Research article

Variation of ABO phenotypes, Rhesus factor and salivary secretor status in chronic periodontitis patients with and without type II diabetes mellitus: A cross sectional study

Samjotha Dharma[1], Sahana Purushotham[1], Sreeraj Surendran[2]

[1]Department of Periodontology, A J Institute of Dental Sciences, Mangalore, Karnataka, India

[2]A J Research Centre, A J Institute of Medical Sciences and Research Centre, Mangalore, Karnataka, India

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Corresponding author: **Sreeraj Surendran.** Email: sreeraj.s@ajims.edu.in

ABSTRACT

Introduction and Aim: While numerous studies have explored the connection between ABO blood groups and disease incidence in various medical contexts, there has been a scarcity of research dedicated to examining the relationship between ABO blood groups and the occurrence of oral diseases with a specific focus on those with and without Type II diabetes mellitus. Understanding the patient's blood group and salivary secretor status might help to create a new qualitative personalized approach in preclinical diagnosis and to develop preventive measures. Hence, this study delves into the correlation between ABO phenotypes, Rhesus factor, and salivary secretor status in chronic periodontitis patients, with and without Type 2 Diabetes Mellitus

Materials and Methods: The study sample comprised 120 subjects aged between 35 and 60 years. Participants were categorized into three groups: Group A, consisting of patients with only chronic periodontitis; Group B, including patients with only Type II Diabetes Mellitus; and Group C, comprising individuals with both chronic periodontitis and Type II Diabetes Mellitus. Salivary secretor status was determined using the absorption elution method.

Results: Group B showed the highest percentage of salivary secretors (97.5%), followed by group A (95%) and group C showed the least percentage of secretors (92.5%). Group B showed the least percentage of non-secretors (2.5%), Group A showed 5% and group C showed the highest percentage of non-secretors (7.5%). The common blood groups in Study Group A were O>B>A>AB. Similarly in Group B, the blood groups were B>O=A>AB and finally in Study Group C, the common blood groups were B>A>O>AB. Majority of the individuals were Rh Positive.

Conclusion: The current study has identified a correlation between ABO phenotype, Rh phenotype, and secretor status among individuals with periodontitis, both with and without type 2 diabetes.

Keywords: Periodontitis; salivary secretor status; blood group; diabetes mellitus.

INTRODUCTION

Periodontitis, a prevalent chronic inflammatory disease, significantly impacts an individual's quality of life by affecting supporting dental structures. Traditionally considered a consequence of microbial infection, the understanding of periodontitis pathogenesis has evolved (1). Plaque, being the primary etiologic agent, recent insights suggest a shift towards host-based risk factors acknowledging the role of environmental influences, genetics, and systemic conditions like diabetes and smoking (2). The interplay between diabetes and periodontitis is particularly noteworthy. Diabetic individuals face an elevated risk of approximately three times compared to non-diabetic individuals when it comes to developing periodontitis. This suggests a significant correlation between diabetes and an increased susceptibility to periodontal disease. Chronic periodontal inflammation adversely affects glycaemic control, while diabetes increases the susceptibility to periodontitis. This bidirectional relationship underscores the complex nature of these health conditions (3).

Karl Landsteiner was the first person to develop the ABO blood group system in the year 1990. Currently, according to the International Society of Blood Transfusion, approximately 33 blood group systems have been listed. After the ABO blood group system, the second most important is the Rhesus-system (4). Apart from the blood, these anti A and anti B haem-agglutinins were also found and analysed in saliva in 1928. However, the medical application of the secretory status specifically as evidence in the medico legal cases were not utilized initially. This could be due to lack of sufficient techniques available initially (5). Subsequently in later years, identification of blood group antigen from body fluids with 100% specificity was achieved by modifying the known techniques. All individuals can be classified as secretors and non-secretors based on the fact that their blood group antigens may be also found in other bodily fluids such as saliva. The distinction between secretors (Se) and non-secretors (se) became crucial, as an individual's secretory status is independent of their blood type.

