INTERNATIONAL JOURNAL OF SCIENCE AND NATURE

www.scienceandnature.org

INDIVIDUAL VARIATIONS IN SECRETORY ABO GROUP ANTIGEN

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ABSTRACT
About 80% of the people have the secretor gene (Se). These people secrete water-soluble antigen in their saliva and other body fluids. The aim of this study was to show if there is any individual variations in secretory ABO group antigen that present in saliva. First determine if a person is a secretor, or non -secretor, Agglutination inhibition is the principle which is used in this study, then determined the degree of agglutination microscopically to show individual variations in secretory ABO group antigen the occurrence of agglutination indicates a negative test and absence the agglutination is translated as a positive result. The difference in degree of agglutination reflects that there are individual variations in secretory ABO group antigen. This study revealed that there are great variations among individuals in the amount of secretory ABO group antigen that present in saliva.

KEY WORDS: secretory antigen, ABO group, agglutination inhibition, saliva.

INTRODUCTION

A secretor is well-defined as a person who secretes their blood type antigens into body fluids and other secretions of the body like the saliva in the mouth, the mucus in the digestive tract and respiratory openings, semen , and urine where traces of the water soluble A, B, or O agglutinogens that determine blood group are found [1]. ABH refers to the “A” and “B” antigens of the ABO blood group system and “H”, the heterogenetic constituent which is discovered in persons of all ABO types including type “O” [2]. The H antigens are indirect gene products stated as fucose-containing glycan units, exist in on glycolipids or glycoproteins of red blood cell membranes or on mucin glycoproteins in their secretions and are the fucosylated glycans substrates for glycosyl transferases that give rise to the epitopes for the A, B blood group antigens [3]. The utmost essential blood-typing system, the ABO blood group, is the determinant for transfusion reactions and organ transplantation. Unlike the other blood-typing systems, the ABO blood type system has significance beyond transfusion and transplantation, as, for example, it controls numerous of the digestive and immunological features of the body[4]. The ABO blood type system comprises of four types of blood group: O, A, B and AB. Blood group O erythrocytes have no actual antigen, but blood serum of O-type individuals carries antibodies to both A and B antigens. Type A and B erythrocytes carry the A and B antigens, correspondingly, and make antibodies to the others. Type AB erythrocytes do not manufacture antibodies to other blood types because they have both A and B antigens [4,5] Anthropologists have used the ABO blood types as a director to the development of advanced humans. Several diseases, particularly digestive disorders, infection, and cancer, show preferences among the ABO blood types[6]. These preferences are not generally assumed or acceptable by physicians or the general population[7]. Landsteiner first described that presence of serologic variances between individuals, permitting him to categorize populations to one of four groups rest on whether their red blood cells included antigen "A," antigen "B," neither A nor B (O) or both A and B (AB). This finding guided to a sequences of genetic, serologic, and immunochemical investigations which are remaining even at the present time [8]. The localization of the tissue the histo- blood group antigens has exposed that the antigens in the tissues match to the RBC blood group, but the expression of the tissue is dependent on the secret category of the individual. Secretor status is secretion of blood group antigens ABO (H), which may be a factor inducing the advancement of systemic oral diseases[9]. Saliva was first analyzed for anti-A and anti-B haemagglutinins in 1928. However it was not used in excessive investigation because of insufficient techniques available at the time. In past few years, many adapted methods have been created and many investigations have shown up to 100% accuracy in detecting blood group from saliva[10]. It was statistically proven that the secretion concentration into saliva is equal in men and women. The individuals with different blood groups were found to have different values. The secretion intensity of all three antigens A, B and H followed a bimodal pattern; therefore, determination of high and low secretion was possible [11]. The aim of this study was to determination if there are any Individual variations in secretory blood group antigen.

MATERIALS AND METHODS

Collection of Saliva

Saliva was collected from thirty apparently healthy person without any systemic disease (male and female) their ages range between (35–55 years) from teaching hospital of dentistry, University of Baghdad. Collection of saliva was performed 2-3 hours after the volunteer usual breakfast time and after thoroughly rinsing the mouth with water. Saliva was collected by standard spitting method using chewing gum then saliva collected in a plane tube,

centrifuged 10 minute at 1500 *e.g.*, and the supernatant liquid was used for study analysis. The saliva was initially assessed for the secretor status by detecting the H antigen in saliva using the anti H antibody. All the secretors and non-secretors were isolated on the basis of the presence or absence of the H antigen in saliva. The blood group specific antigens are present only in the secretors. They are absent in the non-secretors.

