

Correlation of Dental Caries with ABO Blood Group and Salivary Secretor Status among Female Subjects, Makkah City, Saudi Arabia

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ABSTRACT

Background: Salivary secretor status and ABO blood group are important in clinical and forensic medicine. In addition, both may have impact on some oral disease status.

Objective: To assess the correlation between ABO blood group, salivary secretor status and dental caries and to evaluate which type of ABO blood groups is associated with an increased risk for dental caries.

Materials & Methods: Cross-sectional study was carried out on 30 dental caries female patients aged between 20 to 50 years, attending to College of Dentistry, Umm Al-Qura University. Caries experience was assessed by using WHO index and DMFT score. Then, ABO blood grouping was done by slide agglutination method and 1 ml of unstimulated saliva is collected in a sterile test tube. Hemagglutination inhibition test was performed to analyze the salivary secretor status. The collected resulted data were tabulated and statistically analyzed using (SPSS) version 20.0.

Results: DMFT index scores were different among the four ABO blood groups; however, the differences were statistically insignificant ($p > 0.05$). On the other hand, salivary secretor status was different among the four blood groups with highest percentage of secretors were of blood group A (85.7%) and those of non-secretors were of blood group AB (60%). The mean values of DMFT index were higher in non-secretor than secretor status; however, the differences were statistically insignificant ($p > 0.05$).

Conclusion: With the study limitations, it could be concluded that, although higher percentage of ABO blood group non-secretors have dental caries than secretors but the difference was non-significant.

Keywords: Dental caries; ABO blood group; DMFT; secretor status; Makkah.

INTRODUCTION

Dental caries that commonly known as tooth decay; is the most common human chronic disease. When, its treatment ignored, the disease will progress to destruction of the tooth. ^[1] Tooth decay process, has been known for 100 years to be caused by bacteria fermenting foods, producing acids and mineral dissolving tooth. In recent decades, the process of dental caries have been explained from many aspects including microbiology, tooth

composition of minerals, saliva, tooth structure, diffusion processes, demineralization and remineralization process, and factors that are involved in the reversal process. ^[2]

Generally, in developed countries the prevalence of dental caries is decreasing, while in underdeveloped and developing countries, the prevalence is increasing. ^[3] In Saudi Arabia, tooth decay is one of the most important reasons of teeth extraction. ^[4] It was observed that dental caries in the past

few decades, showed an increase in prevalence, which could be due to the change in lifestyle of Saudis, that include increasing the sugary food consumption, and soft drinks, and lack of awareness about proper maintenance of oral and dental health. [3] Tooth decay is a multi-factorial disease that involves many complex risk and protective factors, [1] the three main factors in dental caries are host factor, diet and microorganism. [5]

Over 100 years ago, the ABO blood group discovered. At the beginning of the 20th century first blood group system discovered by Austrian scientist, Karl Landsteiner, when he noted that the red blood cells of some individuals agglutinated with serum from other individuals and he submitted a memorandum of agglutination patterns and derive that the blood can be divided into groups. [6] Blood type has importance in transfusion medicine, paternity suits, forensic science and study of different populations by anthropologists. Difference in ABO blood types vary among different populations, which showed that a certain type of blood group granted a selection advantage such as resistance against an infectious disease. [6]

ABO gene is hereditary and every person carries two copies of the gene coding for the ABO blood group. The A and B blood groups dominant than O blood group type. [7] Any person over the age of 6 months have anti-A and / or anti B antibodies in their blood serum as blood group A have antibody against blood group B in serum and blood group B contain antibody against blood group A in serum, while blood group O have no A/B antigen but carries both antibodies in serum. [8] Rhesus-system (RH) is also an important blood group system that become after ABO; [9] Rhesus (Rh) system determined by the nature of different proteins present on the surface of erythrocytes. [10]

A large percentage of people are secretors, which, indicates that the antigens are present in their blood, it also can be found in other body fluids like saliva. An

individual can be a secretor or a non-secretor and it is completely independent from blood type whether the individual has blood type A, B, AB, or O, such as that individual can be group A secretor or group A non-secretor, group B secretor or group B non-secretor. In simple manner, it is called a secretor's person if secretes their antigens into their body fluids such as the saliva, mucus, while on other hand called a non-secretor's person if does not secrete completely or very little antigens into their body fluids. [11]

