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ST Tabasum
RP Nayak

Salivary blood group antigens and microbial flora

Authors' affiliations:
ST Tabasum, *RP Nayak*, Jaipur Dental College, Kukas Industrial Area, Dhand, Jaipur, Rajastan, India

Correspondence to:
Dr Syeda Tawkhira Tabasum
Kukas Industrial Area Dhand
Jaipur-Delhi N.H.8
Jaipur
Rajasthan 303101
India
Tel.: 01426 234175
Fax: 01412 704229
E-mail: drsyedin@yahoo.co.in

Abstract: *Objective:* Secretor is an individual with ability to produce blood group reactive substances in the exocrine glands and to secrete these substances in the body fluids such as saliva. In saliva the blood reactive antigens are found primarily on mucins which exhibit microheterogenicity and different subtypes of these molecules. This study was done to find out if the salivary blood group antigens have any role in the adherence of certain selected microorganism in the oral cavity. *Methods:* Unstimulated whole saliva and sub gingival plaque samples were collected from subjects with clinically healthy gingival, chronic gingivitis and chronic periodontitis with probing pocket depth ≥4 mm and attachment loss ≥1 mm. Secretor status was determined by using Haemagglutination Inhibition Assay. Unstimulated whole saliva and sub gingival plaque samples were collected to culture and isolate the selected microorganisms. The clinical scores, secretor status and the presence or absence of selected microorganisms were compared within the groups using Chi-square test and students unpaired *t*-test. *Results:* The numbers of secretors were more in the healthy group (22.2%) and non secretors were more in the chronic periodontitis group (22.2%). The clinical scores were higher in the in non secretors compared to the secretors in all the three groups. P intermedia and P gingivalis were prevalent among non secretors in chronic gingivitis group. ($P = 0.075$ and $P = 0.032$) and chronic periodontitis group ($P = 0.068$ and $P = 0.009$).

Key words: blood group antigens; gingivitis; microorganisms; periodontitis; saliva; secretor

Introduction

The oral cavity contains numerous microorganisms. Studies of bacteria in the mouth were the first to provide knowledge that microorganisms attach to tissue in a remarkably selective manner; this is often the first discernable event in the process of colonizing a host. Blood group antigens are oligosaccharides found on the surface of erythrocytes and other cells. An individual with the ability to produce blood group reactive substances in the exocrine glands and who secrete these substances in the body fluids such as saliva and gastric juice is referred to as a secretor. Secretors with the blood group A, B or AB secrete A, B or A and B antigens respectively, but subjects with blood group O secrete H antigen because of the biochemical conversion of the blood group core structure.

Weber and Pastern (1) were the first to study the association of various ABO blood groups with periodontal disease followed by Polivitsky. Later Kaslick *et al.* (2) showed that chronic gingivitis group was significantly different in ABO grouping than the control group (3, 4).

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The salivary blood group antigens play a dual role:

1 They cause bacterial aggregation and clearance from the oral cavity or

2 They cause selective adherence of the bacteria to oral epithelium and mucins in the dental pellicle, which is mediated through blood group glycolipids; thus resulting in their colonization in the oral cavity and formation of dental plaque (5).

Very little is known about a possible correlation between secretor status and the prevalence of specific periodontal pathogens. Thus, this study was carried out to find out if salivary blood group antigens have any role in the adherence and colonization of certain selected microorganisms in the oral cavity.

Aims and objectives

1 To detect the salivary blood group antigens.

2 To analyse the microbial flora in subjects with chronic gingivitis/chronic periodontitis.

3 To formulate a relationship between salivary blood group antigens and the microbial flora of chronic gingivitis/chronic periodontitis.

Materials and methods

Source

The subjects for the study were selected from outpatients visiting the Department of Periodontics, A.B. Shetty Memorial Institute of Dental Sciences, Mangalore.

Sample size

A randomized sample comprising 90 patients between 20 and 55 years of age belonging to both genders was selected. The patients were divided into two groups of 30 patients each with chronic gingivitis/chronic periodontitis. The control group consisted of 30 patients with clinically healthy gingival.

