening in individuals with knee OA. Due to the Non-Selective recruitment of motor units, muscle fibers type 1 & 2 are simultaneously recruited through the NMES, even at the relatively low intensities of stimulation, potentially producing the structure and functional changes in the neuromuscular system (16). The Conventional Exercise helps in decreasing the pain, improving range of motion, strength, and endurance (17). The connective tissue elasticity, as well as exercise, decreases the functional limitation by improving speed, walking, physical activity and gait (18). The exercise decreases the depression and anxiety.

**MATERIALS AND METHODS**

30 patients who reported to outpatient Physiotherapy department ACS Medical College and Hospital were randomly grouped into Group-A, Group-B based on Inclusion and Exclusion criteria. A detailed demography data, past medical history were collected from the patient. After detailed explanation about the treatment procedure, an informed consent was obtained. The outcome measures, VAS, WOMAC was recorded before and after the treatment as pre and post test score. Group-A: Consists of 15 subjects and they received LASER and Conventional Exercise. Group-B: Consists of 15 subjects were treated with LASER, Neuromuscular Electrical Stimulation, and Conventional Exercise which consists of Bicycle ergometer, stretching of hamstrings muscles with the aid of an Thera band, and knee Isometric exercises, straight leg raise, knee extension, hip abduction.

**Data Analysis**

The collected data were tabulated and analyzed using both descriptive and inferential statistics. All the parameters were assessed using statistical package for social science (SPSS) version 24. Paired t-test was adopted to find statistical difference within the groups & Independent t-test (Student t-Test) was adopted to find statistical difference between the groups.

**Table 1: Comparison of VAS Score Between Group – A and Group - B an Pre and Post Test**

<table>
<thead>
<tr>
<th>VAS</th>
<th>GROUP - A</th>
<th>GROUP - B</th>
<th>t-TEST</th>
<th>SIGNIFICANCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRE TEST</td>
<td>5.80</td>
<td>6.06</td>
<td>-.928</td>
<td>.361*</td>
</tr>
<tr>
<td>POST TEST</td>
<td>3.73</td>
<td>1.46</td>
<td>11.15</td>
<td>.000***</td>
</tr>
</tbody>
</table>

(* - P > 0.05) (** - P ≤ 0.001)

The above table reveals the Mean, Standard Deviation (S.D), Student t-test, and p-value of the VAS score between (Group A) & (Group B) in pre test and post test weeks. This table shows that there is no significant difference in pre test values of the VAS score between Group A (5.80) & Group B (6.06) (*P > 0.05). Group-B (1.46) which has the Lower Mean value is more effective than Group-A (3.73)

**Graph I: Comparison of VAS Between Group – A and Group - B in Pre & Post Test**
The above table reveals the Mean, Standard Deviation (S.D), Student t-test, and p-value of the WOMAC score between (Group A) & (Group B) in pre test and post test weeks. This table shows that there is no significant difference in pre test values of the WOMAC score between Group A (39.33) & Group B (40.06) (*P > 0.05). Both the Groups shows highly significant decrease in the post test Means but Group-B (16.13) which has the lower mean value is more effective than Group-A (21.26)

Graph II: Comparison of WOMAC Score between Group – A and Group - B In Pre & Post Test

<table>
<thead>
<tr>
<th>WOMAC</th>
<th>GROUP - A</th>
<th>GROUP - B</th>
<th>t- TEST</th>
<th>SIGNIFICANCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRE TEST</td>
<td>39.33</td>
<td>40.06</td>
<td>-1.28</td>
<td>.209*</td>
</tr>
<tr>
<td>POST TEST</td>
<td>21.26</td>
<td>16.13</td>
<td>9.71</td>
<td>.000***</td>
</tr>
</tbody>
</table>

(*- P > 0.05)(**- P ≤ 0.001)

The above table reveals the Mean, Standard Deviation (S.D), Student t-test, and p-value of the WOMAC score between (Group A) & (Group B) in pre test and post test weeks. This table shows that there is no significant difference in pre test values of the WOMAC score between Group A (39.33) & Group B (40.06) (*P > 0.05). Both the Groups shows highly significant decrease in the post test Means but Group-B (16.13) which has the lower mean value is more effective than Group-A (21.26)

Graph III: Comparison of VAS and WOMAC Score within Group – A Between Pre & Post Test Values

<table>
<thead>
<tr>
<th>VAS</th>
<th>PRE TEST</th>
<th>POST TEST</th>
<th>t- TEST</th>
<th>SIGNIFICANCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRE TEST</td>
<td>5.80</td>
<td>3.73</td>
<td>11.37</td>
<td>.000***</td>
</tr>
<tr>
<td>WOMAC</td>
<td>40.06</td>
<td>16.13</td>
<td>50.62</td>
<td>.000***</td>
</tr>
</tbody>
</table>