Since 1930, investigations started about the relation between blood groups, Rhesus (Rh) factor and dental diseases. [12] Although many studies have been conducted to investigate the relationship between ABO blood group and the incidence of disease in medicine, studies regarding their relationship with the incidence of oral diseases are limited and some researchers could not find a relationship, which might be attributed to the geographical diversity in the population. [13] Moreover, few studies have been conducted to investigate the relationship between dental caries and ABO blood group and salivary secretor status as some studies found the dental caries prevalence was lower in secretors group than in non-secretors group. [14,15] In contrast, Chung et.al. in 1965 found there is no relationship between salivary secretor status and caries score, [16] and Mazumdar et.al. in 2014 did not record any significant differences in correlation between blood group and dental caries. [17]

The objectives of the study are:

To assess the correlation of ABO blood grouping and dental caries as well as with salivary secretor status with further detection of the relationship between salivary secretor status and dental caries.

The significance of the study: The information about the influence of ABO blood grouping and salivary secretor status on dental caries status is not readily

available in the Saudi population; that information is necessary to develop early intervention programs for prevention of tooth decay and controlling their consequences.

MATERIALS AND METHODS

Study design:

Cross-sectional study was conducted to determine the correlation between blood group (ABO), salivary secretor status and dental caries.

Ethical approval

Ethical and consent study approvals were obtained from the Institutional Review Board-IRB (Research Ethics Committee) at Faculty of Dentistry, Umm Al-Qura University. Participation of each participant was voluntary and informed consent was obtained before commencement of the study.

Subjects:

This study was conducted in Makkah city, which located in west of Saudi Arabia, with estimated population of 2 million in 2010.^[18] The study was performed at College of Dentistry, Umm Al-Qura University and was carried on randomly selected 30 female students, employees and patients having dental caries and aged 20-50 years.

Inclusion criteria: Female subjects with dental caries aged 20-50 years, agreed to share in the study after obtaining informed consent were included in the study.

Exclusion criteria: Male subjects, female subjects under 20 years and above 50 years, those with developmental disorders of teeth, systemic diseases and mental disabilities were excluded from the study.

Clinical examination (determination of dental caries status):

- Clinical examination was performed by one examiner, the main author (Al-Hanoof A. Al-Sihli), the examiner was trained and calibrated with the co-author (Sahar M Elmarsafy).
- The examination of the participated subjects was done inside the outpatient

dental clinics of Umm Al-Qura University, under visible light using sterile disposable mouth mirrors and sterilized community periodontal index (CPI) probe to visually examine the caries on the occlusal, incisal, buccal and lingual surfaces.

- Caries experience of each subject was measured by using DMFT index for permanent teeth; which is the method of choice for World Health Organization (WHO) in their basic survey technique.^[19]

Collection of blood and saliva samples:

Under complete aseptic precautions, 2 ml of blood was collected from the antecubital vein of each subject using sterile disposable syringe and was placed in a dry sterile test tube containing EDTA. After rinsing mouth with distilled water and discarding few drops, 2 ml of non-stimulated saliva was collected in a dry sterile container from each subject.

Laboratory Procedures:

All the laboratory works have been done inside the research laboratory of Faculty of Dentistry, Umm Al-Qura University.

I. Determination of blood group: ABO blood group was determined for all subjects by slide agglutination method test using Anti-A, anti-B antisera.

II. Saliva preparation: saliva was prepared by transferring into sterile test tubes that sealed with nonabsorbent gauze and placed in a boiling water bath for 10 min, centrifuged at 1,700 rpm for 10 min and the supernatant was then pipetted, placed in Eppendorf tube and preserved in refrigerator until identification time for secretors and non-secretors status.

III. Preparation of washed 3-5% Red Cell Suspension: Two drops of whole blood were placed into a test tube that then 3/4 filled with saline to re-suspend the cells, centrifuged at 2500 rpm for 5 min to obtain clear supernatant and a definite red cell

button. Supernatant of each tube was then decanted and the red cells were gently re-suspended with saline to a 3-5% red cell suspension.

