The journal
Biomedicine (Print ISSN: 0970 2067), official publication of Indian Association of Biomedical Scientists (IABMS), published quarterly in March, June, September and December. It is an International Journal for Biomedical Sciences. The journal publishes research papers, reviews, special article, case report, book review and letter to the editor related to Anatomy, Physiology, Biochemistry, Microbiology, Toxicology, Endocrinology, Reproductive Biology, Pharmacology and Alternative Systems of Medicines like Siddha, Unani, Ayurveda, Homeopathy and Naturopathy.

Abstracting and indexing information
It is indexed in Excerpta Medica, Scopus, Elsevier Indian Citation Index and Ind.Med.

Information for authors
Minimal printing cost for various categories of accepted manuscripts and color images are charged by Biomedicine. For details see instruction to the authors.
All manuscripts must be submitted by email to biomedicinepreview@gmail.com

Subscription information
A subscription to Biomedicine comprises 4 issues. Prices include postage. Annual subscription for non-members

- Institutional : INR Rs. 4,500 for India [Vol. 35 (1-4) 2015 issues]
  : USD 250 for outside India [Vol. 35 (1-4) 2015 issues]
  : INR Rs. 5,000 for India [Vol. 36 (1-4) 2016 issues]
  : USD 280 for outside India [Vol. 36 (1-4) 2016 issues]
- Personal : INR Rs. 2,800 for India [Vol. 35 (1-4) 2015 issues]
  : USD 160 for outside India [Vol. 35 (1-4) 2015 issues]
  : INR Rs. 3,000 for India [Vol. 36 (1-4) 2016 issues]
  : USD 200 for outside India [Vol. 36 (1-4) 2016 issues]

Mode of payment : Nationalized Bank DD drawn in favor of The Editor-in-chief, Biomedicine, payable at Chennai.
Claims for missing issues will be serviced at no charge if received within 60 days of the cover date for domestic subscribers and 90 days for subscribers outside India. Duplicate copies cannot be sent to replace issues not delivered because of failure to notify change of address to the General Secretary, IABMS or The Editor-in-chief, Biomedicine.
Non members: All change of address information to be sent to biomedicinepreview@gmail.com

Copy right
No part of this journal should be reproduced without getting written permission from the Editor-in-chief.

Disclaimers
The information and opinions presented in the journal reflect the views of the authors and not of the journal or its editorial board or the publisher. Publication does not constitute endorsement by the journal. Neither the journal nor anyone else involved in creating, producing or delivering Biomedicine or the materials contained therein, assumes any liability or responsibility for the accuracy, completeness, or usefulness of any information provided in Biomedicine, nor shall they be liable for any direct, indirect, incidental, special, consequential or punitive damages arising out of the use of Biomedicine. Biomedicine or any other party involved in the preparation of the material contained in Biomedicine represents or warrants that the information contained herein is in every respect accurate or complete and they are not responsible for any errors or omissions or for the results obtained from the use of such material. Readers are encouraged to confirm the information contained herein with other sources.

Address
Dr. E.Padmini, Editor-in-chief, Biomedicine
Associate Professor, Department of Biochemistry, Bharathi Women’s College, Broadway, Chennai, Tamil Nadu, India. Email: biomedicinepreview@gmail.com
Publication
Biomedicine is a quarterly publication of IAMBS. It is indexed in Excerpta Medica, Scopus, Elsevier Indian Citation Index and Ind.Med.

Editorial Office
Dr.E.Padmini, Associate Professor, Department of Biochemistry, Bharathi Women’s College, Chennai-600108
Phone: Board – 9952094777, 9884207570

Editorial Board
Dr. S. Karthikeyan, Dr. ALM PG IBMS, Chennai
Dr. G.K. Pal, JIPMER, Puducherry
Dr. G. Rajagopal, Chennai
Dr. Ajay Kumar Singh, DRDO, New Delhi
Dr. M. A. Hussain, Chennai
Dr. S. Venkataraman, Chennai
Dr. Manjula Shantaram, Mangalore
Dr. S. Jayaram, Puducherry
Dr. V. Madhavachandran, Thiruvananthapuram
Dr. Sachin B Ingle, Latur, Maharashtra
Dr. R. Sheela Devi, Dr.ALM PG IBMS, Chennai
Dr. Victor Rajamanikam, Chennai
Dr. J. Shanmugam, Puducherry
Dr. D. Sakthi Sekaran, Dr. ALM PG IBMS, Chennai
Dr. S.K. Trigun, Banaras Hindu University, Varanasi
Dr. N. Suchetha Kumari, K.S.H.M.A, Karnataka
Dr. Devarajan Thangadurai, Karnataka University
Dr. Subir Kumar Das, COMJNNH, WBUHS, Kalyani
Dr. D.N. Rao, New Delhi

International Advisory Council
Dr.G. Parameswarai, Principal, Bharathi Women’s College, Chennai
Dr. W. Selvamurthy, President, Amity University, Noida
Dr. S. P. Thyagarajan, SRMC, Chennai
Dr. S. Prasanta Gupta, National Medical College, Birgunj, Nepal
Dr. M. Ramachandran, Atlanta, United States of America.
Dr. Sashi Bala Singh, Ministry of Defense, New Delhi.
Dr. V. Anantharaman, Chennai
Dr. S. Ramakrishnan, Chennai.
Dr. Sanguansak Rerksuppaphol, Srinakharinwirot University, Nakhora Nayok, Thailand
Dr. N. Murugesan, Director, CDTL, Chennai
Dr. P. Mohan Kumar, Secretariat, NTRF, Kolkata
Dr. Sheila Leonard, CSI Rainy Multispeciality Hospital, Chennai
Dr. Vijay Kumar Kutala, NIMS, Hyderabad
Dr. Santosh Kumar Sandur, BARC, Mumbai

Editor- in-Chief
Dr.E.Padmini
Associate Professor, Department of biochemistry, Bharathi Women’s College,
Broadway, Chennai, Tamil Nadu, India.

Editors
Dr.T.Thirunala sundari,
Prof & Head, Department of Industrial Biotechnology
Bharathidasan University, Tiruchirappalli-620 024

Dr. D.C. Mathangi,
Professor, Department of Physiology
Chettinad Health City
Kelambakkam, Kancheepuram District-603 103

Language Editor
Dr. V. Jayalakshmi, Chennai

Pagination
G. Suman, Chennai

Disclaimer
The journal is not responsible for any statements made by authors.

Copy Right
No part of this journal should be reproduced without written permission from the Editor-in-Chief.

Communication Address
All correspondence should be addressed to:
Dr. E.Padmini, Editor-in-Chief, Biomedicine.

www.biomedicineonline.org
**BIOMEDICINE**  
Vol. 36 No. 1: (January - March) 2016

## Contents

### Topics and Authors Page(s)

| I | Editorial message | 001 |
| II | Editorial | 002 |
| III | Former President of IABMS | 004 |

### IV Review/Special Articles

1. Chemical and Electronic Sensors used as a Diagnosing Tool for Diseases – A Review  
   Bijithra C, Rajan G and Shanmugasundaram P  
   Page(s): 005

### V Research Papers

2. In-vitro antioxidant and anti-inflammatory activity of ethanolic extracts of Momordica Charantia  
   Shobha CR, Prashant V, Parveen D, Akila P, Suma MN, and Basavanagowdappa H  
   Page(s): 011

3. Inflammatory and oxidative stress markers in Acute myocardial infarction  
   Ashok Prabhu, Sudha K, Kiran Kumar AM, and Rajib Kumar Pandey  
   Page(s): 021

4. A study of CVD risk assessment in post-menopausal women in comparison with pre-menopausal women with serum lipid profile and hs CRP as risk markers.  
   Yasmeen Fatima, Sreekantha, Ramesh, Sadananjali, Saba, and Pallavi  
   Page(s): 026

5. Assessment of serum leptin levels and lipid profile in hypertensive obese cases  
   Keshavamurthy HR, and Sunitha S  
   Page(s): 033

6. Urea reduction rate as dialysis adequacy indicator and serum albumin as mortality indicator in hemodialysis patients.  
   Shanthala D, Indumati V, Krishnaswamy D, Vijay V, Rajeshwari V, Ramesh, and Shilpa A  
   Page(s): 039

7. Study of Oxidative stress and Apolipoprotein A-I in reduction of Reverse Cholesterol Transport in type 2 Diabetes Mellitus  
   Suman D, Shashikant N, Padmina N, and Vishwanath P  
   Page(s): 044

8. Evaluation of serum high molecular weight adiponectin and lipid profiles in predicting the risk of coronary artery disease among cad patients  
   Chitra devi M, Chandra Sekhar M, Sivasubramaniam P  
   Page(s): 049

9. Relationship of BMI and Dental Caries among children in Chennai  
   Visha MG, and Deepa Gurunathan  
   Page(s): 057

10. A study on Prevalence of Hypertension in cardiovascular risk factors among adults in Chittoor district population  
    Bhavani Yamasani, Khadervali Nagoor, and Raziya Dudekula  
    Page(s): 062

11. Lipid peroxidation and antioxidant status in vitiligo patients  
    Pallavi Mishra, Rajkumari Rathore, and Prashant Hisalkar  
    Page(s): 072
12. Effects of threshold inspiratory muscle trainer in bronchial asthma .......................... 077
Shiny S James, Rekha K, Anandh V, Chandrasekar L, and Unnikrishnan R

13. Assessment of serum uric acid in type 2 diabetes mellitus in a middle-aged south Indian population ................................................................. 083
Jyoti John, RajLaxmi Sarangi, Asha Dinakaran, Umadevi SV, Somanath Padhi, and Nitin Ashok John

Sumana M, and Siddhartha D

15. Comparison in between subjects of determinants of oxygen uptake during maximal and submaximal exercise – ventilation and O₂ pulse, determinants of O₂ uptake are less dependent on load in submaximal exercise at ventilator threshold .......................... 098
Thiagarajan KA, Vasanthi C, Parikh T, Madhusudhan Rao V, Arumugam S

16. Is autonomic function test helps to assess the severity of metabolic syndrome: A study on comparison of Frequency-Domain recordings of Heart rate variability (HRV) with the severity of metabolic syndrome ....................................................... 103

17. Antioxidant activity of Phytoformulation 1, a polyherbal formulation, on hyperlipidemicWistar rats ................................................................. 109
Vanaja R, and Mercy Jasmine J

18. Comparative effects of different teaching methods in pharmacology in second MBBS medical students ................................................................. 117
Baswaraj Munge, Kodandaramu Burli, Sindhura Nagisetty, Mamata Bandhopadhyay, and Prasad Naidu

19. Effect of electromagnetic radiation exposure on hematological parameters of swiss albino mice and their modulation by high protein diet ........................................... 121
Debajyoti Bhattacharya, Niladri Ghosh, and Mausumi Sikdar (nee) Bhakta

20. Ethanol enhances lamivudine-induced liver toxicity: Investigation on hepatoprotective properties of silibinin-phosphatidylcholine complex in rats ............................ 128
Balasubramanian Jesudas, Ramanathan Raghu, Ganapathy Bhavani, Devaraj Ezhilarasan, and Sivanesan Karthikeyan

21. Is Internet use Related to Academic Performance in Medical Students? A study from South Indian Medical College ....................................................... 138
Ravi Kishore Polepalli

VI Case Report

22. Raising the index of suspicion for cerebral venous thrombosis: A case report ............ 141
Renata Mazurek, Naveen Ramesh, Kiran PV, and Avita Johnson

23. Oral Squamous papilloma of hard palate ................................................................. 145
Jamin Joseph, and Karpagavalli S
EDITORIAL MESSAGE

Dear Reader,

As the New Editor-in-Chief of the journal Biomedicine, I am writing to invite you to send your most important research papers to the journal. The Journal will not only accommodate traditional, theoretical and experimental biomedical research, but also welcomes research that builds molecular discoveries and medical research that connect to clinical states within the journal’s domain.

The qualitative and timely publication of Volume 36 of our esteemed international journals has brought great joy and happiness to the entire fraternity of the journal and honorable members of the editorial and advisory board. The rich experience and varied expertise of the board members is providing immense succor in propelling the journal to meet a comfortable place in areas of medical research and accentuate its visibility. The aim of the journal is to percolate knowledge in biomedical research with erudition by providing our ecosystem for budding researchers in India.

Large number of research papers have been handed over to me in February this year. They were reviewed and the accepted papers are published in this issue. I extend my heartfelt thanks to the reviewers and members of the editorial board who so carefully perused the papers and carried out justified evaluations in very short time. Based on their evaluation, we could accept 23 research papers. I am sure that these papers will offer qualitative information and thoughtful ideas to our accomplished readers. I also thank all those who profusely conveyed their appreciation and best wishes for future issues. I convey my deep gratitude to the office bearers and members of IABMS who gave me this opportunity to serve as Editor in Chief. The publication of this journal in the planned time frame was possible due to untiring help and support by our previous Editor-in-Chief.

I humbly invite all the authors and their professional colleagues to send their research papers for consideration for publication in our forthcoming issue as per the “Scope and Guidelines to Authors” given in this issue. Immense effort was put by our team to format the research papers right from abstract to reference. Additionally plagiarism and grammar check was performed. I am sure that with organized team efforts, the journal will soon prove its name among best quality national and international journals in biological sciences. I solicit your continuous support, help, guidance and blessing to take Biomedicine to new heights, particularly, in-terms of visibility in Google Scholar in increasing its impact factor.

Dr. E. Padmini
Editor-in-Chief
Biomedicine
An International Journal for Biomedical Scientists
A quarterly journal of IABMS, Chennai, India.

The highest honor bestowed upon me was to interact with Bharat Ratna Dr.A.P.J.Abdul Kalam during Golden Jubilee celebrations at Bharathi Women’s College in April 2015. I pray and seek his blessings for successful completion of my turn as EIC.

www.biomedicineonline.org
EDITORIAL

Xenohormesis and Calorie Restriction

It has long been valued that diets rich in fruits and vegetables promote health, reduce the risk of life killing disease and correlate with increased longevity. This has typically been attributed to the antioxidant properties of plant-derived foods. At the molecular level, many phytochemicals exert their effects in various ways. One is by directly interacting with and modulating specific enzymes or receptors. Polyphenols such as resveratrol (found in grapes) and quercetin (in tea for example), which are produced by stressed plants, activate sirtuin enzymes and extend the lifespan of fungi and animals, apparently by mimicking the beneficial effects of caloric restriction. Caloric restriction (CR) is currently the only way to slow down aging in mammals. It has been well documented that caloric restrictions (CR) delay most diseases of aging and stress tolerance including cancer, metabolism, atherosclerosis, Type 2 diabetes and even neurodegeneration.

As a result, an intense interest was raised to explore how CR works at the molecular level to find small molecules that can mimic its effects. The fact that these foreign molecules that are non-nutritive and seemingly unrelated to any endogenous molecule alter the same biochemical pathways that mediate the response to an energy deficit is an interesting aspect. A possible explanation provided is that the Sirtuin enzymes have evolved to respond to plant stress molecules as indicators of an approaching deterioration of the environment. The Sirtuin family appears to be first arising in module eukaryotes, possibly to help them cope with adverse conditions. Sirtuin genes are unique in their activity, and their level is positively correlated with lifespan. Sirtuins are a class of enzymes that could have beneficial effects on a variety of human diseases. Sirtuins are found in plants, yeast and animals and may underlie the remarkable health benefits of CR. The hormesis hypothesis of CR, therefore, is based on the concept that low calorie intake is itself a mild stress which evokes a general stress response that promote a better health and longer life. It may entirely or partially account for the beneficial effects of phytochemicals. Plant molecules induced by the stress such as resveratrol, butein, and fisetin can evoke defense responses in fungi, flies, fish, and mice, leading to an increased life span. Such molecules are known as “caloric restriction mimetics”. However, it remains to be clearly understood.

Xenohormesis, defined as an adaptive response in regulating the physiology of an organism to molecular cues that acts as neither nutritive nor direct stressors, most likely occurs at some level. The term hormesis refers to a process by which a mild stress provides health benefits by causing the organisms to mount a defense response. The Xenohormesis Hypothesis can be explained using Resveratrol as an example. Resveratrol is a small polyphenol found in grapes and wine. It is identified in a screen for compounds that mimic the effects of Caloric Restriction (CR) by activating the sirtuin enzyme SIRT1. Sirtuins, a family of NAD$^+$-dependent deacetylases, conserved from yeast to humans have been proposed to mediate lifespan extension, that effects Caloric Restriction in lower organisms.

Consistently, resveratrol was found to extend the lifespan of wild type yeast, but not that of mutant strains lacking the yeast sirtuin, Sir2. It depicts that the beneficial effect of resveratrol on yeast cells is attributed to a very specific manipulation of a conserved signaling pathway and not due to a general property such as antioxidant capacity or nutritive value. From this, it was observed that the absence of an endogenous small-molecule activator of Sir2/ SIRT1, prompted the suggestion of a differential way by which plants might influence organisms. It assumes that the evolutionarily conserved survival pathways might respond to chemical cues in the environment or the food supply. For growing a yeast cell on a grape, an increase in the concentration of resveratrol was observed suggesting that it is induced in the plant after injury or infection and serves as a useful marker that the food supply is about to become limiting. Therefore, the yeast cell might gain a selective advantage by responding to resveratrol and activation of sirtuin enzymes in the same way it would to an actual deficit of calories.

The complex relationship between animals and the plants they consume is yet to be understood clearly. Throughout evolution, the flux of various phytochemicals in food sources would have provided a wealth of information to an animal capable of deciphering the signals. The existence of such signals and selective pressure to adapt to environmental changes, like xenohormesis occurs at some level. To what extent this phenomenon might contribute to the beneficial effects of specific phytochemicals in fruits and vegetables, remains to be seen. It has been suggested that a seemingly coordinated beneficial response to a phytochemical such as resveratrol could reflect mimicry of an endogenous signaling molecule. Plants and
animals retain a surprising degree of similarity in certain signaling pathways. Many neurotransmitters, like acetylcholine, histamine, serotonin, glutamate and GABA, are made by plants. Similar signaling molecules might exist in every species. Genistein has been found to interact with the estrogen receptor in some cases and several other polyphenols have been established as bonafide estrogen mimics. No endogenous molecules similar to plant polyphenols have been identified in mammals due to the absence of the biosynthetic pathways for polyphenols. It is hypothesized that stressed plants may contain a host of potentially therapeutic molecules that have yet to be recognized. The timing of therapeutic effects may also play an important role. Direct stresses may induce protective hermetic responses early in life, but have diminished effects later in life. Similarly, there may be an optimal therapeutic window in which a protective Xenohormetic response can be established. Elucidating the mechanism by which plant foods benefit human health has enormous potential to improve the quality of life and prevent or treat major diseases. There are also non-polyphenolic endogenous modulators waiting to be discovered. Importantly, if they are discovered, their existence would not rule out Xenohormesis. Any progress towards the testing of the CR and Xenohormesis hypothesis will be an important challenge in the coming years.

Reference

FORMER PRESIDENT OF IABMS

Prof. G. Victor Rajamanickam the immediate past president of the Indian Association of Biomedical Scientist (2012-2015) hails from a small village called Usilampatti in Madurai. He graduated from the Alagappa college and continued his postgraduation at Annamalai University in Geology. As the eldest son in the family he joined service as SRF in the CSIR laboratory-National Institute of Oceanography.

Prof. Victor as he is known joined the Department of Earth Science at Tamil University, Thanjavur in 1985. He developed the department with several projects and research grants from various funding agencies both nationally and internationally. He was the key player in starting the Ocean Science Technology Cell (OSTC) funded by Department of Ocean Development. He was recognized as Distinguished Professor of OSTC. Based on his initiatives, It was considered to be the best Earth Science laboratory in Tamil Nadu and was recognized as Centre for Excellence by the Ministry of Ocean Development. Off-late the Department of Science and Technology has entrusted the National Coordination of Coastal Resource Management as a Sub-Programme to this Department. During his tenure at Tamil University he has served at various capacities as HOD, Dean, Senate member and Syndicate member and was helpful to anyone who approached him to do research. In this process he guided as many as 38 Ph. Ds and 49 M. Phil candidates in various fields of science ranging from Geology, Geography, Geophysics, Geochemistry, Geomorphology, Geoinformatics, Geomedicine, Pharmacology, Biochemistry, Siddha Medicine, Disaster Management, Remote sensing, Agriculture including History of Science making him a supervisor with multidisciplinary expertise.

Accordingly he has several patents (6) journal publications (254) and books (25) to his credit.

After his retirement at Tamil University his quench to do research and motivate young research minds made him to serve at SASTRA University, Thanjavur as Dean and Coordinator for the Centre for Advanced Research in Indian System of Medicine (CARISM). He developed a good GLP and NABL Accredited laboratory with state of the art technology for drug testing including herbomineral drugs. Later he continued as Director (Research) at Sai Ram group of Institutions and presently as Senior Scientist at VelsUniversity. His enormous teaching and research experience and expertise made him an able administrator too.

He has several awards and distinctions to his credit which includes the DAAD Fellowship (1977), Visiting Professorship to UK and Germany (1982,1985), Tamilnadu Life Time Achievement award in Environmental Science (2011), V.V Swaminathan award (2009),BhramiahSastriMemorial Award (2012) and M.K. Nambiar Oration Award(2015) by the Indian Association of Biomedical Scientists. Even now at the age of 73 he serves the Central Council for Research in Siddha, Ministry of AYUSH as Expert member for Research. He is also an expert member of the Research Board in Kerala. He continues to give invited lectures and radio talks on various aspects of science and technology encouraging multidisciplinary and interdisciplinary research to unravel the mysteries of creation in a scientific manner. Till date he continues to motivate and guide all his students and scholars in all walks of life to achieve great things for this country.
Review Article

Chemical and electronic sensors used as a diagnosing tool for diseases—A review
C. Bijithra¹, G. Rajan¹, and P. Shanmugasundaram¹
¹School of Pharmaceutical Sciences, Vels University (VISTAS), Chennai, India.

(Received: Jan 2016   Accepted: Mar 2016)

Corresponding Author
P. Shanmugasundaram. Email: samsimahe@gmail.com

ABSTRACT

This article discusses the development of various sensor tools based on chemicals and electronics, which are finding a place in recent diagnosis of diseases like Tuberculosis, Vibrio cholera, Dengue fever, yellow fever and Ebola, Cancer, HIV, Liver diseases, Cardio vascular diseases, Skin diseases, Thyroid disease, Depression, Stomach diseases.

Key words: Brain Sensor, Electronic-Nose, E-Nose, Miniature Sensors, Mini Sensors, Mobile Phone-Sensors, Sensor Diagnosis, Sensors

INTRODUCTION

Identification of a condition, disease, disorder, or problem by systematic analysis of the background or history, examination of the signs or symptoms, evaluation of the research or test results, and investigation of the assumed or probable causes. Effective prognosis is not possible without effective diagnosis (Business dictionary). A diagnosis, in the sense of diagnostic procedure, can be regarded as an attempt at classification of an individual’s condition into separate and distinct categories that allow medical decisions about treatment and prognosis to be made. Subsequently, a diagnostic opinion is often described in terms of a disease or other condition, but in the case of a wrong diagnosis, the individual’s actual disease or condition is not the same as the individual’s diagnosis.

Diagnosis is also the identification of the nature and cause of a certain phenomenon. Diagnosis is used in many different disciplines with variations in the use of logics, analytics, and experience to determine “cause and effect.” In systems engineering and computer science, it is typically used to determine the causes of symptoms, mitigations, and solutions.

A diagnostic procedure may be performed by various health care professionals such as a physician, physical therapist, optometrist, healthcare scientist, chiropractor, dentist, podiatrist, nurse practitioner, or physician assistant.

It might be a management-naming or prognosis-naming exercise. It may indicate either degree of abnormality on a continuum or kind of abnormality in a classification. It’s influenced by non-medical factors such as power, ethics and financial incentives for patient or doctor. Traditional and conventional diagnostic tools and Chemicals are very time consuming and costly. With the advent of various sensors applied to individual diseases we find a paradigm shift from the traditional system of diagnosis to that of the modern system of diagnostic tools.

A sensor is a device that detects events that occur in the physical environment (like light, heat, motion, moisture, pressure, and more), and responds with an output, usually an electrical, mechanical or optical signal. A sensor is a transducer whose purpose is to sense (that is, to detect) some characteristic of its environments.
The household mercury thermometer is a simple example of a sensor—it detects temperature and reacts with a measurable expansion of liquid. Sensors are everywhere—they can be found in everyday applications like touch-sensitive elevator buttons and lamp dimmer surfaces that respond to touch, but there are also many kinds of sensors that go unnoticed by most—like sensors that are used in medicine, robotics, aerospace and more. Sensors are divided into two groups: active and passive sensors. Active sensors (such as photconductive cells or light detection sensors) require a power supply while passive ones (radiometers, film photography) do not.

**Application of Sensor Devices in Various Diseases**

There is an urgent need in the medical diagnostics laboratories for accurate, fast and inexpensive devices, which can be routinely used. The reliable and accurate information on the desired biochemical parameters is an essential prerequisite for effective healthcare. In this context, biosensors are considered to provide viable solutions to the problems posed by the contemporary healthcare industry. This is because these biosensing devices offer considerable advantages, such as specificity, small size faster response and cost. It is anticipated that these bioanalytical tools can be used for frequent measurements of metabolites, blood cations and gases, etc. Malhotra and Chaubey in their research article they made an attempt to highlight some of the trends that rule the research and developments of some of the important biosensors that are likely to accelerate the growth of clinical diagnostics industry.

Research is going on in electronic nose (e-nose) devises application in medical diagnosis. Gardner et al. in their research have applied an e-nose to the identification of pathogens from cultures and diagnosing illness from breath samples. These initial results suggest that an e-nose will be able to assist in the diagnosis of diseases in the near future.

Tuberculosis (TB) remains one of the world’s deadliest communicable diseases. In 2013, an estimated 9.0 million people developed TB and 1.5 million died from the disease, 360,000 of whom were HIV-positive. TB is slowly declining each year and it is estimated that 37 million lives were saved between 2000 and 2013 through effective diagnosis and treatment. However, given that most deaths from TB are preventable, the death toll from the disease is still unacceptably high and efforts to combat it must be accelerated if 2015 global targets, set within the context of the Millennium Development Goals (MDGs), are to be met.

A wide spectrum of diagnostic technologies and tools are used to identify the agents causing infectious diseases. In fact, monitoring Volatile Organic Compounds (VOCs) in the breath may soon become an interesting supplement (or even an alternative) to conventional medical diagnostics, thanks to the rapid advances in the techniques for breath collection and gas-analysis.

The diagnosis of active TB is critical for controlling the disease. Diagnosis methods require highly skilled personnel and specialized laboratory infrastructure. There are several diagnostic methods for detection of active tuberculosis (e.g. sputum smear microscopy, tuberculosis culture from sputum and Xpert MTB/RIF). Although these methods are widely used for diagnosing TB, they suffer of specificity and sensitivity limitations and microbiological culture takes several weeks to confirm a clinical diagnosis and at the proof-of-concept stage a breath test using nanomaterial-based sensors are used.

Recently, several studies have speculated that the distinguishing features of each breath print do not arise solely from pathogen metabolism, but are also due to changes of host VOCs, possibly in conjunction with the immunological response.

**Sensor to detect cholera quickly**

Although the bacterium, *V. cholerae*, cannot be sensed directly, remotely sensed data can be used to infer its presence. In the study reported here, satellite data were used to monitor the timing and spread of cholera. Public domain remote sensing data for the Bay of Bengal were compared directly with cholera case data collected in Bangladesh from 1992–1995. The remote sensing data
included sea surface temperature and sea surface height. It was discovered that sea surface temperature shows an annual cycle similar to the cholera case data. Sea surface height may be an indicator of incursion of plankton-laden water inland, e.g., tidal rivers, because it was also found to be correlated with cholera outbreaks. The extensive studies accomplished during the past 25 years, confirming the hypothesis that *V. cholera* is autochthonous to the aquatic environment and is a commensal of zooplankton, i.e., copepods, when combined with the findings of the satellite data analyses, provide strong evidence that cholera epidemics are climate-linked.

A nano-structured Magnesium Oxide (nanoMgO) based electrochemical DNA biosensor is created. It exhibits a sensitivity of 16.80 nA/ng/cm², faster than any available sensor, and has a response time of 2 seconds. It also has a lower detection limit of 59.12 ng/µL with good reproducibility. It is a new sensor that can quickly detect *Vibrio cholerae*, the cholera-causing bug, in clinical samples. Conventional diagnosis methods of cholera are based on culture of bacterium, biochemical reactions and agglutination tests against serogroup-specific antisera. The whole process of *V. cholera* isolation and identification takes at least 2–3 days and during this period, the disease can spread explosively. PCR tests take 3–4 hours and require expertise in molecular biology. Besides this, false positivity of PCR has been reported especially in commercial assays due to cross-reactions. Therefore, rapid and sensitive diagnosis has been the need to detect the infectious disease at an early stage.

**Paper test quickly detects Ebola, dengue and yellow fever**

Researchers in the US have developed a silver nanoparticle-based paper test to simultaneously detect dengue, yellow fever and Ebola. The Ebola epidemic in West Africa underscores an urgent need for rapid diagnostics; quick identification and patient isolation can benefit the sick and the healthy. However, dengue, yellow fever and Ebola all initially manifest as a fever and headache, so are easily mixed up. Now, this huge problem has a tiny solution—an 8 × 3 cm lateral flow test. Lee Gehrke and his team at the Massachusetts Institute of Technology and Harvard Medical School adapted the traditional single marker lateral flow test to diagnose several diseases at once. It costs $2, takes 10 minutes, and there is no need for a power supply, trained specialist or expensive equipment.

The test is made from strips of paper containing antibodies attached to triangular silver nanoparticles of varying size according to the disease they recognize and bind to. Silver nanoparticles appear as different colours according to their size, so when a patient’s serum sample migrates through the device, distinctive colored lines appear on the paper to indicate positive results for Ebola, dengue or yellow fever. This pattern of lines can be analysed by eye but the team are also working on a mobile phone application to aid diagnosis. “An app could be very useful for diseases that are mosquito-spread,” says Gehrke. “It adds a date and geographical stamp to the test results so the spread of disease can be followed in real-time.

**Sensors to Diagnose Skin Diseases**

Color related skin conditions or alterations can be identified accurately using optical instruments. While in the past such skin color measurements were only possible in laboratories with expensive spectrometers or special measurement equipment, nowadays doctors can use handhelds based on multi-spectral color sensors. The demand for precise color measurement in both the area of analysis using instruments for medical diagnostics such as point-of-care (Po Ct) and in dermatological research and applications, and especially for diagnostic documentation, is constantly rising. For example, in pharmacology—skin conditions or changes as a result of disease or environmental influences can be determined and documented via optical measuring instruments. The results of these measurements can be used both for evaluating skin diseases and for preparing treatment or assessing treatment success. Gray scale levels, colors and spectral data followed by a subsequent analysis, color and spectral algorithms as well as application-specific
evaluation are the basis for a broad application of optical measurement methods in the field of diagnosis. Color measuring tasks are performed in different ways. Tristimulus sensors are compact and optimized for large numbers and fast measurement tasks. Using an RGB filter, they are ideal for color detection. True color sensors with XYZ filter are suitable for absolute color measurement based in the Cie 1931 standard for human eye perception. Both sensors work according to the colorimetric principle.

Researchers, including one of Indian-origin, are developing a new wearable vapour sensor that could offer continuous disease monitoring for patients with diabetes, high blood pressure, anemia or lung disease. The new sensor, being developed at the University of Michigan, can detect airborne chemicals either exhaled or released through the skin.

**Smart Phone Sensors to Detect Mental Health**

Depression is a common, burdensome, often recurring mental health disorder that frequently goes undetected and untreated. Mobile phones are ubiquitous and have an increasingly large complement of sensors that can potentially be useful in monitoring behavioral patterns that might be indicative of depressive symptoms. Features extracted from mobile phone sensor data, including GPS and phone usage, provided behavioral markers that were strongly related to depressive symptom severity. While these findings must be replicated in a larger study among participants with confirmed clinical symptoms, they suggest that phone sensors offer numerous clinical opportunities, including continuous monitoring of at-risk populations with little patient burden and interventions that can provide just-in-time outreach.

**Stomach Diseases Identification by Sensors**

Patel et al. in his article applied a short description of key enabling technologies (i.e. sensor technology, communication technology, and data analysis techniques) that have allowed researchers to implement wearable systems is followed by a detailed description of major areas of application of wearable technology. Applications described in this review paper include those that focus on health and wellness, safety, home rehabilitation, assessment of treatment efficacy, and early detection of disorders. The integration of wearable and ambient sensors is discussed in the context of achieving home monitoring of older adults and subjects with chronic conditions. Future work required to advance the field toward clinical deployment of wearable sensors and systems is discussed.

Researchers in Australia are developing a new gas-sensing capsule that can evaluate stomach health and wirelessly transmit results to a smartphone. According to the scientists, the new technology could improve diagnostics and assess gas as a biomarker for overall gut health analysis. A team from the Royal Melbourne Institute of Technology (RMIT) and Monash University, led by RMIT professor Kourosh Kalantar-zadeh, designed the capsule to be swallowed and passed safely through the digestive system. The device features biocompatible cladding, a gas-permeable membrane, and a gas sensor, microprocessor, and wireless transmitter powered by a battery. According to an RMIT press release, the system is designed to communicate results directly to a smartphone. Research shows that intestinal gasses share a link with colon cancer, irritable bowel syndrome, and inflammatory bowel disease. The RMIT team, which published its findings in *Trends in Biotechnology*, indicated that further study of the gasses could link their presence to other health issues.

**Sensor Detection by Epilepsy**

Due to the recent developments in the technology era, and it is perceived that the technical development has improved in every field. Using this development, the world has gained a great deal even in the medical field. In the aware of that, all disorders have being cured by many technical equipments and detected effectively before it occurs. In that case, the Epilepsy disorder can be recognized and
makes an alert to the people using an application in Android smart phones. This kind of application is already exists but it will send unwanted alert message. In this paper, we mainly focused on to avoid the unwanted alert message using brain sensor. It has an in-built function which can trigger alert messages whenever the brain function goes abnormal.

The main reason behind the proposed system is that the brain function of person wills same at ever. It could vary only at the time of any disease attack in the neural system. Hence the false identification can be easily reduced.

A sensor for detecting various fatal diseases in advance, including HIV, has been developed.

Researchers at the Moscow Institute of Physics And Technology are of the opinion that the e sensor—an optical chip—will enable the doctors to identify tumour markers, whose presence in the body signals the emergence and growth of cancerous tumours.

The researchers—Dmitry Fedyanin and Yury Stebunov—are of the opinion that the new sensor will combine high sensitivity with a comparative ease of production and miniature dimensions, allowing it to be used in all portable devices, such as smartphones, wearable electronics.

The researchers further explained that unlike similar devices, their sensor has no complex junctions and can be produced through a standard CMOS process technology used in microelectronics. The sensor doesn’t have a single circuit, and its design is very simple.”

Early therapy conveys a double benefit—not only to improve the health of individuals but at the same time by lowering their viral load, reducing the risk of HIV transmission.

The highly sensitive nanomechanical sensor can analyse the chemical composition of substances and detect viral disease markers which appear when the immune system responds to incurable or hard-to-cure diseases.

The ultrasensitive sensor can track changes of just a few kilodaltons in the mass of a cantilever in real time. One Dalton is roughly the mass of a proton or neutron, and several thousand Daltons are the mass of individual proteins and DNA molecules.

So the new optical sensor will allow for diagnosing diseases long before they can be detected by any other method, which will pave the way for a new-generation of diagnostics.

One chip, several millimetres in size, will be able to accommodate several thousand such sensors, configured to detect different particles or molecules, said the paper published in the journal Scientific Reports.

Mobile Health Care is the integration of mobile computing and health monitoring. It is the application of mobile computing technologies for improving monitoring. It is the application of mobile computing technologies for improving communication among patients, physicians, and other health care workers. As mobile devices have become an inseparable part of our life it can integrate health care more seamlessly to our everyday life. It enables the delivery of accurate medical information anytime anywhere by means of mobile devices. Recent technological advances in sensors, low-power integrated circuits, and wireless communications have enabled the design of low-cost, miniature, lightweight and intelligent bio-sensor nodes. These nodes, capable of sensing, processing, and communicating one or more vital signs, can be seamlessly integrated into wireless personal or body area networks for mobile health monitoring.

CONCLUSION

Although the mobile sensor devices have been shown to be accurate and have clinical utility, they continue to be underutilized in the healthcare industry. Incorporating smart wearable sensors into routine care of patients could augment physician-patient relationships, increase the autonomy and involvement of patients in regards to their healthcare and will provide for novel remote monitoring techniques which will revolutionize healthcare management.
REFERENCES

9. doi:10.1038/nindia.2013.95 Published 19 July 2013.
10. This article is reproduced from Chemistry World by Vicki Davison and ChemistryWorld | February 18, 2015. The article was first published on February 17, 2015 by Scientific American 8/5/2015 Paper Test Quickly Detects Ebola, Dengue and Yellow Fever.
13. Krumbein, F. Employ color sensors to diagnose skin diseases: learn how a non-contact measuring system can be used to assess skin diseases. EE Times India. etindia.com4/3/201302:50PMEDT
Research Paper

**In-vitro antioxidant and anti-inflammatory activity of ethanolic extracts of Momordica charantia: 50% ethanolic extract of MC has good antioxidant activity**

Shobha C.R., Prashant Vishwanath, Parveen Dodamani, Akila Prashant, Suma M.N., and Basavanagowdappa H.

1Department of Biochemistry, Center of Excellence in Molecular Biology and Regenerative Medicine, JSS Medical College, JSS University, Mysore, Karnataka, India.

2Department of Medicine, JSS Medical College, JSS University, Mysore, Karnataka, India.

(Received: Jan 2016   Accepted: Mar 2016)

Corresponding Author

Dr. Prashant Vishwanath. Email: drmvps@gmail.com

**ABSTRACT**

**Introduction and Aim:** Deep-colored vegetables and fruits including bitter melon are good sources of phenolic compounds which are known to possess antioxidant, antimutagen, antitumor, anti-inflammatory, and anticarcinogenic properties. To determine the antioxidant and anti-inflammatory activity in different percentage of ethanolic extract of *Momordica Charantia* (EEMC).

**Materials and Methods:** The paste of *Momordica Charantia* (MC) fruit was lyophilized using freeze dryer and the powder obtained was stored at -80°C in airtight plastic container. EEMC was prepared by graded ethanol fractionation method. The extracts were concentrated, freeze-dried and reconstituted in ethanol for the assessment of (a) antioxidant activity was determined by total phenols—using Folin–Ciocalteu (F–C), total flavanoid by Aluminum Chloride Colorimetric Method, ferric reducing antioxidant power (FRAP), 2,2-diphenyl-1-picrylhydrazyl (DPPH) and reducing power assay; (b) anti-inflammatory activity by red cell membrane stabilization assay.

**Results:** The total phenolic content and total flavanoid content of EEMC decreased with increasing ethanol concentration from 50% to 100%. The 50% EEMC has more potent antioxidant activity when compared to 70% and 100% EEMC by FRAP, DPPH and reducing power assay. The anti-inflammatory activity is more by 50% when compared to 70% and 100%.

**Conclusion:** The data showed that the 50% EEMC contain a high percentage of phenolic content, flavanoid content, antioxidant and anti-inflammatory potential than 70% and 100%. Further studies to identifying the key antioxidants in this extract are under way.

**Key words:** Anti-inflammatory Activity, Antioxidant, DPPH, FRAP, *Momordica charantia*.
INTRODUCTION

Phenolics are the group of phytochemicals that account for most of the antioxidant activity in plants or plant products. It is estimated that there are about eight thousand naturally occurring plant phenolics and about half this number are flavonoids. Phenolic compounds are mainly synthesized from cinnamic acid, which is formed from phenylalanine by the action of L-phenylalanine ammonia-lyase, the branch point enzyme between primary (shikimate pathway) and secondary (phenylpropanoid) metabolism. Phenolic compounds act as radical scavengers, reducing agents and chelators of metal ions and flavonoids act as a free radical scavenger, inhibitor of hydrolytic and oxidative enzymes and also has anti-inflammatory action (1). The antioxidant activities of the phenolic compounds are related to the structure, generally depending on the number and position of hydroxyl groups and glycosylation or other substituents.

The antioxidant test models differ in different aspects. Therefore, it is difficult to compare exactly one method to another one. Antioxidants can deactivate radicals by two major mechanisms, hydrogen atom transfer (HAT) and single electron transfer (SET). The end result of both the mechanism is the same, but kinetics and potential for side reactions differ. HAT-based methods measure the classical ability of an antioxidant to quench free radicals by hydrogen donation and SET-based methods detect the ability of a potential antioxidant to transfer one electron to reduce any compound, including metals, carbonyls, and radicals (2). The 2,2-Diphenyl-1-picrylhydrazyl (DPPH) act by both HAT and SET mechanism whereas Ferric Reducing Antioxidant Power (FRAP) by SET mechanism. Many researchers have used both the DPPH and the FRAP assays in their plant activity screening programs, to obtain a better description of the antioxidant activity. A single method is not suitable for all and there is no shortcut approach to determine antioxidant activity.

It is known that deep–colored vegetables and fruits including Momordica Charantia (MC) are good sources of phenolic compounds which are known as hydrophilic antioxidants, those posses antioxidant, antimutagen, antitumor, anti-inflammatory, and anticarcinogenic properties (3,4). Extraction, as the term is used pharmaceutically, involves the separation of medicinally active portions of plant or animal tissues from the inactive or inert components by using selective solvents in standard extraction procedures. Different solvents like water, acetone, methanol, ethanol, n-butanol, etc. are used for extraction of phytochemical constituents from plants (5,6). The extraction of the phenolic compounds is influenced by their chemical nature, the extraction method, the sample size, time, storage conditions and the presence of interfering substances. The phenolic extracts of plants are selectively soluble in the different solvent. The use of an alcoholic solution provides satisfactory results for the extraction process (7). With these references, we proposed to evaluate phytoconstituent activities and several biological activities of MC using alcohol.

MATERIALS AND METHODS

The study was conducted in Center of Excellence for Molecular Biology and Regenerative Medicine (CEMR) Laboratory, Department of Biochemistry, JSS Medical College, Mysore, Karnataka, India.

Preparation of ethanolic extract of Momordica charantia (EEMC)

EEMC was prepared by graded ethanol fractionation method as described earlier (8). Different percentage (50%, 70%, and 100%) of EEMC was obtained using 50 g of lyophilized powder. The obtained different percentage solutions of EEMC were then concentrated using Rotovapor R-215 (Buchi, Switzerland) which was later filtered using Whatman's filter paper number-1. The concentrated extracts were subjected for lyophilisation using freeze dryer (Alpha 2-4 LD Plus from Christ, GmbH) which was done by dehydrating all the 50%, 70% and 100% concentrated extracts completely at reduced pressure after being frozen at –80°C. Once completely dehydrated, the concentrated lyophilized powder extracts were preserved at –80°C.
For further analysis EEMC stock was prepared by dissolving the lyophilized powder in Phosphate buffered saline (PBS) (HiMedia Laboratories).