Blood Typing Kit# 11: Blood Typing Using Saliva Student Manual

1- Put saliva into a small beaker.
2- Stand the tube upright in a test tube rack in a boiling water path for ten minutes. To denature both salivary as well as the bacterial enzymes.
3- Centrifuge the test tube for several minutes to sediment any coarse precipitate. Use only supernatant fluid for this study.
4- Place six test tubes in arrow of a test tube rack. Label the tube as follows: C, 2, 4, 8, 16, 32. C refers to control tube, to which no saliva will be added.7
5- Using a clean pasture pipette, (or micropipette) to place one drop of saliva into the tube labeled 2. Place one drop of saline into each of six tubes. Titer (dilute) the saliva in tube 2 by mixing it with the saline in the tube and then drawing the contents in the pipette.
6- Expel one drop into the next test tube (test tube 4) and then return the remaining liquid in the pipette to test tube 2.
7- Draw the contents of test tube 4 into the pipette and expel one drop into the test tube 8. Return the remaining liquid to test tube 4. Continue this procedure from one tube to the next.
8- Finally, remove one drop from the tube 32 and discard it. Return the remaining saline-saliva mixture liquid to test tube 32.
9- Add one drop of anti-A serum to each test tube. Shake each tube and let them stand them stand undisturbed for ten to fifteen minutes. Then add one drop of the suspension of group a red blood cells in concentration 5% to each tube, let the tubes stand to for five minutes and then centrifuge them at high speed (about 3500 rpm) for 20 seconds. Inspect he tubes for the presence or absence of red blood cell clumping.
10- Check the degree of aggregation microscopically. To do this, pour the contents of each tube in sequence into the compartmentalized plastic tray. Next to each compartment, mark with a wax pencils the identification symbol or dilution corresponding to each tube. Under low power search the entire area of fluid from each tube.

Note whether all the red blood cells are discrete and evenly distributed in the surrounding fluid. If so, there is no agglutination (mark the tube -0-). If clumps are present, they may range from a few, composed of relatively few cells (mark +); to more, composed of moderate aggregates (++); to many, composed of moderate or large aggregates (+++); to many, composed of very large aggregates (++++) with very few cells remaining UN clumped.

(Repeat the same procedure for anti-B serum and group B red cell and for the anti-H serum and group O red cell)

RESULTS & DISCUSSION

Out of the 30 saliva samples there were 25 secretors and 5 non-secretors. All the 25 secretors were subjected to absorption inhibition method. (Figure 1) score (-): The reaction will be negative for presence of agglutination, but is construed as positive for secretor status. If the person has a secretor antigen, the secretory antigens in the saliva will react with and counteract the antibody in the antiserum that added to it. When suspension of RBC of the appropriate RBC blood group are subsequently added to the mixture, here should be no antibody in free form to clump them if the person is a secretor, because the antibodies have previously reacted with the blood group antigens that present in the saliva. Therefore the reaction for the agglutination will be negative, but is positive for secretor type. That's mean the greater number in agglutinations isolates; the less concentration in soluble secretory antigen in saliva and vise versa.

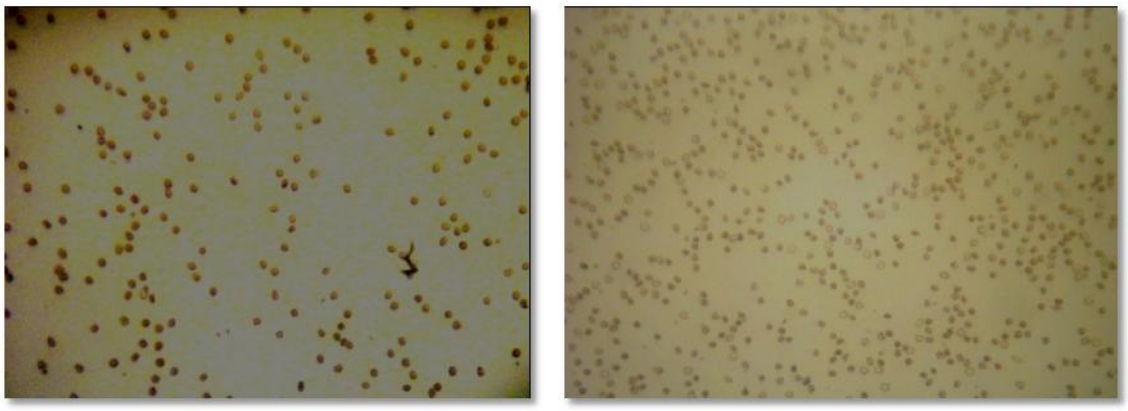

FIGURE 1: Score (-) no agglutination in RBC

Figure 2 show very small agglutination in RBC suspension that is mean score (+).