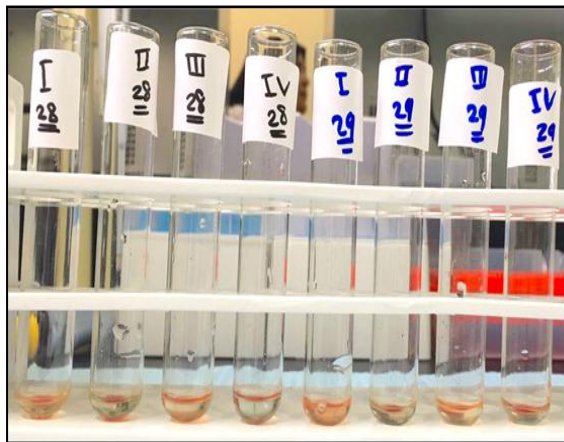


Figure 1: Agglutination seen in some test tubes after one-hour incubation.

IV. Secretor status detection:

Performed by Hemagglutination inhibition test according to Rai et.al. 2015. [21]

1. The A and B antiserum were diluted in a separate test tubes by a saline solution at a ratio of 1:10.
2. Four test tubes were prepared for each patient where diluted antiserum were poured into test tubes labeled from I to IV and added to the prepared her saliva:
 - Test tube I: 1 drop of saliva + 1 drop of anti-B serum.
 - Test tube II: 1 drop of saliva + 1 drop of anti-A serum.
 - Test tube III: 1 drop of saline solution + 1 drop of anti-B serum.
 - Test tube IV: 1 drop of saline solution + 1 drop of anti-A serum.
3. The tubes were left at room temperature (20°C) for 10 minute.
4. One drop of 2-3% A-erythrocyte solution in saline solution was added to test tubes II and IV.
5. One drop of 2-3% B-erythrocyte solution in saline solution was added to test tubes III and I.
6. All the test tubes were shaken and kept at room temperature. The

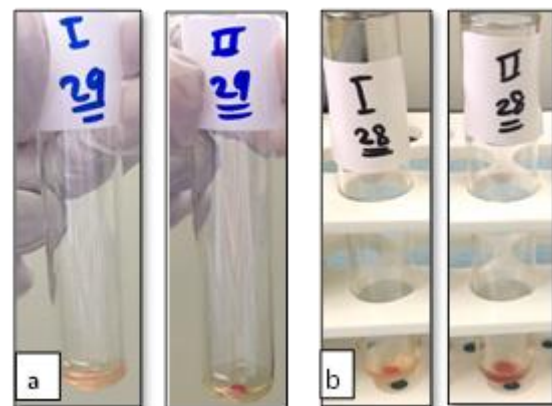
results were recorded after one hour (Figure 1).

V. Interpretation of Secretors and Non-secretors:

The erythrocyte agglutination was anticipated in test-tubes III and IV that considered as controls. While, agglutination in tube I indicated that the person is secretor A due to presence of substance A2 in saliva and agglutination in tube II indicated that the person is secretor B A due to presence of substance B2 in saliva (Figure 2a). On the other hand, absence of agglutination in both tubes I and II indicated that the person is AB secretor having both the antigens in the saliva that lead to interference with agglutination in tubes I and II as antiserum is added before erythrocyte. Agglutination in both tubes I and II at same time indicated that the person is non-secretor because there is no antigen in the saliva, that lead to agglutination (Figure 2b).

Figure 2a: Agglutination in tube II indicating secretor B status.

Figure 2b: Agglutination in both tubes I and II indicating non-



secretor status.

Statistical analysis:

The collected data were tabulated and statistically analyzed using Statistical Package for Social Sciences (SPSS) version 20.0. Descriptive statistical analysis was performed to calculate the frequencies, percentage and means with corresponding standard deviations. One Way Analysis of

Variance (ANOVA test) was used to analyze the effect of blood groups on the DMFT score, Chi-square test was used to analyze the effect of blood groups on the salivary secretor status while t-test was used to analyze the effect of the secretor status on the DMFT score. *P* value of ≤ 0.05 was taken to be statistically significant.

RESULTS

Total number of participants was 30 females; their ABO blood groups, secretor

status, were illustrated in *Figures 3a&3b*. According to ABO blood groups; the majority of participants ($n=10$) having B phenotype (33.3%), while 26.7% ($n=8$) have O phenotype, and 23.3% out of them have A phenotype ($n=7$). AB phenotypes constitute the minority of patients ($n=5$) (16.7%). In respect to the salivary secretor status the results indicate that (63.3%) of the participants are secretor while (36.7%) are non-secretor.