Research exploring the connection between blood groups and their association with health issues has

expanded beyond transfusion-related concerns. Many research highlights the importance of genetic factors in individuals with periodontal disease. Exploring potential associations with innate factors adds another layer to our understanding. If a well-established relationship between blood groups and periodontal disease can be confirmed unequivocally, it would lead to the conclusion that the presence of specific blood group antigens has indeed heightened susceptibility to the disease. This finding would underscore the significance of blood group factors in influencing the likelihood of developing periodontal issues and contribute to a more comprehensive understanding of the interplay between genetic factors, blood groups, and the onset of periodontal disease. Notably, investigations into the potential correlation between ABO and Rh blood groups and diabetes mellitus have yielded inconsistent findings. These studies suggest that blood groups may or may not play a role in influencing susceptibility to diabetes (6).

The objective of our study is to assess ABO phenotypes, Rhesus factor, and salivary secretor status in chronic periodontitis patients, both with and without Type 2 Diabetes mellitus. By investigating these blood-related parameters, we seek to uncover potential associations that could contribute to preventive strategies for periodontal diseases. This research strives to shed light on the intricate interconnections between systemic health factors and periodontal conditions, opening new avenues for tailored prevention and treatment approaches.

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MATERIALS AND METHODS

This study was conducted on subjects who visited the Outpatient Department of Periodontology in A.J. Institute of Dental Sciences, Mangalore. Study sample consisted of 120 subjects of both sexes, male and female, with age ranging from 35-60 years. The evaluation of each subject was done by taking a brief medical and dental history and were clinically examined and distributed into three groups namely, Group A - Patients with only Chronic periodontitis (American Academy of Periodontology 1999 classification) ,Group B - Patients with only Type II Diabetes Mellitus Group C - Chronic periodontitis patients (American Academy of Periodontology 1999 classification) with Type II Diabetes Mellitus. This study was approved by the Institutional ethical committee of A. J. Institute of Health Sciences and written informed consent was obtained from the patients before enrolling them in the study. The following patients were excluded from the study: 1. If any patient has undergone any periodontal surgical or non-surgical therapy since the last 6 months, 2. If any patient has received any chemotherapeutic mouth rinse, oral irrigation and antibiotics and anti-inflammatory drugs since the last 6 months, 3. If any patients had a history of underlying systemic diseases and conditions other than type II diabetes mellitus.

Blood group determination

In the Department of Periodontics, blood groups were identified using a visual slide agglutination technique. The process involved collecting blood samples through a sterile disposable lancet using the finger prick method. A glass slide was divided into three sections, and a drop of blood from the subject was placed on each part. Anti-A, anti-B, and anti-D were then separately mixed with the blood samples. The determination of blood group type and Rhesus D (RhD) status was based on the observed agglutination or clumping patterns, which were assessed visually. Meril Antisera kit (commercially available for laboratory usage) was used for the blood group determination in this study.

Collection of saliva

Patients' unstimulated saliva (5ml) was collected in plastic containers after they were asked to thoroughly rinse the mouth with distilled water. These containers with saliva were stored in an ultra-low temperature freezer at a temperature of -80°C and later transferred to the laboratory for biochemical analysis using an ice carrier box.

Detection of ABO salivary secretor status - absorption inhibition method (6)

The biochemical analysis was carried out at the Research Centre, A.J. Institute of Medical Sciences, Mangalore. Protocol by Waseem *et al*., was followed to perform absorption inhibition assay (6). Briefly, saliva samples were transferred to microcentrifuge tubes, then subjected to a boiling water bath at 100°C for 10 minutes and left to cool at room temperature. Following cooling, the tubes were centrifuged at 3000 rpm for 10 minutes. The supernatant was discarded, and the resulting clear saliva was collected.