**In vitro biochemical analysis**

Different percentages of EEMC were subjected to various biochemical analyses to determine the total phenolic content, reducing sugar, antioxidant activity, anti-inflammatory activity and flavonoids. All the analyses were done in triplicate, and the reagents for each analysis were prepared freshly.

The estimation of total phenol content (TPC) was performed using Folin-Ciocalteu solution and the gallic acid as standard (9). Percentage Total Phenol (%TP) were obtained by back calculating for dried powdered plant materials and expressed as Percentage gram weight (% w/w). The total flavonoid content (TFC) in the extracts were estimated by an aluminium chloride colorimetric method using Quercetin as standard and was expressed as quercetin equivalents (mg of QE/g sample) (10). The estimation of reducing sugar was performed using 3,5-Dinitrosalicylic acid method using D-glucose as standard. Percentage Reducing Sugar (%RS) was obtained by back calculating for dried powdered plant materials and expressed as Percentage Gram weight (% w/w).

**Identification of reducing sugar in EEMC by paper chromatography**

Paper chromatography was done using Butanol: Acetic acid: Distilled Water in the ratio 12:3:5 as mobile phase and 1.66% of Phthalic acid in n-Butanol: Distilled water: Aniline in the ratio 95:4:1 as Visualizing agent. Rf (Relative fraction) for standards including extract were determined by the equation: 

\[ Rf = \frac{\text{Distance travelled by the substance}}{\text{Distance travelled by the solvent front}} \]

Identification of sugars present in 50% EEMC was compared with the Rf values of standards in the same run and was confirmed by a cascade of colorimetric reactions.

The quantitative estimation of Glucose present in the EEMC was determined by Glucose Oxidase and Peroxidase (GOP-POD) Method using D-glucose as standard. Glucose concentration present in 50% EEMC was derived from the standard calibration curve and expressed as the concentration of glucose in mg/dl.

**Preparation of stock of different percentage of EEMC**

Based on the total phenolic content the stock of 50%, 70%, and 100% EEMC was prepared in the concentration of 80 µg/ml, which was further serially diluted up to 1.25 µg/ml in ethanol.

Ferric reducing the ability of plasma (FRAP assay) was used to measure the antioxidant power of the extracts taking FeSO₄ as standard (11). The antioxidant capacity based on the ability to reduce ferric ions to ferrous form was calculated from the linear calibration curve and expressed as mmol of FeSO₄ equivalents (FRAP units). The free radical scavenging activity was estimated by 2,2-diphenyl-1-picrylhydrazyl (DPPH) method using ascorbic acid as standard (12). The radical scavenging activity was expressed as the percentage inhibition (I%) and calculated as per the equation:

\[ I(\%) = \frac{(A_{\text{blank}} - A_{\text{sample}})}{A_{\text{blank}}} \times 100 \]

where \( A_{\text{blank}} \) is the absorbance of the blank reagent with no testing compound, and \( A_{\text{sample}} \) is the absorbance of the test sample with all reagents. The IC-50 value was calculated from the plot of inhibition (%) against the concentration of the extract.

The estimation of Antioxidant Activity of the extracts was performed by Reducing Power Assay using ascorbic acid as standard (13). The anti-inflammatory activity of the extracts was determined by Human Red Blood Corpuscle (HRBC) Membrane Stabilization Assay (Hypotonicity Induced) using diclofenac as positive control (14).

The percentage inhibition of haemolysis or membrane stabilization was calculated using the following equation:

\[ \% \text{Inhibition of haemolysis} = \left( \frac{A_1 - A_2}{A_1} \right) \times 100 \]
where: $A_1$ = Absorbance of hypotonic buffered saline and $A_2$ = Absorbance of the test sample in hyposaline and were compared with the % inhibition of positive control.

**Statistical analysis**

All statistical analysis was performed using GraphPad Prism 5 for Windows, version 5.01. Data were expressed as mean values ± SEM. A one-way ANOVA model was used to compare means between the groups. Each sample was analyzed thrice. Post hoc pairwise multiple comparisons were evaluated using the Bonferroni post-test, after ANOVA. Results were considered significant at $p < 0.05$

**RESULTS**

**TPC of different percentage of EEMC**

The total phenol content in 50%, 70% and 100% EEMC has been reported in our previous study (0.029%, 0.0098% and 0.0022% respectively) (8). Further experiments were planned based on the total phenol content in each of these extracts.

**TFC in different percentage of EEMC**

Fig. 1 Percentage of total flavanoid content in 50%, 70% and 100% EEMC.

50% ethanolic extract had the highest flavanoid content (22%) when compared to 70% and 100% ethanolic extract. The inset shows the standard graph with $R^2 = 0.9985$.

The data obtained showed that, the 50% ethanolic extract had the highest flavanoid content (22%) followed by the 70% (14.5%) and 100% (11%) ethanolic extracts respectively (Fig. 1) ($p = <0.016$) using quercetin as standard ($R^2 = 0.9985$). Since a gradient extraction was followed, most of the water soluble fractions of the flavanoids were found in 50% extracts than 70% and 100% extracts.

**Reducing sugar in different percentage of EEMC**

Fig. 2 Percentage of reducing sugar in 50%, 70% and 100% EEMC.

50% ethanolic extract had the highest percentage of reducing sugar (2.3%) when compared to 70% and 100% ethanolic extract. The inset shows the standard graph with $R^2 = 0.9920$.

The data obtained showed that, the 50% ethanolic extract had the highest percentage of reducing sugar (2.3%) followed by the 70% (0.51%) and 100% (0.06%) ethanolic extracts respectively (Fig. 2) ($p = <0.0001$) using D-glucose as standard ($R^2 = 0.9920$).

**Identification of sugar in 50% EEMC by paper chromatography**
Fig. 3 Ascending paper chromatography for qualitative estimation of reducing sugars in the 50% extract.

The distance moved by the sample corresponds to that of glucose standard.

Table 1 Rf value of extract and the standards from chromatography.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Rf value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xylulose</td>
<td>0.37</td>
</tr>
<tr>
<td>Maltose</td>
<td>0.19</td>
</tr>
<tr>
<td>Glucose</td>
<td>0.31</td>
</tr>
<tr>
<td>Sample(extract)</td>
<td>0.30</td>
</tr>
<tr>
<td>Fructose</td>
<td>0.344</td>
</tr>
</tbody>
</table>

The Rf value of the sample corresponds to that of glucose standard.

In the paper chromatography the distance moved by the sample corresponded to that of the glucose standard (Fig. 3). This was confirmed by calculating the Rf values which is represented in Table 1. So the reducing sugar present in the extract may be Glucose.

The presence of glucose was confirmed by GOD-POD method, which showed 332mg/dl of glucose in the 50% EEMC.

Antioxidant activity of EEMC using FRAP, DPPH and reducing power assay

Fig. 4 Antioxidant activity of 50%, 70% and 100% EEMC by FRAP method.

50% ethanolic extract had the highest antioxidant activity compared to 70% and 100% ethanolic extracts respectively by FRAP (p = 0.004). The inset shows the standard graph with R2 = 0.9926.

Fig. 5 Antioxidant activity of 50%, 70% and 100% EEMC by DPPH method

50% ethanolic extract had the highest antioxidant activity followed by the 70% and 100% ethanolic extracts respectively by DPPH method (p = 0.079). The inset shows the standard graph with R2 = 0.9644.
50% ethanolic extract had the highest antioxidant activity followed by the 70% and 100% ethanolic extracts respectively by Reducing power assay \( (p = 0.010) \). The inset shows the standard graph with \( R^2 = 0.9947 \).

The data obtained showed that, the 50% ethanolic extract had the highest antioxidant activity followed by the 70% and 100% ethanolic extracts respectively by FRAP \( (p = 0.004) \), DPPH \( (p = 0.079) \) and Reducing Power Assay \( (p = 0.010) \), which may be attributed to the presence of higher percentage of phenolic acid and flavanoid content in 50% EEMC (Figs. 4–6).

50% ethanolic extract has lowest IC(50) value \( (11.43 \mu g/ml) \) when compared to 70% and 100% ethanolic extract.

The data obtained from the DPPH method showed that, the 50% \( (11.43 \mu g/ml) \) EEMC has lowest IC(50) value than 70% \( (16.42 \mu g/ml) \) EEMC and 100% \( (175.4 \mu g/ml) \) EEMC (Fig. 7).

**ANTI-INFLAMMATORY ACTIVITY OF EEMC USING HRBC MEMBRANE STABILIZATION ASSAY**

50% ethanolic extract had the highest anti-inflammatory activity followed by the 70% and 100% ethanolic extracts respectively \( (p = 0.001) \) using diclofenac sodium as positive control.

The data obtained showed that, the 50% ethanolic extract had the highest anti-inflammatory activity followed by the 70% and 100% ethanolic extracts respectively \( (p = <0.001) \) using diclofenac sodium as positive control.

**DISCUSSION**

Medicinal value of MC has been attributed to its high antioxidant property which is related to phenols, flavonoids, isoflavones, terpenes, anthraquinones, and glucosinolates. Based on the previous references which have stated that ethanol is a better solvent for extraction of phytochemicals (6,7), we have used ethanol as a solvent for extraction to evaluate phytoconstituent activities and biological activities of MC. Amira et al. have shown that the
pure solvents were inefficient extraction media for antioxidant, and enhanced extraction yields were obtained from solvent containing a higher concentration of water (3). Koffi et al in their work have shown that since the vast majority of polyphenols are not water soluble manufacturers would have to use extraction solvents with a mixture of suitable solvents to obtain fractions rich in polyphenols (7). Based on these studies we have used different percentage of ethanol, that is, 50%, 70% and 100% for extraction and the result obtained in our study goes in accordance to these references.

Phenolic compounds are known to have antioxidant activity, and it is due to the redox properties, which play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides (15). Flavonoids are the largest group of naturally occurring phenolic compounds, which occurs in different parts of the plant both in a free state and as glycosides. The polyphenolic nature of flavanoids acts as an antioxidant which scavenges injurious free radicals such as super oxide and hydroxyl radicals. MC is a good source of phenolic compounds which possess potent antioxidant activity. Semiz and Sen have shown that in-vivo treatment of rats with MC fruit extract enhanced both antioxidant enzyme (AOE) and Glutathione S-Transferase (GST) activities (16). Hamissou et al. have shown that, as a polarity of the solvent increased, extraction yields of total soluble solids and total extractable polyphenolics were higher (18). The data obtained in our work agrees with this study. The result of TPC and TFC shows that the reactivity of phenolic compounds depends upon the polarity of the medium, as there is a decrease in the polarity, reactivity also decreases proportionally showing minimum reactivity with 100% ethanol soluble fractions of phenolic contents. Further phenolic acid profiling using HPLC will be done in order to specify the key compound present in the 50% EEMC.

The reducing sugar was estimated by DNS method using D-Glucose as standard (R² = 0.9920). The result obtained showed that the 50% (2.3%) EEMC is having the highest percentage of reducing sugar followed by 70% (0.51%) and 100% (0.06%) EEMC (Fig. 2). Since the 50% EEMC has shown the highest percentage of reducing sugar qualitative estimation of reducing sugar was done by ascending paper chromatography using glucose, fructose, xylulose and maltose as standard. The result obtained showed the presence of glucose in 50% EEMC (Fig. 3 and Table 1). The presence of glucose was further confirmed by the GOD-POD method using D-glucose as standard, which surprisingly showed 332mg/dl of glucose in the extract. The GOD-POD method is a specific method for estimation of D-Glucose, since the enzyme specifically oxidises only D-Glucose.

There are several methods to determine antioxidant activity of plants. The antioxidant activity of different percentage of EEMC in our study was determined using three methods, that is, FRAP, DPPH and reducing power assay. The phytochemicals which are responsible for the scavenging activity

EEMC is having highest phenol content followed by 70% (0.0098%) and 100% (0.0022%) EEMC. The TFC was estimated using Aluminum Chloride Colorimetric Method using quercetin as standard (R² = 0.9985). The result obtained in our study showed that the 50% (22%) EEMC is having highest flavanoid content followed by 70% (14.5%) and 100% (11%) EEMC (Fig. 1).

Moure et al. showed that, as a polarity of the solvent increased, extraction yields of total soluble solids and total extractable polyphenolics were higher (18). The data obtained in our work agrees with this study. The result of TPC and TFC shows that the reactivity of phenolic compounds depends upon the polarity of the medium, as there is a decrease in the polarity, reactivity also decreases proportionally showing minimum reactivity with 100% ethanol soluble fractions of phenolic contents. Further phenolic acid profiling using HPLC will be done in order to specify the key compound present in the 50% EEMC.

The TPC was estimated using the F-C method using gallic acid as standard. A Linear response was observed between 5 and 60 µg/µl and TPC was expressed as GAE (R² = 0.9236). The result obtained in our study showed that the 50% (0.029%)
are the phenolic and flavonoid content in the extract (19). The Higher absorbance of the reaction mixture indicates higher reductive potential (20). The result obtained in our study showed that the 50% ethanolic extract had the highest antioxidant activity followed by the 70% and 100% ethanolic extracts respectively, in FRAP (Fig. 4), DPPH (Fig. 5) and Reducing Power Assay (Fig. 6), which may be attributed to the presence of higher percentage of phenolic acids and flavanoids.

IC(50) value is defined as the concentration of antioxidant required for 50% scavenging of DPPH radicals, which is a parameter widely used to measure antioxidant activity. Smaller the IC(50) value corresponds to a higher antioxidant activity of the plant extract (21). The data obtained in our study showed that the 50% (11.43 µg/ml) EEMC has lowest IC(50) value than 70% (16.42 µg/ml) EEMC and 100% (175.4 µg/ml) EEMC and attributes to TPC and TFC (Fig. 7). The DPPH radical scavenging activity of EEMC increased gradually as the concentration was increased. Decrease in absorbance of DPPH solution indicated by a change in the colour from purple to yellow which depends on the intrinsic antioxidant activity of antioxidant and on the speed of reaction between DPPH and antioxidant present in the extract.

The anti-inflammatory activity of EEMC was estimated by HRBC Membrane Stabilization Assay using diclofenac sodium as a positive control. The erythrocyte membrane is analogous to the lysosomal membrane and its stabilization by the extract implies that the extract will stabilize lysosomal membranes which will, in turn, limit the inflammatory response by preventing the release of lysosomal constituents. Cioua et al. and Chao et al. have independently shown that MC in sepsis-induced mice reduced expression of proteins associated with inflammation like Cyclooxygenase-2 (COX-2), Inducible nitric oxide Synthase (iNOS), and Nuclear factor-kappaB (NF-kB) and reduced secretions of pro-inflammatory cytokines and other substances, hence reducing organ damage (22,23). Umukoro et al have shown that aqueous leaf extract of MC inhibits the late phase of inflammatory events, namely the release of chemical mediators and thus suggesting that it may offer some beneficial effects in the management of inflammatory conditions (24). In these studies they have used crude extract or single percentage of extract and explained about the anti-inflammatory activity of MC, but in our study we have used different percentage of extract for the anti-inflammatory activity. The data obtained in our study showed that the 50% ethanolic extract had the highest anti-inflammatory activity compared to 70% and 100% ethanolic extracts respectively (Fig. 8). The extracts might have exhibited membrane stabilization effect by inhibiting hypotonicity-induced lyses of erythrocyte membrane. Thus 50% EEMC is having more potent anti-inflammatory activity than 70% and 100% EEMC (Fig. 9).

Thus, the data obtained showed that 50% EEMC is having highest antioxidant activity and anti-inflammatory activity when compared to 70% and 100% EEMC. Further studies will be done to know the key antioxidant compound in the extract by phenolic acid profiling using HPLC.

ACKNOWLEDGMENTS

We thank the Department of Science and technology-Fund for the improvement of science and technology infrastructure for funding the Center of Excellence for Molecular Biology and Regenerative Medicine (CEMR) laboratory and facilitating the project and JSS University for funding the project. We would also like to acknowledge Dr. MVSST SubbaRao, Associate Professor, Department of Biochemistry, JSS Medical College, Mysore for his support and guidance to carry out the biochemical analysis.

REFERENCES


Inflammatory and oxidative stress markers in acute myocardial infarction
Ashok Prabhu¹, K. Sudha¹, A.M. Kiran Kumar¹, and Rajib Kumar Pandey¹

¹Department of Biochemistry, Center For Basic Sciences, Kasturba Medical College, Mangaluru, Manipal University, Manipal, India.

(Received: Feb 2016    Accepted: Mar 2016)

Corresponding Author
Dr. K. Sudha. Email: sudha.k@manipal.edu

ABSTRACT

Introduction and Aim: The present study aims to correlate oxidative stress markers viz., oxidized LDL, Ischemia modified albumin (IMA)and inflammatory marker—matrix metalloproteinase 9 (MMP 9) with the established cardiac markers (CK MB, Troponin T) and to assess their clinical utility in the diagnosis of acute myocardial infarction.

Materials and Methods: Ninety patients complaining of chest pain for the first time, were divided into 2 groups which included 45 patients with acute myocardial infarction and 45 age and sex matched patients with normal ECG and normal troponin T, who served as controls. Serum troponin T, CK MB, were determined by ECLIA, IMA, oxidized LDL spectrophotometrically and MMP-9 was measured by ELISA.

Results: Oxidized LDL and IMA were significantly elevated in MI compared to controls. However, an increase in MMP 9 in AMI patients was not statistically significant. Both specificity and sensitivity of newer markers were lower than gold standard markers viz., CK MB and Troponin T. There was a significant positive correlation between standard markers and oxidized LDL ($p < 0.001$) and an insignificant positive correlation with other newer markers studied.

Conclusion: It can be concluded that usefulness of IMA and MMP9 as predictive cardiac markers remain feeble. However, oxidized LDL may be included in the battery of extended lipid profile as well as in the panel of cardiac markers.

Key words: Cardiac Markers, Ischemia Modified Albumin, Matrix Metalloproteinase

INTRODUCTION

Myocardial infarction (MI), happens to be a prime cause of death and disability worldwide with an ongoing increase in incidence. Coronary artery diseases (CAD), is due to a complex interplay between genetic and environmental risk factors setting into motion an inflammatory cascade of monocyte migration, lipid oxidation and atheromatous plaque formation (1). The most common event of myocardial ischemia is oxidation of unsaturated fatty acids of the cell membrane which may induce proinflammatory pathways, activation of matrix metalloproteinase, vascular smooth muscle cell proliferation and death, endothelial dysfunction and lipid peroxidation (2). Reactive oxygen species have dose dependent action on the heart while small amounts serve as signaling molecules and result in cardiac protection, large amount cause lipid peroxidation and myocardial damage. ROS can cause reversible or irreversible
modifications of cardiac myofibrillar proteins and serum proteins like albumin and LDL (3). ROS produced due to ischemia damage the metal binding site on the amino terminus of albumin and lower metal binding capacity for cobalt (4). Ischemia modified albumin (IMA) increases within minutes of the onset of ischemia and returns to normal within 6–12 hours. LDL is the most vulnerable target of oxidation, and oxidative modification of LDL is a key step in the initiation and progression of atherosclerosis. Oxidized LDL induces local inflammation in the atherosclerotic plaque, leads to endothelial dysfunction and MI (5). Matrix metalloproteinase 9 (MMP 9), a gelatinase secreted by inflammatory cells, macrophages has shown to play a role in the pathogenesis of atherosclerosis and MI (6). The currently preferred biomarkers for MI are Troponin I or T and CK-MB, which have high myocardial tissue specificity as well as high clinical sensitivity. Although current markers have greatly improved the diagnosis and quickened the treatment of AMI patients, there is still scope for improvement, especially in the area of early detection and treatment as these markers assess different pathophysiological mechanisms in myocardial ischemia. Hence, the aim of the present study is to correlate serum IMA and oxidized LDL, the markers of oxidative stress and MMP9, an inflammatory marker with the well-established markers CK MB and Troponin T (TnT) of acute myocardial Infarction.

**MATERIALS AND METHODS**

This study is a case control study comprising of 90 subjects of either sex, of 20–60 age, who presented with a complaint of chest pain for the first time. Patients with previous history of myocardial infarction, malignancy, patients with statins, known cases of kidney and liver diseases were excluded. Selected subjects were diagnosed and classified as controls and MI patients, depending upon the assessment as per the clinical signs, symptoms, TnT levels and the ECG findings at the time of admission. Informed consent was taken from all the subjects and the study was approved by the institutional ethical and research committee. 5ml of whole blood was collected in red cap vacutainer, serum separated was used for the estimation of albumin, CKMB, troponin T, IMA, MMP9, and oxidized LDL. Estimation of serum TnT was done by ECLIA (7) and that of CK-MB was done by immunological UV kinetic method (8) Ischemia modified albumin was determined by cobalt-

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control (N = 45)</th>
<th>Myocardial Infarction (N = 45)</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Troponin T (pg/ml)</td>
<td>8.35 ± 3.04</td>
<td>82.13 ± 78.50</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>CKMB (IU/L)</td>
<td>14.17 ± 5.6</td>
<td>40.2 ± 20.3</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>MMP9 (pg/ml)</td>
<td>11932</td>
<td>17970*</td>
<td>0.200</td>
</tr>
<tr>
<td>OxLDL (µmol/L)</td>
<td>68.8 ± 19.3</td>
<td>95.01 ± 16.02</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>IMA (Abs unit)</td>
<td>0.55 ± 0.21</td>
<td>0.746 ± 0.34</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>AIMA (Abs.unit)</td>
<td>0.52 ± 0.2</td>
<td>0.707 ± 0.4</td>
<td>&lt;0.001*</td>
</tr>
</tbody>
</table>

Table 1 Comparison of study parameters among the groups.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>R</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMP9</td>
<td>0.060</td>
<td>0.577</td>
</tr>
<tr>
<td>OxLDL</td>
<td>0.451*</td>
<td>0.001*</td>
</tr>
<tr>
<td>IMA</td>
<td>0.133</td>
<td>0.213</td>
</tr>
<tr>
<td>ADIMA</td>
<td>0.100</td>
<td>0.350</td>
</tr>
<tr>
<td>CK-MB</td>
<td>0.526*</td>
<td>0.001*</td>
</tr>
</tbody>
</table>

Table 2 Correlation coefficients (r) of TroponinT with Study parameters.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>R</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMP9</td>
<td>0.108</td>
<td>0.313</td>
</tr>
<tr>
<td>OxLDL</td>
<td>0.432</td>
<td>0.001*</td>
</tr>
<tr>
<td>IMA</td>
<td>0.100</td>
<td>0.35</td>
</tr>
<tr>
<td>ADIMA</td>
<td>0.046</td>
<td>0.665</td>
</tr>
</tbody>
</table>

Table 3 Correlation coefficients (r) of CK-MB with study parameter.
albumin binding method (9). Adjusted Ischemia Modified Albumin (AIMA) was calculated by multiplying concentration of albumin with the absorbance of IMA, MMP-9 was assayed by ELISA (10) and that of oxidized LDL was assayed by precipitation method (11). Data were analyzed using IBM SPSS version 17. Student’s t-test was used to determine the significance of variables, p value less than 0.05 was considered statistically significant. The association of analytical markers with clinical outcome was compared with chi-square test. Results of TnT, CKMB, oxdLDL, MMP9, AIMA and IMA were analyzed for clinical sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), positive likely hood ratio (LR+) and negative likely hood ratio (LR−). ROC Curve is generated for individual analytes to find out the cutoff values.

RESULTS

Serum troponin T and CK MB was significantly higher (p < 0.001) in AMI patients compared to patients with normal ECG. Oxidized LDL values were strikingly elevated in patients compared to controls (p < 0.001). Further, a statistically significant increase in IMA and AIMA was observed in AMI patients compared to controls. However, median values of MMP-9 did not differ significantly between the groups (Table 1). Correlation studies on IMA, ADIMA, MMP-9 did not show any statistical significance with gold standard markers CK MB and TnT (Tables 2 and 3). However, oxidized LDL showed a positive correlation (r value 0.452, 0.432) and p < 0.001 with Tropo-

<table>
<thead>
<tr>
<th></th>
<th>TnT</th>
<th>CKMB</th>
<th>MMP9</th>
<th>OxdLDL</th>
<th>AIMA</th>
<th>IMA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>75.6</td>
<td>77.8</td>
<td>57.8</td>
<td>73.3</td>
<td>71.1</td>
<td>57.8</td>
</tr>
<tr>
<td>Specificity</td>
<td>96</td>
<td>89</td>
<td>62.2</td>
<td>80</td>
<td>67</td>
<td>72</td>
</tr>
<tr>
<td>PPV</td>
<td>94.4</td>
<td>87.5</td>
<td>60.4</td>
<td>78.5</td>
<td>68</td>
<td>66.6</td>
</tr>
<tr>
<td>NPV</td>
<td>79.6</td>
<td>80</td>
<td>59.5</td>
<td>75</td>
<td>69.7</td>
<td>63.8</td>
</tr>
<tr>
<td>OR</td>
<td>66.4</td>
<td>28</td>
<td>2.25</td>
<td>11</td>
<td>4.92</td>
<td>3.5</td>
</tr>
<tr>
<td>ROC AUC</td>
<td>0.96</td>
<td>0.91</td>
<td>0.567</td>
<td>0.86</td>
<td>0.72</td>
<td>0.651</td>
</tr>
<tr>
<td>Cut off</td>
<td>14 (pg/l)</td>
<td>25.5 (IU/L)</td>
<td>14398 (pg/ml)</td>
<td>90.1 (µmol/l)</td>
<td>0.58 (ABSU)</td>
<td>0.57 (ABSU)</td>
</tr>
<tr>
<td>LR+</td>
<td>18.9</td>
<td>7</td>
<td>1.5</td>
<td>3.66</td>
<td>2.1</td>
<td>2</td>
</tr>
<tr>
<td>LR−</td>
<td>0.21</td>
<td>0.25</td>
<td>0.67</td>
<td>0.32</td>
<td>0.55</td>
<td>0.42</td>
</tr>
</tbody>
</table>

Table 4 Efficiency of serum TnT, CKMB, MMP9, OxdLDL, IMA and adjusted IMA (AIMA) as marker of cardiac ischemia.

DISCUSSION

ECG and cardiac troponin are the most widely used cardiac markers though ECG can be misleading in 8% of the patients with AMI. Serum proteins markers have become increasingly important to improve the diagnosis of myocardial infarction, in identifying people at risk of having an infarct and in others to predict long term prognosis following MI (12). This study showed significant increase in TnT in patients with AMI, indicating potential relevancy of this
marker for predicting cardiac tissue damage with specificity and sensitivity of 96% and 75.6%, respectively and PPV of 94.4 and positive LR of 18.9 which is in agreement with the findings of Janice et al. (13). The present study proves the fact that CKMB is more sensitive (NPV-80) than TnT which is in concurrence with earlier studies (14,15). Microscopic study of atherosclerotic plaque (both coronary and aortic) in humans had demonstrated excess matrix degrading activity, suggesting that elevated levels of matrix metalloproteinase (MMP) might act as a surrogate marker of atherosclerotic plaque burden. However, elevated levels of MMP had demonstrated no utility in evaluation of acute coronary syndrome patients (ACS) (16). This finding is justified by the present study. However, Benjamin et al showed that MMP-9 was reduced by therapies associated with favorable outcome in atherosclerosis and thus may serve as a surrogate biomarker for treatment efficiency (5). Several studies have demonstrated that oxidative stress markers in plasma are increased in coronary heart disease and in patients with classical risk factors (2). Free radicals cause oxidation of several plasma proteins such as albumin, ceruloplasmin, transferrin. Albumin, which is considered a “sacrificial” antioxidant, via thiol groups, provides tenfold protection in plasma by capturing oxygen radicals. ROS produced due to ischemia, damage the metal binding site on the amino terminus of albumin and lower metal binding capacity leading to increased formation of IMA (4). Although our study presented a significant increase in the serum levels of IMA and AIMA in the MI cases as compared to the controls, there was no significant correlation observed with any of the cardiac markers. However, Chawla et al. (17) reported higher specificity and sensitivity along with a higher positive and negative predictive value for IMA as compared to the CK-MB. Sinha et al. (18) reported that IMA along with either the ECG or TnT measurements, or both, increased the sensitivity for the diagnosis ACS to 90%–95%. IMA increases within minutes of the onset of ischemia and returns to normal within 6–12 hours, is not specific to cardiac tissue, hence several earlier studies have reported contradicting results. Furthermore, our earlier study on MI showed that advanced oxidation protein products (AOPP), predominantly aggregates of albumin damaged by oxidative stress, was significantly elevated in plasma. AOPP also act as inflammatory mediators may serve as prognostic factor of severe forms of cardiovascular diseases (19). LDL is the most vulnerable target of oxidation, and oxidative modification of LDL is a key step in the initiation and progression of atherosclerosis. Oxidized LDL is a pro-inflammatory mediator highly correlated with adverse cardiovascular events. The present study exhibited significant rise in oxidized LDL level in MI cases compared to the controls, the rise significantly correlated with the recognized cardiac markers, TnT and CK-MB. Similar results have been reported in several studies on patients with acute coronary syndromes (20,21).

The study focuses on the role of newer biomolecules as cardiac markers to increase the diagnostic efficiency and to establish the multiple marker approaches towards investigating acute myocardial infarction and for early patient care. It can be concluded that though usefulness of IMA and MMP9 as predictive cardiac markers remain feeble, oxidized LDL may be included in the battery of extended lipid profile as well as in the panel of cardiac markers.

**REFERENCES**

A study of CVD risk assessment in post-menopausal women in comparison with pre-menopausal women with serum lipid profile and hs CRP as risk markers

Yasmeen Fatima,¹ Sreekantha,¹ Ramesh,² Sadananjali,¹ Saba,³ and Pallavi⁴

Department of Biochemistry ¹RIMS, Raichur; ²VIMS, Bellary; ³KBN, Gulbarga; ⁴NMC, Raichur, India.

(Received: March 2016  Accepted: Mar 2016)

Corresponding Author
Yasmeen Fatima. Email: dryasmeenfatima@gmail.com

ABSTRACT

Introduction and Aim: Menopause is the permanent cessation of menstruation. Since the cardio vascular disease is the leading cause of death among post-menopausal women and hs-CRP is an acute phase reac tant involved in the development of atherosclerosis. Thus, addition of hs-CRP to traditional lipid screening improves the ability to accurately predicting global cardiovascular risk among post-menopausal women.

Materials and Methods: Fifty post-menopausal and 50 pre-menopausal women in the reproductive age group were selected in the study. We have measured serum lipid profile [total cholesterol (TC), triglyceride (TG), HDL, LDL, and VLDL] and serum hs CRP level in both the groups. The comparison and correlation of hs-CRP and lipid profile were done using correlation test and the $p$-value less than 0.05 was considered significant. Lipid profile estimated by enzymatic methods and serum hs-CRP by Turbidimetric method.

Results: Study showed statistically significant higher values of serum TC, TG, LDL, VLDL and hs CRP levels in post-menopausal women compared to pre-menopausal women ($p < 0.001$). And statistically significant lower values of HDL in post-menopausal women compared to pre-menopausal women ($p < 0.001$).

Conclusion: Our study showed dyslipidaemia and high hs-CRP in post-menopausal women, justifying that post-menopausal women are at an increased risk for CVD. Thus, addition of hs-CRP to traditional lipid screening improves CVD risk assessment among post-menopausal women. And specific health education is needed for all women to prevent the emerging CVD risk.

Key words: Cardiovascular Disease, HDL, LDL, Post-menopausal, Pre-menopausal

INTRODUCTION

Cardiovascular Disease (CVD) is an emerging dominant chronic disease in the world. At the beginning of the 20th century, CVD accounted for less than 10% of all deaths worldwide. At 21st century, CVD accounts for nearly half of all deaths in the developed world and 25% in the developing world (1). By 2020, it is predicted that CVD will claim 25 million lives annually and that coronary heart disease will surpass infectious disease as the world’s number one cause of death and disability. This will lead to at least one in every three deaths due to CVD in developing countries (2).

Framingham Study suggests that female CVD morbidity rates accelerate more quickly than do those of males after the age of 45 years. Menopause is
defined by The WHO as ‘the permanent cessation of menstruation as a result of the loss of ovarian activity’ (3). Menopause develops as the result of low estrogen and due to the disturbed hormonal cycle of ovulation (4). It occurs at a mean age of 51 years. A woman today will live approximately for about a third of her life beyond the menopause (5).

Many risk factors have been identified as contributory to the development of CVD. Cholesterol as the main culprit in atherosclerosis formation, given the concept of in sudation of lipids as a cause for atherosclerosis (2). Low-Density Lipoprotein (LDL), has been implicated in the development of CVD (5). And HDL protects from atherosclerosis formation by its role in reverse cholesterol transport. As atherosclerosis is a chronic process where inflammation plays an important role not only in triggering but also in promoting atherosclerotic plaque development and complications, C-reactive protein (CRP) has emerged as a novel and potentially clinically useful marker for increased CVD risk (6). It suggests the possibility that subclinical states of atherosclerosis can be associated with an increase in circulating markers of inflammation before acute events occur. Based on data of in vitro studies it has been proposed that CRP is actively contributing to disease progression, and it should be considered as a true risk factor and also as a target for intervention. Studies have also demonstrated a significant association between CRP and future risk of metabolic syndrome, diabetes and hypertension (7). National guidelines for measurement of CRP as a marker of CVD risk have been issued jointly by AHA (American Heart Association) and CDC (Centres for Diseases Control and Prevention). Prospective studies have shown that single hsCRP measurement is a strong predictor of myocardial infarction, stroke, peripheral vascular disease and sudden cardiac death (7). Standard clinical assays of CRP typically have a lower detection limit of 3 to 8 mg/l. Thus, these assays lack sensitivity within the lower normal range. Hence, detection of hsCRP allows a better analysis of CRP distribution within the normal range in general population. Also hsCRP concentrations in healthy humans are neither subjected to diurnal or seasonal variations. hsCRP seems to be a stronger predictor of cardiovascular events than LDL. Further hsCRP testing may also have potential prognostic value among low risk groups (8).

Our study aimed to establish differences Cardiovascular disease risk in post-menopausal women compared to young menstruating women estimating and comparing serum lipid profile and hsCRP in both the groups. This implies that in order to modify risks of CVD in older women, addition of hsCRP testing to traditional lipid screening improves CVD risk prediction and intervention with regard to dyslipidemia should begin early.

MATERIALS AND METHODS

Study was conducted at Raichur Institute of Medical Sciences and Rajiv Gandhi Super Speciality Hospital and Research Centre, Raichur, from September 2014 to September 2015. Women attending outpatient department and those working as house keepers in college were included in the study. The study comprised total 100 women, which included 50 Post-menopausal women who attained menopause at least 1 year before with daily moderate working habits and without any disease and disorder as cases. And 50 healthy pre-menopausal women of reproductive age group having regular periodic menses, with daily moderate working habits and without any disease and disorder as controls. Women with CVD, Hypertension, DM, any systemic disorder, any neoplasia, or any other inflammatory disease, and those who are on exogenous hormones and hypolipidemic drugs were excluded from the study. And also to minimize the effect of lifestyle on lipid profile, smokers, alcoholics, sedentary women and trained athletes or sports women were also excluded from the study.

After 12 hours overnight fasting 6 ml of venous blood samples were collected from both the groups and the samples were centrifuged for the estimation of serum total cholesterol (TC) and serum triglycerides (TG), HDL and high sensitive C-reactive protein (hs CRP). TC level estimated by CHOD-PAP method (9), TG level by GPO Trinder method (10),
HDL level by phosphotungstic acid method (11). And LDL, VLDL values were calculated by applying Friedwald’s equation (8).

\[ \text{VLDL} = \frac{\text{TG}}{5} \text{ and } \text{LDL} = \text{TC} - (\text{VLDL} + \text{HDL}) \]

hs CRP estimated by antigen antibody reaction by the End Point Method (12).

Statistical analysis

The results of all profiles TC, TG, LDL, VLDL, HDL, and hsCRP of 100 samples were expressed as mean ± SD. Comparison between both the groups done by student unpaired t – test. One way analysis of variance (ANOVA) was used to compare mean values in the two groups. Pearson’s correlation was applied to correlate between the parameters. p-Value of less than 0.05 was considered significant. And p-value less than 0.001 was considered highly significant. Data was analysed using Microsoft excel, SPSS 22 version.

RESULTS

Showing-Positive correlation of hs CRP with LDL and negative correlation with HDL in pre-menopausal women.

Showing-Positive correlation of hsCRP with LDL and negative correlation with HDL in post-menopausal women.

DISCUSSION

As CVD is the leading cause of death among the post-menopausal women. We have done this comparative study of serum lipid profile and hs CRP as markers of CVD risk in both pre and post-menopausal women, so that the early intervention can be taken by educating them. Atherosclerosis is a chronic process with an active and ongoing inflammatory component. CRP is a marker of inflammation which can be used to predict the risk of CVD. Detection of hsCRP allows a better analysis of CRP distribution within the normal range in the general population as hsCRP concentrations in healthy humans are neither subjected to diurnal or seasonal variations.
24 years. Gordon et al. noted an increase in incidence and severity of CAD after menopause. The incidences of CAD in postmenopausal women were more than double than those in pre-menopausal women (14).

In our study the TC, TG, LDL, and VLDL levels are increased in postmenopausal women than in premenopausal women and is statistically highly significant (p < 0.001). These findings are in accordance with the studies done by (3,5,13,15–17). TC is an independent risk factor for CVD and Razay et al. (18) showed that 1%, increase in TC is associated with at least 2% increase in the incidence of CAD. They also showed that TC was 19% higher in postmenopausal women compared to premenopausal women. TC level includes levels of HDL, LDL and VLDL. After menopause plasma level of LDL and VLDL increases, but plasma level of HDL decreases so the increase in plasma LDL and VLDL levels is more than decrease in HDL level. Hence the net effect is that plasma level of TC increases after menopause (16). According to the study done by Razay et al., in the postmenopausal women, TG was higher by 31% when compared to premenopausal women.

Circulating estrogen is a regulator of lipoprotein lipase, it catalyses the hydrolysis of VLDL to form IDL and later LDL. Hepatic triglyceride lipase hydrolysates the TG of IDL to produce LDL. After menopause due to estrogen deficiency, there will be increased plasma lipoprotein lipase and hepatic TG lipase activity causing plasma LDL accumulation and also leads to down-regulation of LDL receptors (19), as estrogen stimulates the synthesis of LDL receptors (16).

From our study it is evident that pre-menopausal women are having high HDL than postmenopausal women and is statistically highly significant (p < 0.001). These findings are in accordance with many studies (3,5,13,15–17). Due to estrogen deficiency, postmenopausal women have highest activity of hepatic lipase and enhances the uptake and catabolism of HDL thus decreasing plasma HDL concentration (20). Plasma LCAT activity is greater in the postmenopausal women suggesting that, cholesterol esterification is accelerated in plasma HDL (21). In postmenopausal women there is increased LDL accumulation, so more and more HDL gets esterified. Hence they have lower HDL compared to premenopausal women. For every 10 mg/dl increase in HDL, there is a corresponding 50% decrease in CAD risk (22). So HDL is an independent and better
predictor of CAD risk in women (23).

According to our study the hsCRP values are significantly higher in post-menopausal women 1.68 ± 0.36 mg/l than pre-menopausal women. 0.73 ± 0.24 mg/l. These findings are similar to studies done by Shende et al. (13) and Suguna and Jayarajan (24). Joint guidelines from the Centers for Disease Control and Prevention (CDC) and the American Heart Association (AHA) named hs-CRP as the inflammatory marker of choice to assess CVD risk. The guidelines support the use of hs-CRP in primary prevention and set cut off points according to relative risk categories: low risk (<1.0 mg/l), average risk (1.0–3.0 mg/l), and high risk (>3.0 mg/l) (25).

According to our study post-menopausal women are having increased risk for CVD. Study done by Suguna and Jayarajan the hs CRP levels were not statistically increased among the post-menopausal women compared to pre-menopausal women. However the frequency of elevated hsCRP levels (>1.0 mg/dl) in post-menopausal women was 4.33 times greater than in Pre-menopausal women.

Plasma CRP levels reflect the amount and activity of pro-inflammatory cytokines such as TNF-α, IL-1 and IL-6 which are involved in the process of atherosclerotic plaque formation. CRP synthesis in the liver is largely under the control of IL-6 which is secreted from adipose tissue (13). During menopause changes in the concentration of sex hormones, influence levels of hsCRP, which binds to oxidized LDL, causing increase expression of adhesion molecules enhancing the atherogenicity of LDL (24).

In a study on healthy men by Ridker et al., it was observed that evaluation of CRP added to the predictive value of lipids on risk of first MI. It was seen that patients with highest tertile of both TC and CRP had relative risk of first MI 5.3 times more than that of individuals in lowest tertile of both parameters (26). In our study also both TC and hs CRP are significantly increased.

We observed significant positive correlation between hs CRP with LDL and the negative correlation between hs CRP with HDL level. This suggests that unfavourable lipid profile may facilitate the formation of foam cells in arterial wall increasing the inflammatory activity. Ridker et al. in their study did a comparison of LDL-cholesterol and CRP among apparently healthy women. They found minimal correlation ($r = 0.08$) between LDL cholesterol and CRP (27), suggesting that each level predicted risk in different groups (28).

According to the present study post-menopausal women are having high risk for CVD risk and serum hsCRP along with lipid profile should be used as periodic screening for CVD risk assessment.

**CONCLUSION**

The current study showed significant dyslipidemia and increased hsCRP levels in post-menopausal women compared to pre-menopausal women clearly indicating that post-menopausal women are at increased risk for CVD. Addition of hsCRP testing to traditional lipid screening improves CVD risk prediction. And it should be considered as a routine biochemical parameter in order to prevent the occurrence of CVD and to prolong life in postmenopausal women. And also intervention should begin in premenopausal state which include specific health education, regular exercise, healthy diet, periodic screening of lipid profile and hs CRP, stress free life.

**REFERENCES**

5. Kilim, S.R., and Chandala, S.R. A Comparative Study of Lipid Profile and Oestradiol in Pre-
26. Ridker, P.M., Glynn, R.J., and Hennekens, C.M. C-reactive protein adds to the predictive value of total and HDL cholesterol in determining


Assessment of serum leptin levels and lipid profile in hypertensive obese cases

Keshavamurthy H.R.¹ and Sunitha S.²

¹Department of Biochemistry, Pariyaram Medical College, Kerala University of Health Sciences, Thrissur, Kerala, India.
²Department of Biochemistry, Kempegowda Institute of Medical Sciences, Rajiv Gandhi University of Health Sciences, Bangalore, Karnataka, India.