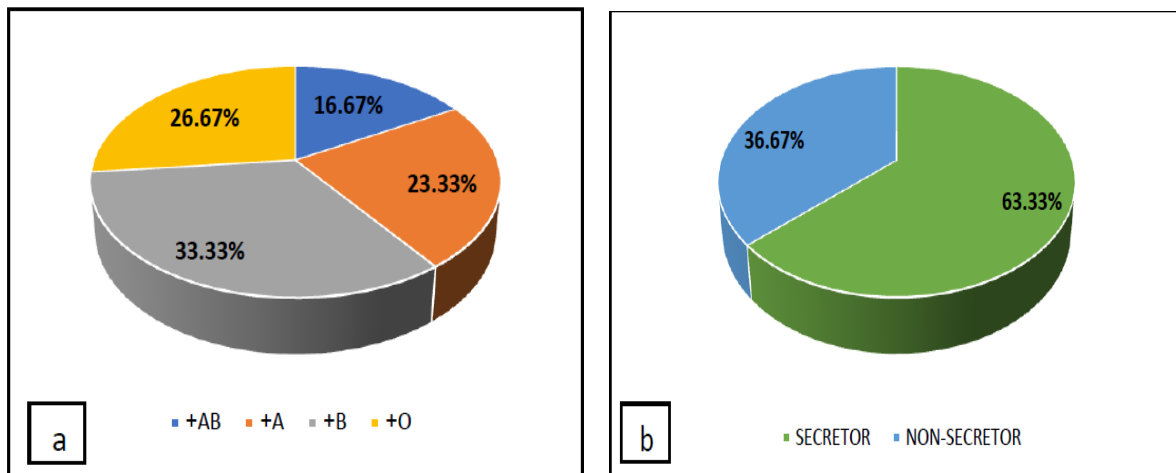


Figure 3a: Pie chart for distribution of individuals according to blood groups.

Figure 3b: Pie chart for distribution of individuals according to salivary secretor status.

Correlation between the blood groups and the salivary secretor status:

As shown in *Table 1* and *Figure 4*, the percentages of secretors in blood groups A, O and B types were 85.7%, 62.5% and 60% respectively, while in blood group AB it is reported as 40%; therefore, blood group A revealed the highest percentage, while blood group AB showed the lowest percentage. In respect to the non-secretor status, the results indicated that the

percentages of non-secretor in blood groups AB, B and O types were 60%, 40% and 37.5% respectively, while in blood group A it reported 14.3% therefore, blood group AB revealed the highest percentage, while blood group A showed the lowest percentage. The results showed that the salivary secretor status were relatively different among the different ABO blood groups but the differences were statistically insignificant where the (*p*-value is > 0.05).

Table 1: The level of salivary secretor status in different ABO blood groups.

		Blood group				Total	Chi - square	P-value
		AB	A	B	O			
Secretor Status	Secretor	40%	85.7%	60%	62.5%	19	2.29	0.520
	Non-secretor	60%	14.3%	40%	37.5%			

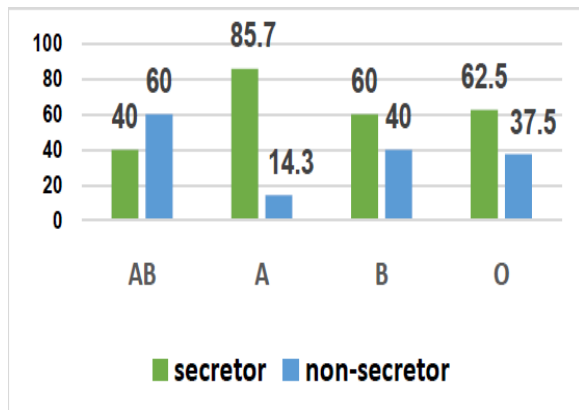


Figure 4: Histogram for the level of salivary secretor status in different ABO blood groups.