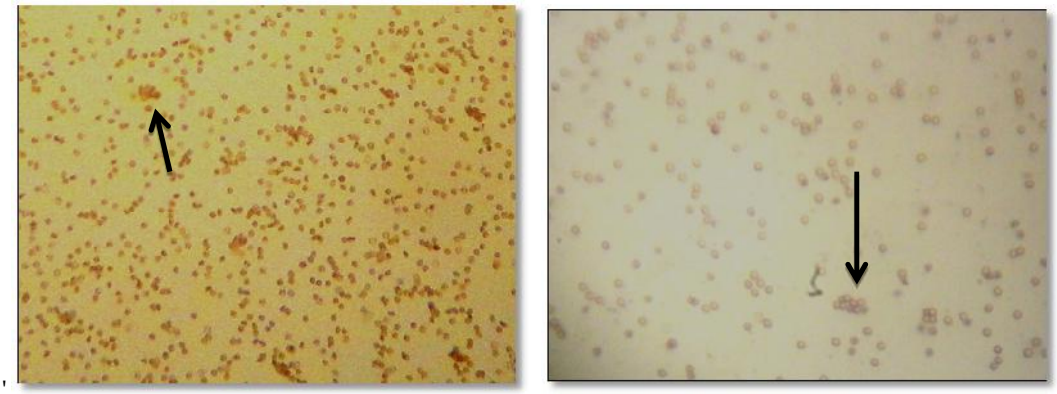

FIGURE 2: Score (+) very small agglutination in RBC

Figure 3 Score (++) moderate agglutination in RBC suspension

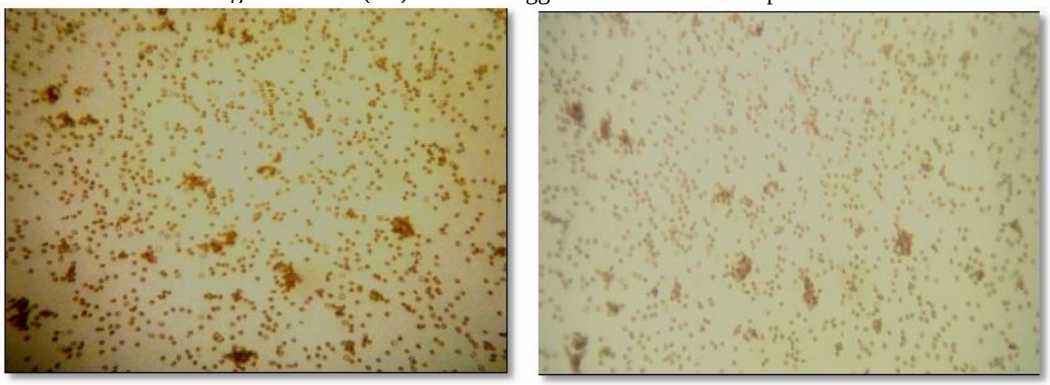

FIGURE 3: Score (++) moderate agglutination in RBC

Figure 4 Score (+++) moderate to large agglutination with small free RBC

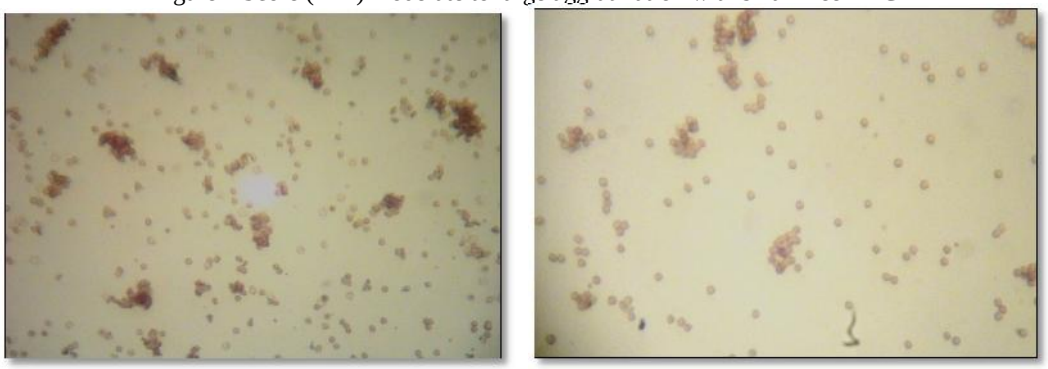

FIGURE 4: Score (+++) moderate to large agglutination in RBC

Figure 5: Score (++++) very large agglutination in RBC with very small free RBC

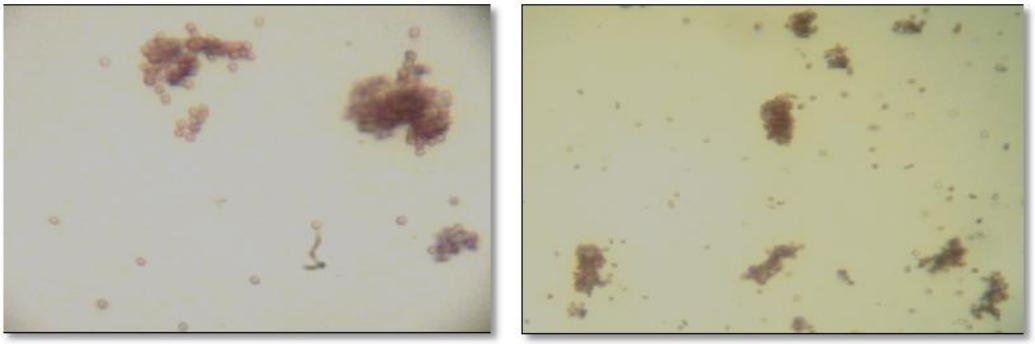

FIGURE 5: Score (++++) very large agglutination in RBC

Table 1 show individual variations in secretory group O antigen for 11 secretory saliva samples in all saliva dilutions. The most concentrated antigen found in 0 where the concentration of saliva not diluted by saline. While the variation began in concentration 2, some samples have a high concentration in secretory antigen as in sample no. 2 and sample no. 10, while sample no. 1 has a lesser concentration the other samples of O antigen in between them. But this individual variations decrease as the dilution for saliva increase until it reach the last dilution 32 the less variations exhibit in this dilution.

TABLE 1: Individual variations in secretory group O antigen

Sample no.	ABO Group	0	2	4	8	16	32
1	O	-	++++	++++	++++	++++	++++
2	O	-	-	-	+	+	++