Four micro-centrifuges were taken among which, two were labelled as TEST (denoted as A and B respectively) and other two as CONTROL (A and B). Control tubes were added to make sure that the antisera were not diluted beyond its ability to agglutinate. A 1:10 dilution of antisera was used for the study. One drop (approx. 25µl) of this diluted antisera was added to all tubes respectively (antisera A to TEST A and CONTROL A tube; antisera B to TEST B and CONTROL B tube). Subsequently, 25µl of clear saliva was added to both "TEST" microtubes whereas a drop of saline was added to both "CONTROL" tubes. The tubes were mixed thoroughly followed by incubation for 10 minutes at room temperature. After the incubation period, a single drop (approximately 25µl) of recently prepared indicator erythrocytes, derived from a combination of A and B pooled cells, were added into each tube labelled as A and B. The tubes were thoroughly mixed and allowed to incubate for 10 minutes at room temperature. Agglutination reaction was recorded after incubation. Negative result samples were re-tested and evaluated

following the same procedure. If negative again, the sample was considered as true negative for secretory status. Clumping/agglutination was considered as a negative result and absence of agglutination as a positive result. All control samples showed agglutination which is due to the absence of antigen. A positive result indicates that an antigen-antibody reaction had taken place between saliva and antisera thus preventing the agglutination.

RESULTS

This study was carried out to assess ABO phenotypes, Rhesus factor, and salivary secretor status in chronic periodontitis patients, both with and without Type 2 Diabetes mellitus. G*Power tool version 3.0.1(Franz Faul universitat, Kiel, Germany) was used to compute statistical power analyses. All statistical analysis was performed using SPSS version 20. (IBM SPASS statistics [IBM corp. released 2011]. Frequency and proportions were used to analyse descriptive statistics for both the explanatory and outcome variables. Qualitative variables were analysed using a Chi-square test, and a 5% level of significance level was applied.

Table 1: Distribution of the subjects based on salivary secretor status

Salivary secretor status		Groups			Total
		CP	Type 2 DM	CP + Type 2 DM	
Non-secretor	Count	2	1	3	6
	%	5.0%	2.5%	7.5%	5.0%
Secretor	Count	38	39	37	114
	%	95.0%	97.5%	92.5%	95.0%
Total	Count	40	40	40	120
	%	100.0%	100.0%	100.0%	100.0%
Chi-square value- 1.05					
p value-0.59					

Table 2: Distribution of the subjects based on ABO phenotype and salivary secretor status

Salivary secretor status	ABO PHENOTYPE		Groups			Total	Chi-square value	p value
			CP	Type 2 DM	CP + Type 2 DM			
Non-secretor	A	Count	1	1	1	3	1.33	0.51
		%	50.00%	100.00%	33.30%	50.00%		
	B	Count	1	0	2	3		
		%	50.00%	0.00%	66.70%	50.00%		
	Total	Count	2	1	3	6		
		%	100.00%	100.00%	100.00%	100.00%		
Secretor	A	Count	10	10	11	31	2.35	0.88
		%	26.30%	25.60%	29.70%	27.20%		
	AB	Count	3	3	5	11		
		%	7.90%	7.70%	13.50%	9.60%		
	B	Count	11	15	11	37		
		%	28.90%	38.50%	29.70%	32.50%		
	O	Count	14	11	10	35		
		%	36.80%	28.20%	27.00%	30.70%		
	Total	Count	38	39	37	114		
		%	100.00%	100.00%	100.00%	100.00%		

Table 1 results indicate that the majority of the individuals have blood group antigens in their saliva and hence are secretors. The chi-square value obtained was 1.05. Table 2 represents the distribution of the subjects based on ABO phenotype and salivary secretor status. The chi-square value obtained was 1.33.