Correlation between the blood groups, salivary secretor status and DMFT index score:

As shown in Table 2 and Figure 5a, the mean values of DMFT in blood groups O, A

and AB types were 13.5, 12.9 and 12.2 respectively while in blood group B it was reported as 9.8. The DMFT index scores were relatively different among the different ABO blood groups, in which blood group O revealed the highest value and blood group B showed the lowest value but the differences were statistically insignificant where the (p- value is > 0.05).

As shown in Table 3 and Figure 5 b, the mean value of DMFT in salivary secretor state was 11.7 while in non-secretor it reported 12.2. The DMFT index scores were relatively differ among the different secretor status but the differences were statistically in significant where the (p- value is > 0.05).

Table 2: DMFT index values in different ABO blood groups (Mean ± SD, Median, Range and p- value).

Blood group	Mean ± SD	Median	Range (Minimum– Maximum)	P- value
AB (n=5)	12.2± 2.7	14	4-19	0.534
A (n=7)	12.9± 1.8	14	5-20	
B (n=10)	9.8± 1.6	9.5	3-19	
O (n=8)	13.5± 2.4	16	0-21	

Table 3: DMFT index values in different salivary secretor status (Mean ± SD, Median, Range and p- value).

		Blood group				Total	Chi – square	P- value
		AB	A	B	O			
Secretor Status	Secretor	40%	85.7%	60%	62.5%	19	2.29	0.520
	Non-secretor	60%	14.3%	40%	37.5%	11		

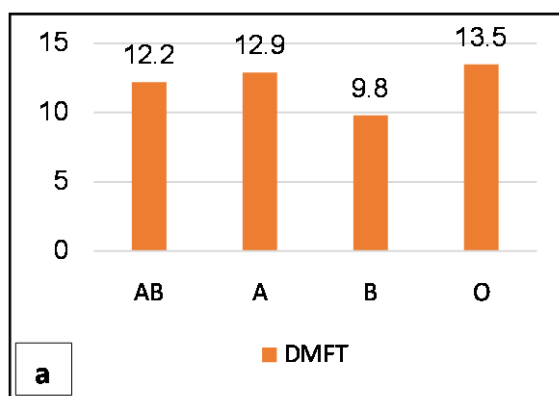


Figure 5a: Histogram for DMFT index mean values in different ABO blood groups.

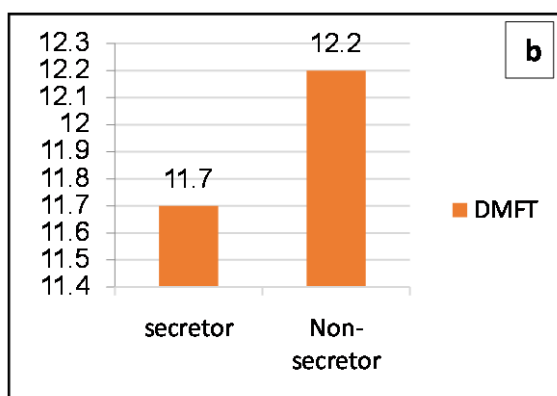


Figure 5b: Histogram for DMFT index mean values in different salivary secretor status.

DISCUSSION

The ABO system is the most exploration erythrocyte antigen system; several studies have been conducted to investigate the relationship between blood groups and different systemic diseases such as various cancers, diabetes mellitus, skin

disease, heart disease, genetic disease and dental caries. [20]

Distributions of ABO blood group and Rh system showed obvious diversity around the world; different regions within the same country showed some differences. Studies revealed that O blood type is most

common in American and Canadian population and B type is most common in Chinese and Indian population while A type is more frequent in Eskimos. [13] The presented study found that, the majority of participants are having B phenotype (33.3%), then O phenotype (26.7%), then A phenotype (23.3%) while AB phenotypes constitute the minority that is (16.7%).