(Received: Dec 2016  Accepted: Mar 2016)

Corresponding Author
Dr. Keshavamurthy H.R. Email: keshavamurthy.calmness@gmail.com

ABSTRACT

Introduction and Aim: Obesity is associated with an increased risk of morbidity and mortality as well as reduced life expectancy. It is well known that obesity is related to hypertension through mechanisms such as sympathetic overactivity and excess renal sodium reabsorption. Obesity and hypertension are also linked by leptin, a peptide produced by the adipocytes that is elevated in obese individuals. The purpose of the study was to estimate and compare Serum Leptin levels and Lipid profile in Hypertensive obese and Normotensive obese subjects.

Materials and Methods: The study was carried out in 45 Hypertensive obese cases, in the age group of 30–60 years and 45 age and gender matched Normotensive obese controls. Fasting blood samples were collected. Serum lipid profile and Leptin were estimated by enzymatic and ELISA methods respectively.

Results: The obese Hypertensive group showed significantly higher values of Leptin ($p < 0.05$), total cholesterol (TC) ($p < 0.05$), TC/HDL ratio ($p < 0.05$), LDL/HDL ratio ($p < 0.05$), SBP ($p < 0.05$) and DBP ($p < 0.05$). The obese Hypertensive group also showed significantly lower values of HDL ($p < 0.05$). Leptin showed a positive significant correlation with BMI in the obese Hypertensive group.

Conclusion: All the above derangements confirm that Leptin correlates with Hypertension and the mechanisms for development of selective Leptin resistance seem to be the main leading cause for the development of obesity related hypertension. And the dyslipidemia along with obesity and hypertension places the patient at a higher risk of metabolic syndrome.

Key words: Hypertension, Leptin, Lipid Profile, Obesity

INTRODUCTION

Obesity has reached epidemic proportions in India in the 21st century with morbid obesity affecting 5% of the country’s population. Obese patients are more prone to develop diseases such as Hypertension, Type II Diabetes, Hyperlipidemia, Cholelithiasis, Arteriosclerosis, cardio-vascular and cerebrovascular diseases, certain type of cancers and osteoarthritis. Hypertension and obesity are common risk factors for the development of cardiovascular diseases, along with diabetes and hyperlipidemia. Almost half of the hypertensive patients are obese and the prevalence of hypertension in
obese individuals is two times higher than in general population (1).

Leptin, an adipocyte derived hormone regulates appetite and enhances energy expenditure by activating sympathetic nerve activity to thermogenic brown adipose tissue (2). However in common obesity leptin loses the ability to inhibit energy intake and increase energy expenditure (3).

Leptin is a possible mediator of obesity related hypertension since it acts in the hypothalamus by stimulating sympathetic nervous system (SNS) centrally, which indirectly causes vasoconstriction and excess renal sodium absorption (4). The purpose of our study was to estimate and compare serum Leptin levels and lipid profile in Hypertensive obese and Normotensive obese subjects.

MATERIALS AND METHODS

The study was carried out on 45 hypertensive obese cases and 45 age and sex matched normotensive obese cases in the age group of 30–60 years. The study was conducted at Kempegowda institute of Medical sciences and hospital. The diagnosis of Obesity was fulfilled as per WHO criteria and the diagnosis of Hypertension was fulfilled as per JNC-7th report. Patients with Diabetes mellitus, endocrinal disorders, family history of hyperlipidemia, cardiovascular disease, patients taking systemic drugs especially lipid lowering agents, smoking, alcohol users and other conditions which may alter lipid profile were excluded from the study. The institutional ethical committee approved the study protocol. Informed consent was obtained from all the participants.

A pre-structured and pre-tested proforma was used to collect the data. Baseline data including age, BMI, detailed medical history, clinical examinations and relevant investigations were included as part of the methodology. Serum Leptin and serum lipid profile were measured in all participants from morning blood samples collected after 12 hours of fasting. Serum Leptin was measured by sandwich ELISA method (Diagnostic Biochem Canada Inc. Cat. No. CAN-L-4260; Version; 8.1; August 2009). Serum Cholesterol was estimated by enzymatic, colorimetric method. Serum triglycerides was estimated by enzymatic colorimetric test. Serum HDL-cholesterol was estimated by homogenous enzymatic colorimetric test. Serum VLDL cholesterol was calculated according to the formula VLDL = TG/5. Serum LDL-cholesterol estimated by homogenous enzymatic colorimetric assay. TC/HDL and LDL/HDL ratios were determined. Body mass index (BMI) was calculated as the ratio of weight (kg) to height squared (m²).

Statistical analysis

Null hypothesis. There is no significant difference in the mean value of parameter between the two groups, that is, mean1 = mean 2.

Alternate hypothesis. There is significant difference in the mean value of parameter between the two groups, that is, mean ≠ mean 2.

Level of significance. α 0.05

SPSS software version 13.0 was used for statistical analysis.

Statistical test used. t-Test and Mann-Whitney test were both used. Correlation analysis between Leptin, BMI and lipid profile were done using Pearson’s rank correlation.

Decision criterion. If p < 0.05, we reject the null hypothesis and accept the alternate hypothesis.

Results

Among the total sample of 90, 16 males (36%) and 29 females (64%) were hypertensive obese cases (Table 1 and Fig. 1). The age ranged from 30 to 60 years and the maximum number of cases were in the age group of 31–39 years but the mean of age between the cases (41.64 ± 8.25) and controls (42.00 ± 7.69) was not statistically significant.

<table>
<thead>
<tr>
<th>Gender</th>
<th>Cases</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>%</td>
</tr>
<tr>
<td>Male</td>
<td>16</td>
<td>36</td>
</tr>
<tr>
<td>Female</td>
<td>29</td>
<td>64</td>
</tr>
<tr>
<td>Total</td>
<td>45</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 1 Gender distribution in the two Table groups
The mean of BMI of the case and controls groups were 33.94 ± 2.99 and 32.62 ± 2.16, respectively which was not found to be statistically significant \((p = 0.051)\) (Table 2 and Fig. 2).

The mean TC levels in the cases and controls were 213.07 ± 43.58 and 191.64 ± 33.30, respectively, which was found to be statistically significant \((p = 0.013)\). The mean TC/HDL ratio in the cases and controls were 5.97 ± 1.45 and 4.71 ± 1.10, respectively which was found to be statistically significant \((p < 0.001)\). There was no significant difference between the case and control group with respect to TG, LDL-C, VLDL-C.

The mean HDL-C levels in the cases and controls were 36.29 ± 4.28 and 41.44 ± 4.53 respectively, which was found to be statistically significant \((p < 0.001)\). The mean LDL/HDL ratio in the cases and controls were 3.96 ± 1.37 and 3.14 ± 0.95, respectively, which was found to be statistically significant \((p = 0.003)\).

Higher mean Leptin was recorded in cases compared to controls and the difference in mean Leptin between the two groups is statistically significant \((p = 0.001)\) (Table 2 and Fig. 2). A significant positive correlation between Leptin levels with BMI \((\rho = 0.231, p = 0.029)\), TG \((\rho = 0.347, p = 0.001)\) and negative correlation with HDL \((\rho = 0.318, p = 0.002)\) has been shown in Table 4.

### Table 2 Comparison of Biochemical results of study subjects

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Case Mean ± SD</th>
<th>Control Mean ± SD</th>
<th>(p)-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI</td>
<td>33.94 ± 2.99</td>
<td>32.62 ± 2.16</td>
<td>0.051</td>
</tr>
<tr>
<td>TC</td>
<td>213.07 ± 43.58</td>
<td>191.64 ± 33.30</td>
<td>0.013*</td>
</tr>
<tr>
<td>TG</td>
<td>175.07 ± 81.06</td>
<td>160.00 ± 74.23</td>
<td>0.337</td>
</tr>
<tr>
<td>LDL</td>
<td>140.84 ± 44.30</td>
<td>127.40 ± 32.47</td>
<td>0.196</td>
</tr>
<tr>
<td>VLDL</td>
<td>38.42 ± 13.55</td>
<td>35.47 ± 6.61</td>
<td>0.591</td>
</tr>
<tr>
<td>HDL</td>
<td>36.29 ± 4.28</td>
<td>41.44 ± 4.53</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>TC/HDL</td>
<td>5.97 ± 1.45</td>
<td>4.71 ± 1.10</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>LDL/HDL</td>
<td>3.96 ± 1.37</td>
<td>3.14 ± 0.95</td>
<td>0.003*</td>
</tr>
<tr>
<td>LEPTIN</td>
<td>46.83 ± 16.31</td>
<td>36.11 ± 12.41</td>
<td>0.001*</td>
</tr>
</tbody>
</table>

* Denotes significant difference.

### Table 4 Correlation between Leptin and other parameters (Pearson’s Rank Correlation)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>(p)</th>
<th>(p)-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI</td>
<td>0.231</td>
<td>0.029*</td>
</tr>
<tr>
<td>TC</td>
<td>0.078</td>
<td>0.468</td>
</tr>
<tr>
<td>TG</td>
<td>0.347</td>
<td>0.001*</td>
</tr>
<tr>
<td>LDL</td>
<td>0.085</td>
<td>0.424</td>
</tr>
<tr>
<td>HDL</td>
<td>−0.318</td>
<td>0.002*</td>
</tr>
<tr>
<td>VLDL</td>
<td>−0.114</td>
<td>0.286</td>
</tr>
<tr>
<td>TC/HDL</td>
<td>0.180</td>
<td>0.090</td>
</tr>
<tr>
<td>LDL/HDL</td>
<td>0.188</td>
<td>0.076</td>
</tr>
</tbody>
</table>

* Denotes significant correlation.

### Discussion

Since Leptin is an adipose tissue-derived hormone, it is possible that its association with BP is partly explained by obesity and weight gain, one of the well-known risk factors for hypertension (5). The results from earlier studies showed strong correlation between leptin and hypertension in humans suggesting that leptin has a significant role in the pathophysiology of obesity induced hypertension, but some studies were not able to confirm this association (6). As previous studies revealed that increased leptin levels combined with decreased...
NO production and enhanced sympathetic activity may contribute to blood pressure elevation in the obese (7). In this study the difference in mean Leptin between the obese hypertensive and obese normotensive is statistically significant. A significant positive correlation of leptin with BMI is present. This was consistent with the study by Vedrana et al. (1) who showed plasma leptin levels were significantly higher in hypertensive obese patients than in normotensive obese patients in both genders. Duanduan et al. (8), Caroline et al. (9), Costas et al. (10), Kawaiji et al. (11), also showed that plasma leptin levels were significantly higher in hypertensive patients than the normotensive patients. This study was in contrast with Freddy et al. (4) who showed that plasma leptin levels were not significantly higher in hypertensive patients compared to normotensive patients.

Leptin has no effect on blood pressure in healthy eutrophic subjects because it has pressor and depressor effects that are in constant balance in healthy individuals (12). In obese hypertensives the balance between those pressor and depressor mechanisms is damaged. The possible explanation is selective leptin resistance (13) where only pressor effects take place and depressor effects of leptin are lost. The mechanism of selective leptin resistance has still not been identified, but theories involve intracellular signaling disruption and saturable transport across blood-brain barrier (14). Due to selective leptin resistance, the preserved sympathoactivation causes pressor effects in the kidneys and blood vessels, thus elevating blood pressure and leading to further cardiovascular complications.

It should be noted that some of the hypertensive patients in our study were treated with antihypertensive medications. Previous studies suggest that antihypertensive agents have metabolic effects beside blood pressure lowering such as increasing level of adiponectin and decreasing leptin. Therefore, the difference between plasma leptin levels in hypertensive and normotensive patients might have been greater if there was no influence of antihypertensive medication (15). Central or abdominal obesity is typical for males and acting through many CHD factors, including disturbances in plasma glucose, insulin, total cholesterol, low-density lipoprotein cholesterol and apolipoproteins A-I and B, can influence the development of atherosclerosis (16). Intraabdominal fat may lower HDL levels by increasing the fractional catabolic rate of high density lipoprotein particles containing apo A-I, suggesting a mechanism, by which central adiposity may be proatherogenic (17).

There was a female preponderance in this study with 36% of the males and 64% of the females were obese hypertensives. In our study it is also shown that the mean BMI is higher in cases than in controls and the difference between them is not statistically significant. This was consistent with the study by Pooja et al. (18). Obesity and dyslipidemia are commonly associated with a recognized risk for the development of Metabolic syndrome. Metabolic syndrome is a complex of interrelated risk factors for cardiovascular disease and diabetes mellitus. These factors include raised blood pressure, elevated glucose, cholesterol and Triglyceride levels and low HDL-C (19). Hypertension is recognized globally as a major public health problem. It is also a well-known risk factor for coronary heart disease, type 2 diabetes mellitus and renal diseases (20). In our study the mean total cholesterol, TC/HDL ratio and LDL/HDL ratio was higher in cases than in controls and the difference between them was found to be statistically significant and this was consistent with the study by Bhatti et al. (21). The mean HDL is lower in cases than in controls and the association is statistically significant and this was also consistent with Bhatti et al. This study did not show a statistically significant difference between cases and controls regarding Triglycerides (TG), LDL-C and VLDL-C and this was consistent with the study by Szczygielska et al. (22).

The parameters such as Leptin, Total Cholesterol, TC/HDL, LDL/HDL were significantly elevated in the study group and HDL was significantly lower. All the above derangements confirm that Leptin correlates with Hypertension and the mechanisms for development of selective Leptin resistance seem to be the main leading cause for the devel-
development of obesity related hypertension. And the dyslipidemia along with obesity and hypertension places the patient at a higher risk of metabolic syndrome. Thus follow-up of these patients with regard to the development of diseases associated with atherosclerosis may be beneficial.

Because our study consisted of a limited number of cases and controls from a single population, further studies with large number of cases will be beneficial in elucidating the relationship between Leptin, obesity, Hypertension, Lipid profile and the atherosclerotic risk factors.

ACKNOWLEDGMENTS

I owe my thanks to Dr. C. Yogitha, Associate Professor, Department of Medicine, Kempegowda Institute of Medical Sciences, Bangalore, for her expert guidance and warm support. I heartily acknowledge Dr. B.V. Ravi, Professor and Head, Department of Biochemistry, Kempegowda Institute of Medical Sciences, Bangalore for his suggestions and support in successful compilation of this work. My sincere thanks to Mr. Tejasvi T.V, who helped me in the statistical analysis of this study and gave it to me on time.

REFERENCES


Urea reduction rate as dialysis adequacy indicator and serum albumin as mortality indicator in hemodialysis patients

Shanthala D.,¹ Indumati V.,¹ Krishnaswamy D.,¹ Vijay Rajeshwari V.,¹ Ramesh,¹ and Shilpa A¹

¹Department of Biochemistry, Vijaynagar Institute of Medical Sciences, Ballari, Karnataka, India.

(Received: Dec 2016  Accepted: Mar 2016)

Corresponding Author
Shanthala D. Email: shanthala.ravikumar@yahoo.com

ABSTRACT

Introduction and Aim: Hemodialysis (HD) provides renal replacement therapy for those with end-stage kidney disease (ESKD). Urea reduction rate (URR) is used as a marker of dialysis adequacy. Hypoalbuminemia is a major risk factor for mortality and morbidity in ESKD patients. Recent studies have shown that both URR and serum albumin levels are predictors of mortality in HD patients. Hence present study was undertaken to assess the achievement of URR as dialysis adequacy predictor and to assess whether serum albumin levels predicts mortality in ESKD patients on HD.

Materials and Methods: We analyzed Blood Urea levels by Urease method, in pre-dialysis and post-dialysis blood samples of 35 patients who were on HD. URR was calculated by \((\text{predialysis urea} - \text{postdialysis urea}) \div \text{predialysis urea}\) and it was expressed in %. Serum albumin was estimated in post-dialysis blood sample by Biuret method. The patients were followed up for a period of 1 year and any mortalities during this period were noted. Data analysis was done by using SPSS, version 17.

Results: There was statistically significant decreased in post dialysis blood urea levels (42.88 ± 9.88 mg/dl) when compared to pre-dialysis blood urea levels (117.91 ± 26.83 mg/dl) with \(p\)-value of <0.001. URR was found to be 63.65% ± 3.35%. The mean serum albumin of HD patients was 2.95 ± 0.35 g/dl. There was no significant change in serum albumin levels in HD patients who died during the course of the study.

Conclusion: The National kidney foundation—kidney disease outcome quality initiative (NKF-K/DOQI) target of the mean urea reduction rate (URR) was almost achieved, thus suggesting the adequacy of the dialysis. Though mean serum albumin is decreased to 2.9 g/dl (<3.5 g/dl), in our study it was not associated with increased mortality in HD patients.

Key words: Chronic Kidney Disease, Hemodialysis, NKF-K/DOQI, URR

INTRODUCTION

Dialysis provides renal replacement therapy for those with end-stage renal disease (ESRD). Dialysis provides removal of unwanted solutes, equilibration of desired solutes and also helps in fluid management. The provision of dialysis requires some assessment of whether the delivered dose is adequate for the patient or not (1).
A number of studies have demonstrated a correlation between the delivered dose of the haemodialysis and the patient mortality and morbidity (2–6). These studies have demonstrated that the mortality among ESRD patients was lower when sufficient hemodialysis (HD) treatments were provided. The clinical signs and symptoms alone were not the reliable indicators of the HD adequacy (7).

Adequacy of dialysis can be assessed in several ways. The most common acceptable methods are—formal urea – kinetic kt/v, urea reduction rate (URR), natural log kt/v and the Daugirdas second generation formula (1). The URR was first popularized by Lowrie and Lew in 1991 as a method of measuring amount of dialysis that correlated with patient outcome (2).

Of the three methods, that is, urea kinetic modelling, kt/v and URR, which are considered appropriate by National kidney Foundation—Kidney Disease Quality Initiative (NKF-K DOQI) for measuring the delivered dose of HD, URR is the simplest and most commonly used parameter to express dialysis dose (8). Urea is a small, readily dialyzed solute which is the bulk catabolite of the dietary protein that constitutes 90% of the waste nitrogen accumulated in body. Although urea is not only the uremic toxin, all current indices of dialysis dose are based on urea measurements, and thus set urea removal as the major goal of haemodialysis (1). The NKF-KDOQI guidelines recommend that the adequacy of the dialysis dose should be measured routinely, typically on a monthly basis with a target of >3.5 g/dl. The guidelines focused on the inverse association between the serum albumin and mortality (9).

Hence the present study was undertaken,
1. To estimate URR and Serum albumin in HD patients.
2. To assess the achievement of URR of 65% as dialysis adequacy predictor as per NKF-K/DOQI guidelines.
3. To assess whether Serum albumin levels predict mortality in CKD patients on HD.

Materials and Methods
Our study included 35 CKD patients who were on HD. They were randomly selected from HD unit of Nephrology, VIMS, Ballari, Karnataka, India. The study was approved by the Institutional ethical committee. An informed consent was obtained from all the 35 patients.

Inclusion criteria
CKD patients on HD aged between 25 and 60 years of age, who were on regular (three times per week) HD sessions.

Exclusion criteria
Liver diseases, chronic alcoholics, malignancies, congenital renal disorders.

Dialysis prescription
All patients were hemodialysed using Fresenius Medical Care (4008S) hemodialyzer machine through permanent arteriovenous fistulas. They were dialyzed with polysulphone low flux dialyzer having
a blood flow rate of 180–200 ml/min and dialysate flow rate of 200–400 ml/min for 3–4 hours. It was performed thrice a week.

**Laboratory Analysis**

Three milliliters of blood sample was collected before initiation and after termination of haemodialysis during midweek HD session, in each month for three consecutive months. Blood urea levels were measured in all the samples using Glutamate dehydrogenase -Urease method and the mean of pre-dialysis and post-dialysis urea levels were calculated. The other parameters measured were Blood glucose by glucose oxidase and peroxidase method, Serum Creatinine by modified Jaffé’s method in pre-dialysis blood sample and Serum albumin by Bromocresol Green method in post-dialysis blood sample.

URR was calculated by the following formula: (pre dialysis urea − post dialysis urea) divided by pre-dialysis urea and it was expressed in percentage. The patients were followed up for a period of 1 year and any mortality during this period was noted. Statistical analysis was performed using SPSS, version 17 using paired two-tailed Student’s t-test for Urea.

Data are expressed as mean ± standard deviation (SD). A p-value of <0.05 was considered statistically significant. The patients were followed up for a period of 1 year and any mortalities during this period were noted.

**Results**

Among the 35 CKD Patients in our study, 3 were diabetic nephropathy, 4 were hypertensive nephropathy and 28 patients were non diabetic CKD. The mean age of the CKD patients in the study was 38.1 ± 12.5 years. Among the 35 patients, 26 (74.6%) were males and 9 (25.6%) were females.

There was statistically significant decrease in post-dialysis blood urea levels (42.88 ± 9.88 mg/dl) when compared to pre-dialysis blood Urea levels (117.91 ± 26.83 mg/dl) with a p-value of <0.001 (Table 1 and Fig. 1).

The Urea reduction rate (URR) in 35 patients was 63.65% ± 3.35%. The mean Serum Albumin levels in HD patients were 2.95 ± 0.35 g/dl. The serum albumin levels in different chronic kidney diseases are shown in Table 2 and Fig. 2.

The mean blood glucose levels were 105.32 ± 17.61 mg/dl, mean Serum Creatinine levels were 8.70 ± 2.4 mg/dl and mean Serum protein levels were 5.75 ± 0.66 g/dl in the pre-dialysis blood sample. During the follow up period of 1 year, three patients died with a mortality rate of 8.5%.

**Discussion**

The National Cooperative Dialysis Study (NCDS)
identified the dose of dialysis delivery as a factor affecting patient morbidity and mortality, and this led to guidelines for prescribing dialysis dose (14). A number of studies have identified that the URR and Serum albumin concentration as powerful predictors of mortality in patients on HD (15).

In our study URR was 63.65% ± 3.35% with a range of 60.3–67%. This shows that URR was almost achieved as per the recommended guideline of NKF-K/DOQI to suggest the adequacy of the dialysis dose. Similar to our study Sunanda et al. (16) reported a URR of 66.4%. Brimble et al. (17) in one study reported URR of 72% and another study (18) by the same another reported URR of 67.1%. Owen et al. (19) reported that a URR of 55–59% has relative risk of 1.28 compared to a URR of 65–69%.

Our study showed that the mean Serum albumin levels were 2.95 ± 0.35 g/dl and it did not reach the target levels (3.5 g/dl) of NKF-K/DOQI. In contrast, Colling et al. (4) reported that patients with Serum albumin levels of less than 3.5 g/dl have a relative mortality risk of 3.13, compared to albumin levels of 4.0 g/dl.

In our study though there was a positive correlation between URR and Serum albumin levels with a strength of 0.08, it was not statistically significant, indicating that Serum albumin cannot be used as a mortality marker. The mortality rate was only 8.5% in our study, though the serum albumin levels (2.95 ± 0.35 g/dl) were less than the target levels of 3.5 g/dl. There was also no significant difference in serum albumin levels in the three patients who died when compared to other patients.

Hertel et al. (15) concluded that high URR and Serum albumin levels are associated with improved mortality statistics in the HD patients. The low albumin levels that are encountered in incident dialysis patients are likely a consequence of a combination of protein-calorie malnutrition, inflammation and plasma volume expansion (20).

**CONCLUSION**

The NKF-KDOQI target of the mean Urea reduction rate (URR) was almost achieved in our study, suggesting the adequacy of the dialysis dose. Though the mean Serum albumin levels were 2.95 ± 0.35 g/dl (less than the target of 3.5 g/dl) in our study, it was not associated with increased mortality in HD patients.
REFERENCES


Study of oxidative stress and apolipoprotein A-I in reduction of reverse cholesterol transport in type 2 diabetes mellitus

Suman Doddamani,¹ Shashikant Nikam,¹ Padmaja Nikam,¹ and Vishwanath Patil²

¹Department of Biochemistry, Belgaum Institute of Medical Sciences, Belagavi, Karnataka, India.
²Department of Medicine, Karnataka Institute of Medical Sciences, Hubli, Karnataka, India.

(Received: Jan 2016 Accepted: Mar 2016)

Corresponding Author
Shashikant Nikam. Email: nikam31@gmail.com

ABSTRACT

Introduction and Aim: Diabetes Mellitus (DM) refers to a group of common metabolic disorders that share the phenotype of hyperglycemia. Oxidative stress plays an important role in the development and progression of diabetes and its complications. The present study has been undertaken to evaluate the effect of oxidative stress on apolipoprotein A-I in newly detected type 2 DM. 200 participants were enrolled in the study.

Methods: 100 with newly detected type 2 DM were taken as study group and 100 age and sex matched healthy participants were taken as control group. Biochemical parameters such as serum Malondialdehyde (MDA), Superoxide dismutase (SOD), Apolipoprotein A-I, Total cholesterol, Triglyceride, HDL, LDL, VLDL and erythrocyte reduced Glutathione levels were analyzed in all the participants.

Results: The patients with type 2 DM showed increased oxidative stress and decreased apolipoprotein A-I and HDL levels.

Conclusion: Hence the present study concludes that increased oxidative stress and glycated apolipoprotein A-I might be responsible for the reduction of reverse cholesterol transport (RCT), which may lead to increased incidence of atherosclerosis and cardiovascular complications in type 2 Diabetes Mellitus.

Key words: Oxidative stress, Apolipoprotein A-I, RCT, Diabetes Mellitus.

INTRODUCTION

Diabetes Mellitus refers to a group of common metabolic disorders that share the phenotype of hyperglycemia (1). Oxidative stress is a condition in which there is either increased rate of free radical production or there is impairment in antioxidant mechanisms (2). Increased oxidative stress is one of the etiologies for the development and progression of Diabetes and its complications (3). Atherosclerosis is one of the major complications associated with type 2 Diabetes. 50% of diabetic patients’ deaths occur due to cardiovascular disease (4). Low HDL is a strong risk factor for the development of atherosclerosis. The cardioprotective role of HDL is related to its role in Reverse Cholesterol Transport (RCT). Apolipoprotein A-I is the most abundant protein in HDL, the concentration of apo A-I is known to be inversely correlated with cardiovascular risk (5). HDL associated apo A-I play a crucial role in cholesterol homeostasis by regulating reverse cholesterol transport delivering it to the liver (5).
The present study was undertaken to study the role of oxidative stress and apolipoprotein A-I in reduction of reverse cholesterol transport in type 2 DM.

**MATERIALS AND METHODS**

The study group was comprised of 100 newly detected type 2 diabetic patients in the age group of 30–60 years visiting medicine Out Patient Department of BIMS (Belgaum Institute of Medical Sciences) Hospital, Belgaum. The diagnosis of Diabetes Mellitus was done by senior physician. The diagnosis of type 2 DM was confirmed by measuring fasting blood glucose (>126 mg/dl) and 2 hour OGTT (>200 mg/dl) values on two occasions as per American Diabetic Association’s revised criteria. 100 age and sex matched healthy participants were taken as control group. The study was conducted from December 2011 to May 2013 at department of Biochemistry, BIMS, Belagavi. All authors hereby declare that the experiments have been examined and approved by institutional ethical committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 declaration of Helsinki.

After obtaining informed written consent, 10 ml of 12 hours fasting venous blood sample was collected from diabetic patients and the control participants under all aseptic conditions. The blood samples were used for measuring various parameters. Estimation of apolipoprotein A-I was done by measurement of antigen-antibody reaction by the end-point method using immunoturbidimetry (6). HDL cholesterol (6) and total cholesterol was measured by CHOD-POD method (6). Triglyceride estimation was done by GPO-PAP method (6). VLDL and LDL cholesterol was calculated by formula (6). Fasting blood glucose was measured by Glucose Oxidase Peroxidase method (7). Serum MDA levels were measured by method of Wilbur et al. (8). Serum SOD was measured by Marklund and Marklund method (9). Erythrocyte reduced glutathione (GSH) measurement was done by method of Beutler et al. (10).

**Exclusion criteria**

Patients on hypolipidemic drugs, antioxidant supplements, steroids and oral contraceptives were excluded. Known cases of hypothyroidism, hyperthyroidism, Cushing’s syndrome, kidney diseases, hepatic diseases, alcoholics, smokers, tobacco chewers and patients with Type 1 Diabetes Mellitus were also excluded from the study.

**Limitations**

The duration of diabetes before the formal diagnosis was unknown.

**Statistical analysis**

The results are expressed as mean ± SD. The results are further subjected to Student’s *t*-test, differences between means are considered significant at *p* < 0.05.

**RESULTS**

Study found that serum MDA, LDL, VLDL, total cholesterol, triglycerides were significantly increased in type 2 DM when compared to control group. Erythrocyte reduced glutathione, serum apolipoprotein A-I, SOD and HDL were significantly decreased in type 2 DM when compared to control group (Table 1).

**DISCUSSION**

Oxidative stress is a widely accepted participant in the development and progression of diabetes and its complications. Diabetes Mellitus is associated with hyperglycemia which may lead to cellular damage; increased extra vascular matrix production and vascular dysfunction which have been implicated in the pathogenesis of vascular disease in type 2 DM (11, 12). This study shows increased oxidative stress in diabetics when compared with the control group.

Present study found that MDA levels were significantly (*p* < 0.05) increased in type 2 DM when compared to the control group (Table 1). These findings are in agreement with the findings of Mahreen et al. (13) and Ozdemir et al. (14). Higher blood glucose level is associated with free radical mediated lipid peroxidation (15). The peroxidative breakdown of phospholipids might lead to accumulation of MDA in type 2 diabetics.
In healthy individuals, oxidative damage to tissue is prevented by a system of defence called antioxidant enzymes. Mechanisms involved in the increased oxidative stress in diabetes include not only oxygen free radical generation due to nonenzymatic glycation, autooxidation of glycation products, but also changes in the tissue content and activity of antioxidant defense systems (16).

Study found that serum SOD activity and concentration of erythrocyte reduced glutathione was significantly (<0.05) decreased in type 2 DM as compared to the control group (Table 1). These findings are in agreement with Abou-Seif and Youssef (17), and Yoshida et al. (18). Reduced glutathione functions as a free radical scavenger, thus with increased oxidative stress the levels of reduced glutathione may be lowered. In diabetic patients, the autoxidation of glucose results in the formation of hydrogen peroxide which inactivates SOD (19) and this accumulated hydrogen peroxide may be one of the reactive oxygen species responsible for lowered activity of SOD and raised levels of MDA in type 2 diabetic patients. Reduced activity of the antioxidant enzymes may increase the susceptibility of diabetic patients to oxidative injury.

This study also found that the levels of apo A-I were significantly reduced (p < 0.001) in newly detected type 2 Diabetes Mellitus (Table 1) when compared with the control group. Gugliucci et Al. concluded that apo A-I levels are decreased in type 2 DM (4). Calvo et. al. also showed glycation of apo A-I resulting in decreased levels of apo A-I in type 2 DM (20). In type 2 Diabetes Mellitus there is over production of reactive oxygen and this is associated with auto-oxidation of glucose which may result in glycation of apolipoprotein A-I. This glycated apo A-I might be responsible for lowered functional capacity of apo A-I.

The levels of HDL cholesterol were significantly lowered (p < 0.01) in newly detected type 2 Diabetes Mellitus in comparison with the control group. (Table 1) Smith and Lall (21) concluded that HDL cholesterol was significantly lower in diabetic subjects as compared to controls. It has been reported that non-enzymatic glycation of apolipoprotein A-I in diabetics alters the functional ability of HDL (22). Recent studies suggest that glycation of apo A-I adversely affects HDL metabolism (23–25) leading to decreased levels of HDL. Decreased levels of apolipoprotein A-I may lead to reduction of reverse cholesterol transport concluded by Doddamani et al. (26).

Thus the present study indicates that lowered HDL cholesterol might lead to reduction of reverse cholesterol transport, this may result in increased risk of atherosclerosis in type 2 DM.

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Parameters</th>
<th>Newly detected type 2 DM (n = 100)</th>
<th>Controls (n = 100)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Serum MDA (nmol/ml)</td>
<td>9.02 ± 1.36*</td>
<td>5.78 ± 0.83*</td>
</tr>
<tr>
<td>2</td>
<td>Serum SOD (units/ml)</td>
<td>2.75 ± 0.98*</td>
<td>3.77 ± 0.79*</td>
</tr>
<tr>
<td>3</td>
<td>Erythrocyte reduced GSH</td>
<td>4.54 ± 0.32*</td>
<td>6.03 ± 0.95*</td>
</tr>
<tr>
<td></td>
<td>(micro mol/g of Hb)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Apolipoprotein-A-I (mg/dl)</td>
<td>133.10 ± 24.32*</td>
<td>188.72 ± 19.49*</td>
</tr>
<tr>
<td>5</td>
<td>HDL (mg/dl)</td>
<td>33.37 ± 4.44*</td>
<td>48.76 ± 16.84*</td>
</tr>
<tr>
<td>6</td>
<td>LDL (mg/dl)</td>
<td>130.57 ± 36.04*</td>
<td>95.98 ± 39.16*</td>
</tr>
<tr>
<td>7</td>
<td>VLDL (mg/dl)</td>
<td>40.38 ± 17.12*</td>
<td>29.74 ± 19.70*</td>
</tr>
<tr>
<td>8</td>
<td>Total cholesterol (mg/dl)</td>
<td>205.43 ± 35.70*</td>
<td>175.07 ± 39.88*</td>
</tr>
<tr>
<td>9</td>
<td>Triacylglycerol (mg/dl)</td>
<td>227.36 ± 106.01*</td>
<td>155.56 ± 107.22*</td>
</tr>
</tbody>
</table>

Table 1 Biochemical parameters in newly detected Type 2 DM and Control participants.

n = number of participants, p < 0.05, significant.
CONCLUSION

Present study concludes that increased oxidative stress and glycated apolipoprotein A-I might cause lowered HDL levels leading to reduction of reverse cholesterol transport which may lead to increased incidence of cardiovascular complications in type 2 DM.

ACKNOWLEDGMENTS

Authors are thankful to the Director, BIMS, Belagavi, for funding the project and to all teaching and non-teaching staff of Biochemistry, General Medicine and Statistics department for their help.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this manuscript.

REFERENCES


Evaluation of serum high molecular weight adiponectin and lipid profiles in predicting the risk of coronary artery disease among CAD patients

Chitra Devi M.,¹ Chandra Sekhar M.,² and Sivasubramaniam P.³

¹Government Dharmapuri Medical College, Dharmapuri, India.
²Meenakshi Medical College & Research Institute, Enathur, Kanchipuram, India.
³Cardiac Care Centre, Dharmapuri, India.

(Received: Feb 2016  Accepted: Mar 2016)

Corresponding Author
Chitra Devi. M. Email: chitrakmc@gmail.com

ABSTRACT

Introduction and Aim: We sought to assess the value of serum High Molecular Weight (HMW) Adiponectin in predicting the risk of coronary artery disease in angiographically proven CAD patients with and without Type 2 diabetes mellitus. It has been postulated that lower concentrations of serum adiponectin can be an effective biomarker which is linked to insulin resistance and endothelial dysfunction in both diabetic and CAD patients.

Materials and Methods: This analytical cross-sectional study was done to evaluate serum HMW Adiponectin by Enzyme Linked Immunosorbant Assay (ELISA) in 40 cases of angiographically proven coronary artery disease (CAD) patients with and without diabetes of both sex, aged 40–60 years.

Results: Lower levels of HMW Adiponectin were observed in CAD patients with type 2 diabetes when compared to CAD patients without Type 2 Diabetes.

Conclusion: Our study confirms that serum HMW adiponectin levels were much lower in CAD patients with Type 2 diabetes in South Indians when compared with the western standards. Hence serum adiponectin level should be considered in the laboratory work-up of Type 2 Diabetic Mellitus patients who are more prone for the risk of CAD.

Keywords: Adipocytokine, Coronary Artery Disease (CAD), Enzyme Linked Immunosorbant Assay (ELISA), HMW Adiponectin, Hypoadiponectinemia

INTRODUCTION

Diabetes is no more considered to be an epidemic disease as it has turned pandemic and appears to be a worldwide public health problem. India is titled as the diabetic capital of the world according to the international journal of diabetes in developing countries (1). Cardiovascular disease remains the biggest cause of the death recent days and the combined effect of the diabetes and CAD has placed a greater strain on the health care providers to ensure a disease free healthy population, which has become complicated with growing sedentary life style habits (2).
Diabetes is considered as a CAD risk equivalent according to the adult treatment panel of the national cholesterol education program guidelines which confirms that diabetes patients have a threat for coronary events similar to that of non diabetic patients who previously had an event (3).

Adiponectin being a protein hormone synthesised exclusively from the white adipose tissue has a number of metabolic functions that includes fatty acid oxidation (4), glucose regulation and also reduces inflammation and vascular injury (5,6). Adiponectin an adipose specific protein belong to collectin family (7,8) and is present profusely in circulation, accounting for approximately 0.01% of total plasma protein (9).

It was documented that adiponectin exists in plasma as trimer—low molecular weight form, hexamer—medium molecular weight, and as multimer—high molecular weight form (10,11) of which the high molecular weight form (HMW) of adiponectin is considered to be the most biologically active form, as it regulates the glucose homeostasis by mediating insulin sensitization (12,13).

Very few epidemiological studies have investigated the association of HMW Adiponectin and the risk of developing CAD in type 2 diabetes. There are strong evidences which had proved that instead of total adiponectin, it is the HMW Adiponectin or the ratio between total and HMW Adiponectin that is strongly related to the development of type 2 diabetes (14)

Low levels of HMW Adiponectin has been identified in CAD patients (15) and in type 2 diabetic patients where the findings has suggested that adiponectin has both anti atherogenic and antidiabetic properties, and it acts as an endogenous mediator of both vascular and metabolic disorders.

It has been reported that adiponectin accumulates in the damaged vascular walls (16) when the endothelium of the carotid arteries is damaged by a balloon catheter in rats, and it is also observed that adiponectin prevents the attachment of monocytes to the endothelial cells (17) which is an early event in atherosclerotic vascular change.

In this study we investigated the role of serum HMW Adiponectin in angiographically proven 40 cases of coronary artery disease (CAD) patients with and without Type 2 diabetes of both sex, aged between 40 and 60 years and in 20 healthy controls and their lipid levels to rule out the possible significant relationship which may contribute to the atherosclerotic vascular complications in both CAD and T2DM patients.

GROUPING OF SUBJECTS

Based on the presence or absence of type 2 diabetes mellitus, CAD (proven by coronary angiography) patients were categorised as the follows;

Group I—Control subjects; Group II—CAD patients with diabetes (CAD+T2DM); Group III—CAD patients without diabetes (CAD-T2DM). Control subjects were selected based on the following criteria. They had no history of T2DM and CAD, they were non smokers and non consumers of alcohol and had no history of endocrinal dysfunctions, hyperlipidaemia, and hypertension and they were not on any medication.

CAD subjects with T2DM included in the study were as under:

T2DM diagnosed according to World Health Organization (2015) criteria and they had undergone coronary angiography for suspected ischaemic heart disease with the following:

The subjects had Positive exercise stress testing in ECG, ECG changes suggesting of CAD, Acute coronary syndrome patients and patients who were admitted previously in the hospital for CAD and who had undergone previous cardiac interventions like percutaneous coronary intervention (PCI), Percutaneous transluminal coronary angioplasty (PTCA), and coronary artery bypass graft (CABG) surgery. CAD subjects without T2DM included patients with all the above said criteria and were non-diabetic.

T2DM Patients were diagnosed when the fasting blood glucose levels were >126 mg/dl or the 2 hours postprandial blood glucose levels were >200 mg/dl.
Exclusion Criteria included Patients with Type I Diabetes, Patients of Chronic alcoholism, Patients who had Gross obesity and Body Mass Index >35 kg/m². Patients with inflammatory diseases, Hypothyroidism, endocrinopathies, dilated cardiomyopathy, valvular disease, pre existing hepatocellular disease, cerebrovascular diseases were excluded. Patients with renal insufficiency and patients taking thiazolidinedione drugs were also excluded.

All the patients in each group after the selection criteria, a detailed history was obtained, followed with a thorough clinical examination. The protocol of the study was approved by the members of institution ethical committee constituted by MMCH and RI as per ICMR guidelines and Informed consent was obtained from each of the study subjects.

BLOOD SAMPLING AND LABORATORY EXAMINATIONS

All samples were collected by venipuncture about 7 ml of venous blood were withdrawn from each subject after 12 hours of fasting for the measurement of HMW Adiponectin, ApolipoproteinA1, Apolipoprotein B, into plain and EDTA treated tubes and the samples are divided as follows. Within 30 minutes, blood was analysed for fasting glucose. 4 ml blood was collected and the blood sample was left to clot, and then centrifuged at 3000/r.p.m for 10 minutes. Serum was separated into two separate aliquots. The first aliquot was used for laboratory analysis of lipid profile; the second aliquot was immediately frozen at -20°C in the deep freezer for analysis of HMW Adiponectin.

Total cholesterol, triglycerides and high density lipoprotein cholesterol were measured by ChemWell® 2910 Automated EIA and Chemistry Analyzer. Low density lipoprotein cholesterol was estimated indirectly using the Friedewald formula (LDL cholesterol = Total cholesterol – HDL cholesterol + 1/5 Triglycerides), for subjects with a serum TG concentration of less than 400 mg/ml. Apolipoprotein A1 and Apolipoprotein B were measured by enzyme linked immunosorbent assay after the serum samples were thawed at room temperature. Serum HMW Adiponectin concentration was measured by enzyme linked immunosorbent assay using commercially available human HMW Adiponectin ELISA kit (Cusabio biotech Co., Ltd., CSB-E13400h). Apolipoprotein A1, was measured by using commercially available human Apolipoprotein A1, ELISA kit (AssayPro, EA5201-1) and Apolipoprotein B was measured by using commercially available human Apolipoprotein B, ELISA kit (AssayPro, EA7001-1).

ANTHROPOMETRIC ASSESSMENTS

Anthropometric indices including height and weight were measured while subjects were in the standing position and wearing light clothing without shoes. Body weight was measured in kilograms to the nearest 0.5 kg. Height was measured in centimeters to the nearest 0.5 cm. Body Mass Index (BMI) was calculated as the body weight in kilogram divided by the square of height in meters (kg/m²).

CAD diagnosis criteria included Patients who had Acute MI based on ECG, (ST elevation of atleast 0.1 mv) in two or more leads, Patients who had Angina pectoris based on clinical history, ECG, Coronary Angiography, Patients who had 50% or greater organic stenosis of at least one major coronary artery (confirmed by coronary Angiogram). Coronary angiography findings (occlusion of a main coronary artery branch with TIMI—Grade flow 0, 1, 2).

Statistical analysis

All data were analyzed and were expressed as Mean ± SE. statistics were performed using SPSS for windows version 17 software. Significant differences between groups were compared by one way analysis of variance (ANOVA) with Duncans test for post hoc comparisons of each group. Pearson correlation coefficients were calculated to evaluate the relationship between serum Adiponectin and all other study variables. $p$ Value $<0.01$ was considered as statistically significant.