Among 120 subjects, 6 subjects were non-secretors and 114 were secretors. Out of the non-secretor subjects with chronic periodontitis one belonged to blood group A and other, blood group B. In the Type 2 Diabetes mellitus group, there was only one non-secretor individual with blood group A. Among those with both chronic periodontitis and diabetes mellitus, two subjects had blood group B, and one had blood group A. Blood group AB and O were not found among the non-secretors. The second section of table 2 represents the distribution of individuals who are secretors. Here the chi-square value obtained was 2.35.The results obtained were as follows. In the chronic periodontitis group, among those who were secretors, there were 10 subjects with blood group A, 3 subjects with blood group AB, 11 subjects with blood group B, and 14 subjects with blood group O. Additionally, among the subjects with Type 2 diabetes mellitus, there were 10 subjects with blood group A, 3 subjects with blood group AB, 15 subjects with blood

group B, and 11 subjects with blood group O. Finally among the subjects having chronic periodontitis and type 2 diabetes, 11 of the subjects had blood group A, 5 of them had blood group AB, 11 of them had blood group B and 10 of the subjects had blood group O.

From the above results the common blood groups among each of the study groups was also determined. The common blood groups in study Group A in the descending order were O>B>A>AB. Similarly in Group B, the blood group was B>O=A>AB and finally in study Group C, the common blood groups were B>A>O>AB. Majority of the individuals were Rh positive and also secretors.

DISCUSSION

Periodontal diseases are linked to multiple risk factors. Although various microorganisms have been associated as potential periodontal pathogens, it has become apparent that for the disease to occur, pathogens are necessary, but not exclusively sufficient. (7). Shifting from a traditional pathogenic view, the multifactorial nature of periodontitis is gaining recognition. Genetic factors have the potential to influence oral ecology and the progression of periodontal diseases. Variations in the genetic makeup can impact the development of immune cells and the way antigens are presented, potentially contributing to an individual's susceptibility to infectious diseases, including periodontal diseases. The genetic differences in immune cell function and antigen presentation highlight the intricate relationship between genetics and the predisposition to oral health issues, particularly those involving infectious factors (8). This study explores the correlation between ABO phenotypes, Rhesus factor, and salivary secretor status in chronic periodontitis patients, with and without type 2 diabetes mellitus, providing valuable insights into potential associations.

The antigens belonging to the ABO system are essential components of the red cell membrane and are additionally present in plasma and various body fluids. These antigens, their presence or absence, have been linked to diverse diseases and anomalies (9). Furthermore, these antigens can function as receptors for infectious agents. Through immunohistochemical studies, it has been observed that spinous cells in the non-keratinized oral epithelium of individuals with blood groups A and B exhibit the A/B antigens. In these cases, basal cells express precursor structures, while the more differentiated spinous cells express either the A or B antigens. On the other hand, individuals with blood group O, lacking the A and B gene-coded glycosyltransferase, express a fucosylated variant (Ley) of the precursor structure. This insight sheds light on the intricate relationship between ABO antigens and oral epithelial cells, offering a deeper understanding of their expression patterns (10).

Yamakami, in the year 1926 first detected the presence of A & B antigens in saliva (11). Subsequently, Lehrs and Putkonin, in the year 1930, noted the existence of secretors and non-secretors as distinct groups (12), with the ability to secrete blood group antigens inherited as a Mendelian dominant character. Furthermore, it is not considered that the genes responsible for secretion are connected to the ABO genes (Race & Sanger 1970) (13). Several studies have indicated that a substantial percentage of individuals fall into the secretor category, indicating the presence of blood group antigens in additional body fluids such as saliva (14).

ABO blood groups and its correlation and susceptibility to various diseases have been studied extensively (15). In a study that linked secretor status to periodontal disease by Kauser *et al.*, found that the secretor subjects were more prone for periodontal disease. However, the underlying mechanisms for the observed association are unknown (16). Modulation of endothelial or inflammation markers by ABO locus is one among the suggested mechanisms. Supporting the suggestions, it has been found that the level of maker such as the factor VIII–von Willebrand factor (vWF) complex was found to be higher in non-O blood group individuals (17). In addition, the association of biological markers such as plasma soluble intercellular adhesion molecule 1 (ICAM-1) and TNF receptor 2 (TNF-R2) levels with increased type 2 diabetes risk also provides a potential explanation for the observed relationship (18).