(***- P ≤ 0.001)

The above table reveals the Mean, Standard Deviation (S.D), t-value and p-value of the VAS & WOMAC score between pre-test and post-test within Group – B. In the VAS score, there is a statistically highly significant difference between the pre test (6.06) and post test values (1.46) (***- P ≤ 0.001). In the WOMAC score, there is statistically highly significant difference between the pre test (40.06) and post test values (16.13) (***- P ≤ 0.001). (Graph-IV)

Graph IV: Comparison of VAS and WOMAC Score within Group – B Between Pre & Post Test Values

<table>
<thead>
<tr>
<th>VAS</th>
<th>PRE TEST</th>
<th>POST TEST</th>
<th>t- TEST</th>
<th>SIGNIFICANCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRE TEST</td>
<td>6.06</td>
<td>1.46</td>
<td>28.16</td>
<td>.000***</td>
</tr>
<tr>
<td>WOMAC</td>
<td>40.06</td>
<td>16.13</td>
<td>50.62</td>
<td>.000***</td>
</tr>
</tbody>
</table>

(***- P ≤ 0.001)

The above table reveals the Mean, Standard Deviation (S.D), t-value and p-value of the VAS & WOMAC score between pre-test and post-test within Group – B. In the VAS score, there is a statistically highly significant difference between the pre test (6.06) and post test values (1.46) (***- P ≤ 0.001). In the WOMAC score, there is statistically highly significant difference between the pre test (40.06) and post test values (16.13) (***- P ≤ 0.001). (Graph-IV)

RESULTS

A Total number of 30 patients with osteoarthritis of the knee joint were taken within the age of 40- 60 years both males and females were included in this study.
Group A - was given LASER with Conventional Exercise and Group B – was given NMES, with Conventional Exercise for 4 weeks. Pre and post test were taken and compared from VAS and WOMAC and after 4 weeks of treatment Group - B proved better improvement than Group A. Hence Null hypothesis is rejected.

DISCUSSION

This study evaluated the effects to compare the effectiveness of LASER and Conventional Exercise versus Neuromuscular Electrical Stimulation and Conventional Exercise in the treatment of subjects with Osteoarthritis of the Knee Joint.

The result of this study showed strong evidence that Neuromuscular Electrical Stimulation with Conventional Exercise, in reducing pain. The result of the study was statistically significant as the descriptive data’s such as mean and standard deviation which indicated that improvement in terms of pain and Functional Activity at the end of the treatment session in both the Groups. Comparing the result obtained from the two Groups, the result of the study showed that Neuromuscular Electrical Stimulation with Conventional Exercise showed significant improvement than LASER and Conventional Exercise. This result supported by Mônica de Oliveira Melo et al. (2016) who concluded that LASER, Neuromuscular Electrical Stimulation with Conventional Exercise are helpful for treating a patient with Osteoarthritis knee Pain, as well as Functional Activity. The pre-test mean value of Visual Analogue Scale (VAS) between Group-A (5.80) and Group-B (6.06) does not show a significant difference. However, the post-test mean value showed a significant difference between Group-A (3.73) and Group-B (1.46). The pre-test mean value of Western Ontario McMaster Osteoarthritis Index (WOMAC) between Group-A (39.33) and Group-B (40.06) does not show a significant difference. At the end of the treatment session, the post-test mean value of WOMAC between Group-A (21.26) and Group-B (16.13) showed a significant difference.

CONCLUSION

The present study concluded that 4-weeks treatment program using LASER and NMES in subjects with Osteoarthritis of the knee Joint showed that both the interventions were effective in reducing pain intensity and improves the functional activities. This study suggested that NMES with Conventional Exercise showed statistically high significant improvement than LASER and Conventional Exercise. The study reveals there is a significant difference in Group-A and Group-B in the treatment of subjects with osteoarthritis of the knee joint.

Ethical Consideration: This study was conducted after obtaining the approval from the Institutional Review Board of faculty of Physiotherapy.