RESULTS

The anthropometric and biochemical characteristics of the patients and control group are presented in Table 1.
Sixty patients include 40 subjects of CAD of which 20 subjects had CAD and T2DM, 20 subjects had CAD without T2DM and 20 control subjects. Patients who were CAD and T2DM had the highest BMI, SBP, DBP, total cholesterol, triglycerides; LDL-C, TC/HDL ratio and they had the lowest level of HMW Adiponectin, Apolipoprotein A1, and HDL-C cholesterol. The results revealed that there was a statistically significant differences in serum Adiponectin levels between CAD without T2DM and control subjects (07.11 ± 0.75 vs. 15.70 ± 1.28 µg/ml, p < 0.01) as shown in Table 1.

Serum High Molecular Weight adiponectin levels were significantly higher in men when compared with women as shown in Table 2.

NS non significant p < 0.05; *mean bearing different superscript in a row differ significantly **p < 0.01.

Statistical analysis done by ANOVA analysis (post hoc test: duncans).

Significance fixed at *p < 0.05, highly significant **p < 0.001.

Table 1 Anthropometric and biochemical characteristics of patients and control subjects.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Group-I (Control)</th>
<th>Group-II (CAD+T2DM)</th>
<th>Group-III (CAD–T2DM)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>48.45 ± 1.57</td>
<td>51.75 ± 1.40</td>
<td>51.10 ± 1.44</td>
<td>0.253</td>
</tr>
<tr>
<td>BMI, kg/m&lt;sup&gt;2&lt;/sup&gt;&lt;sup&gt;**&lt;/sup&gt;</td>
<td>22.62 ± 0.35</td>
<td>29.08 ± 0.43</td>
<td>26.62 ± 0.50</td>
<td>0.000</td>
</tr>
<tr>
<td>SBP, mmHg&lt;sup&gt;**&lt;/sup&gt;</td>
<td>115.05 ± 0.97</td>
<td>135.85 ± 1.14</td>
<td>132.95 ± 1.51</td>
<td>0.000</td>
</tr>
<tr>
<td>DBP, mmHg&lt;sup&gt;**&lt;/sup&gt;</td>
<td>72.35 ± 0.75</td>
<td>80.80 ± 1.07</td>
<td>75.75 ± 0.64</td>
<td>0.000</td>
</tr>
<tr>
<td>TGL, mg/dl&lt;sup&gt;**&lt;/sup&gt;</td>
<td>131.10 ± 3.08</td>
<td>207.85 ± 5.81</td>
<td>162.00 ± 5.10</td>
<td>0.000</td>
</tr>
<tr>
<td>TC, mg/dl&lt;sup&gt;**&lt;/sup&gt;</td>
<td>179.60 ± 1.96</td>
<td>200.50 ± 2.82</td>
<td>126.00 ± 1.80</td>
<td>0.000</td>
</tr>
<tr>
<td>HDL, mg/dl&lt;sup&gt;**&lt;/sup&gt;</td>
<td>48.35 ± 1.21</td>
<td>33.00 ± 0.59</td>
<td>41.15 ± 1.75</td>
<td>0.000</td>
</tr>
<tr>
<td>TC/HDL&lt;sup&gt;**&lt;/sup&gt;</td>
<td>3.76 ± 0.11</td>
<td>6.12 ± 0.15</td>
<td>3.20 ± 0.19</td>
<td>0.000</td>
</tr>
<tr>
<td>LDL, mg/dl&lt;sup&gt;**&lt;/sup&gt;</td>
<td>105.65 ± 2.55</td>
<td>143.90 ± 5.70</td>
<td>119.26 ± 4.12</td>
<td>0.000</td>
</tr>
<tr>
<td>HMW ADP, µg/ml&lt;sup&gt;**&lt;/sup&gt;</td>
<td>15.70 ± 1.28</td>
<td>7.11 ± 0.75</td>
<td>12.78 ± 1.78</td>
<td>0.000</td>
</tr>
<tr>
<td>APO A1, mg/dl&lt;sup&gt;**&lt;/sup&gt;</td>
<td>133.05 ± 1.38</td>
<td>102.90 ± 0.79</td>
<td>117.70 ± 1.08</td>
<td>0.000</td>
</tr>
<tr>
<td>APO B, mg/dl&lt;sup&gt;**&lt;/sup&gt;</td>
<td>82.75 ± 1.94</td>
<td>165.10 ± 1.46</td>
<td>167.20 ± 1.75</td>
<td>0.000</td>
</tr>
<tr>
<td>APO B/APO A1&lt;sup&gt;**&lt;/sup&gt;</td>
<td>0.62 ± 0.02</td>
<td>1.61 ± 0.02</td>
<td>1.43 ± 0.02</td>
<td>0.000</td>
</tr>
</tbody>
</table>

Table 2 Serum high molecular weight adiponectin levels between male and female subjects.
Table 3 Correlation between serum Adiponectin levels and various cardiovascular risk factors.

<table>
<thead>
<tr>
<th>Description</th>
<th>r Value</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>−0.011</td>
<td>0.934</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>−0.421**</td>
<td>0.001</td>
</tr>
<tr>
<td>SBP, mmHg</td>
<td>−0.470**</td>
<td>0.000</td>
</tr>
<tr>
<td>DBP, mmHg</td>
<td>−0.242</td>
<td>0.062</td>
</tr>
<tr>
<td>Triglyceride, mg/dl</td>
<td>−0.398**</td>
<td>0.002</td>
</tr>
<tr>
<td>Total cholesterol, mg/dl</td>
<td>−0.264*</td>
<td>0.041</td>
</tr>
<tr>
<td>HDL-C, mg/dl</td>
<td>0.391**</td>
<td>0.002</td>
</tr>
<tr>
<td>TC/HDLC</td>
<td>−0.464**</td>
<td>0.000</td>
</tr>
<tr>
<td>LDL-C, mg/dl</td>
<td>−0.206</td>
<td>0.114</td>
</tr>
<tr>
<td>APO-A1, mg/dl</td>
<td>0.512**</td>
<td>0.000</td>
</tr>
<tr>
<td>APO-B, mg/dl</td>
<td>−0.406**</td>
<td>0.001</td>
</tr>
<tr>
<td>APOB/APOA1</td>
<td>−0.460**</td>
<td>0.000</td>
</tr>
</tbody>
</table>

* Correlation is significant at the 0.05 level (2-tailed).

**Correlation is significant at the 0.01 level (2-tailed).

Results revealed that there was a negative correlation between serum HMW Adiponectin level and BMI ($r = −0.421, p = 0.001$), SBP ($r = −0.470, p = 0.000$), DBP ($r = −0.242, p = 0.062$), triglycerides ($r = −0.398, p = 0.002$), total cholesterol ($r = −0.264, p = 0.041$), low density lipoprotein cholesterol ($r = −0.206, p = 0.114$), Apo lipoprotein B ($r = −0.406, p = 0.001$), ApoB /ApoA1 ratio ($r = −0.460, p = 0.000$), TC/HDL ratio ($r = −0.464, p = 0.000$). There was a positive correlation between serum High Molecular Weight Adiponectin levels and serum HDL cholesterol ($r = 0.391, p = 0.002$), Apolipoprotein A1 ($r = 0.512, p = 0.000$), among the study groups.

**DISCUSSION**

Advanced research in adipose tissue has given us strong evidence that it is not just an energy storage depot, but an active endocrine organ secreting a variety of biological substances called adipokines. Adipokines secreted by the adipose tissue are adipins, aphelin, visfatin, leptin, resistin, interleukins, TNF-α, CRP, angiotensin II, estrogen, Plasminogen Activator Inhibitor-1 (PAI-1).

Among all these adipokines secreted, adiponectin is the only adipokine considered to be an anti diabetic and anti atherogenic and hence not surprising to have gained attraction in the midst of other adipokines.

Adiponectin as a protein hormone modulates glucose regulation and fatty acid oxidation and hence any rapid change in the adiponectin levels might lead to insulin resistance and dyslipidemia causing endothelial dysfunction leading to atherosclerosis. This could be a plausible provoking factor for the cardiovascular complications in T2DM patients.

Our main finding is that there were a significant lower levels of serum HMW Adiponectin in CAD with T2DM patients when compared to CAD alone and normal subjects. Our study supports the findings of previous studies that lower levels of serum adiponectin is definitely associated with the coronary artery disease risk factors in diabetes. Decreased levels of serum Adiponectin in coronary artery disease patients (18,19) concurs with our study and it also coincides with the study of 20. Yaturu et al. (20), who has previously reported that the levels of serum Adiponectin is lower in both Type II diabetic and CAD patients. The study is in line with the study of Hotta et al. and Kumada et al., who had also already reported that adiponectin concentration were significantly lower in patients with coronary artery disease when compared to control subjects.

Our study is controversial with the study of Monika et al., who had concluded that there was no statistically significant difference found among diabetic patients with or without CAD. When concerned with systolic and diastolic BP, both were found to be significantly higher in CAD patients with Type 2 DM and are negatively correlated with serum adiponectin. Ouchi et al., has reported that serum adiponectin is independently correlated with vasodilator response to reactive hyperaemia, and assessing the serum concentration of adiponectin could be an independent parameter for predicting endothelial dysfunction in CAD patients.
There was a significant difference in the Adiponectin concentration of men and women in control subjects. Cnop et al. (21), has given a possible reason that the sex differences in adiponectin levels may be due to the different numbers and size of the fat cells distribution in the individual sexes. In our study women had lower levels of adiponectin which may be because of the fact of more adipose tissue deposition. Few studies have reported that women had a higher serum adiponectin levels when compared to men which is controversial with our study. Nishizawa et al. (22), has indicated that androgen has the capability of decreasing serum adiponectin level and the androgen induced hypoadiponectinemia could be related to a high risk of insulin resistance and atherosclerosis in men. In our study there was no significant difference observed among CAD patients with and without T2DM except in control subjects.

When considering the lipid profile, HDL cholesterol, Apo A1 were positively correlated with serum HMW Adiponectin in CAD patients with T2DM and were negatively correlated with triglycerides, total cholesterol, LDL cholesterol, Apolipoprotein B, TC/HDL ratio, Apolipoprotein B, Apo B/ApoA1 ratio and this is in concurrence with the work done in Japanese patients (23) The strongest correlation is present between Adiponectin, HDL and Apo A1. It may be postulated that adiponectin may directly stimulate the lipoprotein lipase expression, which might increase the production of HDL Cholesterol levels and it may also be postulated that an “adipovascular” axis (24) may contribute to the increased risk of cardiovascular events in CAD patients.

The probable reason for the low adiponectin levels may be related with the reduced expression of Nitric oxide and increased expression of angiotensin II and cellular adhesion molecules from the Endothelium (17) where studies have suggested that adiponectin modulates the inflammatory vascular response by inhibiting the expression of adhesion molecules on endothelial cells (15), and inhibits endothelial cells nuclear factor kappa B signalling (25) and suppressing the macrophage function (26).

**CONCLUSION**

The study reports that HMW adiponectin as an antidiabetic, antiatherogenic adipocytokine regulates insulin resistance and dyslipidemia. This study suggests that, presence of lower levels of HMW adiponectin, Apo A1 and HDL-C could be strongly associated with endothelial dysfunction. This mechanism may lead to atherosclerosis progressing to coronary artery disease in Type 2 DM patients. Despite the smaller sample size, our study provides evidence for an inverse association between serum HMW Adiponectin and the lipid profiles which might lead to subsequent risk of developing CAD among Type 2 DM in the age group between 40-60. Hence individuals with a low level of HMW Adiponectin, low HDL-C and low levels of Apo A1 are more prone for an increased risk of developing both CAD and T2DM. This opens a new research for the underlying mechanism of evaluating HMW Adiponectin in CAD and in T2DM patients.

Therefore, lower HMW Adiponectin levels cannot just be a bio-marker but it could also be a causal risk factor for CAD in T2DM patients and hence additional measurement of HMW Adiponectin in laboratory set up might help in identifying a highly prevalent subgroup that are at an increased risk of developing Coronary Artery Disease.

**REFERENCES**


Relationship of BMI and dental caries among children in Chennai

Visha M.G.¹ and Deepa Gurunathan¹

¹Department of Paediatric Dentistry, Saveetha Dental College, Chennai, India.

(Received: Jan 2016  Accepted: Mar 2016)

Corresponding Author
Deepa Gurunathan. Email: drgdeepa@yahoo.co.in

ABSTRACT

Introduction and Aim: Dental caries (tooth decay) is a breakdown of teeth due to the activities of bacteria. Obesity is a medical condition in which excess fat has accumulated to the extent that it has negative effects on health. It is measured by BMI. One of the key factors for causing dental caries is diet and obesity is also associated with dietary intake. Both obesity and dental caries are major health issues in children. To determine the association of obesity and dental caries among children.

Materials and Methods: The study population consisted of 151 randomly selected children in Chennai population. The children were categorised based on the BMI percentage (under weight, normal, over weight and obese). On co-relation of caries index by Klein, Palmer and Knutson (1938) with BMI (body mass index) of each child the results were calculated by Metric method.

Results: A total of 151 (88 girls and 63 boys) children were examined and it was found that underweight children were 27.2%, 27.8% children were normal, 25.8% were overweight and 19.2% of children were obese. In 151 children, 22.5% had permanent tooth decay (DT) and 49.0% had primary tooth decay (dt). Based on the significant BMI groups; underweight children had 7.3% of permanent tooth dental caries and 22.0% had primary tooth decay. Children with normal BMI had 14.3% of permanent tooth decay and 19.0% had primary tooth decay. Overweight children had 38.5% of permanent tooth decay and 82.1% had primary tooth decay. The children subjected to have higher values of BMI being obese had 34.5% of permanent tooth dental caries and 86.2% had primary tooth decay. The presence of carious lesions was more prevalent in overweight and obese children when compared to underweight and normal children.

Conclusion: In this study from the statistical analysis of BMI and dental caries it is concluded that there is an association of obesity and dental caries as the prevalence of dental caries was found to be more in obese and overweight children than normal and underweight children.

Key words: Body Mass Index, Dental Caries, Obesity

INTRODUCTION

Childhood obesity is a major public health problem which is increasing at a high rate in both developed and developing countries (1). Obesity refers to a medical condition in which excess body fat has accumulated to the extent that it may have a negative effect on health, leading to reduced life expectancy and other health problems (2,3). An individual is considered obese when their body mass index (BMI) (4) exceeds 30 kg/m²,
with the range 25–30 kg/m² defined as overweight. The measurement obtained by dividing a person’s weight by the square of the person’s height. BMI categories include: underweight, normal, overweight and obese. Dental caries is defined as a localized, progressively destructive disease of the teeth that starts at the external surface (usually the enamel) with the apparent dissolution of the inorganic components by organic acids that are produced in immediate proximity to the tooth by the enzymatic action of masses of microorganisms (in the bacterial plaque) on carbohydrates; the initial demineralization is followed by an enzymatic destruction of the protein matrix with subsequent cavitation and direct bacterial invasion; in the dentin, demineralization of the walls of the tubules is followed by bacterial invasion and destruction of the organic matrix. The correlation between dental caries and BMI consists of various contributing factors which include biological, genetic, socioeconomic, cultural, dietary, and environmental and sedentary lifestyle concern (5). If the highest incidence of caries, overweight and obesity are found in a particular group such as those who consume foods and beverages with a high content of refined carbohydrates (6–11), then it is possible to establish a relationship between the two conditions. Although community water fluoridation has significantly reduced the prevalence of smooth surface caries, dental caries remains to be a common chronic childhood disease. Obesity and dental caries are two highly prevalent health problems in both children and adolescent. The goal of this study was in investigating the association between dental caries and obesity in children.

MATERIALS AND METHODS

The Study sample consisted of 151 (88 girls and 63 boys) randomly selected children among Chennai population. Children of varying ages were examined. A Questionnaire was composed and was used in the examination of randomly selected children in Chennai. An informed consent from parents was obtained. The completed Questionnaire consisted of demographic data such as Age, Sex (12) and Height, Weight were recorded and BMI was calculated. Intra oral examination was done as the second part of the study. The hard tissue examination consisted of DMF(S) and DMF (T) caries index which was introduced by Klein, Palmer and Knutson in 1938 and modified by WHO. BMI for each individual was calculated based on the height and weight measured by Metric method of calculation.

According to WHO, the ranges of BMI are categorised as follows:
1. BMI less than 18.5—Underweight
2. BMI range of 18.5–24.9—Normal
3. BMI range of 25–29.9—overweight
4. BMI range of 30 and above—Obese.

Outcome variables included: (1) measures of dental caries prevalence; and (2) severity of caries in primary and permanent dentitions; (3) measures of overweight and obese prevalence.

Descriptive statistics were used to summarize demographic data. The collected data was analysed with SPSS 16.0 version. To A chi-square test was used to investigate the relationship between Obesity and BMI at the examination describe about the data descriptive statistics frequency analysis, percentage analysis, mean and S.D. were used. To find the significance between the variables Pearson’s Chi-Square test was used. In the above statistical tool, the probability value 0.05 was considered as significant level.

RESULTS

The study sample consisted of 151 (88 girls and 63 boys) randomly selected children among Chennai
Based on the significant BMI groups; underweight children had 7.3% of permanent tooth dental caries and 22.0% had primary tooth decay. Children with normal BMI had 14.3% of permanent tooth decay and 19.0% had primary tooth decay. Overweight children had 38.5% of permanent tooth decay and 82.1% had primary tooth decay. The children subjected to have higher values of BMI being obese had 34.5% of permanent tooth dental caries and 86.2% had primary tooth decay (Table 1 and Fig. 3).

Based on the collected study sample, on comparison with the percentage of caries associated with the significant BMI categories, it was found that the prevalence of dental caries is greater in overweight and obese children.

**DISCUSSION**

The study of dental caries is always a challenging task as the onset of carious lesion includes various of factors such as maintenance of oral cleanliness, diet intake frequency and composition, socioeconomic status, salivary immunoglobulins, bacterial load and fluoride intake (12). Obesity is described by the energy and metabolism imbalance and is responsible for various complications (13,14). Dental caries and childhood obesity are also affected by Socio-economic status. The interaction between genetic and environmental factors develops dental caries. The process involves the destruction of hard tissues by acidic by-products from bacterial carbohydrate fermentation (15). Sugar composition in soft drinks and snacks are considered highly cariogenic.

In 2001, a study by Macek and Mitola was published to decrease and prevent obesity and overweight

<table>
<thead>
<tr>
<th>Body mass index</th>
<th>Permanent teeth</th>
<th>Primary teeth</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No Decay N (%)</td>
<td>Decay N (%)</td>
</tr>
<tr>
<td>Underweight</td>
<td>38 (92.7)</td>
<td>3 (7.3)</td>
</tr>
<tr>
<td>Normal</td>
<td>36 (85.7)</td>
<td>6 (14.3)</td>
</tr>
<tr>
<td>Overweight</td>
<td>24 (61.5)</td>
<td>15 (38.5)</td>
</tr>
<tr>
<td>Obese</td>
<td>19 (65.5)</td>
<td>10 (34.5)</td>
</tr>
<tr>
<td>Total</td>
<td>117 (77.5)</td>
<td>34 (22.5)</td>
</tr>
</tbody>
</table>

Table 1 Represents the prevalence of caries based on the BMI categories.
as dental professionals have an important role in influencing eating habits and food choices (16). A study conducted at the school of Dental Medicine, University of Pennsylvania had 142 subjects was reported in a paediatric clinic. BMI percentile was used instead of BMI for weight classification, and the number of decayed surfaces on primary and permanent teeth was used as the caries index. Caries prevalence in that sample was low, and therefore, no association was found between obesity and caries (17). The low prevalence may be due to local factors such as access to fluoridated water or fluoride programs in the school system. Another study was done in Brazil that included 2,651 preschool children which also found no association between obesity and caries. The study found more caries in children in public schools vs. private schools which suggested that SES may be associated with caries prevalence (18). Another study associating obesity and dental caries was conducted at the University of Dammam, Kingdom of Saudi Arabia found that participants who consumed fast foods, soft drinks and sweet beverages on a daily basis were more prevalent to obesity than the participants who consumed home cooked food for the daily meal. Hence a correlation exists between diet and obesity.

CONCLUSION

It can be concluded that there appears to be an association between obesity and dental caries as the prevalence of dental caries was found to be more in obese and overweight children than normal and underweight children.

RECOMMENDATIONS

Future research with a larger sample and over a bigger geographical area should be used to assess whether a direct association between childhood obesity and dental caries does or does not exist.

ACKNOWLEDGEMENT

I would like to convey my sincere regards to the participants and their parents for their co-operation and also the statistician for the support.

POTENTIAL CONFLICT OF INTEREST

No.

REFERENCES


A study on prevalence of hypertension in cardiovascular risk factors among adults in Chittoor district population

Bhavani Yamasani,1 Khadervali Nagoor,2 and Raziya Dudekula3

Departments of 1Community Medicine, 2Community Medicine, and 3Physiology, Sri Padmavathi Medical College (W), SVIMS, Tirupathi, India.

(Received: Jan 2016 Accepted: Mar 2016)

Corresponding Author
Dr. Bhavani Yamasani. Email: svims.faculty@gmail.com

ABSTRACT

Introduction and Aim: Socio-demographic patterning of cardiovascular disease risk factors information is useful for predicting the future course of the epidemic and designing community-based interventions to reduce risk factors in the rural population.

Materials and Methods: A total of 734 subjects aged 30 years and above of both sexes were selected for the study.

Results: Prevalence of the hypertension was found to be 28.2%. The prevalence was found to be significantly higher in those with extra intake of salt (66.7%), previous cardiovascular/cerebrovascular events (61.9%), family history of hypertension (47.4%), stress (44.2%), current smoking (41.1%), current alcohol intake (39.0%), age 50 years and above (38.8%), other than unskilled occupation (35.3%), upper and middle socioeconomic status (34.3%), males (33.2%). Contrastingly significantly higher prevalence was found in those who do physical exercise regularly (44.8%) and who were taking oils rich in PUFA (41.0%). It is found that the prevalence of medium and high risk was found to be significantly higher in males (41.8%) than in females (5.5%).

Conclusion: The present study has found a significant proportion of undiagnosed and inadequately treated hypertension. The cardiovascular risk has been found to be significantly higher in males than females. The study revealed that there is enormous scope for intervention in the form of reduction of modifiable risk factors of cardiovascular diseases.

Key words: Alcohol, Cardiovascular Diseases, Hypertension, Stress

INTRODUCTION

Cardiovascular diseases (CVD) have been leading cause of morbidity and mortality in India. Recent trends indicate that the disease has escalated to younger age group. It has a significant presence in males and females in both urban and rural population (1). The risk factors are classified as non-modifiable and modifiable. Non-modifiable risk factors include age, sex, genetic factors and family history. Modifiable risk factors include smoking, high blood pressure, elevated serum cholesterol, diabetes, obesity, physical inactivity, stress, poor eating habits, excessive drinking, tobacco chewing (2). Hypertension is the pressure exerted by the flow of blood on the walls of blood vessels. Due to increased pressure, the heart is over burdened, and work load is increased. Uncontrolled hypertension will lead to cardiovascular complications such as myocardial infarction, heart failure, peripheral arterial disease, renal complications...
like chronic renal failure, end-stage kidney disease, neurological complications like cerebrovascular accidents such as stroke, haemorrhage, retinal complications and aortic Aneurysm (3). The present study was undertaken to provide the data on the prevalence of hypertension in cardiovascular disease risk factors among adults aged 30 years and above in rural Tirupati of Chittor district population.

MATERIALS AND METHODS

This study was conducted in a rural area of Tirupati under the jurisdiction of PHC Mangalam. Most of the people are daily labourers, vegetable and fruit vendors, dhobis and a majority of the women are homemakers. A total of 734 subjects in the age group of 30 years and above age group were selected from the study areas to estimate the prevalence. Subjects were included in the cross-sectional study by using 20 cluster sampling technique. Thus, all the sectors, all the sub-centers within the sectors and all the villages/habitations within the subcentres are listed in alphabetical order and cumulative population. The study subjects have explained the purpose of the study and informed consent was taken from the subjects. Within each village/habitation, the investigator went to the centre of the village, and all houses to the right side are included until the required number covered. One subject from each house aged 30 years and above is interviewed who is selected randomly from those available at home at the time of the study. On an average, four families were examined during each day of the visit. Reassurance was given regarding the confidentiality of their responses. Ethical clearance for this study was accorded by Institutional ethical committee, Sri Venkateswara Medical College, Tirupati. Height was measured by using portable stadiometer. Weight was measured with calibrated weighing machine. Body mass Index was calculated by using the formula: weight (kg)/height (m²). Measurement of Blood pressure was carried out on each participant by using the standard technique (4). The diagnosis and classification of Hypertension were done according to the JNC-VII report (Table 1). As per the cardiovascular risk categorization by the American Heart Association, 2008 based on the prevalence of risk factors like age, smoking, systolic blood pressure treated and not treated, and body mass index which were given points for cardiovascular risk was recorded. We have considered smoking, alcohol, extra salt intake and cooking oil, stress, physical exercise and some other parameters to study the prevalence of hypertension in cardiovascular risk diseases.

<table>
<thead>
<tr>
<th>JNC 6 category</th>
<th>SBP/DBP</th>
<th>JNC 7 category</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>&lt;120/80</td>
<td>Normal</td>
</tr>
<tr>
<td>Normal</td>
<td>120–129/80–84</td>
<td>Prehypertension</td>
</tr>
<tr>
<td>Borderline</td>
<td>130–139/85–89</td>
<td>Hypertension</td>
</tr>
<tr>
<td>Hypertension</td>
<td>≥140/90</td>
<td>Stage 1</td>
</tr>
<tr>
<td>Stage 1</td>
<td>140–159/90–99</td>
<td>Stage 2</td>
</tr>
<tr>
<td>Stage 2</td>
<td>160–179/100–109</td>
<td>Stage 3</td>
</tr>
<tr>
<td>Stage 3</td>
<td>≥180/110</td>
<td></td>
</tr>
</tbody>
</table>

Table 1 Joint National Committee on Prevention, Detection, Evaluation and Treatment of High Blood Pressure (JNC) report has introduced a new classification.

Results

The socio-economic scale based on the per-capita monthly income of B.G. Prasad Classification (Table 2), it was found that most of them belong to upper lower category 40.2% followed by lower middle category 30.9% (5). It was found that 15.7% are known hypertensive. The duration of hypertension was found to be less than 5 years in a majority of cases 73.9% followed by 5–10 years 21.7%. Out of the 115 known hypertensive, 106 (92.2%) is receiving treatment (Table 3). It was found that the prevalence of hypertension was significantly higher in 50 years and above 38.8% compared to those aged less than 50 years 23.1%. Similarly, the prevalence was found to be significantly higher in males 33.2% compared to that in females 23.0%. Although it was found that the prevalence is higher in those with secondary and above educational level 28.5% compared to those educated up to primary level 27.9%, the difference is found to be not statistically significant (P = 0.86; NS). Those subjects with other than unskilled occupation had significantly higher
prevalence of hypertension 35.3% compared to unskilled occupations 24.0% (Table 4). Higher prevalence of hypertension was found among those unmarried/widowed/divorced group 35.8% compared to married group 27.1%, but however the difference is not statistically significant (P = 0.08; NS). An insignificantly higher prevalence is found in Hindu religion 28.4% compared to other religions 25.0%. Similarly, a higher prevalence is found in those belonging to other castes and backward classes 29.9% than in scheduled caste and tribe 22.2%, but the difference is not statistically significant (P = 0.053; NS). A significantly higher prevalence was found in those belonging to upper and middle socioeconomic status 34.3% than lower socioeconomic status group 21.3% (Table 5). A significantly higher prevalence of hypertension was found in those with a positive family history of hypertension 47.4% than those without positive family history 24.6% (Table 6). A significantly higher prevalence of hypertension is found in current smokers 41.1% compared to non-smokers 25.6% and current alcoholics 39.0% compared to 25.5% (Table 7). With regard to tobacco chewing however, no association was found with the difference being not statistically significant (P = 0.77; NS). A significantly higher prevalence of hypertension was found in those taking extra salt 66.7% than those not taking extra salt 25.0%. With regard to the type of cooking oil, however, a significantly higher prevalence was observed in those taking oils rich in PUFA 41.0% compared to those taking other type of oils 23.6% (Table 8). It was found that the prevalence of hypertension was higher with all grades of Holmes’ categorization of stress compared to those without stress, and the difference is also statistically significant (P < 0.001; S) (Table 9). The prevalence of hypertension was found to be slightly higher in those subjects with sedentary life style 29.5% compared to those who were engaged in the moderate and severe type of work 26.1% but the difference, however, is not statistically significant (P = 0.32; NS). Contrastingly, significantly higher prevalence was found in those who do regular physical exercise 44.8% compared to those who do not do exercise regularly 24.5% (Table 10). It was found that most of the male subjects belong to lower category of cardiovascular risk 58.5% while there were 98 subjects 26.3% with severe risk and 58 subjects 15.5% with medium risk. It was found that most of the female subjects belong to lower category of cardiovascular risk 94.4% while there were 14 subjects 3.9% with moderate risk and 6 subjects 1.7% with severe risk. It was found that overall, 76% belong to low risk followed by 14.2% with severe risk and 9.8% with medium risk. It can be seen that the prevalence of medium risk was found to be higher in males 15.5% than in females 3.9% (Table 11). Similarly, the prevalence of high risk is also found to be higher in males 26.3% compared to that in females 1.7%, and the differences are also found to be statistically significant (P < 0.001; S).

**Table 2.** B.G. Prasad’s socio economic status scale was used to classify study subjects based on per capita monthly income.

<table>
<thead>
<tr>
<th>Socio-economic status</th>
<th>Per capita monthly income (range in rupees)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Upper</td>
<td>5433 and above</td>
</tr>
<tr>
<td>Upper middle</td>
<td>2716–5432</td>
</tr>
<tr>
<td>Lower middle</td>
<td>1630–2715</td>
</tr>
<tr>
<td>Upper lower</td>
<td>815–1629</td>
</tr>
<tr>
<td>Lower</td>
<td>&lt;815</td>
</tr>
</tbody>
</table>

**Table 3.** Details of Hypertension among subjects (N = 734).

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Parameter</th>
<th>No. of subjects</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Known hypertension</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(a)</td>
<td>Yes</td>
<td>115</td>
<td>15.7</td>
</tr>
<tr>
<td>(b)</td>
<td>No</td>
<td>619</td>
<td>84.3</td>
</tr>
<tr>
<td>2</td>
<td>Duration of hypertension (N = 115)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(a)</td>
<td>Less than 5 years</td>
<td>85</td>
<td>73.9</td>
</tr>
<tr>
<td>(b)</td>
<td>5–10 years</td>
<td>25</td>
<td>21.7</td>
</tr>
<tr>
<td>(c)</td>
<td>More than 10 years</td>
<td>5</td>
<td>4.4</td>
</tr>
<tr>
<td>3</td>
<td>Treatment of hypertension (N = 115)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(a)</td>
<td>Yes</td>
<td>106</td>
<td>92.2</td>
</tr>
<tr>
<td>(b)</td>
<td>No</td>
<td>9</td>
<td>7.8</td>
</tr>
<tr>
<td>S. No.</td>
<td>Risk factor</td>
<td>Prevalence of hypertension (%)</td>
<td>Odds ratio and 95% CI</td>
</tr>
<tr>
<td>--------</td>
<td>----------------------</td>
<td>-------------------------------</td>
<td>------------------------</td>
</tr>
<tr>
<td>1</td>
<td>Age group</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(a)</td>
<td>50 and above years 92/237 (38.8)</td>
<td>2.10 (1.50–2.94)</td>
<td>$\chi^2 = 19.5$; $P &lt; 0.001$; S</td>
</tr>
<tr>
<td>(b)</td>
<td>Less than 50 years 115/497 (23.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(a)</td>
<td>Male 124/373 (33.2)</td>
<td>1.66 (1.20–2.31)</td>
<td>$\chi^2 = 9.52$; $P = 0.002$; S</td>
</tr>
<tr>
<td>(b)</td>
<td>Female 83/361 (23.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Level of education</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(a)</td>
<td>Secondary and above 102/358 (28.5)</td>
<td>1.02 (0.74–1.41)</td>
<td>$\chi^2 = 0.02$; $P = 0.86$; NS</td>
</tr>
<tr>
<td>(b)</td>
<td>Up to primary 105/376 (27.9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Occupation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(a)</td>
<td>Not unskilled 96/272 (35.3)</td>
<td>1.72 (1.24–2.39)</td>
<td>$\chi^2 = 10.7$; $P &lt; 0.001$; S</td>
</tr>
<tr>
<td>(b)</td>
<td>Unskilled 111/462 (24.0)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 4 Relationship between hypertension and various socio-demographic factors (N = 734).
<table>
<thead>
<tr>
<th>S. No.</th>
<th>Risk factor</th>
<th>Prevalence of hypertension (%)</th>
<th>Odds ratio and 95% CI</th>
<th>Statistical significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Marital status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(a) Unmarried/Divorced/Separated</td>
<td>34/95 (35.8)</td>
<td>1.50 (0.95–2.36)</td>
<td>$\chi^2 = 3.10; P = 0.08; NS$</td>
</tr>
<tr>
<td></td>
<td>(b) Married</td>
<td>173/639 (27.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Religion</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(a) Hindu</td>
<td>196/690 (28.4)</td>
<td>1.19 (0.57–2.56)</td>
<td>$\chi^2 = 0.24; P = 0.62; NS$</td>
</tr>
<tr>
<td></td>
<td>(b) Others</td>
<td>11/44 (25.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Type of family</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(a) Joint/Extended</td>
<td>81/264 (30.7)</td>
<td>1.20 (0.86–1.68)</td>
<td>$\chi^2 = 1.25; P = 0.23; NS$</td>
</tr>
<tr>
<td></td>
<td>(b) Nuclear</td>
<td>126/470 (26.8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Social status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(a) Other castes and Backward castes</td>
<td>171/572 (29.9)</td>
<td>1.49 (0.97–2.30)</td>
<td>$\chi^2 = 3.67; P = 0.053; NS$</td>
</tr>
<tr>
<td></td>
<td>(b) Scheduled Caste and tribe</td>
<td>36/162 (22.2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Social economic status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(a) Upper and middle</td>
<td>134/391 (34.3)</td>
<td>1.92 (1.38–2.68)</td>
<td>$\chi^2 = 15.2; P &lt; 0.001; NS$</td>
</tr>
<tr>
<td></td>
<td>(b) Lower</td>
<td>73/343 (21.3)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 5 Relationship between hypertension and various socio-demographic factors ($N = 734$).
<table>
<thead>
<tr>
<th>S. No</th>
<th>Risk factor</th>
<th>Prevalence of hypertension (%)</th>
<th>Odds ratio and 95% CI</th>
<th>Statistical significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Family history of hypertension</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(a) Yes</td>
<td>55/116 (47.4)</td>
<td>2.76 (1.80–4.24)</td>
<td>$\chi^2 = 25.1; P &lt; 0.001; S$</td>
</tr>
<tr>
<td></td>
<td>(b) No</td>
<td>152/618 (24.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(b) No</td>
<td>194/713 (27.2)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 6: Relationship between hypertension and family history of hypertension subjects.

<table>
<thead>
<tr>
<th>S. No</th>
<th>Risk factor</th>
<th>Prevalence of hypertension (%)</th>
<th>Odds ratio and 95% CI</th>
<th>Statistical significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Current smoking</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(a) Yes</td>
<td>51/124 (41.1)</td>
<td>2.03 (1.33–3.10)</td>
<td>$\chi^2 = 12.3; P &lt; 0.001; S$</td>
</tr>
<tr>
<td></td>
<td>(b) No</td>
<td>156/610 (25.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Current alcohol</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(a) Yes</td>
<td>57/146 (39.0)</td>
<td>1.87 (1.26–2.78)</td>
<td>$\chi^2 = 10.6; P &lt; 0.001; S$</td>
</tr>
<tr>
<td></td>
<td>(b) No</td>
<td>150/588 (25.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Current tobacco chewing</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(a) Yes</td>
<td>17/70 (24.3)</td>
<td>0.80 (0.43–1.46)</td>
<td>$\chi^2 = 0.58; P = 0.77; NS$</td>
</tr>
<tr>
<td></td>
<td>(b) No</td>
<td>190/664 (28.6)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 7: Relationship between hypertension and current smoking, alcohol and tobacco intake.
### Table 8: Relationship between hypertension and extra salt intake and type of cooking oil.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Risk factor</th>
<th>Prevalence of hypertension (%)</th>
<th>Odds ratio and 95% CI</th>
<th>Statistical significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Extra salt intake</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(a) Yes</td>
<td>38/57 (66.7)</td>
<td>6.01 (3.26–11.2)</td>
<td>$\chi^2 = 45.2; P &lt; 0.001; S$</td>
</tr>
<tr>
<td></td>
<td>(b) No</td>
<td>169/677 (25.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Type of cooking oil</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(a) Other type of oils</td>
<td>127/539 (23.6)</td>
<td>0.44 (0.31–0.63)</td>
<td>$\chi^2 = 21.6; P &lt; 0.001; S$</td>
</tr>
<tr>
<td></td>
<td>(b) Oil rich in PUFA</td>
<td>80/195 (41.0)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Table 9: Relationship between hypertension and Holmes’ stress categories (N = 734).

<table>
<thead>
<tr>
<th>Holmes Stress category</th>
<th>Prevalence of hypertension (%)</th>
<th>Statistical significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nil</td>
<td>161/630 (25.6)</td>
<td>$\chi^2 = 15.6; df: 2; P &lt; 0.001; S$</td>
</tr>
<tr>
<td>Mild</td>
<td>39/90 (43.4)</td>
<td></td>
</tr>
<tr>
<td>Moderate</td>
<td>5/12 (41.7)</td>
<td></td>
</tr>
<tr>
<td>Severe</td>
<td>2/2 (100.0)</td>
<td></td>
</tr>
<tr>
<td>S. No.</td>
<td>Risk factor</td>
<td>Prevalence of hypertension (%)</td>
</tr>
<tr>
<td>-------</td>
<td>-------------------</td>
<td>---------------------------------</td>
</tr>
<tr>
<td>1</td>
<td>Nature of work</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(a) Sedentary</td>
<td>133/451 (29.5)</td>
</tr>
<tr>
<td></td>
<td>(b) Moderate and severe</td>
<td>74/283 (26.1)</td>
</tr>
<tr>
<td>2</td>
<td>Physical exercise</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(a) No</td>
<td>147/600 (24.5)</td>
</tr>
</tbody>
</table>

Table 10  Relationship between hypertension and nature of work and physical exercise.

<table>
<thead>
<tr>
<th>Cardiovascular risk category</th>
<th>Males (%)</th>
<th>Females (%)</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low (less than 10%)</td>
<td>217 (58.5)</td>
<td>341 (94.4)</td>
<td>558 (76.0)</td>
</tr>
<tr>
<td>Medium (10–20%)</td>
<td>58 (15.5)</td>
<td>14 (3.9)</td>
<td>72 (9.8)</td>
</tr>
<tr>
<td>High (Above 20%)</td>
<td>98 (26.3)</td>
<td>6 (1.7)</td>
<td>104 (14.2)</td>
</tr>
<tr>
<td>Total</td>
<td>373 (100.0)</td>
<td>361 (100.0)</td>
<td>734 (100.0)</td>
</tr>
</tbody>
</table>

Table 11  Cardiovascular risk categorization compared between male and female subjects.
Discussion

Studies have shown that a most cost-effective approach to containing emerging epidemics of these diseases were to reduce the prevalence of these modifiable risk factors (2). Prevalence of hypertension (including those who are known hypertensives and newly diagnosed hypertensives whose blood pressure exceeded the cutoff level of 140/90 mm Hg) is found to be 28.2%. A comparable prevalence of 30.0% was reported in Delhi industry study; rural Bareilly study 27.4% and Assam study 33.3% (6–8). A higher prevalence 37.3% was reported in Jaipur study (9). A lower prevalence was reported in a study in Chennai 25.4% and another study in Andhra Pradesh 20.3% (10,11). Similar lower prevalence was reported in rural Meerut study (15.7%). A very low prevalence of 7.2% was reported in a study in rural Maharashtra (12,13). A similar lower prevalence of 8.6% was reported in Tirupati (14). A lower prevalence of 4.6% was reported in rural north India study (15). The differences in the prevalence of hypertension among various studies may be linked to geographical differences, dietary patterns, behavioural factors, differing time periods, urban and rural differences as well as differing classifications adopted (16). It was found in the present study that only 55.6% are aware of their hypertension. Out of those aware, 92.2% are currently taking treatment. Out of those treated 57.5% are adequately treated with blood pressure under control. Thus only 61 out of 207 hypertensives (29.5%) are diagnosed and adequately treated. This finding follows the known principle of the rule of halves in hypertension to a major extent. In Chennai study also, it was found that 55.3% of known hypertensives are adequately treated while Tirupati study also found that only 41.7% are adequately treated (11,14). It was found that significantly higher prevalence of hypertension was found in those with extra salt consumption 66.7%, positive family history of hypertension 47.4%, stress 44.2%, 50 years and above 38.8%, other than unskilled occupation 35.3%, upper and middle socioeconomic status 34.3%, males 33.2%. The prevalence of hypertension was also found in those unmarried/widowed/divorced 35.8%, other castes and backward classes 29.9%, and sedentary life style 29.5%, higher secondary and above education 28.5%, Hindu religion 28.4% but the differences is not statistically significant. Contrastingly significant level of hypertension was found in those doing regular physical exercise 44.8% and those taking oils rich in PUFA 41.0%. This may be due to the fact that most hypertensives after being diagnosed start doing regular physical exercise and consume oils rich in PUFA out of knowledge acquired by self or from other sources (2). Contrastingly lower prevalence was found in those with tobacco chewing 24.3% which however not statistically significant. The prevalence of high risk is also found to be higher in males 26.3% compared to that in females 1.7% and the differences are also found to be statistically significant which indicating the cardiovascular risk has been found to be significantly higher in males than females. Treating hypertension has been associated with 15% reduction in the incidence of myocardial infarction and 40% reduction of stroke (17).

CONCLUSION

The present study has found a significant proportion of undiagnosed and inadequately treated hypertension. The cardiovascular risk has been found to be significantly higher in males than females. The study revealed that there is enormous scope for intervention in the form of reduction of modifiable risk factors of cardiovascular diseases.

ACKNOWLEDGMENTS

Authors acknowledge the valuable suggestions received from Prof. G. Raviprabhu, Department of Community Medicine, ACSR Medical College, during this work.

CONFLICT OF INTEREST

Nil.

REFERENCES


Lipid peroxidation and antioxidant status in vitiligo patients

Pallavi Mishra¹, Rajkumari Rathore¹, and Prashant Hisalkar²

¹RKDF Medical College Hospital and Research Center, Bhopal, Madhya Pradesh, India.
²People’s College of Medical Science & Research Center, Bhopal, Madhya Pradesh, India.