Selection criteria

1 Subjects should be free of systemic disease.

2 Subjects should not be on antibiotics/corticosteroids prior to the commencement of the trial.

3 Subjects should have a minimum of 20 permanent teeth and should not have undergone any periodontal treatment for at least 6 months prior to the study.

4 Pregnant and lactating subjects were excluded.

Criteria for group division

Ninety subjects as per the inclusion criteria were selected and explained the nature and design of the clinical trial. The consent to participation was obtained from each patient. The subjects were divided into three groups of 30 each.

Group I: It consists of subjects with clinically healthy gingival with gingival index score 0–0.4 (Loe And Silness).

Group II: This group had subjects with chronic gingivitis with gingival index score 1–2 (Loe And Silness).

Group III: This group consisted of subjects with chronic periodontitis with probing pocket depth ≥4 mm and attachment loss ≥1 mm.

Procedure

Prior to the clinical examination, 1 ml of unstimulated saliva was collected. The patients were instructed to pool the saliva in the mouth and were asked to collect it in two 5 ml sterile bottles, which were then sealed tightly and sent to the laboratory. Clinical evaluation was performed using plaque (Silness and Loe) and gingivitis (Loe and Silness) indices on the Ramford teeth, and probing pocket depth was determined on all the teeth and attachment loss was measured at sites with probing depth ≥4 mm.

Method of collection of subgingival microbial samples

After removing the supra gingival calculus and plaque with Gracey curette, the paper points (Size 40; DENTSPLY India Pvt. Ltd, Mumbai, India), were inserted into the depth of the gingival sulcus/pocket and left for 10 seconds. They were then transferred to 2 ml of preheated thioglycolate and Robertson's cooked meat media.

Table 1. **Comparison between blood group and secretor status: blood group versus secretor status**

Blood group versus secretor status	X^2	*P*-value	Significance
Group I	3.681	0.298	NS
Group II	2.269	0.518	NS
Group III	3.393	0.335	NS

NS, not significant.

Table 2. **Comparison of microorganisms and secretor status (secretor versus non-secretor): group I-clinically healthy gingiva**

	Subgingival plaque			Saliva		
Species	*t*	*P*	Significance	*t*	*P*	Significance
Streptococcu mutans	2.126	0.045	S	2.250	0.032	S
Streptococcus sanguis	1.285	0.210	NS	1.216	0.237	NS
Fusobacterium nucleatum	0.976	0.374	NS	0.136	0.473	NS
Peptostreptococcus micros	1.742	0.125	NS	1.742	0.125	NS

S, significant; NS, not significant.

A total of 1 ml of blood was drawn from the antecubital vein from all the subjects using 5 ml disposable syringes under sterile conditions and transferred into 5 ml EDTA bottles. Blood was used to determine the blood group and to prepare 3% human erythrocyte suspension in phosphate buffered saline.

Microbiological procedure

The paper points, which were used to collect the subgingival plaque samples and unstimulated whole saliva, were transported to the microbiological laboratory to culture and isolate selected microorganisms.

Specific bacterial examination included culture for the following microorganisms.

Gram positive microorganisms:

Actinomyces viscosus, *Streptococcus mutans*, *Streptococcus sanguis* and *Peptostreptococcus micros*.

Gram negative microorganisms:

Actinobacillus actinomycetem comitans, *Campylobacter rectus*, *Fusobacterium nucleatum*, *Porphyromonas gingivalis* and *Prevotella intermedia*.

1 Trypticase Soy Agar supplemented with bacitracin, vancomycin, horse serum and yeast extract was used for the isolation of *Actinobacillus actinomycetem comitans*.

2 Columbia Blood Agar, +0.05% hemin +0.01% menadione, was used for the isolation of *Fusobacterium nucleatum*, *Porphyromonas gingivalis*, *Prevotella intermedia* and *Peptostreptococcus micros*.

3 Skirrow's Medium was used for the isolation of *Campylobacter rectus*.

4 Mitis Salivarius Agar was used for the isolation *of Actinomyces viscosus*, *Streptococcus mutans* and *Streptococcus sanguis*.