The purpose of this study was to correlate the variation of ABO phenotypes, Rhesus factor and salivary secretor status in chronic periodontitis patients with and without type 2 diabetes mellitus. One among the initial research by Pradhan *et al.*, who investigated the correlation between periodontal disease, blood groups, and secretor status identified a statistically significant association between periodontal disease and blood groups. However, no significant correlation was observed with secretor status. This finding suggests a potential genetic basis for the etiopathogenesis of periodontal disease (19).

Over the years, numerous studies have reported a positive correlation between blood groups and periodontal disease. A study conducted by Vivek *et al*., revealed that individuals with blood group O (65.8%) and Rh-positive status (73.33%) exhibited a higher susceptibility to periodontitis. Consistent with these findings, the current study also observed a higher prevalence of blood group O among individuals with chronic periodontitis (2). In a comparable study conducted by Arati *et al*., it was revealed that a greater proportion of individuals with gingivitis belonged to blood group A, while those with periodontitis predominantly exhibited blood group O. Blood group AB showed the least association with periodontal diseases. Additionally, all groups exhibited a

significantly higher prevalence of Rh-positive individuals, aligning with the findings obtained in the current study (20). In contrast, a study conducted by Demir *et al*., found no significant difference in the distribution of the Rh factor. The same study revealed significant differences in the rates of colonization of various periodontal pathogens among different ABO blood groups (21).

In the present study the association of blood groups and presence of diabetes mellitus was also assessed. Sandhya *et al*, studied the ABO blood frequency among the diabetics and observed their order to be, blood group B > O > A > AB, in both types (I & II) of Diabetic patients. The results obtained in the present study were similar and the most common blood group observed among the diabetic individuals was blood group B (22). Agarwal *et al.*, found a statistically significant association between blood group AB and type 2 diabetes mellitus individuals and increased frequency of Rh positive blood group in diabetes patients (96.15%) whose results were similar to the study by Ghani *et al.*, (13, 23). In contrast to these studies, no association of blood group and Rh factor with diabetes mellitus was observed by Shailaza *et al.*, who aimed to determine the association of diabetes mellitus with ABO and Rhesus (Rh) blood groups (24).

The results obtained from the current studies are as follows. The common blood groups among individuals with chronic periodontitis in descending order were O>B>A>AB. Similarly among the individuals with diabetes mellitus, the blood group was B>O=A>AB and finally among the individuals having chronic periodontitis and diabetes mellitus, the common blood groups were B>A>O>AB. The percentage of distribution of the subjects based on ABO phenotype and salivary secretor status are enumerated in Table 2. Majority of the individuals were Rh Positive. This parameter could not be statistically assessed as there were only two individuals who belonged to Rh-negative blood type under the three study Groups A, B and C.

Salivary secretor status, assessed in this study, indicated that 95% of individuals were secretors. Although not statistically significant, the predominance of secretors in the chronic periodontitis and diabetes group suggests a potential association. The study's limitations include a small sample size for Rh factor analysis and a lack of exploration into specific genetic markers. Despite these constraints, the findings contribute to the evolving understanding of chronic periodontitis and its interplay with systemic conditions, particularly diabetes, about which not much has been explored. The study emphasizes the importance of personalized preventive and treatment strategies considering an individual's blood group and secretor status

CONCLUSION

In conclusion, this research explores the intricate relationship between ABO phenotypes, Rhesus factor, and salivary secretor status in the context of chronic periodontitis and type 2 diabetes mellitus. Further investigations are required to further understand the genetic and molecular aspects of these associations, providing a foundation for targeted therapeutic interventions and preventive measures tailored to an individual's unique biological profile.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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