(Received: Nov 2016  Accepted: Mar 2016)

Corresponding Author

Pallavi Mishra. Email: mishra17pallavi@gmail.com

ABSTRACT

Introduction and Aim: Vitiligo is a skin disorder or discoloration disease. When the immune system turns against itself (autoimmune disease) then immune cells of the body attack the color-producing cells and in reaction to these abnormalities white patches develop on the skin. These white patches are vitiligo skin disorder. Etiology of vitiligo and cause of melanocyte death are not clear. Three pathogenic mechanisms – immunological, neural and biochemical have been suggested.


Results: P-MDA, S-SOD and S-Cu were found to be significantly higher level (P<0.001) in vitiligo patients as compared to control. But the S-Zn was found to be significantly lower level in vitiligo patients than controls.

Conclusion: In oxidative stress, there is insufficient antioxidant activity leading to excessive accumulation of free radicals which damage cellular compounds such as proteins, carbohydrates, DNA and lipids. This result confirmed that oxidative stress may play an important role in melanocyte death.

Key Words: Antioxidants, Free radicals, MDA, SOD, Vitiligo

INTRODUCTION

The etiology is still unknown, so there are several hypothesis concerning the pathogenesis of vitiligo: genetic, autoimmune, neural, autotoxic, biochemical and oxidative stress theories (1–5). It presents in childhood or adult life. It often involves the hands, feet, wrists, axilla, periorbital, perioral and anogenital skin (6). Patients with vitiligo present with one to several amelanotic macules that appear chalky or milky white in color. The macules are round and/or oval in shape often with scalloped margins (1). Vitiligo is classified as focal, segmental, acrofacial, generalized, mucosal and universal vitiligo (7).

In general oxidative stress is caused by an imbalance between the production of reactive oxygen and a biological systems ability to readily detoxify the reactive intermediates or easily repair the resulting damage (9,10). Reactive oxygen species (ROS) are produced as byproducts of melanogenesis in melanocyte and controlled in the epidermis by several antioxidant enzymes such as superoxide-dismutase.
Oxidative stress is the initial pathogenic event in melanocyte destruction. In oxidative stress, there is insufficient antioxidant activity leading to excessive accumulation of free radicals which damage cellular compounds such as proteins, carbohydrates, DNA, and lipids.

Copper is an essential mineral that is a component of several important enzymes in the body and is essential for good health. Copper deficiency leads to the variety of abnormalities including anemia, skeletal defects, degeneration of the nervous system, reproductive failure, pronounced cardiovascular lesions, elevated blood cholesterol, impaired immunity and defects in the pigmentation and structure of hair. More than a dozen enzymes have been found to contain copper. The best studied are superoxide dismutase (SOD), cytochrome C oxidase, catalase, dopamine hydroxylase, uricase, tryptophane dioxygenase, lecithinase and other monoamine and diamine oxidase. Melanin, the normal body pigment is synthesized from the essential amino acid L-phenylalanine by an enzyme system dependent on copper, vitamin B6, vitamin C.

**MATERIALS AND METHODS**

This study was conducted in the Department of Biochemistry, Gandhi Medical College, Bhopal M.P. in association with Department of Skin and V.D. Hamidia Hospital, Bhopal M.P. The study included 50 vitiligo cases and 50 normal healthy subjects. This study was undergoing according to sex wise distribution (n = 32 males and n = 18 females), types of vitiligo, duration of vitiligo, a progression of disease and diet. There were three sub groups in this study generalized vitiligo (n = 30), localized vitiligo (n = 15) and segmental vitiligo (n = 05) occurs.

Vitiligo patients were divided into two groups who were suffering from vitiligo more than 1 year and who were suffering from vitiligo less than 1 year. Patients with vitiligo not undergoing treatment were considered for the study and patients suffering from other chronic diseases like renal diseases, liver diseases, heart diseases, diabetes mellitus, hypopigmented scars due to injury or burn, leprosy and vitiligo undergoing treatment were excluded from the study. n = 25 progressive vitiligo cases and n = 25 stable vitiligo cases. n = 27 vitiligo patients were
taking vegetarian diet and n = 23 cases were taking vegetarian and non vegetarian diet.

Plasma malondialdehyde (P-MDA) by Jean et al. (1983), Serum superoxide dismutase (S-SOD) by Mishra and Fridovich (1972), Serum copper (S-Cu) by Sodium diethyldithiocarbamate method (Eden and Green, 1940; Ventura and King, 1951) tests were performed on the blood samples of patients and control.

Statistical analysis

Data analysis was performed using the online t ÔÇô test calculator with a value of p < 0.001 and p < 0.05 considered highly significant and significant respectively. Comparison of two groups was done by the Student’sÔÇôs paired or unpaired t-test. Results are expressed as mean ± SD.

RESULTS

The P-MDA, S-SOD, and S-Cu were found to be significantly higher level in vitiligo cases as compared to controls because fresh cases were selected for the study. Therefore initially oxidative stress occur to which suppress antioxidant level (S-SOD and S-Cu) were also increased (Table 1).

According to sex wise distribution when compared between male cases and female cases the P-MDA, S-SOD and S-Cu were not found to be statistically significant. Vitiligo affects both the sex equally.

According to the type of vitiligo, it was divided into three groups: the first group was generalized vitiligo cases, the second group having localized vitiligo cases and third group comprises of segmental vitiligo cases. Among all the parameters, P-MDA was found to be statistically highly significant in generalized vitiligo cases (p < 0.001) (Fig. 1).

According to the duration of vitiligo, among all the parameters the P-MDA was found to be statistically highly significant in patients having vitiligo more than 1 year (Table 3).

According to the progression of the disease, P-MDA was found to be statistically highly significant in progressive vitiligo cases. Progression of disease increases oxidative stress (Table 4).

According to the diet wise comparison between cases, there was no statistically significant difference was found in any parameters in patients taking vegetarian diet and non-vegetarian diet which shown diet has no role in vitiligo (Table 5).

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Parameters</th>
<th>Stable cases (Mean ± SD)</th>
<th>Progressive cases (Mean ± SD)</th>
<th>t-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>P-MDA</td>
<td>5.031 ± 0.796</td>
<td>5.768 ± 0.726</td>
<td>3.422*</td>
</tr>
<tr>
<td>2</td>
<td>S-SOD</td>
<td>32.896 ± 11.648</td>
<td>33.234 ± 12.579</td>
<td>0.098**</td>
</tr>
<tr>
<td>3</td>
<td>S-Cu</td>
<td>129.271 ± 20.319</td>
<td>129.842 ± 15.774</td>
<td>0.111**</td>
</tr>
</tbody>
</table>

Table 4 Comparison between stable cases and progressive cases

* p < 0.001 shown highly significant result and ** p > 0.1 shown not significant result.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Parameters</th>
<th>Patients taking Veg diet (Mean ± SD)</th>
<th>Patients taking Veg and Non Veg diet (Mean ± SD)</th>
<th>t-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>P-MDA</td>
<td>5.447 ± 0.789</td>
<td>5.344 ± 0.913</td>
<td>0.428**</td>
</tr>
<tr>
<td>2</td>
<td>S-SOD</td>
<td>30.764 ± 12.488</td>
<td>35.766 ± 11.057</td>
<td>1.487**</td>
</tr>
<tr>
<td>3</td>
<td>S-Cu</td>
<td>133.230 ± 18.683</td>
<td>125.244 ± 16.536</td>
<td>1.587**</td>
</tr>
</tbody>
</table>

Table 5 Comparison between two different diet groups in cases

** p > 0.1 shown not significant result.
DISCUSSION

The etiology of vitiligo is still unknown. So there are many hypotheses to explain its pathogenesis. One of the hypotheses to explain vitiligo is the self-destructive theory of melanocytes, which suggests a role for oxidative stress (9–13). The observations and results show that the P-MDA, S-SOD, and S-Cu were found to be a significantly higher level in vitiligo patients as compared to control. This is comparable to the study of Yildirim et al. (2003), Yousry et al. (2007), Jain et al. (2008). Recently many studies have reported accumulation of free radicals (oxidative stress) in the epidermal layers of the affected skin (14,15) and blood of vitiligo patients (16). Oxidative stress could be an important phenomenon leading to melanocyte death in vitiligo. Damage caused by free radicals could be a possible pathogenic factor for vitiligo (17).

This study is also supported by the O. Arican, E.B. Kurutas, which was found significantly higher levels of SOD activity of erythrocytes in patients with active localized vitiligo. In addition, high SOD activities were correlated with high immune competence (18). P.V. Sravani, N. Kishore Babu, K.V.T. Gopal et al. in his study the levels of SOD in the vitiliginous skin of vitiligo patients were found to be higher than the levels of SOD in normal skin of control. So this study concluded that oxidative stress plays an important role in the pathogenesis of vitiligo.

ACKNOWLEDGMENTS

The authors are thankful to Dr. B.K. Agrawal (Ex-HOD) Department of Biochemistry and Dr. Anna Alex (HOD) Department of Skin and V.D. Gandhi Medical College Bhopal MP for providing facilities to carry out this work and proper guidance.

REFERENCES

10. Maresca, V., Roccella, M., Roccella, F., et al. Increased sensitivity to peroxidative agents as


Effects of threshold inspiratory muscle trainer in bronchial asthma

Shiny S. James,1 K. Rekha,1 Vaiyapuri Anandh,2 L. Chandrasekar,2 And Radhakrishnan Unnikrishnan2

1Department of Cardio-Pulmonary, Saveetha College of Physiotherapy, Chennai, India.
2Department of Physical Therapy, Applied Medical Sciences College, Majmaah University, Majmaah, Saudi Arabia.

(Received: Jan 2016 Accepted: Mar 2016)

Corresponding Author
K. Rekha. Email: futurdreams88@gmail.com

ABSTRACT

Introduction and Aim: Many asthma patients develop weakness of their chest and diaphragm muscles, this further contributes to symptoms like shortness of breath or an impaired capability to exercise. Loss of respiratory muscle strength could occur in asthma. If this weakness is substantial, then it is necessary to strengthen the respiratory muscles to improve their functions. Thereby this study is proposed to strengthen the respiratory muscles in asthma patients.

Materials and Methods: A randomized placebo-controlled study performed in Bronchial asthma to strengthen Inspiratory Muscles using Threshold Inspiratory Muscle trainer. Subjects were randomized into 2 groups experimental and control group. Subjects of both the groups performed FEV1, PEFR and 6 minutes walk test as a pretest following which subjects were treated with Threshold device with resistance for experimental group and control group were treated with threshold device without resistance for 30 minutes. Three days a week for a period of 1 month following which post test was done.

Results: Difference between the groups shows that group A is statistically significant with the p value > 0.0001 for all the variables FEV1, PEFR and 6 min walk test.

Conclusion: Threshold Inspiratory muscle training with resistance proved to be effective for improving the Inspiratory muscle strength and Endurance.

Key words: Bronchial Asthma, Inspiratory Muscle Training, Threshold Inspiratory Muscle Trainer

INTRODUCTION

Asthma is a global health problem with raising prevalence rate in the world. According to WHO 300 million people are known to have Bronchial asthma. 255,000 have died due to asthma in 2005. It has been estimated that in India prevalence rate is about 3% which is around 30 million patients consisting of 2.4% adults and 4%–20% is children (5).

Asthma is defined as a “chronic inflammatory disorder of the airways in which many cells and cellular elements play a role.” The chronic inflammation causes an associated increased resistance of airways and hyperinflation that leads to recurrent epi-
sodes of wheezing, breathlessness, chest tightness, and coughing, particularly at night or in the early morning (3), lung places the respiratory muscles at mechanical disadvantage (1). Generalized loss of respiratory muscle bulk occurs in COPD commonly, in asthma patients also it occurs due to the compounded effects of steroid therapy, asthma also shows relevant respiratory muscle weakness (1). Airways smooth muscle also increases in size along with an increase in the numbers of mucous glands. Other cell types involved include: T lymphocytes, macrophages, and neutrophils. There may also be involvement of other components of the immune system including: cytokines, chemokines, histamine, and leukotrienes among others (2).

Asthma patients can be treated with both medical and non-medical intervention, the aim of the treatment being to prevent recurrent asthma attacks, optimal lung function and normal breathing pattern and well-tolerated exercise capacity. Chronic bronchoconstriction in asthmatics is associated with increased inspiratory muscle work, thereby strengthening the inspiratory muscles among asthma could reduce dyspnea and improve exercise tolerance level (3).

Threshold Inspiratory muscle trainers is a device which was designed to provide resistance during breathing; that is independent of in Inspiratory flow rate. The study has concluded that patients trained with threshold inspiratory muscle trainer were able to increase their Inspiratory muscle strength and endurance (4). Threshold loading enhances the velocity of inspiratory muscle contraction and provides a favorable alteration for breathing pattern and time (9).

In the previous studies, it has been proved that the Threshold Inspiratory muscle trainer has trained both strength and endurances in normal subjects as well as in patients with COPD quadriplegics (7), patients with cystic fibrosis (8). Inspiratory muscle strength training have also been investigated in COPD patients, and evidence states that the stimulus or load placed on the respiratory muscles during training was enough to strengthen the inspiratory muscles as a part of the pulmonary rehabilitation program and reduced the duration of hospitalization (12).

Six minute walk test is a simple test easy to administer, better tolerated and reflective of activities of daily living than the other walk tests. It is a test developed to check the physical fitness level of the healthy individual (13). In this study, we perform 6 minute walk test to assess the exercise tolerance level of the asthma patients.

Among bronchial asthma, it is known that Inspiratory muscles are impaired due to hyperinflation of lung and diaphragm become flatten and shorten. Many techniques (breathing exercise, incentive spirometer, PI flex, etc.) which are used to strength the Inspiratory muscle are commonly used. Whereas Threshold Inspiratory muscle trainer (IMT) device is not commonly used in India due to lack of awareness, thereby this study aim to create awareness and make use of the threshold Inspiratory muscle trainer device as it provides resistive breathing which helps in improving the Inspiratory Muscle strength and endurance and there by reduces dyspnea. This study proposed to find out the effect of Threshold Inspiratory Muscle Trainer (IMT) in Bronchial Asthma.

MATERIALS AND METHODS

A placebo controlled study was undertaken at Saveetha Medical College and hospital with 79 Bronchial asthma subjects. Ethical approval and informed consent have been obtained prior to the study. Both males and females subjects with mild and moderate Bronchial asthma (FEV 79% to 40%) between 20 and 60 years of age, using bronchodilators were included in the study. Subjects were excluded if they had status asthmatics, perceptual disorders, and Unstable cardiovascular condition.

Subjects were asked to rest in a chair for approximately 10 minutes after arriving outpatient department. Forced Expiratory volume in one second (FEV1) and Peak Expiratory flow rate (PEFR) were performed and measured using the digital spirometer, 3 trials were performed, and the best trial was recorded (14). Following which 6 Minute Walk Distance was monitored (13). Patients were instructed to walk for 6 minutes in a corridor for a measured distance at their fastest pace and cover the longest possible distance under the
supervision of the physiotherapist (13). And the distance walked will be recorded, all the tests were performed at the baseline as a pretest and at the end of 1 month as a posttest.

Seventy-nine subjects were randomized into two groups, Group A—Experimental group and Group B—Control group. Subjects were not aware in which group they were placed, and subjects were new to the use of Threshold device. Group A consists of 40 subjects were trained with Threshold Inspiratory Muscle trainer (Respironics product REF HS730, USA) in sitting position. The subject was asked place their lips tightly around the mouthpiece, asked to inhale (breathe in) through threshold Inspiratory muscle trainer against the spring loaded valve that provides resistance following which subject was asked to Exhale (breathe out) normally. Threshold Inspiratory muscle trainer with resistance trained for 5 sets per day each set consists of 10 repetitions with 2 minutes rest between each sets, 3 days a week, 1 session per day, each session consisted of 30 mins intervention period for 1 month. Intensity: 12 cm H$_2$O (20% of MIP) in the first week, 16 cm H$_2$O (30% of MIP) in the second week, 20 cm H$_2$O (50% of MIP) in the third week and 24 cm H$_2$O (70% of MIP) in the fourth week (21).

Group B consists of 39 subjects they were trained with Threshold Inspiratory muscle trainer without resistance, subjects were asked to Inhale with

<table>
<thead>
<tr>
<th>Table 1 Characteristics of Participants.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Outcome measure</td>
</tr>
<tr>
<td>-----------------</td>
</tr>
<tr>
<td>FEV1 (%)</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>PEFR (%)</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>6 MWD (ft)</td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 2 Observations of Group A.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Outcome measure</td>
</tr>
<tr>
<td>-----------------</td>
</tr>
<tr>
<td>FEV1 (%)</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>PEFR (%)</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>6 MWT (ft)</td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 3 Observations of Group B.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Outcome measure</td>
</tr>
<tr>
<td>-----------------</td>
</tr>
<tr>
<td>FEV1 (%)</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>PEFR (%)</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>6 MWD (ft)</td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>
their lips sealed around the mouth piece of Threshold device and exhale normally no resistance was provided against Inspiration. Subjects were asked to perform inspiration and expiration with that Threshold device for 10 repetitions with 2 minutes rest between each set. Five sets with 1 session per day lasting for 30 minutes, 3 days a week and so on for a total duration of 1 month.

RESULTS

Statistical significance of Pretest and posttest data for all the outcome measures are analyzed using the paired \( t \)-test within the group and unpaired \( t \) test for between the groups. \( p \)-value <0.05 is considered to be statistically significant.

DISCUSSION

The study shows that Inspiratory muscle strength was impaired in patients with asthma which contributed to other factors like increased dyspnea and reduced exercise tolerance. Weiner et al. has demonstrated that both inspiratory and expiratory muscle weakness among COPD patients in which he specifically trained both the group of muscles and concluded that improving inspiratory muscle strength alone improves 6 minute walk distance and reduces dyspnea, and he found that no special benefits were observed in the combined treatment of both inspiratory and expiratory muscle strength (15).

In our study we used Inspiratory Threshold Muscle trainer, This device had been used for Both the groups but Group A it had been used with the resistance dial which provides resistive breathing exercise, and for Group B the device was used without resistance dial it does not provide resistance against breathing. The intervention was given for 1 month duration 3 times a week with 30 minutes a day. Group A showed significant differences that Threshold Inspiratory Muscle training with resistance is more effective in patients with Bronchial Asthma, which increased the Inspiratory muscle strength and endurance, increased exercise tolerance capacity by reducing dyspnea while performance. Whereas group B also showed slight improvement as it was also a form of breathing exercise but it did not show significance compared to Group A.

Romer et al. stated that inspiratory airflow is proportional to the velocity of muscle shortening, an inspiratory pressure is proportional to force generation, training with high flow loads and resistive loads are known to increase maximum static pressure and maximal inspiratory flow rate (16). Respiratory muscles could be trained similarly to other skeletal muscles which significantly

Fig. 1 Comparison of FEV1 between Group A and Group B.

Fig. 2 Comparison of PEFR between Group A and Group B.

Fig. 3 Comparison of 6 Minute walk Distance between Group A and Group B.
improves respiratory muscle performance. The study suggests that men could generate maximal inspiratory and expiratory pressure than women for a given resistance during breathing (17). Resistance during inhalation comes from constant pressure loading that takes account of inspiratory flow, the valve allows the load to be altered according to the desired level of the patient (10).

Turner et al. demonstrated increase in inspiratory muscle strength following inspiratory muscle training, the results showed an increase in diaphragm thickness and hypertrophy of type 1 and type 2 muscle fibers of external intercostals muscles (18) and increased pulmonary function variables FEV1 and FVC. Hill et al. performed high-intensity inspiratory muscle training for COPD and demonstrated that inspiratory muscle strength increased to the greater extent resulting from the reduction in respiratory motor output and perception of inspiratory effort (19). Loss of fat free mass causes a reduction in skeletal muscle mass including inspiratory muscles and it is associated with impaired inspiratory muscle function. High-intensity inspiratory muscle training produces an increase in inspiratory muscle function which increases diaphragm thickness and lung volumes (20). Martyn et al. demonstrated that ventilator muscle performance allows patients to develop breathing strategies to handle high inspiratory loads. By increasing loads during inspiration, there was a fall in minute ventilation, increased oxygen consumption (21).

Inspiratory muscle training and breathing exercises were effective to promote biomechanical factors of respiratory muscle function including muscles of external intercostals for accessory participation during expiration caused greater thoraco-abdominal mobility. The study showed that inspiratory muscle training performed in children with asthma showed significant improvement by increasing Maximal Inspiratory Pressure, and Maximal Expiratory Pressure and reduced airway obstruction consequently improved activities of daily living (22).

As we know Bronchial Asthma had been a Problem of any age group in recent years, many suffer due to this problem. Threshold Inspiratory Muscle training among Bronchial Asthma improved Inspiratory Muscle Strength and Endurance by improving FEV1, and PEFR and also Improved 6 minute walk Distance by reducing dyspnea by enhancing the daily activities of the individual.

CONCLUSION

Inspiratory muscle training using Threshold device with the resistance dial had proven to be effective when compared with the Threshold device without resistance among the Bronchial Asthma, which had significant effects in improving the FEV1, PEFR, 6 Minute walk Distance and reduced dyspnea.

ACKNOWLEDGEMENT

We thank all the participants for their cooperation and interest delivered in this study.

CONFLICT OF INTEREST

None.

REFERENCES

Assessment of serum uric acid in type 2 diabetes mellitus in a middle-aged south Indian population

Jyoti John,1 RajLaxmi Sarangi,1 Asha Dinakaran,1 S.V. Umadevi,2 Somanath Padhi,3 and Nitin Ashok John,2

Departments of 1Biochemistry and 3Pathology, Pondicherry Institute of Medical Sciences, Pondicherry, India.

2Department of Physiology, Indira Gandhi Medical College and Research Institute, Pondicherry, India.

(Received: Feb 2016  Accepted: Mar 2016)

Corresponding Author

Dr. Jyoti John. Email: jyotijohn7@gmail.com

ABSTRACT

Introduction and Aim: Several studies in recent past have postulated the role of high serum uric acid levels in the pathophysiology of the insulin resistance, metabolic syndrome, and type 2 diabetes mellitus (T2DM). However, controversy persists whether hyperuricemia has a causal association with T2DM; and requires confirmation by further studies.

Materials and Methods: In this case-control study, fasting plasma glucose (FPG), serum uric acid (UA), plasma insulin, glycosylated hemoglobin (HbA1c), HOMA-IR and HOMA-B were measured among 70 T2DM patients (both males and females, age group; 30–80 years); and compared with 50 healthy age-matched controls.

Results: Compared to controls, patients with T2DM had higher FPG (90.62 ± 6.84 vs. 165.81 ± 61.96 mg/dl, p < 0.001), HOMA-IR (2.29 ± 1.44 vs. 5.16 ± 4.41, p < 0.001), plasma insulin (10.04 ± 6.03 μU/ml vs. 12.71 ± 10.77 μU/ml, p = 0.420) and serum uric acid levels (4.77 ± 1.51 mg/dl vs. 5.33 ± 1.94 mg/dl, p = 0.126) but lower beta cell function (131.10% ± 75.76% vs. 59.92% ± 57.13%, p < 0.001). Uric acid correlated negatively with levels of fasting plasma glucose (r = -0.243; p < 0.05) in cases but not controls. The levels of uric acid are correlated positively with the calculated beta cell function (HOMA-B) (r = 0.274; p < 0.05).

Conclusion: Our patients with type 2 diabetes mellitus had significantly higher fasting plasma glucose and HOMA-IR but significantly lower beta cell function as compared to controls. Uric acid shows a significant negative correlation with fasting plasma glucose but a significant positive correlation with beta cell function in our cases.

Key words: Beta Cell Function, HbA1c, HOMA-B, HOMA-IR, Insulin Resistance, Type 2 Diabetes Mellitus, Uric Acid

INTRODUCTION

Uric acid is the end product of purine metabolism in humans. This is because humans lack the enzyme uricase which converts uric acid into allantoin. As a result, humans have higher uric acid levels than most other mammals having the enzyme uricase. An important role of uric acid is that it can function as an antioxidant in the
plasma (1,2). Urate (the soluble form of uric acid in the blood) is a scavenger of super oxide and hydroxyl radical, and can also chelate transition metals. But at increased concentrations, it can also act as a prooxidant and may be a marker of oxidative stress (3). Increased oxidative stress is known to play a potential role in the development of diabetes mellitus and its complications (4).

Uric acid has recently been implicated in the pathophysiology of type 2 diabetes mellitus (T2DM). There is a complex relationship between uric acid and hyperinsulinaemia. Increased serum uric acid levels are seen before the development of insulin resistance and diabetes mellitus (5). Hyperinsulinaemia, in turn, leads to decreased renal excretion of urate (6,7). While the increased uric acid may be due to the increased uric acid absorption in the proximal tubule secondary to hyperinsulinaemia, a growing number of researchers have found that uric acid may predict the development of metabolic syndrome, obesity, and diabetes (8–10).

Though increased uric acid levels could be one of the risk factors for diabetes mellitus, controversy exists on whether uric acid plays a causal role. There are studies showing positive association while some studies show an inverse association. Review of the available literature shows that there is ambiguity regarding association between serum urate levels and diabetes mellitus. Moreover to the best of our knowledge, very few studies have been done in this part of South India evaluating the role of uric acid in diabetes mellitus. With this background, we decided to carry out the present study. The objectives of the study were to measure the levels of serum uric acid, plasma glucose, plasma insulin and glycosylated haemoglobin in cases of diabetes mellitus and controls and to ascertain whether there is any association between serum uric acid and insulin resistance in patients of diabetes mellitus.

MATERIALS AND METHODS

This was a cross-sectional study carried out at our institute between the period January 2014 to July 2014. The study was approved by our Institutional Ethical Committee (IEC number: RC/13/128). A total of 70 patients with type 2 diabetes mellitus both males and females between the age group of 30–80 years were included in our study. Those patients with a history of Hypertension/Gout/ Rheumatoid arthritis/ Kidney disease/Liver disease/ Acute myocardial infarction/ Stroke/Alcoholism were excluded from the study. Patients with any other Acute or Chronic illnesses were also excluded from the study. We also included 50 healthy controls that were in the same age group. A single blood sample (5 ml) was drawn from the subjects and analysed for fasting plasma glucose, serum uric acid, plasma insulin and glycated haemoglobin levels. Diagnostic criterion for cases was fasting plasma glucose >126 mg/dl or HbA1C >6.5%. The fasting plasma glucose (FPG) levels were measured using Cobas Integra 400+ Autoanalyser (Roche). The fasting plasma insulin levels were measured using Cobas e 411 Autoanalyser (Roche). The serum uric acid levels were measured by uricase-peroxidase enzymatic end point method using semi-autoanalyser (Kit by Accurex). The glycated haemoglobin levels were measured by latex enhanced Immuno turbidimetry (Kit by Agappe). The HOMA-IR (homeostasis model assessment of insulin resistance) and HOMA-B (homeostasis model assessment of β-cell function) were calculated based on the formula:

\[
\text{HOMA-IR} = \frac{\text{fasting plasma glucose (mg/dl)} \times \text{fasting plasma insulin (µU/ml)}}{405}
\]

\[
\text{HOMA-B} = \frac{360 \times \text{fasting plasma insulin (µU/ml)}}{\text{fasting plasma glucose (mg/dl)} - 63}
\]

Statistical analysis

The quantitative data was presented as a mean and standard deviation. Mann-Whitney U test was done to assess the statistical significance of the difference between mean values amongst cases and controls. Spearman’s correlation co-efficient was used to analyze the association between biochemical parameters. A value of \( p < 0.05 \) was taken as statistical significance. Analysis was done using IBM SPSS software (version 20.0).

Observations
RESULTS

The mean fasting plasma glucose level in cases was 165.81 ± 61.96 mg/dl and that in controls were 90.62 ± 6.84 mg/dl. The difference is statistically significant (p < 0.001). The plasma insulin levels in cases were 12.71 ± 10.77 μU/ml and that in controls were 10.04 ± 6.03 μU/ml (p = 0.420). The levels of serum uric acid were higher in cases (5.33 ± 1.94 mg/dl) as compared to controls (4.77 ± 1.51 mg/dl) (p = 0.126). The HOMA-IR in cases was 5.16 ± 4.41 and that in controls were 2.29 ± 1.44. The difference is statistically significant (p < 0.001). The HOMA-B in cases was 59.92 ± 57.13 and that in controls were 131.10 ± 75.76. The difference is statistically significant (p < 0.001).

In this study, we found that the levels of fasting plasma glucose correlated negatively with levels of serum uric acid (r = -0.243; p < 0.05). We also found that the levels of fasting plasma glucose correlated negatively with HOMA-B (r = -0.636; p < 0.001). The levels of fasting plasma glucose correlated positively with levels of HbA1c (r = 0.573; p < 0.001) and HOMA-IR (r = -0.359; p = 0.002) in our cases.

We also found that the levels of uric acid correlated positively with the calculated beta cell function (HOMA-B) (r = 0.274; p < 0.05).

DISCUSSION

In our study, patients with type 2 diabetes mellitus had significantly higher fasting plasma glucose and HOMA-IR indicating insulin resistance in our cases. Moreover, our cases had significantly lower beta cell function as compared to controls.

Our results also showed higher levels of uric acid in cases as compared to controls though the difference is not statistically significant. Our findings similar to the findings of Bakshi et al. (11). Gill et al. (12) also found that HbA1c, serum insulin, and serum uric acid were increased in the patients with Type 2 diabetes mellitus as compared to controls. Wasada et al. (13) found that elevated serum uric acid is a feature of insulin resistance syndrome. One study showed that uric acid was found to have prognostic significance in type 2 diabetes patients along with an increased mortality risk (14).

Our results also show that uric acid has a negative correlation with fasting plasma glucose in patients with type 2 diabetes mellitus. The difference is statistically significant (p < 0.05). Our findings similar to the findings of other researchers who found an inverse association between uric acid levels and type 2 diabetes mellitus (Bhandaru and Shankar (15), Oda et al. (16–22).

A negative correlation of uric acid was observed with fasting plasma glucose levels. There is a complex relationship between uric acid and insulin resistance. Serum uric acid levels are known to increase before the development of insulin resistance and diabetes mellitus (5). Increased uric acid may be due to the increased uric acid absorption in the proximal tubule secondary to hyperinsulinemia. Once hyperinsulinemia develops, this, in turn, leads to decreased renal excretion of urate (6,7). Hence,
<table>
<thead>
<tr>
<th>Spearman’s rho</th>
<th>FBS</th>
<th>Insulin</th>
<th>Uric acid</th>
<th>HOMA-IR</th>
<th>Beta cell</th>
<th>HbA1c</th>
<th>Age</th>
</tr>
</thead>
<tbody>
<tr>
<td>FBS</td>
<td>Correlation Coefficient</td>
<td>1.000</td>
<td>0.063</td>
<td>−0.243*</td>
<td>0.359**</td>
<td>−0.636**</td>
<td>0.573**</td>
</tr>
<tr>
<td>Sig. (2-tailed)</td>
<td></td>
<td></td>
<td>0.607</td>
<td>0.042</td>
<td>0.002</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>N</td>
<td>70</td>
<td>70</td>
<td>70</td>
<td>70</td>
<td>70</td>
<td>70</td>
<td>70</td>
</tr>
<tr>
<td>Insulin</td>
<td>Correlation Coefficient</td>
<td>0.063</td>
<td>1.000</td>
<td>0.189</td>
<td>0.929**</td>
<td>0.678**</td>
<td>0.097</td>
</tr>
<tr>
<td>Sig. (2-tailed)</td>
<td></td>
<td>0.607</td>
<td>.</td>
<td>0.117</td>
<td>0.000</td>
<td>0.000</td>
<td>0.424</td>
</tr>
<tr>
<td>N</td>
<td>70</td>
<td>70</td>
<td>70</td>
<td>70</td>
<td>70</td>
<td>70</td>
<td>70</td>
</tr>
<tr>
<td>Uric acid</td>
<td>Correlation Coefficient</td>
<td>−0.243*</td>
<td>0.189</td>
<td>1.000</td>
<td>0.094</td>
<td>0.274*</td>
<td>−0.016</td>
</tr>
<tr>
<td>Sig. (2-tailed)</td>
<td></td>
<td>0.042</td>
<td>0.117</td>
<td>.</td>
<td>0.439</td>
<td>0.022</td>
<td>0.896</td>
</tr>
<tr>
<td>N</td>
<td>70</td>
<td>70</td>
<td>70</td>
<td>70</td>
<td>70</td>
<td>70</td>
<td>70</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>Correlation Coefficient</td>
<td>0.359**</td>
<td>0.929**</td>
<td>0.094</td>
<td>1.000</td>
<td>0.423**</td>
<td>0.232</td>
</tr>
<tr>
<td>Sig. (2-tailed)</td>
<td></td>
<td>0.002</td>
<td>0.000</td>
<td>0.439</td>
<td>.</td>
<td>0.000</td>
<td>0.053</td>
</tr>
<tr>
<td>N</td>
<td>70</td>
<td>70</td>
<td>70</td>
<td>70</td>
<td>70</td>
<td>70</td>
<td>70</td>
</tr>
<tr>
<td>Beta cell</td>
<td>Correlation Coefficient</td>
<td>−0.636**</td>
<td>.678**</td>
<td>.274*</td>
<td>.423**</td>
<td>1.000</td>
<td>−0.301*</td>
</tr>
<tr>
<td>Sig. (2-tailed)</td>
<td></td>
<td>0.000</td>
<td>0.000</td>
<td>0.022</td>
<td>0.000</td>
<td>.</td>
<td>0.011</td>
</tr>
<tr>
<td>N</td>
<td>70</td>
<td>70</td>
<td>70</td>
<td>70</td>
<td>70</td>
<td>70</td>
<td>70</td>
</tr>
<tr>
<td>HbA1c</td>
<td>Correlation Coefficient</td>
<td>0.573**</td>
<td>0.097</td>
<td>−0.016</td>
<td>0.232</td>
<td>−0.301*</td>
<td>1.000</td>
</tr>
<tr>
<td>Sig. (2-tailed)</td>
<td></td>
<td>0.000</td>
<td>0.424</td>
<td>0.896</td>
<td>0.053</td>
<td>0.011</td>
<td>.</td>
</tr>
<tr>
<td>N</td>
<td>70</td>
<td>70</td>
<td>70</td>
<td>70</td>
<td>70</td>
<td>70</td>
<td>70</td>
</tr>
<tr>
<td>Age</td>
<td>Correlation Coefficient</td>
<td>−0.156</td>
<td>−0.185</td>
<td>.120</td>
<td>−0.221</td>
<td>−0.016</td>
<td>−0.051</td>
</tr>
<tr>
<td>Sig. (2-tailed)</td>
<td></td>
<td>0.197</td>
<td>0.125</td>
<td>0.324</td>
<td>0.066</td>
<td>0.897</td>
<td>0.675</td>
</tr>
<tr>
<td>N</td>
<td>70</td>
<td>70</td>
<td>70</td>
<td>70</td>
<td>70</td>
<td>70</td>
<td>70</td>
</tr>
</tbody>
</table>

Table 2 Spearman’s correlations coefficient in patients of type 2 diabetes mellitus (n = 70).

*Correlation is significant at the 0.05 level (2-tailed).

**Correlation is significant at the 0.01 level (2-tailed).
the inverse relationship between serum uric acid and fasting plasma glucose observed in our diabetic subjects may probably be caused by the increased renal excretion of uric acid in the presence of hyperglycaemia.

The hyperglycemia seen in type 2 diabetes mellitus may lead to generation of free radicals, disturbing the endogenous antioxidant defense system including its effect on uric acid homeostasis. In addition, hyperuricemia induces reactive oxygen species production inside adipose cells (23,24). Since oxidative stress in adipocytes has been shown to lead to insulin resistance, uric acid-induced oxidative stress in adipose tissue might play a key role in insulin resistance.

Our study showed that the levels of uric acid correlated positively with the calculated beta cell function (HOMA-B) in our cases, and this was statistically significant \( p < 0.05 \). In one study by Wei et al. type 2 diabetic patients with higher serum uric acid level were found to have better insulin secretion, but their residual beta cell function seemed to decay more quickly (25). In another study Robles-Cervantes et al. found that the serum concentration of uric acid showed a positive relationship with the total phase of insulin secretion and concluded that even in states prior to hyperuricemia, uric acid can play an important role in the function of the beta cell in patients with type 2 diabetes mellitus (26).

The limitation of our study was that it was a cross sectional study, and a causal role cannot be assigned to uric acid. Further, the sample size was small and further prospective studies are needed to elucidate the relationship between uric acid and diabetes mellitus.

**CONCLUSION**

Our patients with type 2 diabetes mellitus had significantly higher fasting plasma glucose and HOMA-IR but significantly lower beta cell function as compared to controls. Uric acid shows a significant negative correlation with fasting plasma glucose in our cases. Though a strong relationship exists between serum uric acid and diabetes mellitus, the exact etiopathogenetic mechanism by which uric acid contributes to this disease are yet to be unravelled. It is clear that further research in this field is needed to fully comprehend the role of uric acid in diabetes mellitus.

**ACKNOWLEDGEMENTS**

We acknowledge the help received from our colleague Dr. Lopamudra Ray, Assistant Professor and Mr. Tamizharasan, Technician, Dept of Biochemistry, PIMS and also from Dr. K Ravichandran, Statistician, PIMS.

**CONFLICT OF INTEREST**

There is no conflict of interest.

**REFERENCES**

A comparative study in search of the best treatment for non-metastatic locally-advanced squamous cell carcinoma of head and neck

Sumana Maiti¹ and Siddhartha Das²

¹Department of Radio-Therapy, Institute of Post-graduate Medical Education and Research (IPGMER), Kolkata, West Bengal, India.
²Department of Physiology, Bankura Sammilani Medical College, Bankura, West Bengal, India.

(Received: Jan 2016 Accepted: Mar 2016)

Corresponding Author
Dr. Siddhartha Das. Email: das.siddhartha01@gmail.com

ABSTRACT

Introduction and Aim: Treatment of locally advanced head neck carcinomas has been the matter of dispute over years. The specific objectives of this study was to compare the efficacy and toxicity profiles of three different radiotherapy regimens namely, hypo-fractionated Christie regimen, conventional pure accelerated radiotherapy, and concurrent chemo-radiotherapy.

Materials and Methods: Sixty-nine patients were divided in three arms with 23 patients receiving “Christie” regimen, 24 patients pure accelerated regimen, and 22 patients concurrent chemo-radiation. The response rates (using RECIST criteria) and toxicity patterns—both acute and late (by RTOG/EORTC criteria) were assessed and statistically analyzed.

Results: Overall response rates did not show statistically significant difference amongst three arms. Acute grade 2 and grade 3 skin and mucosal toxicities were highest in concurrent chemo-radiation arm (p value 0.014 and 0.016 respectively) whereas acute salivary gland toxicity was highest in “Christie” regimen arm (p value 0.029). However there were no statistically significant differences in late toxicities amongst three arms. Improved disease free survival (DFS) was observed in concurrent chemo-radiation arm (p value 0.043).

Conclusions: Concurrent chemo-radiation is the best treatment option in terms of loco-regional control and disease free survival with acceptable increase in acute toxicities in locally advanced squamous cell carcinoma of head and neck.

Key words: Chemo-Radiation, Christie Regimen, Head Neck Carcinoma.

INTRODUCTION

Carcinoma of head and neck is more common in male than female due to indiscriminate use of tobacco in various forms and alcohol. Nearly 60% of newly diagnosed patients present with locally advanced but non-metastatic disease (1).

Head and neck cancer is best managed in a multi-disciplinary setting. Surgery, radiation therapy, chemotherapy and, more recently, biologic therapy are often employed in various combinations in an attempt to eradicate both clinically apparent and occult diseases. The goals of treatment include maximizing tumor control while maintaining function and quality of life.
Historically, patients with unresectable head and neck squamous cell carcinomas used to be treated by radiotherapy (RT) alone. The radiation dose fractionation has evolved from once daily treatment to hyper-fractionation and accelerated fractionation (2). Nonetheless, even the most effective (RT) regimens result in local controls rate of 50%–70% and disease free survivals of 30%–40%. These circumstances lead to the combined modality-concurrent chemo-radiotherapy as the standard care of treatment for locally advanced head and neck cancer (3).

The optimum treatment time for loco-regional control is unclear. The possible cause for treatment resistance could be radiation induced accelerated proliferation of clonogenic tumour cells. The worsening in the loco-regional control by lengthening the treatment time has been clinically and biologically documented (4). Furthermore, several clinical studies showed reduction in total treatment time has improved tumour control (5). A shorter treatment time may be accomplished by applying a higher dose per fraction or by increasing the weekly number of radiation fractions.

The benefit of an increased tumour cell kill because of large fraction size in a short overall treatment time is counteracted from the radiobiological point of view, by an increased potential for late side effects (6). However late toxicity is often less relevant in patients treated in advanced setting.

The Christie Hospital in Manchester developed a 3-week schedule of RT during World War II when RT facilities were limited. Results were found not to be different from the conventional schedules used in the previous treatment periods in terms of local control and toxicity (7). This schedule was, therefore, adopted by Christie hospital and number of other British cancer centers as a standard RT schedule for early-stage laryngeal cancer. Many randomized and non-controlled trials have also shown no difference in local control between conventional and hypo-fractionated schedules. Surprisingly, many of these schedules gave less severe late normal tissue reaction than expected given the short overall treatment time and the high fraction dose (8).

The effort of this study was to compare the response patterns in non-metastatic locally advanced head and neck cancer with squamous cell histology by three modes of radiotherapy namely: the hypo-fractionated “Christie” regimen; pure accelerated radiation (6 fractions per week); and concurrent chemotherapy with conventional radiation.

The specific aim of this study was as follows:

1. To compare the response rates in terms of complete response, partial response and stable disease in different arms by Response Evaluation Criteria in Solid Tumors (RECIST) criteria (9).
2. To determine and compare the acute and chronic toxicities according to the RTOG/EORTC (Radiation Therapy Oncology Group/ European Organization for Research and Treatment of Cancer) Acute and Chronic Radiation Morbidity Criteria.
3. To assess the disease free survival (DFS) rates in different regimens.

MATERIALS AND METHODS

This study was done upon ethical clearance by the Institutional Ethics Committee and as per Helsinki declaration.

Study design

A single-institutional, prospective, open labeled, longitudinal, randomized controlled study.

Patients attending the Radiotherapy OPD, Department of Radiotherapy, IPGMER, Kolkata were selected for study.

Inclusion criteria

1. Age 18–70 years
2. Histologically proven non-metastatic stage III, IVA and IVB Squamous cell carcinoma of head and neck (staging according to Prognostic Groups, American Joint Committee on Cancer, AJCC, 6th edition.)
3. No previous history of treatment of cancer
4. Creatinine clearance more than or equal to 60 ml/minute.

**Exclusion criteria**

1. Histopathology other than squamous cell carcinoma.
2. Patients with stage I or II diseases
3. Evidence of distant metastases by clinical or radiological examination.
4. Prior Radiotherapy for any reason to head and neck region.
5. No other primary malignancies.