Conflict of Interest: None

Fund: Self funded project

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REFERENCES


To Analyse the Effectiveness of Yoga, Pilates and Tai Chi Exercise for Chronic Mechanical Neck Pain -A Randomized Controlled Trial

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ABSTRACT

Introduction and Aim: Chronic mechanical neck pain is a common medical complaint with a high socio-economic impact. Approximately 70% of individuals at some point in their lives experience chronic mechanical neck pain. Life-time prevalence of chronic mechanical neck pain is approximately 50% and is associated with substantial societal and individual burden. Recent studies estimated its print of prevalence to be between 6 and 22% which increases with age, although symptoms of neck pain are common among the population with the age group of 35-55 years. Conservative treatment such as exercise, massage, stretching, physical therapy, local anaesthetic filtration has been found beneficial for chronic mechanical neck pain. Alternative medicinal approach such as Yoga, Pilates, Tai chi have found efficacious for chronic mechanical neck pain. The aim of the study is to analyse and compare the effectiveness of Yoga, Pilates and Tai chi exercise for chronic mechanical neck pain.

Materials and Methods: Chronic mechanical neck pain is a common medical complaint with a high socio-economic impact. Approximately 70% of individuals at some point in their lives experience chronic mechanical neck pain. 40 Samples from 60 volunteers were selected based on the inclusion criteria Painful restriction of cervical spine, Neck pain, Age between 35-55 years, more than 40% of Tampa scale for kinesiophobia and Northwick pain park questionnaire. The study excluded those with Whiplash injury, frozen shoulder syndrome, Prolapse or protrusion, invasive treatment (surgery, nerve blocks, and neurotomy) within last 6 weeks, Spinal stenosis, Herniated vertebral disc. 40 samples were equally divided into 4 groups 10 in each. Group A received yoga, B –Tai Chi, C- Pilates, D-Control group. Participant will be given a consent form, Northwick pain park questionnaire and Tampa scale for kinesiophobia for final screening of inclusion and also used as pre–test. Post–test done after 3 weeks of intervention.

Results: The analysis was randomized experimental study for the subjects with chronic mechanical neck pain. Statistical analysis was done using SPSS software, All the four Groups showed improvement comparing Pre and Post treatment mean value, while Group A Yoga post-test NPPQ (24.60) and TSK (55.20) showed significant difference compared to Group B Pilates post-test NPPQ (29.20) and TSK (58.10)and Group C Tai chi post-test NPPQ (48.80) and TSK (60.80) and Group D Control Group post-test NPPQ (56.70) and TSK (63.70) in reduction of pain by statistically comparing the mean values of Northwick pain park questionnaire and Tampa scale for kinesiophobia of four treatment groups indicated that Yoga as more efficient than the Pilates, Tai chi and Control group.

Conclusion: The study concluded that the Yoga is more effective than the Pilates and Tai chi and Control Group exercise for chronic mechanical neck pain while Pilates, Tai chi and control Group exercise even showed a considerable decrease in symptoms when comparing the post-test mean values. Yoga is effective in reducing pain, disability, quality of life and fear of movements in subjects with mechanical neck pain. Yoga proved to be equally efficacious and safe, it may be considered a suitable alternative in the treatment for subjects with neck pain.

Key Words: Yoga, Pilates, Tai Chi, Control group, Chronic Mechanical Neck pain, Northwick pain park questionnaire, Tampa scale for kinesiophobia.

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INTRODUCTION

Mechanical neck pain is defined as the pain in the anatomical region of neck for which it is not possible to identify a specific pathological cause of pain. It generally includes neck pain, with or without pain in the upper limb which may or may not interfere with activities of daily living. Chronic mechanical neck pain in the spine is a musculoskeletal disorder in modern society with public health and economic impact. It is also defined as the pain caused by placing abnormal stress or strain on the muscles of vertebral column.

Research shows that spinal pain has become the largest category of medical claim, placing a major burden on the individuals and health care system. A recent study estimates its point prevalence to be between 6 and 22% which increases with age 12 months prevalence is estimated to be between 30 and 50%(1). About 19% of population may suffer from chronic mechanical neck pain at any given time, creating substantial societal burden. Chronic mechanical neck pain is more common in females than the males (2).

Significant amount of money spend in today health care environment is used for the treatment of chronic health conditions. This includes neck pain and other related diseases. These lifestyle related conditions are usually the result of stubborn behaviour which immensely contribute to morbidity and mortality of population such as lack of physical activity, over eating, improper diet, cigarette smoking, excessive alcohol consumption, socio economic stress, low social support, poor sleep etc.

Neck pain results from bad habits such as poor posture, poorly designed seating and incorrect bending and lifting motions. These habits can be corrected by maintaining proper posture, adapting proper lifting and bending techniques.

For mechanical neck disorder with or without headache, it appears to be most beneficial, manual therapies should be done with exercise for improving pain and patient satisfaction. It can also be corrected by inhibiting exercises. The exercises which show effective in chronic mechanical neck pain include Yoga, Pilates and Tai chi exercises (3).