Bacteria were speculated on the basis of colony morphology, aero tolerance and biochemical tests. Isolates were tested for saccharolysis, indole and catalase production, esculin and starch hydrolysis, haemagglutinating activity and carbohydrate end products.

Secretor status

The blood group typing was performed before detecting the secretor status. The secretor status was determined using haemagglutination inhibition assay.

Haemagglutination negative – Secretor.

Haemagglutination positive – Non-secretor.

Secretor	Blood group
A antigen	A
B antigen	B
A&B antigen	AB
H antigen	O

H antigen because of biochemical conversion of blood group core structure.

Statistical analysis

Statistical analysis was carried out in P.C. statistical package SPSS 7.0 version (SPSS Inc., Chicago, IL, USA).

1 Chi-square (χ^2) test: This was used to compare the blood groups with the secretors and non-secretors in the three groups.

2 Students unpaired *t*-test: This test was used to compare the various clinical scores and the presence of microorganisms and the secretor and the non-secretors in the three groups.

Discussion

Secretor is an individual with the ability to produce blood group reactive substances (antigens) in exocrine glands and to secrete these substances in the body fluids such as saliva. The synthesis of blood groups' antigens depends on the action of glycosyl transferase. The gene coding for these enzymes is common to a large group of population, but varies for A, B, secretor and Lewis gene within the population (7, 8). According to Gibbons and Spinell (9) and Hay *et al.* (10), molecules present in the dental plaque bind to the bacteria forming an important component that enables bacteria to accumulate in large numbers on the teeth. These important components are either antibodies or high molecular weight mucins, which possess blood group reactive moieties (antigens) helping in bacterial aggregation.

Of the 90 subjects, 44 (49.0%) were secretors and 46 (51.0%) were non-secretors, irrespective of the group to which they belonged (Table 1). The majority of the secretors belonged to the healthy group – 20 (22.2%), whereas periodontitis group had the least number of secretors – 10 (11.1%). The majority of the non-secretors belonged to the periodontitis group – 20 (22.2%), whereas the healthy group had the least number of non-secretors – 10 (11.1%). Gingivitis patients had approximately equal proportion of secretors – 14 (15.6%) and non-secretors – 16 (17.7%). Thus, it can be inferred from this

Table 3. **Giblett comparison between clinical scores and secretor status: (secretor versus non-secretor): Plaque Index versus secretor status**

Plaque scores versus secretor status	*t*	*P*	Significance
Group I	2.315	0.048	S
Group II	2.315	0.048	S
Group III	2.235	0.031	S

S, significant.

Table 4. **Comparison between clinical scores and secretor status: (secretor versus non-secretor): Gingival Index versus secretor status**

Gingival scores versus secretor status	*t*	*P*	Significance
Group I	2.219	0.048	S
Group II	2.218	0.048	S
Group III	2.241	0.031	S

S, significant.

study that secretors were more in the healthy group and non-secretors in the periodontitis group. This may be because of the lack of glucosyl transferase in the salivary glands of non-secretors resulting in the absence of H, A or B antigen in the saliva (11). In Group I, only *Streptococcus mutans* showed a significant relationship of its presence amongst the secretors and the non-secretors, probably suggesting its role as the initial colonizer in plaque formation (Table 2).

In all the three groups, the plaque and gingival scores had a higher value in non-secretors when compared with the secretors. The difference in the mean plaque and gingival scores in the secretors and non-secretors was statistically significant (Tables 3–5). This indicates that the non-secretors were more predisposed to periodontal disease. In the group with chronic periodontitis, the probing pocket depth and attachment loss were higher in the non-secretors than the secretors (Table 5). This may be supported by the fact that attachment loss is mainly attributed to the increased prevalence of anaerobic bacteria, such as *Porphyromonas gingivalis*, which is an indicator of attachment loss as proposed by Christersson *et al.* (12).