**Study period**

January 2010 to August 2012.

**Sample size**

Fifty (50).

**Detailed procedure of study**

Before the inception of the study an application was submitted to the Institutional Ethics Committee (IEC); IEC after proper scrutiny and detailed deliberation review approved the research proposal.

**Pre-treatment assessment**

1. Detailed history, clinical examination with ENT examination
2. Biopsy from primary site and/or FNAC from lymph node
3. Contrast enhanced CT scan of head and neck
4. Routine investigations—hematological, biochemical, chest skiagram

**Radiation therapy—External beam radiation therapy (EBRT)**

Instrument used—cobalt 60-ATC-C9 (Picker manufacture)

**Treatment plan**

Treatment was given using megavoltage equipment with Cobalt-60, energy 1.17, 1.33 MeV (average 1.25 MeV). The minimum Source-Surface Distance (SSD) was 80 cm.

Patients in Arm A (Christie Regimen) received External Beam Radiotherapy(EBRT) with dose of 50 Gy (Gray) in 15 fractions, single fraction per day, 5 fractions per week, 3.33 Gy per fraction, total duration 3 weeks.

Arm B received 66 Gy in 33 fractions, 2 Gy per fraction, 6 fractions per week, single fraction per day, total duration 51/2 weeks.

Arm C received 66 Gy in 33 fractions, 2 Gy per fraction, single fraction per day, total duration 61/2 weeks with IV inj cisplatin (30 mg/m²) weekly. Inj Cisplatin was given from the first day of start of EBRT.

Patients in arm A and C were assigned five fractions per week, given one fraction daily from Monday to Friday. Patients in arm B were assigned six fractions per week, one fraction daily, from Monday to Friday, and the sixth fraction given on Saturday.

**After completion of treatment**

Six to 8 weeks after completion of treatment, a detailed ENT examination and Contrast enhanced CT scan of head and neck were done to assess the response. Biopsies from suspicious clinical and /or radiological residual disease of primary site and/or nodal area were performed. Those with residual disease were sent for surgery if feasible.

The primary end point of the study was loco-regional control in terms of complete response, partial response, stable disease and progressive disease after EBRT in three different arms using recently published “Response Evaluation Criteria in Solid Tumors” (RECIST) criteria (9).

The acute and late toxicity of the patients in all arms were assessed during the treatment and during the
<table>
<thead>
<tr>
<th>Age (years)</th>
<th>GR A (n = 23), n %</th>
<th>GR B (n = 24), n %</th>
<th>GR C (n = 22), n %</th>
</tr>
</thead>
<tbody>
<tr>
<td>40–49</td>
<td>6 (26)</td>
<td>7 (29.2)</td>
<td>5 (22.7)</td>
</tr>
<tr>
<td>50–59</td>
<td>8 (34.8)</td>
<td>7 (29.2)</td>
<td>9 (40.9)</td>
</tr>
<tr>
<td>60–69</td>
<td>9 (39.1)</td>
<td>10 (41.7)</td>
<td>8 (36.4)</td>
</tr>
</tbody>
</table>

Table 1 Number of patients in the treatment protocol.

ANOVA test p-value = 0.740 (not significant).

<table>
<thead>
<tr>
<th>Responses</th>
<th>Groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group A: Christie regimen, n (%)</td>
</tr>
<tr>
<td>Complete response</td>
<td>11 (47.8)</td>
</tr>
<tr>
<td>Partial response</td>
<td>7 (30.4)</td>
</tr>
<tr>
<td>Stable</td>
<td>3 (13.0)</td>
</tr>
</tbody>
</table>

Table 2 Response assessment by RECIST, 6–8 weeks post treatment.

<table>
<thead>
<tr>
<th>Acute radiation induced changes</th>
<th>Groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group A: Christie regimen, n (%)</td>
</tr>
<tr>
<td>Skin</td>
<td></td>
</tr>
<tr>
<td>Grade 0</td>
<td>6 (26.1)</td>
</tr>
<tr>
<td>Grade 1</td>
<td>3 (13.0)</td>
</tr>
<tr>
<td>Grade 2</td>
<td>8 (34.8)</td>
</tr>
<tr>
<td>Grade 3</td>
<td>6 (26.1)</td>
</tr>
<tr>
<td>Mucosal</td>
<td></td>
</tr>
<tr>
<td>Grade 0</td>
<td>2 (8.7)</td>
</tr>
<tr>
<td>Grade 1</td>
<td>3 (13.0)</td>
</tr>
<tr>
<td>Grade 2</td>
<td>11 (47.8)</td>
</tr>
<tr>
<td>Grade 3</td>
<td>7 (30.4)</td>
</tr>
</tbody>
</table>

Table 3 Radiation induced acute toxicities.

<table>
<thead>
<tr>
<th>Salivary gland</th>
<th>Groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group A: Christie regimen, n (%)</td>
</tr>
<tr>
<td>Acute</td>
<td></td>
</tr>
<tr>
<td>Grade 1</td>
<td>5 (21.7)</td>
</tr>
<tr>
<td>Grade 2</td>
<td>18 (78.3)</td>
</tr>
<tr>
<td>Late</td>
<td></td>
</tr>
<tr>
<td>Grade 1</td>
<td>9 (39.1)</td>
</tr>
<tr>
<td>Grade 2</td>
<td>12 (52.2)</td>
</tr>
<tr>
<td>Grade 3</td>
<td>2 (8.7)</td>
</tr>
</tbody>
</table>

Table 4 Radiation induced Acute and Late Salivary gland changes.

www.biomedicineonline.org
follow-up period using clinical status, laboratory tests and the grade according to the RTOG/EORTC (Radiation Therapy Oncology Group/European Organization for Research and Treatment of Cancer) Acute and Chronic Radiation Morbidity Criteria.

Statistical analysis was done using SPSS version 17. The ANOVA test was used for comparing baseline profiles, the response rates and toxicities among patients of three treatment arms, with \( p \) value <0.05 as significant. Disease free survival (DFS) was measured from the date of completion of treatment to the date of first relapse (loco-regional or distant metastasis) or death. The disease free survival was determined using the Kaplan Meier survival analysis with Log Rank test for comparing the DFS.

**RESULT AND ANALYSIS**

The study was designed as single institutional, prospective, open labeled, longitudinal, randomized control study, conducted from January 2010 to July 2012.

Sixty-nine patients were randomly selected after fulfilling the eligibility criteria with 23 patients in “Christie Regimen” (group A), 24 in “Pure accelerated radiotherapy” (group B) and 22 patients in “Concurrent chemo-radiation” (group C).

Comparison of demographic profiles amongst different treatment arms showed no statistically significant difference.

ANOVA analysis showed no statistical difference in Overall response (CR+PR) rates among the three treatment arms (\( p \) value ~0.945) analysis showed no statistical difference in Overall response (CR+PR) rates among the three treatment arms (\( p \) value ~0.945) treatment arms (\( p \) value ~0.945).

Sixty-nine patients were evaluated for response at stipulated 6–8 weeks post treatment using RECIST criteria. Overall response rates (CR+PR) were 78.2% in Arm A—Christie arm, 70.8% in Arm B—pure accelerated radiotherapy schedule and 77.3% in arm C—concurrent chemoradiation with ANOVA analysis showed no significant statistical difference (\( p \) value ~0.945).

The skin toxicity assessed by RTOG Acute Morbidity Scoring was significantly high in chemo-radiation arm showing highest grade 2 and 3 toxicities of 90.9% versus 66.7% in pure accelerated regimen arm and 60.9% in “Christie regimen” arm with ANOVA test \( df = 2.66, f = 4.560 \) and \( p \) value 0.014 (significant). (Table 3)

The skin toxicity assessed by RTOG Acute Morbidity Scoring was significantly high in chemo-radiation arm showing highest grade 2 and 3 toxicities of 90.9% versus 66.7% in pure accelerated regimen arm and 60.9% in “Christie regimen” arm with ANOVA test \( df = 2.66, f = 4.560 \) and \( p \) value 0.014 (significant). (Table 3)

The acute mucosal toxicity assessed by RTOG Acute Morbidity Scoring was significantly high in chemo-radiation arm showing highest grade 2 and 3 toxicities of 86.3% versus 54.2% in pure accelerated radiation arm and 78.2% in “Christie regimen” arm, with ANOVA test \( df = 2.66, f = 4.376 \) and \( p \) value 0.016 (significant) (Table 3). The acute and late radiation induced toxicities of salivary gland were more in

<table>
<thead>
<tr>
<th>Late radiation induced changes</th>
<th>Groups</th>
<th>Groups</th>
<th>Groups</th>
<th>Groups</th>
<th>Groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group A: Christie regimen, ( n ) (%)</td>
<td>Group B: Pure accelerated radiation, ( n ) (%)</td>
<td>GROUP C: Cisplatin chemoradiation, ( n ) (%)</td>
<td>( p ) Value</td>
<td></td>
</tr>
<tr>
<td>Skin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grade 0</td>
<td>3</td>
<td>13.0</td>
<td>3</td>
<td>12.5</td>
<td>1</td>
</tr>
<tr>
<td>Grade 1</td>
<td>10</td>
<td>43.5</td>
<td>14</td>
<td>58.3</td>
<td>11</td>
</tr>
<tr>
<td>Grade 2</td>
<td>6</td>
<td>26.1</td>
<td>6</td>
<td>25.0</td>
<td>9</td>
</tr>
<tr>
<td>Grade 3</td>
<td>4</td>
<td>17.4</td>
<td>1</td>
<td>4.2</td>
<td>1</td>
</tr>
<tr>
<td>Mucosal</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grade 0</td>
<td>6</td>
<td>26.1</td>
<td>2</td>
<td>8.3</td>
<td>0</td>
</tr>
<tr>
<td>Grade 1</td>
<td>10</td>
<td>43.5</td>
<td>15</td>
<td>62.5</td>
<td>15</td>
</tr>
<tr>
<td>Grade 2</td>
<td>5</td>
<td>21.7</td>
<td>6</td>
<td>25.0</td>
<td>4</td>
</tr>
<tr>
<td>Grade 3</td>
<td>2</td>
<td>8.7</td>
<td>1</td>
<td>4.2</td>
<td>3</td>
</tr>
</tbody>
</table>

Table 5 Radiation induced late toxicities.
In spite of high toxicities in all the arms, there were no statistical difference in late effects in any of the treatment arms (Table 5).

At a median disease free survival of 11 months, the disease free survival using Kaplan Meier survival analysis with adjustment for stage, was significantly better in chemo-radiation arm with Log Rank analysis Chi Square 6.544, df 2 and p value 0.043 (Tables 6 and 7).

**DISCUSSION**

Locally advanced head and neck cancer is usually associated with a poor prognosis because of high recurrence rate despite aggressive management with surgery followed by post-operative radiation (10). In an attempt to improve the prognosis, concurrent chemo-radiation was introduced, chemotherapy acting as a radio-sensitizer. The combination of chemotheraphy and radiation may improve the local control and survival rate because of the additive or synergistic effect of chemo-radiation. Altered fractionation improves loco-regional control in patients with contraindication for chemotherapy (11).

This study aims to compare the outcome of three different radiotherapy regimens—hypo-fractionated “Christie regimen,” conventional pure accelerated radiotherapy and concurrent chemo-radiation using weekly inj cisplatin (30 mg/m²). No such comparative study is available in the existing literature. Very few study are available in literature using “Christie regimen” in definitive settings.

The present study results corroborates with previous studies with regard to the response rates along with significantly increased acute skin toxicities and acute mucosal toxicities in cases treated with concurrent chemo-radiotherapy using weekly cisplatin (10,12).

But head and neck cancer patients commonly carry excessive co-morbidities due to chronic tobacco and alcohol use and many of them would not meet the basic eligibility criteria for extensive chemo-radiotherapy with existing co-morbid conditions like renal failure, hearing difficulties or neuropathy. Instead, pure accelerated radiation and hypo-fractionated “Christie regimens” are possible treatment options.

The well documented relationship between acceleration by 1 week and improved local control has also been established in the present study, where overall response rate is better, though the toxicities were high but could be managed conservatively and there were no incidence of treatment break.
The results of our study showed insignificant statistical difference between the responses and late toxicities among the three treatment arms (Tables 2, 4, and 5), despite significantly increased acute skin and mucosal toxicities in concurrent chemo-radiation arm and acute salivary gland toxicity in Christie regimen arm.

Whithers et al. reported that the total dose of RT needed to control 50% of head and neck carcinomas progressively increased with time whenever radiation therapy treatment was prolonged beyond 1 month (4). This increase was attributed to accelerated repopulation, and it was estimated to ~0.6 Gy/day, but may be as high as 1 Gy/day. Extension of overall duration of therapy results in decrease in tumour control rate by 1%–2% each day after the lag period of 4 weeks (13).

Biological Equivalent Dose (BED) corrected for time in concurrent chemo-radiation arm (treatment duration of 45 days) is 58.4 Gy (α/β = 10 Gy) and for pure accelerated arm is 61.6 Gy assuming α/β = 10 Gy, total treatment duration of 38 days (14). Net results should show that maximum benefit would be seen in pure accelerated arm when comparing only radiation.

However, addition of chemotherapy with its additive effects (cytotoxicity) and supra additive effects (described by 5th “R” of radiobiology, i.e., radiosensitivity), the resultant BED is increased by 9–11 Gy BED equivalent. So from radiobiological point of view, concurrent chemo-radiotherapy is always a better option. The benefit of chemotherapy was seen in improved disease free survival.

It was also well established that fraction size would be the dominant factor in determining late effects; overall treatment time has little influence. However, fraction size and overall treatment time both determine the response of acutely responding tissues (15).

Despite highest acute salivary gland toxicity in Christie regimen, no statistically significant difference in chronic toxicity was observed amongst three arms (Table 4). Possible explanation may be shorter follow up period or salivary glands received more than maximum tolerance dose (TD5/5 = 26 Gy) in all three arms due to conventional planning of application.

However, there were several pitfalls of the study.
1. Our sample size was small, so any statistical data was to be interpreted with caution.
2. Locally advanced head and neck cancer in Indian population is probably different from those of the Western World, establishing the basic concept of pharmaco-genomics and its impact on cancer.

**CONCLUSION OF THE STUDY**

Concurrent chemo-radiation is the best option in terms of loco-regional control and disease free survival with acceptable increase in toxicity in case of locally advanced squamous cell carcinoma of head and neck.

With co-morbidities limiting the aggressive use of chemotherapeutic agents in many patients, altered radiation fractionations, that is, pure accelerated and hypo-fractionated “Christie” radiation regimens remain viable alternatives to chemo-radiation, with similar response rates and acceptable toxicities, but definitively inferior disease free survival.

Further studies with larger sample size and longer follow up period are required for establishing these observations.

**REFERENCES**


Comparison in between subjects of determinants of oxygen uptake during maximal and submaximal exercise: ventilation and O2 pulse, determinants of O2 uptake are less dependent on load in submaximal exercise at ventilatory threshold

Thiagarajan K.A., Vasanthi C., Parikh T., Madhusudhan Rao V., and Arumugam S.

Department of Arthroscopy and Sports Medicine, Sri Ramachandra Medical College and Research Institute, Sri Ramachandra University, Porur, Chennai, India.

(Received: Jan 2016  Accepted: Mar 2016)

Corresponding Author

V. Madhusudhan Rao. Email: madhuoxon@hotmail.com

ABSTRACT

Introduction and Aim: Stroke volume (SV), heart rate (HR), (a – v)O2 and ventilation (Ve) determine the rate of Oxygen uptake (VO2). These, in turn, are determined by chemical, non-chemical, psychological factors and load (work rate). The present study was undertaken to study if load determined factors determining O2 uptake viz minute ventilation (Ve) and O2 pulse (an indicator of SV and (a – v)O2) during maximal and submaximal exercise. Also examined was the relationship between VO2 and Ve, O2 pulse to elucidate influences other than load acting on Ve and O2 pulse.

Materials and Methods: The study conducted on young healthy male volunteers. Load at maximal exercise was computed from the load at VO2 max and load at submaximal exercise was computed from the load at ventilatory threshold (50%-80%VO2max). Data was collected from 15 out 25 subjects studied, excluding data which showed artifacts, incomplete data and spurious values. Subjects exercised breathing through a mask on a treadmill to exhaustion as the load was increased by changing incline and speed every 2.5 minutes. Data analysed by Oxycon Pro analyser connected to a computer. Load, O2 uptake, O2 pulse, minute ventilation (Ve), EqO2 and dO2/dW were computed for maximal and submaximal exercise. Correlation coefficients were calculated for maximal and submaximal exercise for VO2 vs O2pulse, VO2 vs Ve and O2 pulse vs Load, Ve vs Load and VO2 vs Load. Paired t-test was done for EqO2 and dO2/dW.

Results: VO2 was correlated with O2 pulse and Ve for both maximal and submaximal exercise. O2 pulse, Ve and VO2 (max) were correlated with Load for maximal exercise. There was less correlation of O2 pulse, Ve with load at submaximal exercise. EqO2 was significantly different. dO2/dW was not significantly different.

Conclusion: Cardiac output (O2 pulse-SV and (a – v)O2) and ventilation (Ve) are less determined by the load during submaximal exercise, chemical factors being important in driving ventilation and central command independent of the load for increasing cardiac output. In contrast, cardiac output and ventilation are determined by Load during maximal exercise. However dO2/dW is similar, and Load correlated with VO2 during both indicating that load has a role.

Key words: EqO2, Load, Oxygen Pulse, Oxygen Uptake, Workrate
INTRODUCTION

The determinants of O2 uptake (VO2) are ventilation and cardiac output. It has been found that work rate (load) and O2 uptake (VO2) show a linear relationship at all intensities (1). However, it is not known what the relationship is between load and determinants of VO2 between subjects. The present study was conducted to examine between subjects the relationship between load (work rate) and determinants of VO2 viz Ve and O2 pulse which is discussed next. From work on hypoxia it has been found that pulmonary gas exchange since PaO2 is lower and cardiac output, since SV and HR are lower than normal, contribute to VO2 in normals (2). The factors that determine pulmonary gas exchange and cardiac output are ventilation (Ve) and O2 pulse. Ventilation determines the supply of O2 and O2 pulse (O2/HR) derived from the Fick equation, which is a measure of SV and \((a - v)O2\) determines cardiac output and extraction of O2. These viz Ve and O2 pulse are the determinants of VO2 and are controlled in turn by chemical, nonchemical, psychological factors (5) and load.

It is possible to study the effect of the load during incremental exercise on Ve, O2 pulse and VO2 at ventilatory threshold (VT) for submaximal exercise and at VO2max which provides data for maximal exercise. VT was chosen to study submaximal exercise as it enables comparison between subjects for submaximal exercise (VT) and data at VO2max provide a comparison between subjects for maximal exercise (ME).

The present study also examined the relationship between VO2 on one hand and Ve and O2 pulse on the other to elucidate influences other than load acting on Ve and O2 pulse.

MATERIALS AND METHODS

The study was carried out on normal young healthy male volunteers performing incremental exercise on a treadmill. Ethical clearance and consent were obtained for the study. Subjects breathed through a mask and exercised to exhaustion on a treadmill while incline and speed were increased every 2.5 min. Data were analysed by Oxycon Pro analyser connected to a computer. Load (work rate), VO2 (oxygen uptake), O2 pulse (O2/HR), Ve (ventilation), EqO2 (a ventilatory equivalent of O2 uptake – Ve/VO2) and dO2/dW were computed at VT (ventilatory threshold 50%–80% VO2max) (6) for submaximal and at VO2max for maximal exercise. Data were studied from 15 out of 25 subjects excluding data with artefacts, incomplete data and spurious values.

Results

The data are tabulated in Table 1. As indicated EqO2 is significantly less \((p = 0.0000)\) at VT indicating that Ve is relatively lower at VT. Ve is less correlated with load at VT \((r = 0.5\) at VT and \(r = 0.74\) at ME) and O2 pulse is less correlated with load at VT \((r = 0.63\) at VT and 0.73 at ME) indicating that the determinants of VO2 viz Ve and O2 pulse are less dependent at VT on load which acts through cortical influences and that load is maximally effective at maximal exercise. VO2 at VT as %VO2 max is 63.8 ± 10.4 (CV 16%) indicating that cortical influences are variable at VT. VO2 is correlated with load both at VT and ME \((r = 0.67\) at VT and 0.74 at ME) This is consistent with the result that dO2/dW is not significantly different at VT and ME \((p = 0.068)\) indicating that load also has a role.

Despite the fact that at VT, Ve and O2 pulse are less correlated with load at submaximal exercise, both during submaximal as well and maximal exercise, VO2 is correlated with Ve \((r = 0.87\) at VT and \(r = 0.57\) at ME) and with O2 pulse \((r = 0.67\) at VT and \(r = 0.82\) at ME) indicating that chemical influences for Ve and non load dependent central command activation of sympathetic for O2 pulse are important at VT given that cortical influences through load at submaximal exercise are submaximal. At maximal exercise load is maximally active in determining Ve and O2 pulse.

Discussion

Load as determinant of Ve and EqO2 vs chemical influences.

If ventilation is matched with O2 uptake (VO2) throughout an incremental exercise, one should
<table>
<thead>
<tr>
<th>Name</th>
<th>Age, years</th>
<th>BM, kg</th>
<th>Ht, cm</th>
<th>EqO2</th>
<th>dO2/dW</th>
<th>Load, W</th>
<th>Ve, l/mi</th>
<th>O2/HR, ml</th>
<th>VO2, ml</th>
<th>VT% VO2 max</th>
</tr>
</thead>
<tbody>
<tr>
<td>MM</td>
<td>22</td>
<td>53</td>
<td>172</td>
<td>20.6</td>
<td>31</td>
<td>10.5</td>
<td>10</td>
<td>51</td>
<td>148</td>
<td>23</td>
</tr>
<tr>
<td>LR</td>
<td>18</td>
<td>60</td>
<td>175</td>
<td>25.6</td>
<td>45</td>
<td>10.5</td>
<td>10</td>
<td>171</td>
<td>313</td>
<td>64</td>
</tr>
<tr>
<td>AJ</td>
<td>19</td>
<td>65</td>
<td>172</td>
<td>28.3</td>
<td>38</td>
<td>7.9</td>
<td>7.7</td>
<td>186</td>
<td>258</td>
<td>64</td>
</tr>
<tr>
<td>SG</td>
<td>19</td>
<td>51</td>
<td>171</td>
<td>22.8</td>
<td>32.6</td>
<td>11.9</td>
<td>11.1</td>
<td>90</td>
<td>200</td>
<td>40</td>
</tr>
<tr>
<td>GG</td>
<td>19</td>
<td>74</td>
<td>172</td>
<td>18.4</td>
<td>28</td>
<td>8</td>
<td>9.8</td>
<td>135</td>
<td>296</td>
<td>35</td>
</tr>
<tr>
<td>MD</td>
<td>19</td>
<td>80</td>
<td>183</td>
<td>23.4</td>
<td>29</td>
<td>9.7</td>
<td>11.1</td>
<td>147</td>
<td>232</td>
<td>55</td>
</tr>
<tr>
<td>SM</td>
<td>21</td>
<td>91</td>
<td>189</td>
<td>21</td>
<td>35.8</td>
<td>8.87</td>
<td>8.63</td>
<td>172</td>
<td>367</td>
<td>46</td>
</tr>
<tr>
<td>KR</td>
<td>21</td>
<td>76</td>
<td>176</td>
<td>23.3</td>
<td>38.2</td>
<td>7.2</td>
<td>9.86</td>
<td>220</td>
<td>308</td>
<td>54</td>
</tr>
<tr>
<td>ZA</td>
<td>19</td>
<td>50</td>
<td>165</td>
<td>24.6</td>
<td>31.5</td>
<td>6.23</td>
<td>7.63</td>
<td>196</td>
<td>240</td>
<td>43</td>
</tr>
<tr>
<td>PH</td>
<td>19</td>
<td>50</td>
<td>178</td>
<td>23.3</td>
<td>35.7</td>
<td>6.96</td>
<td>9.13</td>
<td>139</td>
<td>196</td>
<td>42</td>
</tr>
<tr>
<td>ASK</td>
<td>26</td>
<td>77</td>
<td>180</td>
<td>22.1</td>
<td>33.6</td>
<td>6.48</td>
<td>7.63</td>
<td>223</td>
<td>391</td>
<td>47</td>
</tr>
<tr>
<td>TS</td>
<td>19</td>
<td>63</td>
<td>177</td>
<td>28.1</td>
<td>35.6</td>
<td>15.4</td>
<td>15</td>
<td>112</td>
<td>183</td>
<td>65</td>
</tr>
<tr>
<td>YS</td>
<td>19</td>
<td>60</td>
<td>172</td>
<td>23.2</td>
<td>36.2</td>
<td>8.52</td>
<td>9.1</td>
<td>169</td>
<td>237</td>
<td>50</td>
</tr>
<tr>
<td>SKT</td>
<td>28</td>
<td>70</td>
<td>161</td>
<td>18.5</td>
<td>32.5</td>
<td>7.15</td>
<td>9.8</td>
<td>130</td>
<td>200</td>
<td>33</td>
</tr>
<tr>
<td>RV</td>
<td>19</td>
<td>68</td>
<td>175</td>
<td>23.1</td>
<td>36</td>
<td>8.28</td>
<td>9.4</td>
<td>125</td>
<td>270</td>
<td>44</td>
</tr>
</tbody>
</table>

Table 1: Data from 15 subjects. VT—data at Ventilatory Threshold (Submaximal Exercise); M—data at VO2max (Maximal Exercise).
find that EqO2 (a ventilatory equivalent of O2 uptake – Ve/VO2) is unchanged. However EqO2 is lower at VT, also noted in an earlier study (7) indicating that Ve is relatively less at submaximal exercise than at maximal exercise. Since Ve is poorly correlated with load \( r = 0.5 \) at VT and since load acts through cortical mechanisms it suggests that psychological factors are lower in submaximal exercise. It is possible that load is not great enough and that chemical influences are more important in driving ventilation below VT. Inadequate supply of O2 leads to lactic acid accumulation by anaerobic metabolism. H+ ions drive ventilation which increases disproportionately to O2 uptake\((\text{VO2})\) beyond VT (8). However, there is a correlation between load (work rate) and \( \text{VO2} \) as well at submaximal exercise \( r = 0.67 \) and at maximal exercise \( r = 0.74 \) indicating that load has a role.

**Load as a determinant of O2 pulse**

The other factor that determines \( \text{O2} \) uptake is the cardiovascular response indicated by \( \text{O2 pulse} (\text{O2/HR}) \) which is a measure of stroke volume (SV) and \((\text{a – v})\text{O2}\). SV depends on EDV and to a greater extent on inotropic effect and \((\text{a – v})\text{O2}\) depends on muscle metabolic activity. Cortical influence through Central Command seemingly only in part activated by the load during exercise increases inotropic and chronotropic effects by stimulation of sympathetic increasing SV and HR. O2 pulse which indicates SV is less dependent on load in submaximal exercise since O2 pulse is less correlated with load \( r = 0.63 \) than in maximal exercise \( r = 0.73 \). This suggests that cortical influences are not maximally active during submaximal exercise. Increase in \((\text{a – v})\text{O2}\) due to muscle metabolism along with higher SV could account for the greater O2 pulse at higher intensities of exercise.

**Mechanism of action of load**

Since load acts through cortical mechanisms, the findings suggest that psychological factors in submaximal exercise are not uniform between subjects. The finding that CV is large-16\%, for \( \text{VO2} \) at VT expressed as \% of \( \text{VO2max} \) also indicates that the effect of load is variable. In contrast in maximal exercise, the psychological effort exerted is maximal and dependent on load between subjects. This is analogous to the phenomenon that HR varies between individuals in submaximal exercise, but HR max is about the same for all (9). Since the load is determined by body mass and psychological effect is maximal at \( \text{VO2max} \), along with chemical influences, psychological effort multiplied by load leads to the finding that Ve and O2 pulse, the central command being maximally activated are correlated with load at maximal exercise.

**Determinants of VO2**

Despite the fact that Ve and O2 pulse are less correlated with a load in submaximal exercise, \( \text{VO2} \) is correlated with both. This shows that \( \text{VO2} \) is matched with a supply of O2 and extraction of O2 throughout an incremental exercise. As mentioned earlier Ve and O2 pulse are determined by chemical and nonchemical influences. Peripheral chemoreceptors have been implicated in ventilatory response (6). Non-chemical control viz cortical outflow (motivation) and proprioceptor stimulation for ventilatory response and central command activation independent of load to stimulate sympathetic activity resulting in an increase in cardiac output through an increase in SV and HR seem to be important influences in both submaximal and maximal exercise. Opinion is divided the relative contribution of SV and HR to increase in cardiac output. The contribution of SV reportedly levels off (10) or increases (11) till \( \text{VO2max} \). This is relevant while considering the relative contributions of SV and \((\text{a – v})\text{O2}\) to O2 pulse which increases throughout an incremental exercise. It has been found that after training, an increase in \( \text{VO2} \) is due to increase in cardiac output not \((\text{a – v})\text{O2}\) (12).

**CONCLUSION**

The determinants of \( \text{VO2} \) (oxygen uptake) viz Ve and \( \text{O2 pulse} \) (cardiac output) are less dependent on load at submaximal exercise. In contrast, load determines Ve and O2 pulse at maximal exercise.
However, VO2 is correlated with Ve and O2 pulse both at submaximal and maximal exercise. This shows that cortical influences act through two mechanisms viz load independent and load dependent and that chemical influences contribute to driving ventilation especially at submaximal exercise leading to a ventilatory threshold.

REFERENCES

Is autonomic function test helps to assess the severity of metabolic syndrome: A study on comparison of Frequency-Domain recordings of Heart rate variability (HRV) with the severity of metabolic syndrome

Bhagyashree N.,¹ C. Ramaswamy,² Ganesh M.,³ and Udaya Ganesh B.⁴

¹Assistant Professor, ACS Medical College & Hospital, and Ph.D scholar, Saveetha University, Chennai, India.
²Ph.D Guide, Saveetha University, Chennai, India.
³ACS Medical College & Hospital, Chennai, India.
⁴Sri Jayendra Saraswathi Ayurveda College & Hospital, Chennai, India.

(Received: Jan 2016   Accepted: Mar 2016)

Corresponding Author
Mrs. Bhagyashree N. Email: bhagyashivanugraha@gmail.com

ABSTRACT

Introduction and Aim: Metabolic syndrome constitutes a bouquet of three or more of any five components like obesity, hypertension, diabetes, hypertriglyceridemia and low-HDL concentration in an individual and cardiovascular disease which is being the center. To find out the relationship between HRV parameters with the severity of the metabolic syndrome.

Materials and Methods: A total of ninety patients were divided into 3 groups as a group I with those having only 3 components of the metabolic syndrome, group II with more than 3 components of the metabolic syndrome and group III with less than three components of the metabolic syndrome. The group I served as normal MS people, the group II as severe MS and group III as a control group. The Frequency-domain HRV parameters like TP, LF, HF and LF/HF ratio were recorded in those subjects and were analyzed.

Result: The result showed that group II has high LF and low HF than the group I and III. The LF/HF ratio of group II was higher among them. All these parameters of group 1 and 3 were comparable. The TP among the groups showed no significant difference.

Conclusion: Once metabolic syndrome set in, cardiac reflexes are activated and proportionate to severity, the sympathetic activity increases, and the parasympathetic activity decreases causing reduced cardiac autonomic control. The LF/HF ratio increases proportionately with the severity of the diseases. Hence, the LF/HF ratio may be a better prognostic tool towards the management of metabolic syndrome.

Key words: Cardiac Autonomic Modulation, Frequency–Domain, HRV, Metabolic Syndrome

INTRODUCTION

Metabolic syndrome (MS) also known as syndrome X is characterized by hypertriglyceridemia, low concentration of high density lipoprotein (HDL) cholesterol (dyslipidemia), elevated blood pressure, impaired glucose tolerance and central obesity (1). The metabolic syndrome carries an increased risk for cardiovascular disease and diabetes (2) is associated with alterations in the function of numerous elements of the cardiovascular system. The autonomic nervous system plays a central role in the cardiovascular
regulation (3). Cardiovascular disease is the leading cause of death and disability worldwide (4). Heart rate variability (HRV) represents cardiac autonomic control (5,6) and it has emerged as a practical, noninvasive tool to quantitatively investigate cardiac autonomic dysregulation and it has been proposed as a predictor of increased risk for cardiovascular mortality (4). Reduced HRV significantly increases cardiovascular mortality (5). The high-frequency component (HF, 0.15–0.4 Hz) of the HRV spectrum reflects vagal modulation of the sinus node; the low-frequency component (LF, 0.04–0.14 Hz) represents either sympathetic modulation or a combination of sympathetic and vagal influences. The ratio between LF and HF components (LF/HF ratio) is considered to reflect sympathovagal influences on heart rate control (6). Although many attempts have been done to study the cardiac autonomic functions with each of the individual components of metabolic syndrome, the relationship between severities of the metabolic syndrome with the cardiac autonomic dysfunction is not available in the literature. Hence, the present study was conducted to find out the cardiac autonomic changes in subjects with the number of metabolic abnormalities that constitute metabolic syndrome and we hypothesized that significant difference in cardiac autonomic dysfunction is seen in subjects with more number of metabolic abnormalities.

**OBJECTIVE**

To find out the relationship between the frequency–domain parameters of HRV with the severity of metabolic syndrome proportionately indicated by the number of constellation of diseases that constitute metabolic syndrome.

**MATERIALS AND METHODS**

The study was carried out among 90 subjects in three groups, each comprising 30 subjects. Age-matched, sex-matched subjects were recruited for the study.

Group I: Subjects with any three components of metabolic syndrome (normal MS group)

Group II: Subjects with more than three components of metabolic syndrome (severe MS group)

Group III: Subjects with less than three components of metabolic syndrome (Control group)

The diagnosed metabolic syndrome patients were recruited for the present study. The diagnostic criteria for metabolic syndrome were according to National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP III) criteria (≥3 of 5 risk factors) (7) and with the inclusion and exclusion criteria as detailed below.

**Inclusion criteria**

Subjects with –

- Waist circumference ≥102 cm (40 inches) in men and ≥88 cm (35 inches) in women.
- Triglyceride ≥150 mg/dl or history of drug consumption for hypertriglyceridemia.
- HDL ≤40 mg/dl in men and ≤50 mg/dl in women or history of drug consumption.
- Systolic BP ≥130 mmHg and diastolic BP ≥85 mmHg or history of antihypertensive drug consumption.
- Fasting blood glucose ≥100 mg/dl, history of diabetes mellitus or using antidiabetic drug.

**Exclusion criteria**

Pregnant women, patients suffering from the neurological defect, patients suffering from the urological defect, patients having cancer, patients with acute coronary syndromes within 3 months, patients with atrial fibrillation, subjects with the past/present history of RS, CVS disorders.

The study was carried out in the department of Physiology, A C S Medical College and Hospital, Chennai. The participants were from A C S Medical College and Hospital, Saveetha Medical College and Hospital, Chennai. The study commenced after getting the approval from the Institutional Human Ethical Committee (IHEC), Saveetha University, Chennai.

The data were collected from the participants after giving a detailed explanation about the procedure.
and their cooperation and willingness were obtained with informed consent. A detailed clinical history of all the subjects was taken. Relevant past history, family history, any drug history, personal history like smoking, alcoholism etc. was taken.

The ECG recording was taken for 5 minutes after 10 minutes of seated rest, under standardized conditions to minimize artifacts. ECG signal was obtained using limb lead II and analysis was performed using HRV analysis software version 1.1, Biomedical Signal Analysis Group, Department of Applied physics, University of Kuopio, Finland. The recording was done in the morning hours between 9 AM and 11 AM. The subjects were instructed to avoid coffee or alcohol 24 hours prior to testing and to avoid food two hours prior to testing. While recording, the subjects were instructed to close the eyes, and to avoid talking, coughing, moving of hands, shaking the legs and body, and sleeping during the test.

The data is analyzed between the groups statistically by using Student’s $t$-test and the $p < 0.05$ was considered as significant.

### RESULT

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I</th>
<th>Group II</th>
<th>$p$-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total power (ms²)</td>
<td>684.03 ± 1694.69</td>
<td>542.03 ± 1308.58</td>
<td>0.717 NS</td>
</tr>
<tr>
<td>LF (%)</td>
<td>50.34 ± 30.10</td>
<td>70.76 ± 29.95</td>
<td>0.010*</td>
</tr>
<tr>
<td>HF (%)</td>
<td>16.79 ± 12.95</td>
<td>8.39 ± 8.16</td>
<td>0.003*</td>
</tr>
<tr>
<td>LF/HF</td>
<td>4.11 ± 3.74</td>
<td>11.18 ± 6.95</td>
<td>&lt;0.0001#</td>
</tr>
</tbody>
</table>

Table 1: Comparison of Frequency-domain parameters of HRV among Group I and Group II (Values expressed as mean ± SD).

NS, not significant; *$p = <0.05$ (significant); **$p = <0.001$ (highly significant).

The result of the present study (Table 1) showed that there is no significant difference with the total power (TP) spectral component of HRV between group 1 and II. But, the LF and HF percentage difference was significant between group 1 and II ($p<0.05$) and the ratio between LF and HF was highly significant between group 1 and II ($p = <0.0001$).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group II</th>
<th>Group III</th>
<th>$p$-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total power (ms²)</td>
<td>542.03 ± 1308.58</td>
<td>364.16 ± 1079.95</td>
<td>0.568 NS</td>
</tr>
<tr>
<td>LF (%)</td>
<td>70.76 ± 29.95</td>
<td>53.91 ± 32.51</td>
<td>0.041*</td>
</tr>
<tr>
<td>HF (%)</td>
<td>8.39 ± 8.16</td>
<td>16.70 ± 13.34</td>
<td>0.005*</td>
</tr>
<tr>
<td>LF/HF</td>
<td>11.18 ± 6.95</td>
<td>4.03 ± 3.09</td>
<td>&lt;0.0001#</td>
</tr>
</tbody>
</table>

Table 2: Comparison of Frequency-domain parameters of HRV among Group II and Group III (Values expressed as mean ± SD).

NS, not significant; *$p = <0.05$ (significant); **$p = <0.001$ (highly significant).

The result of the present study (Table 2) showed that there is no significant difference with the total power spectral component of HRV between group II and III. But, the LF and HF percentage difference was significant between group II and III ($p < 0.05$) and the ratio between LF and HF was highly significant between group II and III ($p = <0.0001$).
The result of the present study (Table 3) showed no significant difference with TP, LF, HF and LF/HF between group I and III.

**DISCUSSION**

Metabolic syndrome constitutes three or more among the constellation of diseases comprising obesity, hypertension, diabetes mellitus, hypertriglyceridemia and low HDL concentration. Though metabolic syndrome people are treated, tool is not available to determine the prognosis. As the cardiac diseases invariable form the importance among the metabolic syndrome, attempt was made in this study to link the measurement of HRV (heart rate variability) with metabolic syndrome and its severity. In this study, the groups were made in such a way that the subjects with any of the three components mentioned above were grouped as group I (normal MS group); patients with more than three components as group II (severe MS group) and people with less than three components as group III and considered as control group. The frequency-domain spectrum of HRV which includes TP (total power spectrum), LF (low frequency), HF (high frequency) and LF/HF ratio were recorded on these people and the results were analyzed.

In the present study, the TP which studies the cardio-stabilizing reflex activities, though statistically not significant among the groups, showed maximum effect in group I and least in group III. This indicates that though autonomic modulation takes place in metabolic syndrome people, overall changes evident in both sympathetic and parasympathetic activity more or less nullified each other variations. Further, in this study the LF values which reflects mainly the sympathetic activity and also parasympathetic activity via baroreceptor reflex was 50.34 ± 30.10; 70.76 ± 29.95 and 53.91 ± 32.51 respectively of group I; II and III. This indicates that the sympathetic activity increases with the severity of the metabolic syndrome and also as group I showed less LF values than even control group (group III) probably because metabolic syndrome initially affect baroreceptor activity followed by proportionate with the severity increasable affects the other cardiac reflexes which enhance sympathetic activity as in group II. This argument gets supports from the HF values which are associated with the vagal activity obtained in this study. The HF value was less in group II whereas the HF value between group I and III showed no significant difference though HF value is bit more in group I (metabolic syndrome group) than group III (control) and the LF/HF ratio was more in group II in this study. Assoumou *et al.* (8), Chang *et al.* (9) showed that there were no differences in frequency domain measures of HRV until at least three Metabolic syndrome risk factors were present in agreement with the above result.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I</th>
<th>Group III</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total power (ms²)</td>
<td>684.03 ± 1694.69</td>
<td>364.16 ± 1079.95</td>
<td>0.386 NS</td>
</tr>
<tr>
<td>LF (%)</td>
<td>50.34 ± 30.10</td>
<td>53.91 ± 32.51</td>
<td>0.660 NS</td>
</tr>
<tr>
<td>HF (%)</td>
<td>16.79 ± 12.95</td>
<td>16.70 ± 13.34</td>
<td>0.978 NS</td>
</tr>
<tr>
<td>LF/HF</td>
<td>4.11 ± 3.74</td>
<td>4.03 ± 3.09</td>
<td>0.928 NS</td>
</tr>
</tbody>
</table>

Table 3  **Comparison of Frequency-domain parameters of HRV among Group I and Group III (Values expressed as mean ± SD).**

NS, not significant.
Thus, the results clearly suggest that in metabolic syndrome people, among the cardiac reflexes, the first one which is affected may be the activities of baroreceptor and then proportionate to severity other cardiac reflexes which enhance sympathetic activity. So the LF/HF ratio increases proportionate to the severity of metabolic syndrome may serve as a predictable tool to gauge the degree of severity of the metabolic syndrome. Chang et al. (9), Min et al. (10), Min et al. (11) have shown that in a general population, HF and LF were reduced in subjects with metabolic syndrome (MetS+) compared to subjects without metabolic syndrome (MetS-). Min et al. (11) reported reductions in HRV with increasing number of metabolic syndrome components which is in agreement with the result of the present study. Contrary to these results, Brunner et al. (12) have shown that, in a smaller cohort of middle-aged men (aged 45–63 years) TP, LF and HF were reduced with no differences in LF/HF whereas, Koskinen et al. (6) have studied metabolic syndrome and short-term heart rate variability in young adults and showed that young MetS+ women had reduced HF nu and increased LF nu and LF/HF, but there was no difference in men also supports the present result though that study showed gender differentiation unlike our study which did not show neither gender nor age specific.