Yoga, Pilates and Tai chi are different types of exercises they all have something in common. They can help to alleviate pain and improve quality of life. However there is difference between the forms.

Yoga considered a branch of classical philosophy in India, uses meditation, exercise and breathing practices to improve overall health (4-7).

Pilates focuses on the core postural muscles which help keeps the body balanced and are essential to providing support for the spine. These group of exercise helps to treat the chronic condition including neck pain (8,9).

Tai chi, a Chinese soft martial art form, uses gentle, slow movements and posture to keep the body in constant motion while the person meditates. Tai chi may contribute to the psychological well-being among healthy adults (10).

A few specific outcome measurement tools that are available for assessing neck pain includes Northwick park pain questionnaire (11) and Tampa scale for kinesiophobia. Specific outcome measurement tool for neck pain that reflects the local cultural practice. It is important that the outcome measurement tool must demonstrate reliability (consistency), validity (trueness) and responsiveness (the ability to detect change).

MATERIALS AND METHODS

The design of this study was experimental study. This study was done in Outpatient department of Physiotherapy at A.C.S. Medical College and Hospital. The period of this study was twice in a day for 3 weeks. The study group included 40 patients; patients were selected using simple random sampling method. The study includes only 40 patients of both males and females with the age group between 35 and 55 years, painful restriction of cervical spine, neck pain, more than 40% of Tampa scale for kinesiophobia and Northwick pain park questionnaire. Patients with Whiplash injury, frozen shoulder syndrome prolapsed or protrusion, invasive treatment within last 6 weeks, spinal stenosis, and herniated vertebral disc were excluded in this study. Northwick pain park questionnaire and Tampa scale for kinesiophobia were used as a outcome measure.

Once the study is approved by institution review board. 60 volunteers were recruited from the out-patient physiotherapy department with chronic mechanical neck pain. Out of which 40 samples are selected based on the inclusion criteria, they were fully explained about the study and asked to fill consent form, Northwick pain park questionnaire and Tampa scale for kinesiophobia for final screening of participating in the study.
In this study, the patients were allocated into 4 groups. Group A received yoga, Group B received Pilates, Group C received tai chi exercise and Group D received Control group exercise. All the exercises were given for 5 repetitions, 2 session/day for 6 days/week for 3 weeks, progressed to 10 and 15 repetition in 2nd and 3rd week respectively.


**Group C:** Tai Chi (1. Head Roll, 2. Carrying Moon, 3. Picking Fruit, 4. Dancing With Rainbow, 5. Spinning Wheel)

**Group D:** In this Group isometric neck exercise was given. All other 3 Groups also receive isometric neck exercise as a common intervention.

**Data Analysis**

The collected data were tabulated and analyzed using both descriptive and inferential statistics. All the parameters were assessed using statistical package for social science (SPSS) version 24. One Way ANOVA includes of following tests (Test of Homogeneity of Variance, ANOVA, Robust Equality of Means, Post Hoc test Tukey HSD) (multiple comparison) was adopted to find statistical difference between four groups.

**Table 1:** Comparison of pre npq score using test of homogeneity of variance and one ANOVA test between Group A, Group B, Group C and Group D

<table>
<thead>
<tr>
<th>TEST</th>
<th>GROUPS</th>
<th>MEAN</th>
<th>S.D</th>
<th>df1</th>
<th>df2</th>
<th>F-VALUE</th>
<th>SIGNIFICANCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRE NPQ</td>
<td>GROUP-A</td>
<td>57.3</td>
<td>8.32</td>
<td>3</td>
<td>36</td>
<td>0.29</td>
<td>.993*</td>
</tr>
<tr>
<td></td>
<td>GROUP-B</td>
<td>56.4</td>
<td>13.36</td>
<td>3</td>
<td>36</td>
<td>0.29</td>
<td>.993*</td>
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<tr>
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<td>GROUP-C</td>
<td>56.8</td>
<td>6.87</td>
<td>3</td>
<td>36</td>
<td>0.29</td>
<td>.993*</td>
</tr>
<tr>
<td></td>
<td>GROUP-D</td>
<td>57.6</td>
<td>9.86</td>
<td>3</td>
<td>36</td>
<td>0.29</td>
<td>.993*</td>
</tr>
</tbody>
</table>

**Table 2:** Comparison of post npq score using test of homogeneity of variance and one ANOVA test between Group A, Group B, Group C and Group D