Increased prevalence of *Prevotella intermedia* in the subgingival plaque at sites of periodontal destruction was proposed by Lie M *et al.* (12). The difference in the presence of *Prevotella intermedia* in the subgingival plaque and saliva of secretors and non-secretors was statistically significant in group II and III (Tables 6 and 7), suggesting a possible role of salivary blood group antigens on colonization of *Prevotella intermedia*. This may be supported by the study carried out by Falker *et al.* in 1981 (6) who showed that *Actinomyces naeslundi*, *Porphyromonas gingivalis* and *Prevotella intermedia* are able to bind to blood group reactive substances. This is in variance with the study carried out by Lie M.A *et al.* (13) who found *Prevotella intermedia* at interproximal sites with attachment loss irrespective of the secretor status.

Table 5. **Comparison between clinical scores and secretor status: (secretor versus non-secretor): probing pocket depth/attachment loss versus secretor status**

Probing pocket depth versus secretor status	*t*	*P*	Significance
Group III	2.840	0.010	HS
Attachment loss versus secretor status			
Group III	3.116	0.009	HS

HS, highly significant.

Table 6. **Comparison of microorganisms and secretor status (secretor versus non-secretor): chronic gingivitis**

	Subgingival plaque			Saliva		
Species	*t*	*P*	Significance	*t*	*P*	Significance
Streptococcus mutans	1.018	0.317	NS	1.026	0.314	NS
Streptococcus sanguis	1.216	0.237	NS	1.537	0.136	NS
Actinomyces viscosus	1.643	0.216	NS	0.617	0.547	NS
Campylobacter rectus	–	–	–	0.683	0.516	NS
Peptostreptococcus micros	0.326	0.726	NS	0.184	0.857	NS
Prevotella intermedia	2.229	0.024	S	2.316	0.075	S
Porphyromonas gingivalis	2.800	0.447	S	2.250	0.32	S

NS, not significant; S, significant.

Table 7. **Comparison of microorganisms and secretor status (secretor versus non-secretor): Group III- chronic periodontitis**

	Subgingival plaque			Saliva		
Species	*t*	*P*	Significance	*t*	*P*	Significance
Streptococcus mutans	0.617	0.547	NS	0.526	0.605	NS
Streptococcus sanguis	0.532	0.600	NS	1.417	0.815	NS
Actinomyces viscosus	0.010	1.000	NS	1.296	0.208	NS
Campylobacter rectus	1.593	0.183	NS	1.528	0.141	NS
Fusobacterium nucleatum	1.528	0.149	NS	1.458	0.177	NS
Peptostreptococcus micros	1.537	0.136	NS	1.688	0.121	NS
Prevotella intermedia	2.651	0.025	S	1.491	0.068	S
Porphyromonas gingivalis	3.528	0.008	HS	3.528	0.009	HS

$P < 0.05$ – significant (S).
$P < 0.01$ – highly significant (HS).
$P < 0.001$ – very highly significant.
$P > 0.05$ – significant (S).
NS, not significant.

The difference in the presence of *Porphyromonas gingivalis* in the subgingival plaque and saliva was significant in group II, but highly significant in group III. This may be because of a possible role of salivary blood group antigens on colonization of *Porphyromonas gingivalis*, which is in agreement with the study carried out by Slots and Gibbons (14) who showed that the attachment of *Porphyromonas gingivalis* to the epithelial cells can be inhibited by blood group reactive mucins.

There was a statistically significant relationship between the presence of *Prevotella intermedia* in the subgingival plaque and the saliva with the secretor status (Table 7). Based on the results of this study, it may be proposed that salivary blood group antigens in the saliva promote the clearance of the microorganisms from the oral cavity.

Periodontal diseases are multifactorial. Bacteria alone should not be considered as a causative agent, although bacteria may play a prime role. Other factors such as genetic factors may play an important role in the aetiology and pathogenesis of periodontal disease.

Conclusion

Thus, it can be suggested that the salivary blood group antigens H, A and B may be contributing factors in the aetiology and progression of periodontal disease. They may reduce if not inhibit the aggregation of the pathogenic bacteria thereby decreasing the severity of periodontal disease process. Further studies with emphasis on the other periodontal pathogens and their relationship between the secretor statuses need to be carried out. Moreover, longitudinal studies may help to get more confirmatory results to show the inter-relationship between the secretor status and periodontal disease.

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