CONCLUSION

Metabolic syndrome adversely affects cardiac autonomic control at first through baroreceptor activity followed by other cardiac reflexes which are enhancing the sympathetic activity and deteriorating parasympathetic activity causing abnormal cardiac autonomic control. So, with the severity of metabolic syndrome, cardiac autonomic dysfunction is more pronounced. Hence, the LF/HF ratio may be a better prognostic tool towards the management of metabolic syndrome. Mehmet et al. (13) have quoted that, since HRV measurement is a noninvasive and relatively cheap technique that may be used for the early diagnosis of cardiovascular diseases, and valuable data may be obtained with short recording times. So, early identification and screening of population with the metabolic syndrome for cardiac autonomic function by HRV can be effective step towards the management of metabolic syndrome which helps to reduce the cardiovascular disease burden too. Further, in-depth studies are needed in this direction to confirm the findings of the study.

ACKNOWLEDGMENTS

Authors would like to deliver sincere thanks to the Department of Physiology, ACS Medical College & Hospital and Saveetha Medical College & Hospital, Chennai for providing the facilities to carry out research work.

REFERENCES

5. Park, L.E., Choi, J., Park, J., and Gi, c. Heart rate variability and metabolic syndrome in hos-


Antioxidant activity of Phytoformulation 1, a polyherbal formulation, on hyperlipidemic Wistar rats

R. Vanaja¹ and J. Mercy Jasmine²

¹Department of Biochemistry, Shri Sathya Sai Medical College and Research Institute, Nellikuppam, India.
²Institute of Biochemistry, Madras Medical College, Chennai, India.

(Received: Feb 2016    Accepted: Mar 2016)

Corresponding Author

J. Mercy Jasmine. Email: jasmine.mercy@gmail.com

ABSTRACT

Introduction and Aim: The antioxidant activity of Phytoformulation 1, on the hyperlipidemic Wistar rats was examined.

Materials and Methods: Hyperlipidemia was induced by atherogenic diet. The induced animals were segregated into control and treatment groups. The control group was treated with Atorvastatin (10 mg/kg) and the treatment groups were administered with 250 and 500 mg/kg of Phytoformulation 1 respectively. The level of antioxidants such as Super Oxide Dismutase (SOD) (serum), Catalase (CAT) (hemolysate), Malonaldehyde (MDA), Vitamin E, Vitamin C (plasma) was measured in the hyperlipidemic rats.

Results: Animals treated with atorvastatin showed increased levels of catalase, SOD, vitamin E and vitamin C. There was a decrease in the lipid peroxidation when compared with the hyperlipidemic group. Similarly, there was an increase in the antioxidant activity among the groups treated with 250 and 500 mg/kg phytoformulation 1 with an increased level of catalase, SOD, vitamin E, vitamin C and decreased lipid peroxidation.

Conclusion: The increased SOD and CAT levels expressed in Phytoformulation 1 treated groups showed the antioxidant activity of polyherbal formulations.

Keywords: Antioxidant activity, Hyperlipidemia, Polyherbal formulations

INTRODUCTION

Hyperlipidemia is a condition characterized by abnormal elevation of lipids (triglyceride and cholesterol) and lipoproteins (LDL, VLDL) levels in the blood (1). Human HMGR (E.C 1.1.1.34) is a 97-kDa, transmembrane glycoprotein, situated on the endoplasmic reticulum and peroxisomes (2) and is responsible for catalyzing the NADPH-dependent, two-step reduction of HMG-CoA to mevalonate. The inhibition of this enzyme results in the significant decrease in cholesterol levels (3,4). This is a highly regulated process within the cholesterol biosynthetic pathway and as a result an attractive target for intervention in the treatment of hypercholesterolemia. HMG-CoA Reductase blockers known as statins prevent the synthesis of cholesterol at the mevalonate and provide significant protection against coronary artery disease (5,6).

ROLE OF OXIDATIVE STRESS IN HYPERLIPIDEMIA

Consumption of high-cholesterol diet (HCD) and reduced physical activity to dissipate the energy
leads to hyperlipidemia (7). Oxidative stress in hyperlipidemia is thought to be an important factor in the development of atherosclerotic plaques (8). Accumulation of lipid peroxides generated by free radicals, from fatty acids lead to atherosclerosis and coronary heart disease (9). Hypolipoproteinemia alters lipid composition of cell membranes and extracellular matrix and are thus prone to oxidation by free radicals (10). Free radicals are highly reactive, short-lived molecules that have one or more unpaired electrons and can damage lipids, proteins, carbohydrates and DNA (11). They are constantly generated in our body because of oxidative stress, through leakage of electrons from the electron transport chain and by the activities of oxidoreductase enzymes (12). It is suggested that antioxidants could be used as new, preventive and therapeutic agents to prevent oxidative stress-related disorders (13,14). Hence, there has been an increased interest in the development of functional foods and nutraceuticals to prevent oxidative stress (15) and the oxidation of lipids (16).

### Table 1 Composition of atherogenic diet.

<table>
<thead>
<tr>
<th>Constituents</th>
<th>Normal diet (%)</th>
<th>High fat diet (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrates</td>
<td>67.3</td>
<td>57.3</td>
</tr>
<tr>
<td>Protein</td>
<td>22</td>
<td>22</td>
</tr>
<tr>
<td>Fat</td>
<td>5</td>
<td>15</td>
</tr>
<tr>
<td>Minerals</td>
<td>4.5</td>
<td>4.5</td>
</tr>
<tr>
<td>Vitamin mix</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>—</td>
<td>1</td>
</tr>
</tbody>
</table>

**Plant based compounds as HMG-CoA reductase inhibitors**

Many plant-derived products have shown to have potent inhibitory properties towards HMG-CoA Reductase enzyme. Policosanol safely down-regulates HMG-CoA Reductase – potential as a component of the Esselstyn regimen (17). The tocotrienols derived from barley are widely distributed in the plant kingdom and differ from tocopherols (Vitamin E) only in three double bonds in the isoprenoid chain which appears to be essential for the inhibition of cholesterogenesis (18). It is currently accepted that the consumption of fruit-derived antioxidants such as Vitamin C, Carotenoids, and Flavonoids provides a preventive effect against cardiovascular disease. Kiwifruit has potential cardiovascular protective properties *in vitro* (19). The tetralin derivatives and salts were proved to inhibit HMG-CoA Reductase and so inhibit cholesterol biosynthesis.

Phytoformulation 1 is a polyherbal formulation that contains extracts of plants with renowned hypolipidemic and hyper antioxidant properties such as *Terminalia arjuna* (20,21), *Emberica officinalis* (22), *Plectranthusbarbatus* (23), *Allium sativum* (24). The phytochemicals present in the plant extracts contribute to the protective effect of the plants towards a broad spectrum of clinical conditions. Thus, the development of novel and sophisticated screening processes can be used to recognize the numerous applications of natural products and the introduction of natural product chemicals for treating disease could result in life-saving drugs.
MATERIALS AND METHODS

Plant material

Fresh plant/plant parts were purchased, taxonomic identities were confirmed and the voucher specimen numbers of the plants were deposited at Phytopharma testing lab, T. Stanes Company Ltd. Coimbatore. Fresh plant material was washed under running tap water, air dried and then homogenized to fine powder and stored in airtight bottles.

Plant extraction procedure

Ten grams of air-dried powder of the plant constituents was added to distilled water [1:10] and boiled on slow heat for 2 h and centrifuged at 5000 rpm for 10 mins. The supernatant was collected and the above procedure was repeated thrice. After 6 hours, the supernatant collected at an interval of every 2 hours were pooled together and concentrated to make the final volume one-fourth of the original volume (25). It was then autoclaved at 121°C and 15 lbs pressure and stored at 4°C. The resulted powder was collected for every individual plant. These powders in equal proportions were mixed, homogenized in distilled water [1:20] at 60°C to form a concoction and used for the experiment.

Animals

Healthy Wistar albino rats both male (200–230 g) and female (140–150 g) were used for the experiment. They were housed in plastic cages with filter tops under controlled conditions of a 12 h light/12 h dark cycle, 50% humidity and 28°C. The animals were fed standard rats chow and water ad libitum. The experiment was conducted after obtaining the approval of the institutional animal ethical committee clearance (IAEC approval No: 110/PHARMA/SCRI, 2011). The composition of atherogenic diet used during the study was given in Table 1.

Experimental design

In order to induce hyperlipidemia, the method reported by Bopanna et al. was followed (26).

Animals were divided into six groups, 3 male and 3 female rats each and they received the following diets with or without treatment for 12 weeks.

Bodyweight and the food intake of the animals were monitored once a week throughout the experiment. At the end of the 12th week, the rats were fasted overnight and blood was drawn from retro-orbital plexus and the animals were sacrificed by cervical decapitation. Serum, hemolysate and plasma were

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Group</th>
<th>SOD (units/ml)</th>
<th>CAT (µmol H₂O₂/min/ml)</th>
<th>MDA (nmol/ml)</th>
<th>Vit. E (µg/ml)</th>
<th>Vit. C (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Normal control</td>
<td>5.13 ± 0.09</td>
<td>75.56 ± 0.55</td>
<td>8.18 ± 0.16</td>
<td>6.11 ± 0.03</td>
<td>0.95 ± 0.02</td>
</tr>
<tr>
<td>2.</td>
<td>Hyperlipidemic control</td>
<td>3.01 ± 0.04****</td>
<td>37.62 ± 0.40****</td>
<td>17.04 ± 0.25****</td>
<td>3.14 ± 0.04****</td>
<td>0.31 ± 0.09****</td>
</tr>
<tr>
<td>3.</td>
<td>Atorvastatin (10 mg/kg)</td>
<td>5.09 ± 0.07****</td>
<td>71.25 ± 0.35****</td>
<td>8.38 ± 0.13****</td>
<td>6.08 ± 0.10****</td>
<td>0.92 ± 0.02****</td>
</tr>
<tr>
<td>4.</td>
<td>Phytoformulation 1 (250 mg/kg)</td>
<td>4.68 ± 0.12****</td>
<td>48.41 ± 0.46****</td>
<td>11.98 ± 0.15****</td>
<td>5.09 ± 0.11****</td>
<td>0.77 ± 0.10****</td>
</tr>
<tr>
<td>5.</td>
<td>Phytoformulation 1 (500 mg/kg)</td>
<td>5.05 ± 0.04****</td>
<td>58.20 ± 0.55****</td>
<td>9.71 ± 0.18****</td>
<td>6.00 ± 0.02****</td>
<td>0.89 ± 0.01****</td>
</tr>
</tbody>
</table>

Table 2 Findings observed in the levels of antioxidants [SOD (serum), CAT (hemolysate), MDA, Vit. E, Vit. C (plasma)].

Values are mean ± SD; Number of animals in each group = 6; “a” as compared to normal control; “b” as compared to hyperlipidemic control. One way ANOVA followed by Dunnnett’s multiple comparison tests. p-value ****<0.0001, ***,<0.001, **,<0.01, *,<0.05.
preparing for biochemical analysis.

The assay of superoxide dismutase is devised on the method followed by Mustafa et al. (27). The extent of lipid peroxidation was estimated according to the method of Rotruck et al. (28), Okhawa et al. (29). Catalase activity was assayed following the method of Kaur et al. (30). Vitamin C was analyzed by the spectrophotometric method described by Omaye et al. (31). Tocopherol was estimated as reported by Baker et al. (32).

Statistical analysis

One way analysis of variance (ANOVA) followed by Dunnett’s multiple comparison tests was performed using GraphPad Prism version 6.00. The limit statistical significance was set at $p \leq 0.05$.

RESULTS AND DISCUSSION

Changes in antioxidant levels after treatment with Atorvastatin and phytoformulation 1

The high fat diet increased the free radicals level which was indicated by the decreased catalase, SOD, vitamin E, vitamin C levels and increased level of lipid peroxidation as evidenced by the increased levels of MDA in comparison to the animals with a normal diet. Atorvastatin treated group showed an increase in the level of catalase, SOD, vitamin E and vitamin C. There was a decrease in the lipid peroxidation when compared with the hyperlipidemic group. Similarly, there was an increase in the antioxidant activity among the groups treated with 250 and 500 mg/kg phytoformulation 1 with an increased level of catalase, SOD, vitamin E, vitamin C and decreased lipid peroxidation.

The previous study by Gesquiere et al. (33), showed that the free radicals released due to the oxidative stress cause cellular cholesterol accumulation by increasing the cholesterol biosynthesis. Hence, the elevation in the cholesterol levels monitored in the study may be due to the accelerated cholesterol biosynthesis or reduction in the cholesterol esters hydrolysis. A high-fat diet affects the antioxidant defense mechanism against the process of lipid peroxidation. Oxidative stress in hyperlipidemia as shown by reduced levels of antioxidant enzymes was thought to be a factor in the development of atherosclerotic plaques (34). Antioxidant activity was reported in the extracts of medicinal plants that contain alkaloids (35), catalase activity predominated in Piper longum Linn. (36). Epidemiological studies have shown that the consumption of foods and beverages rich in phenolic content can reduce the risk of heart disease (37). Phytochemicals can directly react with superoxide anions and lipid peroxyl radicals and consequently inhibit/break the chain of lipid peroxidation (38).

High cholesterol in cell membrane and plasma lipoprotein render them highly susceptible to peroxidation mediated by reactive oxygen species (ROS). Membrane lipids are susceptible to oxidation due to their high polyunsaturated fatty acid content. The enzymatic and non-enzymatic systems associated with the membrane lipids generate free radicals. Free radicals oxidize the membrane lipoproteins to produce by-products such as malondialdehyde (MDA), which is the end product of lipid peroxidation and is a marker for free radical mediated damage and oxidative stress (39). Lipid peroxidation is a free radical related process that causes cellular damage as a result of oxidative stress (40). Increased levels of MDA served as a marker for accelerated lipid peroxidation in the plasma caused by the high-fat diet. Superoxide dismutase (SOD) and catalase (CAT) are inherent antioxidant defense enzymes that scavenge the superoxides and lipid peroxides. It was reported by Lu and Chiang (41) that cholesterol feeding decreased the activity of SOD and CAT, enhancing the lipid peroxides which corroborated well with the present study. A decreased activity of these enzymes may make the cell predisposed to free radical damage (42). The increased SOD and CAT levels expressed in the Decholestrate and phytoformulation 1 treated groups showed the antioxidant activity of polyherbal formulations.

Vitamin E is the main lipid-soluble antioxidant in the body. Vitamin E prevents the propagation of free radical reactions in the cell membrane (43). Hyperlipidemic condition results in the increased LDL
levels and there by increased transport of LDL particles into the artery wall, which were prone to oxidative damage (44). Vitamin E, mainly α-tocopherol, is the major fat-soluble antioxidant present in the LDL particle. On average, 5–9 vitamin E molecules are carried by each LDL particle and are believed to protect LDL from oxidative damage. In vivo, free radicals generated by endothelial cells of the arterial wall and activated macrophages are thought to oxidize LDL particles (45). Before being completely oxidised the LDL particles undergo some modifications. Initially, the LDL particles contain one intact polypeptide- apolipoprotein B-100 (apo B-100), enriched with poly unsaturated fatty acids (PUFA) and antioxidants. Then it was minimally oxidised resulting in the formation of oxidized phospholipids on the surface (46). α-Tocopherol is confined to the lipid phase of LDL and undergoes oxidation to form α-Toc-O• (Tocopheroxy radical) at the surface of the particle by peroxyl radicals that are confined to an aqueous phase. α-Toc-O• reacts with lipid hydroperoxide (LOOH), initiating the tocopherol-mediated peroxidation. Vitamin C can interrupt α-Toc-O• by exporting the radical from the lipid phase back to the aqueous phase. The oxidized LDL particles are recognized by macrophage scavenger receptors and taken up by the macrophages, forming lipid-laden foam cells in the fatty streak lesions. Vitamin E supplementation has been reported to suppress macrophage uptake of oxidized LDL in human arterial lesions (47). Thus, the increased LDL concentration causes increased lipid peroxidation. Animals treated with phytoformulation 1 showed reduced LDL thus proving the antioxidant potential of the drug.

The depletion of SOD, Catalase, LPO, Vitamin E and Vitamin C level in the hyperlipidemic control group might be due to a depressed antioxidant defence system. Decreased levels of vitamins C and E were observed in the atherogenic rats group. Reduced vitamin C level may be due to increased utilization to trap the reactive oxygen species (ROS) or could be due to the possible reduction in the concentration of glutathione reductase (GSH) that regulates the vitamin C levels. Reduced vitamin E levels may be due to the increased utilization in scavenging the radicals or could be due to the decreased vitamin C because there is a well-established synergism between vitamin C and E (48). Buettner (49) suggested that vitamin E and vitamin C cooperate to protect lipids against peroxidation. Vitamin C repairs the tocopheroxyl radical of vitamin E, thereby permitting vitamin E to function again as a free radical chain breaking antioxidant. The polyherbal formulation phytoformulation 1 has the plant extracts such as Terminalia arjuna, Plectranthus barbatus, Curcuma longa, Piper nigrum, Allium sativum with proven potent antioxidant activity (24,50–53). This further substantiates the free radical scavenging activity of the test drugs.

CONCLUSION

Considering the results of the present study, it may be concluded that the aqueous extract of phytoformulation 1 exhibit possible protective mechanism against the development of coronary heart disease. It also prevents hyperlipidemic complications due to lipid peroxidation and failure in antioxidant systems.

REFERENCES


Comparative effects of different teaching methods in pharmacology in second MBBS medical students

Baswaraj Munge,¹ Kodandaramu Burli,¹ Sindhura Nagisetty,¹ Mamata Bandhopadhyay,¹ and M. Prasad Naidu²

¹Department of Pharmacology, Maharajah’s Institute of Medical Sciences (MIMS), Vizianagaram, Andhra Pradesh, India.
²Department of Biochemistry, Narayana Medical College and Hospital, Nellore, Andhra Pradesh, India.

Corresponding Author
M. Prasad Naidu. Email: m.prasadnaidu@ymail.com

ABSTRACT

Introduction and Aim: A good teaching involves a good communication. It is a complex process and has five main components that are the sender (source/teacher), receiver (audience/students), message (content/lecture), channel (medium/chalk and talk, PPT, practical application, etc.) and feedback. The superiority of these aids over one another has been proven partially. The present study was conducted to evaluate the impact of the chalkboard, PPT and therapeutic problem-based lectures in pharmacology teaching on second MBBS medical students.

Materials and Methods: A cross-sectional study was conducted at Department of Pharmacology, Maharajah’s Institute of Medical Sciences, Nellimarla, Vizianagaram, Andhra Pradesh in 2015. One hundred twenty-seven second year MBBS medical students (n = 127) were divided into two groups.

Results: Shows, there is no significance difference between pre and post test scores in the first group with PPT teaching methods (p value 0.22). And Comparison and assessment of pre and post test scores of Therapeutic problem (Practical based learning) in the chalk and board group. In our study all the three types of teaching shows significant improvement in the similar extent.

Conclusion: All teaching modules are equally effective, lack of regular reading habit of students may be a cause for retaining of knowledge and most of the students preferred combination methods.

Key words: Pharmacology and Medical Students, Teaching Methods

INTRODUCTION

A good teaching involves a good communication. It is a complex process and has five main components that are the sender (source/teacher), receiver (audience/students), a message (content/lecture), channel (medium/chalk and talk, PPT, practical application, etc.) and feedback.
<table>
<thead>
<tr>
<th></th>
<th>n (sample size)</th>
<th>M (mean)</th>
<th>SD (standard deviation)</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pretest score</td>
<td>64</td>
<td>0.63</td>
<td>0.58</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Posttest score</td>
<td>64</td>
<td>3.92</td>
<td>1.11</td>
<td></td>
</tr>
</tbody>
</table>

Table 1  Comparison and assessment of pre and post test scores with chalk and board teaching method.

<table>
<thead>
<tr>
<th></th>
<th>n (sample size)</th>
<th>M (mean)</th>
<th>SD (standard deviation)</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pretest score</td>
<td>63</td>
<td>0.55</td>
<td>0.31</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Posttest score</td>
<td>63</td>
<td>3.6</td>
<td>1.46</td>
<td></td>
</tr>
</tbody>
</table>

Table 2  Comparison and assessment of pre and post test scores with PPT (Powerpoint presentation) teaching method.

<table>
<thead>
<tr>
<th></th>
<th>n (sample size)</th>
<th>M (mean)</th>
<th>SD (standard deviation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chalk board</td>
<td>64</td>
<td>3.9</td>
<td>1.11</td>
</tr>
<tr>
<td>Posttest score</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PPT Posttest</td>
<td>63</td>
<td>3.6</td>
<td>1.46</td>
</tr>
</tbody>
</table>

Table 3  Comparison and assessment of post test scores in the two groups with two teaching methods (chalk board and PPT).

<table>
<thead>
<tr>
<th></th>
<th>n (sample size)</th>
<th>M (mean)</th>
<th>SD (standard deviation)</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pretest score</td>
<td>60</td>
<td>0.55</td>
<td>0.83</td>
<td>0.001</td>
</tr>
<tr>
<td>Posttest score</td>
<td>60</td>
<td>4.23</td>
<td>1.66</td>
<td></td>
</tr>
</tbody>
</table>

Table 4  Comparison and assessment of pre and post test scores of Therapeutic problem (Practical based learning) in the chalk and board group.

<table>
<thead>
<tr>
<th></th>
<th>n (sample size)</th>
<th>M (mean)</th>
<th>SD (standard deviation)</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pretest score</td>
<td>65</td>
<td>0.27</td>
<td>0.41</td>
<td>0.001</td>
</tr>
<tr>
<td>Posttest score</td>
<td>65</td>
<td>3.5</td>
<td>1.77</td>
<td></td>
</tr>
</tbody>
</table>

Table 5  Comparison and assessment of pre and post test scores of therapeutic problem (Practical based learning) in PPT group.

<table>
<thead>
<tr>
<th></th>
<th>n (sample size)</th>
<th>M (mean)</th>
<th>SD (standard deviation)</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Posttest score</td>
<td>60</td>
<td>4.23</td>
<td>1.66</td>
<td>0.02</td>
</tr>
<tr>
<td>Posttest score</td>
<td>65</td>
<td>3.5</td>
<td>1.77</td>
<td></td>
</tr>
</tbody>
</table>

Table 6  Comparison and assessment of post test scores of Therapeutic problem (Practical based learning) in the chalkboard and PPT group.

<table>
<thead>
<tr>
<th></th>
<th>n (sample size)</th>
<th>M (mean)</th>
<th>SD (standard deviation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chalk board</td>
<td>64</td>
<td>3.92</td>
<td>1.11</td>
</tr>
<tr>
<td>Pretest score</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pretest score</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 7  Comparison and assessment of post test of Chalk board and pre test of Therapeutic problem (Practical based learning).

<table>
<thead>
<tr>
<th></th>
<th>n (sample size)</th>
<th>M (mean)</th>
<th>SD (standard deviation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chalk board</td>
<td>64</td>
<td>3.92</td>
<td>1.11</td>
</tr>
<tr>
<td>Posttest score</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 8  Comparison and assessment of post test PPT score and pre test of Therapeutic problem (Practical based learning).
and is not dependent on electricity. But it is time-consuming; one cannot go back to what has been erased and is not so effective for a large number of students.

PPT has the advantage of using colours, fonts, diagrams and animation. Its disadvantage is that dim light causes loss of eye contact; note-taking is difficult, has a tendency to overload information and needs electricity.

Problem based teaching gives the clinical application of knowledge acquired and makes the student write a rational prescription for a particular case and helps solving problems in real therapeutic situations. Disadvantages are until proper basic knowledge about drug’s uses, and adverse effects are understood, the application cannot be done.

The present study is planned to compare the effect of each type in pharmacology teaching by conducting pre and post tests by allotting scores for each question.

MATERIALS AND METHODS

A cross-sectional study was conducted at Department of Pharmacology, Maharajah’s Institute of Medical Sciences, Nellimarrla, Vizianagaram, Andhra Pradesh in 2015. One hundred twenty-seven second year MBBS medical students (n = 127) were divided into two groups. First group consisting of 64 students, to whom 1-hour lecture was delivered on Insulin by using chalk and board followed by a second group of 63 students to whom the same content was taught in 1 hour by using a PowerPoint. Pre-tested objective type questionnaire consisting of 6 questions each having one mark was given for evaluation in both pre and post test. There after two didactic lecture classes were taken for the students on a treatment of diabetes mellitus, before the teaching of a therapeutic problem. While assessing on problem-based learning, all students were dealt together, and all faculty members helped as facilitators. A similar pre and post test was conducted for evaluation by giving 7 similar questions to all students. At the end, the students were given one separate question for giving their choice on different modules by grading them in 6 categories and result analysed.

The difference in the marks obtained in the three groups was analyzed by independent Student’s t-test using the Statistical Package for Social Sciences (SPSS) version 17.

RESULTS

To evaluate the effectiveness of lectures by Chalkboard, Powerpoint presentation (PPT) and practical application by solving therapeutic problems. This study conducted at Department of Pharmacology, Maharajah’s Institute of Medical Sciences, Nellimarla, Vizianagaram, Andhra Pradesh in 2015.

Table 3 shows, there is no significance difference between pre and post test scores in the first group with PPT teaching methods (p-value 0.22).

Above table shows most of the students preferred combination teaching method.

DISCUSSION

Chalkboard—(Table 1): The CB method gives a significant improvement in scores from pre to post which correlates with Dhaliwal (1), Banerjee et al. (2), Bandyopadhyay (3) Dantu Padmasree (4) studies. PPT—(Table 2): There is significant improvement in post-test scores compared to pretest scores.
with PPT aiding the lecture which correlates with Galvis et al. (5) Savoy et al. (6), Erdremir (7), Seth et al. (8) studies. Chalkboard/PPT—(Table 3): Post-test scores of PPT and CB classes did not show any statistical significance, correlates with Ricer et al. study (9). Problem-based learning (Therapeutic problem). There is no evidence available to evaluate the impact of a problem-based learning process. In our study, all the three types of teaching show significant improvement in the similar extent (Tables 4–6).

Most of the students preferred combination methods, which correlates with Kumar et al. (10) and Mohan et al. (11) studies. CB Group and PPT group could not retain knowledge to solve the therapeutic problem may be due to lack of regular reading (Tables 7 and 8).

CONCLUSION

All teaching modules are equally effective, lack of regular reading habit of students may be a cause for retaining of knowledge and most of the students preferred combination methods.

ACKNOWLEDGEMENTS

Cooperation with Department of Pharmacology, Maharajah’s Institute of Medical Sciences, Nellimarla, Vizianagaram, Andhra Pradesh.

CONFLICT OF INTEREST

None declared.

REFERENCES

Effect of electromagnetic radiation exposure on hematological parameters of Swiss Albino mice and their modulation by high protein diet

Debajyoti Bhattacharya,¹ Niladri Ghosh,² and Mausumi Sikdar (nee) Bhakta¹

¹Physiology Unit, Department of Biological Sciences, Presidency University, Kolkata, India. ²Department of Physiology, University of Calcutta; Kolkata, India

(Received: March 2016  Accepted: Mar 2016)

Corresponding Author:
Dr. Mausumi Sikdar (nee) Bhakta. Email: mausumi.dbs@presiuniv.ac.in

ABSTRACT

Introduction and Aim: Research suggests that mobile phone radiation has adverse effects on haematological parameters such as blood cell count, haemoglobin concentration, etc. The purpose of this study is to find out whether high protein diet can reverse the ill effects caused by mobile phone radiation.

Materials and methods: For this study, 24 male Swiss Albino mice were divided into 4 groups (A, B, C, D). Gr A animals were fed normal diet (5% Casein). Gr B and C animals were exposed to microwave radiation emitted with average whole body Specific Absorption Rate (SAR) 0.1790 W/kg daily for 3 hours per day for 60 days. In addition to the radiation doses, Gr B animals received normal diet containing 5% casein whereas Gr C animals received a high protein diet containing 20% casein. Gr D animals were given only High protein diet (HPD) containing 20% casein. All animal experiments were performed according to the ethical guidelines of the Institutional Animal Ethics Committee (IAEC) of Presidency University and Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Culture, Government of India. After 60 days, blood samples were drawn and various hematological parameters were studied.

Results: Microwave exposure from mobile phone resulted in a significant decrease in (p < 0.05) in total R.B.C. count, haemoglobin content, whereas, total W.B.C. count, eosinophil, neutrophil and monocyte percentage increased significantly (p < 0.05). SEM studies demonstrated the presence of distorted R.B.C. structure in microwave exposed group animals. Signs of improvement in the haematological parameters were recorded in Group C animals, which were supplemented with HPD.

Conclusion: The study concludes that electromagnetic exposure from mobile phone has adverse effects on haematological parameters which may be ameliorated by supplementation with HPD containing casein.

Key words: High Protein Diet, Electromagnetic Waves, Haemoglobin, Lymphocytes

INTRODUCTION

Increased use of Electromagnetic (EM) principles for domestic and industrial purposes proves that EMF plays an important role in our daily life. Nowadays, mobile phones are not only used for making and receiving calls, but it also has many other applications, such as banking transactions, social networking, and web browsing. The world economic boom also benefitted from these technologies. According to International Telecommunication Union (ITU), the number of
mobile phone subscribers in the world was estimated to about 5.6 billion in 2014, and it is expected to reach 6 billion by the end of 2015. This increased usage and growing popularity of wireless technologies in RF (Radio Frequency) EMF range represents one of the fastest growing environmental influences (1).

Electromagnetic radiation (EMR) from artificial sources like power distribution networks, cell phones in daily life exceeds the natural electromagnetic fields by thousand folds. Use of cell phones by the public is responsible for “Electropollution.”

At high power density levels, with high microwave energy deposition rates, thermal effects are observed. Some of these effects can be explained on the basis of heat induced thermal stress mechanisms. EMR have been found to cause cellular heat–stress responses far more easily than other kinds of stress including stress caused by heat (2).

Casein, found in milk is a first class protein, containing all the essential amino acids. In vitro studies done to evaluate the role of casein and casein derived peptides reveals its role as an antioxidant agent. Casein and its peptides inhibit enzymatic and non enzymatic lipid peroxidation. The antioxidant activity is not lost with dephosphorylation or proteolysis of casein. Casein thus acts as an anti stress factor (3). But there is no such evidence where casein acts in vivo as an antioxidant agent.

It has been observed that long term use of microwave devices, such as mobile phones has negative impact on haematological parameters in mammals (4, 5, 6). Several studies highlight the role played by reactive oxygen species (ROS) in mediating the effects of microwave radiation emitted by mobile phones (4, 5, 6).

Researches on health effects of RF EMF are quite numerous and diverse. It cuts across many disciplines of engineering, physics, biology and medicine (7). Not only animals, the electromagnetic irradiation can also affect the growth of plants and microorganisms. Therefore, the present investigation has been undertaken to study the effects of microwave exposure on haematological parameters of Swiss albino mice and the possible modulatory role of High Protein diet against these induced changes.

**MATERIALS AND METHODS**

**Selection of Animals**

Twenty-four Swiss Albino male mice (*Mus musculus*) weighing 20 ± 10 g were used. They were maintained in a 12 hours light/dark cycle at 25°C to 27°C. All animal experiments were performed according to the ethical guidelines suggested by the Institutional Animal Ethics Committee (IAEC) of Presidency University and Committee for the Purpose of Control and Supervision of Experiments on Animals (CPC-SEA), Ministry of Culture, Government of India.

<table>
<thead>
<tr>
<th>Name of the group</th>
<th>No. of animals</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td>6</td>
<td>Animals of group A were given only normal diet.</td>
</tr>
<tr>
<td>Group B</td>
<td>6</td>
<td>Animals of group B were given normal diet exposed to mobile phone radiation at a frequency of 0.9 GHz.</td>
</tr>
<tr>
<td>Group C</td>
<td>6</td>
<td>Animals of group C were given High Protein Diet exposed to mobile phone radiation at same frequency (0.9 GHz)</td>
</tr>
<tr>
<td>Group D</td>
<td>6</td>
<td>Animals of group D were given only High Protein Diet.</td>
</tr>
</tbody>
</table>
Selection of dose of radiation

GSM like frequencies of 0.9 GHz was used for 3 hours continuously per day on the exposed groups and connected to a directional antennas (8,9).

Preparation of diet

All the animals were fed ad libium on two series of diets. One hundred grams of first series of diet contained 5 g casein, 38.5 g wheat meal, 46.5 g chick pea, 5 g corn oil, 1 g vitamin mixture, 4 g salt mixture and it was referred to the normal diet. One hundred grams of second series of diet contained 20 g casein, 39 g wheat meal, 31 g chick pea, 5 g corn oil, 1 g vitamin mixture, 4 g salt mixture and this diet is considered as High Protein Diet (HPD) (10).

Experimental design

All the animals were divided randomly into four groups (group A-D) according to the following table:

At the end of the 60 days, blood was drawn from all the animals from each group (A-D) and the following parameters were studied:

Hemoglobin estimation and total RBC and WBC count

Hemoglobin was estimated by Sahli’s hemoglobinometer, total RBC, and WBC were counted with Neuber’s Hemocytometer kit with Hayem’s fluid composed of 0.5% (w/v) Sodium Chloride, 0.25% (w/v) Sodium Sulphate, 0.25% (w/v) Mercuric Chloride (11,12).

Differential count (DC)

A drop blood was taken on a clean glass slide and smear was drawn. It was stained with Leishman solution, and DC was done under the compound microscope (13).

Sample preparation for scanning electron microscopy

Blood was collected from the middle caudal vein of the animals, and a drop of it was placed on the cover slip and gold coated. Then it was examined in SEM (ZEISS EVO-MA 10 scanning microscope) (14).

Statistical analysis

All data was expressed in terms of mean ± SD (n = 6). Multiple co-relation and One Way ANOVA was performed to determine the level of significance. p-Values less than 0.05 were considered as moderately significant and 0.01 were as highly significant.

RESULTS

In the study the effect of electromagnetic radiation on different blood parameters were studied:

Table 1 and Figure 2 depicts that there was significant decrease in the total R.B.C. count (p<0.05) in the animals exposed to mobile phone radiation (Gr B) in comparison to the control group (Gr A).

In Group C animals (treated with High Protein diet along with exposure to mobile phone radiation) there was significant increase (p< 0.01) in R.B.C. count and it reached almost to the control value. But there was no significant difference (p>0.05) the R.B.C. count between the control group (Gr A) and the group treated with only High Protein diet (Gr D).

Table 1 and Figure 3 depicts there was significant increase in the total W. B.C. count (p<0.01) in the animals exposed to mobile phone radiation (Gr B) in comparison to the control group (Gr A).

In Group C animals (treated with High Protein diet along with exposure to mobile phone radiation) there was significant decrease (p< 0.01) in W.B.C. count and it reached almost to the control value. But there was no significant difference (p>0.05) the W.B.C. count between the control group (Gr A) and the group treated with only High Protein diet (Gr D).

Table 1 and Figure 4 depicts there was significant decrease in the haemoglobin concentration (p<0.05) in the animals exposed to mobile phone radiation (Gr B) in comparison to the control group (Gr A). In Group C animals (treated with High Protein diet along with exposure to mobile phone radiation)

---

there was significant increase \((p < 0.01)\) in R.B.C. count and it reached almost to the control value. But there was no significant difference \((p > 0.05)\) the R.B.C. count between the control group (Gr A) and the group treated with only High Protein diet (Gr D).

Table 1 and Figure 3 depicts there was significant increase in the total W. B.C. count \((p < 0.01)\) in the animals exposed to mobile phone radiation (Gr B) in comparison to the control group (Gr A).

In Group C animals (treated with High Protein diet along with exposure to mobile phone radiation) there was significant decrease \((p < 0.01)\) in W.B.C. count and it reached almost to the control value. But there was no significant difference \((p > 0.05)\) the W.B.C. count between the control group (Gr A) and the group treated with only High Protein diet (Gr D).

Table 1 and Figure 4 depicts there was significant decrease in the haemoglobin concentration \((p < 0.05)\) in the animals exposed to mobile phone radiation (Gr B) in comparison to the control group (Gr A). In Group C animals (treated with High Protein diet along with exposure to mobile phone radiation) there was no significant increase in Haemoglobin concentration with respect to Gr B and there was no significant difference \((p > 0.05)\) between the control group (Gr A) and the group treated with only High Protein diet (Gr D).

Table 1: Effect of high casein diet on total count of R.B.C., W.B.C. and Hemoglobin percentage in Albino mice exposed to mobile phone radiation.

All values are expressed as mean ± S.D.
Significance level * = highly significant differences at \(p < 0.01\).
**Significant differences at \(p < 0.05\).
***Nonsignificant differences at \(p > 0.05\).

Table 2: Effect of high casein diet on Differential count of W.B.C. in mice exposed to mobile phone radiation.

neu, Neutrophil; eos, eosinophil, mono, monocyte; lymp, lymphocyte; baso, basophil.

Table 2: Effect of high casein diet on Differential count of W.B.C. in mice exposed to mobile phone radiation.

neu, Neutrophil; eos, eosinophil, mono, monocyte; lymp, lymphocyte; baso, basophil.

there was significant increase \((p < 0.01)\) in R.B.C. count and it reached almost to the control value. But there was no significant difference \((p > 0.05)\) the R.B.C. count between the control group (Gr A) and the group treated with only High Protein diet (Gr D).

Table 1 and Figure 3 depicts there was significant increase in the total W. B.C. count \((p < 0.01)\) in the animals exposed to mobile phone radiation (Gr B) in comparison to the control group (Gr A).

In Group C animals (treated with High Protein diet along with exposure to mobile phone radiation) there was significant decrease \((p < 0.01)\) in W.B.C. count and it reached almost to the control value. But there was no significant difference \((p > 0.05)\) the W.B.C. count between the control group (Gr A) and the group treated with only High Protein diet (Gr D).

Table 1 and Figure 4 depicts there was significant decrease in the haemoglobin concentration \((p < 0.05)\) in the animals exposed to mobile phone radiation (Gr B) in comparison to the control group (Gr A). In Group C animals (treated with High Protein diet along with exposure to mobile phone radiation) there was no significant increase in Haemoglobin concentration with respect to Gr B and there was no significant difference \((p > 0.05)\) between the control group (Gr A) and the group treated with only High Protein diet (Gr D).
respect to Gr B and there was no significant difference $(p > 0.05)$ between the control group (Gr A) and the group treated with only High Protein diet (Gr D).

As evident from Table 2 and Figure 5, there was no significant difference in the percentage of numbers of monocytes and basophil. But there was a significant increase in neutrophil percentage in Gr B Animals (exposed to electromagnetic radiation) (35.4%) in comparison to Gr A animals, eosinophil percentage is also much higher in Gr B animals (33.3%) in comparison to Gr A animals. Animals in Gr C have almost same eosinophil percentage like Gr A. There was a very low number of lymphocytes is present in Gr B animals (8.3%) in comparison to Gr A (22.5%). The animals in Gr C (treated with high casein diet exposed to mobile phone radiation) have almost back to the normal lymphocyte percentage.

Effect of high casein diet on R.B.C. surface structure exposed to mobile phone radiation.

Figure 6A depicts the scanning electron microscopic image of the R.B.C. of mice treated with normal diet. The normal bi concave shape and size of the cells are seen. The number of cells present in the film is also normal.

Figure 6B depicts the electron microscopic image of the R.B.C. of mice exposed to mobile phone radiation treated with normal diet. The cells are distorted in shape, are clumped, total number of cells are also less in the film in comparison to group A.

Figure 6C depicts the electron microscopic structure of the R.B.C. of mice exposed to mobile phone radiation treated with High Protein diet. The amount of cell distortion is much less in comparison to group B animals. The arrangements of the cells are also normal.

Figure 6D depicts the electron microscopic image of the R.B.C. of mice treated with High Protein diet. The cell shows normal size and shape. No structural deformities are seen.

DISCUSSION

Result indicates that the total number of R.B.C. is decreased, and deformed structure is seen in animals exposed to EMR receiving normal diet (Gr B animals) in comparison to the control group. There
may be some apoptotic pathway is responsible for the decreasing number of R.B.C. The mechanistic details of apoptosis of R.B.C. caused by EMR are not known, but studies indicate that prooxidant molecules released from the R.B.C. might be responsible for such changes. It accelerates the destruction of R.B.C. which correlates well with poor antioxidant protection within R.B.C. and higher rate of conversion of haemoglobin to methemoglobin. Formation of methemoglobin within R.B.C. triggers the development of oxidative stress and causes structural membrane defects (15,16). As casein has antioxidant property, it prevents the conversion of haemoglobin to methemoglobin and development of oxidative stress. So the total R.B.C. count and structure is restored in animals receiving high casein diet along with radiation.

The total haemoglobin concentration was significantly decreased in microwave radiation exposed group (GR B) animals, which may be due to low RBC count in the same group. This could be attributed to the interaction between the iron of haem and the electromagnetic field produced due to mobile phone induced radiation exposure in all the vital organs like spleen, bone marrow etc. (17) As casein plays an important role in cell signalling pathways that enhance the growth and division of cells relevant to cell proliferation, so in High Protein diet Group (Gr C animals), the R.B.C. count was restored towards normal (18). But in radiation exposed animals, the electromagnetic radiation exposure may have induced certain structural changes in haemoglobin which it cannot be restored by High Protein diet. It may be possible that the new R.B.C. formed in the group of animals exposed to mobile phone radiation, but fed a High Protein diet (Gr C), may have haemoglobin with deformed structure or in lesser amounts.

From the present study, it can be said that increased eosinophil and neutrophil count indicates that there may be certain stress induced tissue damage in the body caused by electromagnetic radiation (19). Stress induced by radiation activates caspase 9 and induces apoptosis of lymphocytes, as a result of which, the percentage of lymphocytes decreases in the blood (20). Casein is a major component in the High Protein diet, which has some antioxidant properties that can ameliorate the ill effects of radiation and the altered W.B.C. count can be restored back towards normal.

The SEM images of R.B.C. of the exposed group showed that the R.B.Cs have distorted shape and stick next to each other to form a rouleaux. In HPD treated group the R.B.C. showed normal biconcave shape.

**CONCLUSION**

From the present study, it can be concluded that mobile phone radiation-induced changes in blood parameters can be reduced by High Protein diet (High Casein). High Protein diet may have some protective action which can reduce the ill effects of mobile phone radiation on blood parameters.

**ACKNOWLEDGEMENT**

Authors acknowledge the FRPDF grant of Presidency University to the corresponding author.

**ABBREVIATIONS USED**

HPD = High Protein diet  
Rad = Radiation  
SEM = Scanning electron microscopy

**REFERENCES**

4. Adebayo Akeem Otitolou, Vintcent O. Osunkalu, Ruth Oduware, Idowu Ayisat Obe, Adekala Olajide Adewale, “Haematological effects of


Ethanol enhances lamivudine-induced liver toxicity: Investigation on hepatoprotective properties of silibinin-phosphatidylcholine complex in rats

Balasubramanian Jesudas,¹ Ramanathan Raghu,¹ Ganapathy Bhavani,¹ Devaraj Ezhilarasan,¹ and Sivanesan Karthikeyan¹

¹Department of Pharmacology and Environmental Toxicology, Dr. ALM PGIBMS, University of Madras, Taramani Campus, Chennai, Tamil Nadu, India.