With the development in researches throughout the years, it has been understood that, certain unknown factors did play a role in the progress of dental caries regardless from the common etiological agents and environmental factors. There are a lot of evidence suggests that blood groups have a significant role in capability or resistance to different infectious and non-infectious diseases; [21] however, there are no consistent results. [20] Moreover, studies showing the association between ABO blood grouping and dental caries are limited. [17]

In our study the mean scores for DMFT index in O, A and AB types were 13.5, 12.9, 12.2 respectively while in B types it was reported as 9.8. Which indicates that DMFT index in O blood group revealed the highest value, while B blood group showed the lowest value; however, these differences were statistically insignificant where the (p- value is > 0.05). This was in agreement with Mazumdar et.al. in 2014 whom revealed that there is no correlation between blood group and dental caries. [17] On the other hand, Salih et.al. in 2015 have found that, DMFT values were significantly ($P < 0.05$) different among the different ABO blood groups, in which B blood group revealed the highest DMFT value, while AB blood group showed the lowest DMFT. [21]

Many factors could contribute to the incidence of dental caries such as dietary factor, oral health behavior, oral hygiene practices, lifestyle habits and socioeconomic status. [22] One of the suggestions that may lead to increased risk of disease is the patient's secretor status. [20] Genetically the salivary secretor status of the individual determined by a pair of

allomorphic genes: Se and se; Se is dominant over se. When homozygous Se-Se genes or heterozygous Se-se genes occurs, the individuals called secretors while homozygous se-se genes called non-secretors. Se allele is dominant and it regulates the H transferase in certain glands and secretions. In non-secretors individual, the red cells and plasma fully express the specifications determined by their H and ABO genes, but the saliva and other tissue fluids contain no H transferase and, therefore, no A, B and H substance while in all secretors the H antigen should have been exist in their secretions. [20]

In the present study, the frequency of ABH secretor status in the studied population (63.3%) was higher than non-secretor status (36.7%). Similar to our study, Salih et. al., in 2015 revealed that, the secretor status was higher than non-secretor status, [21] and Rai et. al., in 2015 concluded that about 80% of the populations are secretors. [20] Several previous studies showed that, O blood group and ABH secretors were the most prevalent in different geographical and ethnic populations. [20,23] In this study, levels of salivary secretor in A, O and B types were 85.7%, 62.5%, 60% respectively while in AB types it was reported as 40%, in which A blood group revealed the highest percentage and AB blood group showed the lowest percentage. Which indicated that the secretors' status was relatively different among the different ABO blood groups, but the differences were statistically significant where the (p- value is > 0.05).

Available evidence suggests that there are some diseases related to secretor status and some with non-secretors. [24] When blood group capable to secreting antigens in their fluid; it has a significant role in the natural resistance of the microorganisms to infections. [20] Previous studies found that the non-secretors are more susceptible to certain diseases such as, autoimmune diseases, [25] vaginal candidiasis, [26] oral changes like periodontal disease [27] and oral submucous fibrosis. [28]

The results of this study showed that the mean value of DMFT index was more in the non-secretors (12.2) than secretors (11.7) but the differences were statistically insignificant where the (p- value is > 0.05). Similar to our study, Chung et. al., in 1965 did not find any strong correlation between secretor status and development of dental caries. [16] On the other hand, Kárpáti et.al., in 2014 found that, the mean DMFT values were significantly lower in the secretor group (2.1 ± 0.52), as compared to the non-secretor group (3.8 ± 0.93 ; $p < 0.05$, Mann-Whitney U test). [15] Moreover in contrast to our results, Salih et.al., in 2015 showed that the frequency of DMFT values was significantly ($P < 0.05$) higher in the secretors as compared to non-secretors. [21]

The strong correlation between blood group, secretor status and dental caries could be explained by the suggestion of Mazumdar et.al., in 2014, [17] that ABO blood group substances secreted in saliva leads to assemblage of microorganisms and their removal from the oral cavity. So, this indicate that, the secretion of ABO(H) antigens into saliva may inhibits the capability of bacteria to adhere to the tooth surface. [17]

CONCLUSIONS

- With the study limitations, it could be concluded that, although higher percentage of blood group non-secretors have dental caries than secretors but the difference was non-significant. No correlations were found either between blood group and salivary secretor status or between ABO blood grouping, salivary secretor status and dental caries.

The study limitations

- The sample size is small according to the total number of population in Makkah city due to difficulty in obtaining and accessing to an appropriate larger number.
- This study was only restricted to female patients due to difficulty of access to male patients.

So, it is recommended to conduct another future study on larger scale randomly selected population, with both genders, different cultures and ethnically diverse inhabitants, to obtain definitive results and to investigate whether secretor status could be used to check an individual's susceptibility to dental caries.

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