<table>
<thead>
<tr>
<th>TEST</th>
<th>GROUPS</th>
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<th>S.D</th>
<th>df1</th>
<th>df2</th>
<th>F-VALUE</th>
<th>SIGNIFICANCE</th>
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</thead>
<tbody>
<tr>
<td>POST NPQ</td>
<td>GROUP-A</td>
<td>24.60</td>
<td>4.35</td>
<td>3</td>
<td>36</td>
<td>73.59</td>
<td>.000***</td>
</tr>
<tr>
<td></td>
<td>GROUP-B</td>
<td>29.20</td>
<td>5.53</td>
<td>3</td>
<td>36</td>
<td>73.59</td>
<td>.000***</td>
</tr>
<tr>
<td></td>
<td>GROUP-C</td>
<td>48.80</td>
<td>7.03</td>
<td>3</td>
<td>36</td>
<td>73.59</td>
<td>.000***</td>
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<td></td>
<td>GROUP-D</td>
<td>56.70</td>
<td>5.43</td>
<td>3</td>
<td>36</td>
<td>73.59</td>
<td>.000***</td>
</tr>
</tbody>
</table>
RESULTS

The analysis was randomized experimental study for the subjects with chronic mechanical neck pain. Statistical analysis was done using SPSS software. All the four Groups showed improvement comparing Pre and Post treatment mean value, while Group A Yoga post-test NPPQ (24.60) and TSK (55.20) showed significant difference compared to Group B Pilates post-test NPPQ (29.20) and TSK (55.20). The mean value of Group B Pre test NPPQ (56.4) and the post test NPPQ (29.20). Group C pre test mean NPPQ (56.8) and post test NPPQ (48.80) and Group D pre test NPPQ (57.6) and post test NPPQ (56.70) from the data analysis: it shows that there was significant decrease in pain (NPPQ) within Group A, Group B and Group C and Group D at (p ≤0.001)

When the intra group mean value of Northwick pain park questionnaire was analyzed .Group A pre test mean NPPQ (57.3) and post test NPPQ (24.60). The mean value of Group B Pre test NPPQ (56.4) and the post test NPPQ (29.20). Group C pre test mean NPPQ (56.8) and post test NPPQ (48.80) and Group D pre test NPPQ (57.6) and post test NPPQ (56.70) from the data analysis: it shows that there was significant decrease in pain (NPPQ) within Group A, Group B and Group C and Group D at (p ≤0.001)

When the intra Group mean value of Tampa scale for Kinesiophobia was analyzed .Group A pre test mean TSK (65.9) and post test TSK (55.2). The mean value of Group B Pre test TSK (68.6) and the post test TSK (58.10). Group C pre test mean TSK (67.2) and post test TSK (60.80) and Group D pre test TSK (67.0) and post test TSK (63.70) from the data analysis: it shows that there was significant decrease in pain (TSK) within Group A, Group B, Group C, and Group D at (p ≤0.001)

When Standard deviation values of NPQ was analyzed. Group A post standard deviation NPPQ (4.35), Group B post standard deviation NPPQ (5.53) Group C post standard deviation NPPQ (7.03) Group D post standard deviation NPPQ (5.43) clearly indicated that there was equal reduction in pain intensity (NPQ) at P>0.05.

When Standard deviation values of TSK was analyzed. Group A post standard deviation TSK (2.29) Group B post standard deviation TSK (3.29) Group C post standard deviation TSK (3.17) Group D post standard deviation TSK (4.31) Group D post standard deviation TSK (3.80) clearly indicated that there was equal reduction in pain intensity (TSK) at P>0.05 shows statistically significant improvements.

DISCUSSION

The present study was done to compare the effectiveness of Yoga, Pilates, Tai chi exercise along with control group in patients with chronic mechanical neck pain.

Bussing A, ostermann et al, 2012 - effect of Yoga intervention on pain and pain associated disability ‘had concluded that Yoga is useful for several pain associated disorders.

Michalsen - 2012 - ‘Yoga for chronic neck pain ‘had concluded that yoga appears to be an effective treatment in chronic neck pain with additional effects on..
psychological well being and quality of life.

Sang - Dol Kim - 2016 -Effects of Yoga on chronic neck pain – A systemic review of RCT had discussed about benefits of yoga in chronic neck pain and it was found to provide a safe, effective therapy for chronic neck pain relief.

CONCLUSION

The study concluded that the Yoga is more effective than the Pilates and Tai chi and Control Group exercise for chronic mechanical neck pain while Pilates. Tai chi and control Group exercise even showed a considerable decrease in symptoms when comparing the post test mean values.Yoga is effective in reducing pain, disability, quality of life and fear of movements in subjects with mechanical neck pain. Yoga proved to be equally efficacious and safe, it may be considered a suitable alternative in the treatment for subjects with neck pain.

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