(Received: Feb 2016    Accepted: Mar 2016)

Corresponding Author

Dr. Sivanesan Karthikeyan. Email: karthik48y@yahoo.co.in; hepatotoxicology.lab@gmail.com

ABSTRACT

Introduction and Aim: Lamivudine (2'-deoxy-3'thacytidine, 3TC), when advocated with several anti-retroviral drugs for the treatment of HIV infections in human is reported to induce liver toxicity. There is a paucity of data on the influence of ethanol (EtOH) intake (alcoholism) on 3TC alone induced toxic insults of the liver. This study evaluates the time-course onset of 3TC alone, EtOH alone and EtOH + 3TC-induced toxic insults of the liver and the mitigating properties of Silibinin-Phosphatidylcholine complex (SPC) against EtOH + 3TC-induced hepatotoxicity in rats.

Materials and Methods: Control rats (Group I) received saline from day 1 till day 14 and from day 15 till day 60, they were treated propylene glycol (vehicle). Group II rats (3TC alone Group) were given saline from day 1 till day 14 and from day 15 till day 60, they received 3TC alone (100 mg/kg; p.o.). Group III rats (EtOH alone Group) were administered EtOH from day one till day 14 at 4 gm/kg/day (p.o.) and its dose was reduced to 2 gm/kg/day from day 15 till day 60, to maintain alcoholic status. Group IV and V rats received EtOH on par with Group III. Additionally, Group IV rats (EtOH + 3TC Group) were treated 3TC (100 mg/kg/day; p.o.) along with EtOH, from day 15 till day 60. Similarly, Group V rats (EtOH + 3TC + SPC Group) received 3TC (100 mg/kg/day; p.o.) + SPC (100 mg/kg/day; p.o.) along with EtOH from day 15 till day 60. Group VI rats (SPC alone Group) received saline from day 1 till day 14 and from day 15 till day 60, they were treated SPC alone (100 mg/kg/day; p.o.). Marker enzymes of liver toxicity (AST, ALP, γ-GT, ASAL), bilirubin (BIL) and protein were investigated in serum; and various lipid parameters (TL, TG, CHO, PL, FFA) were evaluated in plasma, on days 15, 30, 45 and 60.

Results: 3TC alone, EtOH alone as well as EtOH + 3TC administrations caused a highly significant ($p < 0.001$) progressive increase in marker enzymes (AST, ALP, γ-GT, ASAL), BIL and protein from day 30 till day 60 of their respective treatments. These toxicants also caused significant ($p < 0.001$) progressive elevation of all the lipid parameters (TL, TG, CHO, PL, FFA) investigated in plasma. Hepatotoxicity and hyperlipidemia were more pronounced in rats receiving EtOH + 3TC treatments as compared to rats receiving 3TC alone and EtOH alone. Simultaneous administration of SPC in rats receiving EtOH + 3TC, protected against the hepatotoxicity and hyperlipidemia induced by EtOH + 3TC treatments.

Conclusion: SPC administration mitigates hepatocellular necrosis, cholestasis and hyperlipidemia induced by EtOH + 3TC treatments and this beneficial effect could be attributed to enhanced bioavailability of silibinin.
INTRODUCTION

Lamivudine (2’-deoxy-3’-thiacytidine, 3TC) is a synthetic cytidine 2’,3’-dideoxynucleoside analogue and it belongs to nucleoside reverse transcriptase class of antiretroviral drugs. It was initially approved by FDA for the treatment of chronic hepatitis-B viral infections in adult patients and later its use was extended for the therapy to improve asymptomatic and symptomatic human immune deficiency virus (HIV) infections, either alone or in its combination with other antiretroviral drugs, including zidovudine (1). One of the most common toxic manifestations of 3TC in humans is the development of hepatotoxicity, which is shown by elevation in the marker enzymes of liver toxicity, bilirubin, hepatocellular necrosis and other degenerative changes in the liver. Hepatic necrosis is reported to occur in 6% to 31% of recipients of 3TC when administered with other antiretroviral drugs and hence modifications in dosage or discontinuation of therapy is recommended to reduce its liver toxicity (2,3).

Several workers have reported on the problems of chronic alcohol consumption in HIV patients even under medical care (4). Ethanol (EtOH) consumption in HIV-positive patients accelerated liver damage leading to injury, inflammation, alcoholic steatohepatitis, liver fibrosis and cirrhosis. Though, alcohol drinking is reported to have an impact on liver fibrosis and on the activities of aspartate aminotransferase enzyme, some investigators have concluded that the problem of alcohol abuse in HIV-positive patients is not adequately addressed and the mechanism by which HIV infection effects liver remains somewhat elusive (5,6). It should be emphasized that reports regarding the toxic potentials of 3TC monotherapy in the liver are not well documented. Moreover, data on the hepatotoxic potentials of 3TC in alcoholics are scanty and remedial measures employed to counteract this adversity is poorly defined.

Silibinin a standardized extract obtained from the seeds of Silybum marianum (L.) Gaertn (Carduus marianus L., Asteraceae), is an effective antioxidant, membrane stabilizing and hepatoprotective agent against several drugs and toxins-induced liver damage (7,8). It is stated that silibinin has low bioavailability and hence its absorption from the gastrointestinal tract is limited by the lipid-rich outer membrane of the small intestine (9). It is proposed that the bioavailability of silibinin could be enhanced when it is admixed with phosphatidylcholine as silibinin-phosphatidylcholine-complex (SPC). Pharmacokinetic studies have reported that SPC polysomes enhance the absorption of silibinin by five times, in the bile as well as in plasma, which increases the hepatoprotective ability of SPC. Hence, recent studies recommend usage of SPC complex or its polysomes for enhancing their hepatoprotective, antioxidant and anti-hyperlipidemic properties of silibinin against drugs and toxins-induced liver damage (10). In view of these reports, in the current study, we investigated hepatoprotective potentials of SPC treatment against EtOH + 3TC-induced time-course onset of toxic insults of the liver in rats.

MATERIALS AND METHODS

Animals

Wistar albino rats of both sexes weighing 175 ± 20 gms, procured from Institutional animal house facility were used in this study. They were housed in polypropylene cages under standard conditions (temp: 27°C ± 2°C; relative humidity: 50-70%; 12 h light/dark cycle) and fed standard pellet and water ad lib. The experimental protocol was approved by Institutional Animal Ethical Committee (IAEC no. 01/27/2012), Govt. of India. Constituted for the purpose as per CPCSEA guidelines.
Drugs and chemicals

Silibinin, phosphatidylcholine, argininosuccinylase disodium salt and triolein were purchased from Sigma-Aldrich Chemicals Pvt. Ltd., USA. Lami

Drug treatments and experimental design

Control rats (Group I) received saline from day 1 till day 14 and from day 15 till day 60, they were treated propylene glycol (vehicle). Group II rats (3TC alone Group) were given saline from day 1 till day 14 and from day 15 till day 60, they received 3TC alone (100 mg/kg; p.o.). Group III rats (EtOH alone Group) were administered EtOH from day 1 till day 14 at 4 gm/kg/day (p.o.) as described previously (11) and its dose was reduced to 2 gm/kg/day from day 15 till day 60, to maintain EtOH-induced liver damage, without producing mortality of rats. Group IV and V rats received EtOH on par with Group III. Additionally, Group IV rats (EtOH + 3TC Group) were treated 3TC (100 mg/kg/ day; p.o.) along with EtOH, from day 15 till day 60. Similarly, Group V rats (EtOH + 3TC + SPC Group) received 3TC (100 mg/kg/day; p.o.) + SPC (100 mg/kg/day; p.o.) along with EtOH from day 15 till day 60. Group VI rats (SPC alone Group) received saline from day 1 till day 14 and from day 15 till day 60, they were treated SPC alone (100 mg/kg/day; p.o.).

3TC was dissolved in saline and was prepared fresh before use. Silibinin-Phosphatidylcholine Complex (SPC) was prepared by taking equal quantities of silibinin and phosphatidylcholine at a ratio of (1:1) and suspending them in propylene glycol. The volume of above administrations was maintained at 0.3 to 0.5 ml/100 gm b.w. of the rat.

Collection of serum and plasma

Serum and plasma samples were collected separately from all the treatment Groups on days 15, 30, 45 and 60. Blood was withdrawn by the retro-orbital puncture in the overnight fasted rats under mild ether anesthesia. For separation of serum, blood was collected (1 to 1.5 ml) in a clean tube and it was allowed to clot for 20 min at cold (8°C). Serum was subsequently separated by centrifuging the clotted blood at 2,500 rpm for 20 min. The clear supernatant serum was separated and stored in vials under refrigerated condition (8°C to 10°C) until further analysis. For plasma separation, whole blood (0.75 to 1 ml) was collected in clean tubes which were previously coated with 1% EDTA to prevent clotting and was centrifuged subsequently at 2,500 rpm for 20 min. The clear supernatant plasma was separated and stored in vials under refrigerated condition (8°C to 10°C) until further analysis. All the biochemical evaluations were performed within 48 h after sample collection.

Assay of markers of hepatotoxicity in serum

Serum aspartate transaminase (AST) activity was quantified as described by Reitman and Frankel (12). Briefly, the enzyme liberates pyruvate in the presence of the substrate DL-aspartic acid and α-ketoglutaric acid upon reaction with 2,4-dinitrophenyl hydrazine and sodium hydroxide (NaOH) to form brownish-orange colored hydrazone derivative, whose intensity was measured at 540 nm using the spectrophotometer. Phenol liberated by the enzymatic hydrolysis in the presence of the substrate disodium phenyl phosphate was reacted with Folin-Ciocalteu’s phenol reagent and NaOH to yield blue color, whose optical density was measured at 640 nm, for the assay of alkaline phosphatase (ALP) as described by King (13). The p-nitroaniline liberated by the enzyme γ-glutamyl transpeptidase (γ-GT) in the presence of the substrate L-γ-glutamyl-p-nitroanilide was made to produce a yellow color, whose intensity was measured spectrophotometrically at 420 nm as described by Rosalki and Rau (14). The enzyme argininosuccinic acid lyase mediates the degradation of argininosuccinic acid to arginine and fumicaric acid. The arginine released from the substrate sodium argininosuccinate was allowed to react with sodium hypochlorite to form a pink color, whose intensity was measured at 515 nm for assay of ASAL activi-
ity (15). For the assay of total bilirubin (BIL), the serum was initially diluted with water, and methanol was added in sufficient quantity to permit precipitation of protein and to allow the bilirubin to react with diazo reagent (by Vanden-Berg reaction) to form a purple compound i.e., azobilirubin, and its intensity was measured at 540 nm against blank using spectrophotometer as described by Malloy and Evelyn (16). The total protein was estimated spectrophotometrically by the standard method of Lowry et al. (17).

**Assay of plasma lipid profiles**

The plasma triglyceride (TG) was estimated as described by Varley et al. (18). Briefly, the phospholipids were separated using a mixture of isopropanol and alumina and was subjected to saponification reaction with potassium hydroxide, to liberate glycerol which was subsequently reacted with acetyl acetone to yield a yellow color, whose intensity was quantified spectrophotometrically at 405 nm. For assay of phospholipids (PL), the protein was initially precipitated using trichloro acetic acid and digested with perchloric acid to liberate phosphorus as described by Ziversmit and Davis (19). This liberated phosphorous was treated with molybdate reagent to form phospho-molybdic acid and subsequently, reduced with 1-amino-2-naphthol-4-sulphonic acid, to give a blue coloured complex (20) and its intensity was measured against the blank at 797 nm using spectrophotometer. The total cholesterol (CHO) in plasma was estimated according to the method of Varley (21) by subjecting the samples to a reaction mixture containing acetic acid, ferric chloride and sulphuric acid to yield pink color and its optical density was read at 560 nm using spectrophotometer. The free fatty acids (FFA) were extracted using stable copper reagent to form fattyacid-copper soap (22), and was coloured with sodium diethyldithio carbamate (23) whose intensity was measured spectrophotometrically at 437 nm against blank. The total lipid (TL) was estimated by treating the sample with vanillin, sulphuric acid and phosphoric acid to yield pink color and its intensity was measured at 540 nm using spectrophotometer as described by Frings and Dunn (24).

**Statistical analysis**

Experimental results are expressed as mean ± S.D. The data was subjected to one-way ANOVA and post-Hoc multiple comparisons were done by Tukey’s test to evaluate the significance of difference between treatment groups at various time points, using SPSS software (Version 16.0). Statistical significance was considered when $p$-value was $<0.05$.

**RESULTS**

Data on time-course evaluation of various marker enzymes of hepatotoxicity, BIL and protein in serum of rats are presented in Fig. 1 (a to f). Administration of 3TC alone (Group II), EtOH alone (Group III), as well as EtOH + 3TC (Group IV) produced a progressive and highly significant increase ($p < 0.001$) in the activities of all the marker enzymes of hepatotoxicity (AST, ALP, γ-GT, ASAL) and BIL in serum of rats when their respective values were compared to the vehicle treated control (Group I) from day 30 till day 60, indicating the progression of hepatotoxicity. The degree of hepatotoxicity was more than two-times the normal levels of control in rats receiving EtOH as well as EtOH + 3TC treatments. The liver toxicity was much more pronounced in the later group as compared to the former. The total protein in serum was elevated highly significantly in EtOH + 3TC as well as EtOH alone groups on days 45 and 60 and their values were almost near normal in 3TC alone treated rats. SPC post-treatment in rats receiving EtOH + 3TC (Group-V) significantly ($p < 0.001$) protected against all the above adversities on all the days of evaluation, revealing its protective property against EtOH + 3TC-induced toxic insults of the liver. Administration of SPC alone (Group VI) did not produce any change in the status of all the parameters evaluated in serum and their respective values were on par with the vehicle-treated control (Group I).

3TC alone, EtOH alone and EtOH + 3TC treatments produced a highly significant ($p < 0.001$) progressive increase in TL, TG, CHO, PL and FFA from day 30 till day 60 in plasma of rats. The disruption in lipid metabolism and its homeostasis
observed in EtOH + 3TC treated rats (Group IV) was on par with EtOH alone treatment (Group III) on days 45 and 60. 3TC alone treatment (Group II) shows mild elevations in most of the above lipid parameters on days 30 to 60. Derangement in lipid homeostasis seen in EtOH + 3TC (Group
IV) treated rats was significantly mitigated and reversed back towards normalcy in rats post-treated SPC simultaneously with the above hepatotoxic agents and these results show the (Group V), anti-hyperlipidemic activity of SPC against EtOH + 3TC-induced hyperlipidemia. SPC alone treatment (Group VI) did not produce any change in all the lipid profiles investigated in plasma and their respective values were comparable to those of vehicle-treated controls (Group I). Data on alterations in plasma lipid profiles described above are presented in Fig. 2(a–e).

Fig. 2 Effect of SPC treatment on EtOH + 3TC-induced time-course alteration on lipid parameters in serum of rats.

Data represents mean ± S.D. of 8 nos. of rats in each group. Treatment schedule for each group is described under section materials and methods. Data was subjected to one-way ANOVA and post-Hoc multiple comparison was done by Tukey’s test. a—Group I compared to Groups II to VI. b—Group IV compared to Group V. *p < 0.001.
DISCUSSION

3TC is believed to have a low frequency of liver toxicity. However, some studies have recorded elevations in AST, ALT and BIL in patients receiving 3TC (25,26). The two-fold elevations observed in the status of AST and BIL in this study are in accordance with these reports. In the current investigation, 3TC treatment enhanced the activities of ASAL and γ-GT from day 30 till day 60 and similar studies have not been reported previously. Chronic alcoholism is well demonstrated to enhance the activities of transaminases in serum, ALP and γ-GT in experimental animals and human (27–29) and our present results are in agreement with these findings. Data on the status of protein in serum of 3TC alone treated rats are scanty. Previous studies conducted in rats have shown that EtOH treatment interferes with protein metabolism due to its astringent activity (30) and this could be the probable reason for the fall in protein observed in serum of rats in this study. We have shown 2 to 4-fold increase in the activities of all the marker enzymes of liver toxicity in EtOH + 3TC treated rats as compared to 3TC alone and EtOH alone treatments (Fig. 1-a–e) and these results clearly demonstrates that alcoholism enhances 3TC-induced toxic insults in liver of rats.

Markers of hepatocellular injury and their elevation is said to occur consequent to hepatocellular necrosis, leading to their leakage into serum upon toxins-induced liver injury (31). Elevations of ALP, BIL and protein are shown to occur during intra and extra hepatic cholestasis, disruption of protein metabolism and metabolism of non-essential aminoacids (32). Administration of several antiretroviral drugs including zidovudine have been documented to induce cholestatic injury associated with elevation of transaminases (33). γ-GT is the most sensitive indicator of hepatobiliary disease and its elevation is a known marker of EtOH-induced liver injury (30). The enzyme ASAL is primarily located in parenchymal cells and is not present in any other cells of the liver. Its elevation in serum is considered as the precise indicator of hepatitis, cholestasis and liver cell damage (15,34). We have previously shown elevation in the activity of ASAL in the antiretroviral drug zidovudine alone treated rats (33). In the current investigation, we have demonstrated elevations in the activities of marker enzymes of liver toxicity (AST, ALP, γ-GT, ASAL), BIL and protein in serum of 3TC alone, EtOH alone and EtOH + 3TC treated rats and cumulatively these results reveal that these toxicants induce cholestasis, hepatocellular damage and disruption of protein metabolism in liver of rats.

Previous studies have demonstrated the development of lactic acidosis and hepatic lesions, characterized by microvesicular or macrovesicular fatty liver upon administration of antiretroviral drugs in human. However, abnormalities of lipid metabolism in plasma consequent to administration of 3TC alone treatment has not been reported previously. In the current investigation, we observed that 3TC alone treatment caused hyperlipidemia and disruption of lipid homeostasis. The mechanisms of 3TC-induced hyperlipidemia could not be explained due to paucity of previous reports. It should be highlighted that administration of antiretroviral drugs cause mitochondrial damage, which could result in focal necrosis, cholestasis, proliferation of biliary ducts and deposition of mallory bodies in the liver (35), and this could be the probable reason for the observation of 3TC-induced hyperlipidemia in plasma of rats. Incidentally, induction of mitochondrial damage upon 3TC administration has been documented earlier (36). It is proved that chronic alcoholism cause accumulation of lipids and affects lipid metabolism by elevating CHO, TG and PL in liver and plasma, leading to micro and macrovesicular hepatic steatosis (30,37,38). In agreement with these reports, in the current study, there was an a highly significant increase in the status of all the lipid parameters (TL, TG, CHO, PL, FFA) investigated in plasma of EtOH alone as well as an EtOH + 3TC treated rats (Fig. 2-a–e).

In this study, elevations in the activities of marker enzymes of liver toxicity BIL, protein and various lipid parameters in rats receiving EtOH + 3TC, was significantly (p < 0.001) mitigated and reversed back almost towards normalcy in SPC post-treated rats (Fig. 1,2). These results demonstrate its hepatopro-
tective and anti-hyperlipidemic properties of SPC against EtOH + 3TC-induced hepatocellular damage, cholestasis and hyperlipidemia. It is hypothesized that flavanoid molecules like silibinin (which has poor water solubility and oral absorption), when mixed with phosphatidylcholine (a carrier phospholipid moiety), a bond is presumed to be formed between these two molecules that makes it merge into lipid phase of outer biological cell membranes (7–10,39). It is likely that such a mechanism would have contributed for the greater bioavailability of silibinin and enhanced the mitigating properties of SPC against EtOH + 3TC-induced toxic insults of the liver that is observed in the current study. The hepatoprotective, antioxidant and free radical scavenging properties of silibinin against drug-induced hepatotoxicity are well documented in the previous literature (7,8,40). Similarly, several studies have reported that phosphatidylcholine treatment has clinical efficacy for protecting various liver diseases including alcoholic hepatic steatosis, drug-induced liver damage and hepatitis (8,10,39).

In conclusion, our current results demonstrate that SPC post-treatment mitigates EtOH + 3TC-induced hepatic necrosis, cholestasis and hyperlipidemia in rats. This hepatoprotective ability could be attributed to the enhanced bioavailability of silibinin. Our results suggest that SPC could be a better candidate for the treatment of ethanol and 3TC-induced liver damage. However, it is cautioned that in-depth Phase-III clinical studies are warranted before implementation of SPC in clinical practice.

REFERENCES


and L. Deleve (Eds.), Academic Press (Elsevier Ins.), Tokyo, 2013; 505–518.


Is Internet use related to academic performance in medical students? A study from South Indian Medical College

Ravi Kishore Polepalli

1Department of Biochemistry, MVJ Medical College and Research Hospital, Hoskote, Bangalore, India.

(Received: Jan 2016 Accepted: Mar 2016)

Corresponding Author

Dr. Ravi Kishore Polepalli. Email: writekishore@gmail.com; writekishore@yahoo.co.in

ABSTRACT

Introduction and Aim: Indian Medical students spend a substantial amount of time accessing the internet for curricular and non-curricular purposes. However, the effect of internet use on academic performance is not clear. The purpose of this study is to evaluate the relationship between internet use and academic performance in 1st year Medical students in India.

Materials and Methods: One hundred thirty-two students studying in 1st year undergraduate medical course at MVJ Medical College and Research Hospital, Bangalore participated in the study. Data related to internet use and academic performance was captured using a pretested questionnaire. Statistical analysis was performed using ANOVA test.

Results: Time spent accessing the internet for overall (curricular and non-curricular) use and curricular use did not significantly differ between low, mid-and high-level academically performing groups ($p = 0.67$ and $p = 0.64$). However high performers spend significantly less time chatting/messaging as compared to low- and mid-level performers ($p = 0.006$).

Conclusion: Students with high-level academic performance tend to spend less time on chatting/messaging using applications like Whatsapp/Snapchat when compared to mid-level performers. However, there was no difference between high, mid- and low-level groups in overall and curricular use of the internet.

Key words: Academic Performance, Internet Use, Medical Students

INTRODUCTION

The Internet has become a source of recent advances, a platform for innovative, simplified and self-paced learning methods and tool to independently assess oneself in Medical knowledge. Medical students have been shown to spend significant time in accessing the internet both for curricular and non-curricular (communication, entertainment, etc.) purposes (1–3). However, to the best of our knowledge, there is a lack of evidence about the relationship between internet use and academic performance in Indian Medical students. The objective of this study is to examine the relationship between internet use and academic performance in 1st year Medical students in India.

MATERIALS AND METHODS

A cross-sectional design was employed in this study in which Internet usage and its relationship
with the academic performance was evaluated in 1st year Medical students of MVJ Medical college and Research Hospital, Bangalore. This study was conducted at Biochemistry department, MVJ Medical College and Research Hospital, Bangalore. Ethical approval for the study was obtained from Institutional Ethical Review board. One hundred thirty-two students of 1st year medical undergraduate course participated in the study. Informed consent was obtained from the participants. Pretested anonymous Questionnaire consisting of multiple choice and open ended questions was used to record the data. Self-reported scores of Internal assessment examination in Biochemistry was taken as a measure of academic performance. Based on the academic performance in Internal assessment examination in Biochemistry was taken as a measure of academic performance. The mean duration of Internet use (curricular and non-curricular) was 11.1 hrs per week which was substantially higher when compared to previous studies by Unnikrishnan et al. and Maroof et al. where it was less than 3 hours per week (1,2). To the best of our knowledge, there have been no studies on the relationship between internet use and academic performance in Medical students in India. In a study of Malaysian Medical students, it was found that higher academic performance was associated with higher internet usage (4). However, such a relationship was not observed in our study. One plausible explanation is that hardcopies of reference textbooks may have served as an exclusive source of preparatory material for exams. A novel finding in our study was that low-and mid-level performers spent more time on Internet chatting/messaging on applications like Whatsapp/Snapchat than high performers, hinting at the distraction caused by frequent messaging/chatting (5). An unusual finding was that internet chatting time for low performers was only marginally but significantly higher than high performers; a possible reason could be low performers could be indulging in other non-curricular activities (like

<table>
<thead>
<tr>
<th>Academic performance groups</th>
<th>Time spent on Internet for chatting/messaging in hours per week, Mean (sd)</th>
<th>$F$-statistic</th>
<th>Statistical Significance ($p$-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low ($n = 44$)</td>
<td>5.6 (4.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mid ($n = 41$)</td>
<td>8.5 (7.5)</td>
<td>5.275</td>
<td>0.006*</td>
</tr>
<tr>
<td>High ($n = 45$)</td>
<td>4.8 (3.9)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 1. One way ANOVA analysis of relationship between academic performance and Internet usage (chatting/messaging) in 1st year Medical students in India.

*Significant $p$-values ($p < 0.05$ considered significant).
television) leading to low performance. A limitation of this study was that though we related performance in Biochemistry exams to curricular internet use, it should be emphasized that this curricular use was for all the preclinical subjects (Anatomy, Physiology and Biochemistry) put together, since it may be difficult to delineate accurately internet use among above subjects. After considering the above findings, it may be prudent to guide students in the appropriate use of the internet for achieving best academic outcomes.

CONCLUSION

Ist year Medical students spend a substantial amount of time using the internet for curricular and non-curricular purposes. Students with high-level academic performance tend to spend less time on chatting/messaging using applications like Whatsapp/Snapchat when compared to mid-level performers. However, there was no difference between high, mid- and low-level groups in overall and curricular use of the internet.

ACKNOWLEDGEMENT

I would like to thank Mr Suresh, Assistant Professor and Statistician, Department of Community Medicine for helping with Statistical analysis.

REFERENCES

Case Report

Raising the index of suspicion for cerebral venous thrombosis: A case report

Renata Mazurek,1 Naveen Ramesh,2 Kiran P.V.,3 and Avita Johnson2

1Medical School for International Health, Ben-Gurion University of the Negev, Be’er Sheva, Israel.
Departments of 2Community Health and 3Emergency Medicine, St John’s Medical College, Bangalore, Karnataka, India.

(Received: Jan 2016 Accepted: Mar 2016)

Corresponding Author
Dr. Naveen Ramesh. Email: drnaveenr@gmail.com

ABSTRACT

Cerebral venous thrombosis (CVT) is an important form of stroke in India that if missed can lead to therapeutic mismanagement and devastating consequences whereas recognition typically follows with good prognosis. Predominantly occurring in younger patients, known associations include dehydration, the peripartum period, infection, other hypercoagulable states and risk factors such as alcohol and OCP use. Unrecognized CVT may result in neurologic impairment, recurrence, brain oedema and herniation. However, at onset, CVT often lacks classic signs, as with arterial stroke. While neurologic signs may present, non-specific signs and symptoms are most common, making consideration of CVT in the differential diagnosis critical in the emergency setting. A 43-year-old patient presented to our department with an acute progression of a headache, nausea, vomiting, hemiparesis and unresponsiveness. Despite initial symptom relief, her worsening condition and altered mental status (AMS) prompted hospital arrival. History was significant only for levothyroxine for hypothyroidism and long-term OCP use for dysmenorrhoea. Brain MRI diagnosed CVT, initiating treatment. During recovery, further possible underlying causes were investigated. Recognition and appropriate referral for CVT are crucial for managing outcomes and addressing causes. Knowledge of risk factors and an index of suspicion when evaluating younger adults can prevent oversight of this critical diagnosis.

Key words: Altered Mental Status, Cerebral Venous Thrombosis, Hemiparesis, Oral Contraceptive Pill.

INTRODUCTION

Cerebral venous thrombosis (CVT) accounts for up to 1% of all strokes overall, yet represents 10-20% of strokes in young people in India (1), and can result in significant residual morbidity or mortality. Though not an uncommon medical presentation, CVT can be easily misdiagnosed if not considered. Headache is the most prevalent symptom (1,2), and other common symptoms, such as vomiting or seizures, as well as neurologic signs, paresis, speech disturbance, or confusion, are not specific to CVT. Furthermore, CVT may be secondary to infection, making it important to distinguish and rule out the presence of a venous thrombus if symptoms emerge, even in a backdrop of infection. Besides, thyroid conditions, hypercoagulable disorders, and puerperal association should be taken into account when assessing for CVT. While history may provide important clues to its development, it is also critical to evaluate for CVT under the differential diagnosis of its non-specific signs and symptoms, particularly in young adults to middle-aged adults. Here we describe a case of a patient brought to the emergency room following a three-day history of deteriorating
neurologic function.

Case

The patient is a 43-year-old female with a history of hypothyroidism and dysmenorrhea, who acutely developed a severe, pounding headache over the whole head associated with nausea and relieved by a high dose of an unknown medication and sleep. The next day, she began to have vomiting and right-sided upper and lower extremity weakness such that she could not use her right arm and dragged her right leg. Her family also noted that she became less responsive, using few words, with slurred speech and inappropriate emotional expression. By the following day, her speech had reduced to monosyllabic responses and then further to the lack of speech; she required force to move; she refused to eat or drink, and she was agitated and disturbed in sleep. Sleeping medication was given to her at home and she was brought in by her family members. On initial survey at presentation, the patient was drowsy but afebrile. She had a blood pressure of 140/90, heart rate 100 bpm, respiratory rate 20 breaths/min, and oxygen saturation at 99%. No headache, head injury, loss of consciousness, seizure activity, fever, chills, rash, chest pain, cough, dyspnoea, constipation, dysuria, or evidence of bleeding were noted. The patient’s history was negative for toxins and showed no personal or family history of the neurologic, hematologic, or systemic disorder. Her only medications were levothyroxine for hypothyroidism and an OCP for dysmenorrhea. On examination, the patient’s alertness and orientation could not be assessed; the rest of her general exam was unremarkable other than for pallor. Of note, she had good skin turgor and moist mucous membranes, her pupils were equally round and reactive, and there was no appreciable thyroid gland enlargement. Cardiac, pulmonary, and abdominal exams were normal. Her extremities were warm and well-perfused with palpable pulses, and non-oedematous. On primary neurological evaluation, her GCS score was 11. Given her otherwise stable condition, a brain MRI was done and demonstrated a left frontal hemorrhagic infarct suggestive of cerebral venous thrombosis with evidence of extension into the anterior aspect of the superior sagittal sinus and a midline shift of 2.5mm. Treatment began with mannitol. Corticosteroids were added for control of intracranial pressure (ICP), and additionally Levipil (Levetiracetam) started as seizure prophylaxis, along with Clexane (Enoxaparin) for anticoagulation; OCP medication was stopped. Upon further neurological exam, her extraocular movements were intact; facial nerve weakness was found, along with reduced power in the right upper extremities compared to all other extremities despite normal tone and normal deep tendon reflexes. Right hand grip was absent, and right plantar movement was decreased. Coordination could not be assessed but the patient localized to pain. ECG monitoring was consistent only with sinus tachycardia. CBC, BMP, and LFTs did not show any abnormalities; VBG results were within normal limits. A coagulation panel was taken, including ANA, APLA, and Proteins C and S, along with tests for homocysteine, vitamin B12, and TSH, and pending results revealed to be normal. The patient showed expressive aphasia in the early course of CVT management, however, all of her neurological signs improved during treatment in the emergency ICU, and she was referred to the neurology department upon stabilization for further work-up till discharge.

Discussion

Altered mental status (AMS) in a young patient may be due to some causes that include neurologic or metabolic disorders though cerebrovascular involvement should not be overlooked. Even without a history of the cardiovascular or hematologic disease, an acute presentation with focal neurological defects precipitated by a severe headache may suggest stroke. Wasay et al. have reported that the age of stroke onset is less in South Asian countries, including India (3). Additionally, it is important to denote the significance of venous stroke. Although stroke overall is much less common in younger populations as compared to older populations, CVT is a critical diagnosis that should be ruled out especially in younger patients. The puerperal period has been commonly cited in association with CVT (1,3,4) and a presentation of a headache and seizure in a postpartum patient may signify CVT and would be the indication for evaluation. Symptoms associated with CVT may often not
Renata Mazurek et al.: Raising the index ……………….. thrombosis

immediately or obviously suggest the diagnosis and require raising an index of suspicion. Headache and vomiting are the most common symptoms (2,4) while neurological deficits or seizures are not necessarily present or may take longer to develop. Dehydration accounts for a major proportion of CVT cases (1,4), and can compound on the relatively hypercoagulable state of pregnancy in the immediate postpartum period or other hypercoagulable states. Despite the patient’s pallor, probably due to her acute lack of food and drink, dehydration was not evident upon assessment at arrival. However, in non-puerperal patients, OCP use in women and alcohol use in men have more recently been identified as important causes for CVT (1,2,4). At another tertiary care centre, Patil et al. found OCP consumption to be the most common risk factor in patients with CVT in the superior sagittal sinus (1), the most frequent location of CVT (2,4) and the main sinus affected in our patient. In the evaluation of our patient, long-term OCP use for dysmenorrhea was considered to be the most likely cause for her CVT. After further investigation, other diagnostic tests performed in the metabolic, autoimmune, and coagulation panels returned normal. CVT has also been reported both in patients with hypo- or hyperthyroidism, and thyrotoxicosis, though a rare cause, can be associated with a hypercoagulation (5,6). With our patient’s history of hypothyroidism, thyroid function tests were performed as a confirmation and were within normal limits as expected for patients controlled with thyroid medication. Less evident causes, including autoimmune disorders, hypercoagulable disorders such as factor V Leiden mutation or Protein C or S deficiencies, and homocystinuria are also possible underlying causes (1,4) and should be accounted for in the diagnosis and management of CVT, even if other identifiable causes are present. Infection has also previously been reported as a major cause of CVT (1) and may be preceded by headache and vomiting. Suspecting CVT in the setting of infection with AMS may be particularly challenging. Here, imaging is needed to clarify the presence of neurologic infection and CVT. With our patient, the absence of fever, elevated cell counts, and lack of sick contacts made an infectious cause less plausible. However, in all cases, a definitive diagnosis can be established best by an MRI-venogram (MRV) showing the thrombosed venous sinus. If large enough or late presentation, a brain MRI (as in this case) may be able to detect the distribution of the venous infarct, as can a head CT in very severe cases. The decision to send a patient directly for MRV is case-dependent on CVT being the primary differential diagnosis. Our patient underwent MRI to rule out the possibility of other causes. However, an MRV would have been done upon a negative MRI to look for CVT. As CVT cannot be diagnosed only on clinical grounds, it is a diagnosis that can be easily missed if not thought of, particularly in hospitals and centres without imaging modalities. Given the prevalence of CVT in India, it is important at all levels of the healthcare system to consider CVT in younger populations when presented with symptoms and possible risk factors, and to send for imaging or to refer to a higher centre for imaging if suspected. The treatment for CVT involves the initiation of fluids to prevent and treat dehydration. Anticoagulation has been under some debate due to concerns with hemorrhagic strokes. However the benefit of anticoagulation on morbidity and mortality observed, even after resolution of the CVT, is determined to be greater than the risk in patients with hemorrhagic stroke (7). Elevated intracranial pressure (ICP) may accompany CVT and can be detected on the fundoscopic exam in cooperative patients. Mannitol is a recommended therapy for patients presenting with acute deterioration as this suggests worsening brain oedema and the use of other ICP-lowering treatments varies by clinical scenario (7). Due to the state of our patient and her deterioration, mannitol was started to relieve the suspected elevation of ICP, and corticosteroids were added. Our patient’s intake of sleeping medication was unlikely to account for the extent of her drowsiness, and a midline shift on MRI, though minimal, provided clinical evidence towards reducing ICP both therapeutically and prophylactically against further worsening. Antiseizure medication is given to patients who have a history of seizure or who present with seizure, but may also be given routinely for CVT (7) or otherwise to patients with neurological signs on presentation, as was our case. The question of continuing anticoagulation has favoured the use of a low-molecular-weight heparin over unfractionated heparin (8) as a bridge to warfarin. The long-term use of anticoagulation should depend upon the underly-
ving cause of CVT, such as in patients with chronic hypercoagulable states (7). Of note in patients on OCPs for medical conditions, such as dysmenorrhea in our patient, it is necessary to evaluate and provide alternative treatment beyond initially stopping OCPs in the hospital. Recurrence is uncommon, but the risk is higher if patients resume OCPs (4). Sagittal sinus thrombosis and multiple sinus involvements are associated with the highest mortality rate (1). CVT mortality and morbidity is related to irresolution of elevated ICP resulting in herniation or brain oedema, complications due to infection or underlying causes, recurrence and residual neurological deficits (1,4). Therefore, early recognition remains imperative in the course of CVT management. Our patient’s neurologic presentation markedly improved upon administration of ICP-lowering agents and she recovered with treatment in our department before continued management in the neurology department. Overall, it is important to be aware of both the modifiable and non-modifiable risk factors in deciding further consultations; however imaging, preferably MRV, should not be delayed in confirming a diagnosis, as this allows for initiating the appropriate treatment. In hospitals lacking imaging modalities, prompt referral to a higher centre upon suspicion of CVT is critical. Anticoagulation, even if available, should not be blindly started without imaging confirmation of venous thrombosis. While the prognosis is affected by causes of CVT and time to treatment, diagnostic confirmation allows the best chance in any case for influencing outcomes towards prevention of mortality, worsening morbidity, and recurrence, as well as addressing underlying factors.

Acknowledgments
We would like to thank the patient, and to thank Dr. Shakuntala Murty in the Department of Emergency Medicine at St. John’s Medical College Hospital for guidance in the evaluation of the case and diagnosis.

REFERENCES
Oral squamous papilloma of hard palate: A case report

Jamin Joseph¹ and S. Karpagavalli¹

¹Department of Oral Medicine and Radiology, Saveetha Dental College and Hospitals, Saveetha University, Chennai, India.

(Received: Jan 2016   Accepted: Mar 2016)

Corresponding Author

Jamin Joseph. Email: jaminpj2@yahoo.com

ABSTRACT

Oral squamous papilloma is a benign epithelial proliferation which results in exophytic papillary growth. Most of the cases are induced by human papilloma virus, and some are not. These lesions appear as pedunculated, white or normal coloured cauliflower like projections that arise from the mucosal surface. Conservative surgical excision is the treatment of choice. Here we present a case of the benign squamous papilla of the hard palate in a 16 years old male. An excisional biopsy was performed and diagnosed as squamous papilloma after a histological investigation.

Key words: Excisional Biopsy, Human Papilloma Virus, Squamous Papilloma

INTRODUCTION

Oral squamous papilloma (OSP) is a benign proliferation of the stratified squamous epithelium, which results in papillary or verrucous exophytic mass induced by human papilloma virus (1). It is the fourth most common oral mucosal mass and found in 4 of every 1,000 and accounts for 3-4% of all biopsied oral tissue lesions (2). These are common lesions of the oral cavity with a predilection for the mucosa of hard and soft palate including the uvula and the vermillion border of lips (3). Its pathogenesis is related to human papilloma virus types 6 and 11. The occurrence of these lesions influenced by smoking, co-existing infections, dietary deficiencies, and hormonal imbalance (4). These lesions are exophytic, papillary mass, usually pedunculated and soft in texture. Lesions of the condyloma acuminatum, verruca vulgaris, focal epithelial hyperplasia and verrucous carcinoma may mimic lesions of squamous papilloma. Surgical excision is the preferred modality of management. Recurrence is uncommon except for lesions in patients infected with human immunodeficiency virus (HIV) (4).

CASE REPORT

A 16 year old male visited the Department of Oral Medicine and Radiology with a chief complaint of painless growth on the hard palate since 6 months. History revealed that the growth was initially small and had gradually increased to attain the present size. The patient was not suffering from any other systemic diseases. The patient had not undergone any dental procedures. The patient was calm and quite. There was no history of pain and paraesthesia associated with the growth. Intra-oral examination revealed the presence of a solitary, well-defined exophytic growth on the hard palate (Fig. 1). The lesion had a cauliflower-like appearance which measures 2 × 2 cm in size (Fig. 2). The overlying mucosa was normal in appearance without any secondary changes. On palpation, the mass was pedunculated,
firm in consistency, non-tender and no discharge. A provisional diagnosis of oral papilloma was made, and the patient advised special investigation of ELISA. The ELISA test was negative. Surgical excision of the lesion was performed without any post-operative complications. The excised lesion was sent for histological examination, and diagnosis of squamous papilloma is confirmed. Post-surgical follow up has done. The one week follows up shows adequate healing. Six months follow up shows no evidence of recurrence.

Histopathological examination shows para keratinised stratified squamous epithelium of variable thickness with few areas of broad rete ridges. There is evidence of few areas of parakeratin plugging. Many long thin fingers like projections extend above the surface of the mucosa. Correlating the history, clinical and histological features the final diagnosis squamous papilloma was made.

**DISCUSSION**

Oral squamous papilloma is a generic term used for papillary and verrucous growth composed of benign epithelium and minor amounts of connective tissue (4). The squamous papilloma is seemed to be associated with papilloma virus, the one commonly incriminated as causative in skin warts (2). The association of HPV and squamous cell lesions at the various site of the body including oral cavity was first described by Syrjanen et al. (5). Squamous papilloma is the fourth most common oral mucosal mass and forms 3-4% of all biopsied oral soft tissue lesions (6). It has been shown that the class of HPV’s is very large and that individually these viruses are associated with many conditions of squamous papilloma (7). Human Papilloma virus is most commonly associated with the lesion with HPV 6 and 11 in squamous papilloma and condyloma acuminatum, while HPV 2 and 57 were more prevalent in verruca vulgaris lesions (6). Squamous papilloma are classified as two types: Isolated solitary and multiple recurring. Isolated solitary is usually found in adult’s oral cavity while recurring occurs commonly in children (3). The present case was an isolated, solitary exophytic lesion.

Squamous papilloma is an exophytic growth made of numerous, small finger like projections which result in a lesion with a roughened, verrucous or cauliflower like surface. It is nearly always a well circumscribed pedunculated tumour, occasionally
sessile. It is painless, usually white but sometimes pink in colour (2). The present case had the same clinical picture which was pink in colour.

Intraorally it is found most commonly on the tongue, lips, buccal mucosa, gingiva and palate, particularly that area adjacent to the uvula (2). The papilloma is usually solitary and enlarges rapidly to a maximum size of about 0.5 cm, with little or no changes thereafter. However lesions as large as 3.0 cm in greatest diameter have been reported (7). In the present case, a lesion was present on the hard palate which measures 2 cm in diameter.

Histologically these lesions present as many long, thin fingers like projections extending above the mucosal surface. Each finger-like projection lined by stratified squamous epithelium and connective tissue centrally. The spinous cell proliferates in a papillary pattern. Koilocytes-HPV altered cells may be observed. The upper epithelial layer shows pyknotic nuclei, often surrounded by edematous or optically clear zone, the so called koilocytic cell (4).

The differential diagnosis of oral squamous papilloma includes verruciform xanthoma, papillary hyperplasia, condyloma acuminatum, proliferative verrucous leukoplatikia (3,6). Often squamous papilloma may be clinically and microscopically indistinguishable from verruca vulgaris which is virus induce focal papillary hyperplasia of the epidermis (6).

Surgical removal is the treatment of choice for oral squamous papilloma, either by surgical or electrocautery excision, cryosurgery, intralesional injections of interferon or lase ablasion. The recurrence rate is low for the solitary type compared with multiple lesions (1,3,4).

REFERENCES