3	O	-	+++	++++	++++	++++	++++
4	O	-	++	+++	+++	+++	+++
5	O	-	++	+++	+++	++++	++++
6	O	-	++	+++	++++	++++	++++
7	O	-	+	++	+++	++++	++++
8	O	-	++	++	+++	++++	++++
9	O	-	++	+++	++++	++++	++++
10	O	-	-	+	++	+++	++++
11	O	-	+++	+++	++++	++++	++++

Table 2 show individual variations in secretory group an antigen for 10 secretory saliva samples. Approximately the same results of individual variations in secretory A antigen in saliva. But the concentrations of antigen is more in secretory A group as compared with secretory O group.

TABLE 2: Individual variations in secretory group A antigen

Sample no.	ABO Group	0	2	4	8	16	32
12	A	–	++	++++	++++	++++	++++
13	A	–	+	++	+++	++++	++++
14	A	–	+	+++	++++	++++	++++
15	A	–	++++	++++	++++	++++	++++
16	A	–	++	++	+++	++++	++++
17	A	–	_	+	+	+++	++++
18	A	–	_	+	++	+++	++++
19	A	–	+	++	+++	++++	++++
20	A	–	_	+	++	+++	+++
21	A	–	_	_	+	++	+++

Table 3: Individual variations in secretory group B antigen for 3 secretory saliva samples. While in table 4 only one secretory saliva sample that is not comparative to other saliva sample to show the variations. In secretory **B** and AB the individual variations not appear obviously because the present number was not enough to make comparison.

TABLE 3: Individual variations in secretory group B antigen

Sample no.	ABO Group	0	2	4	8	16	32
22	B	–	+++	+++	+++	++++	++++
23	B	–	+++	++++	++++	++++	++++
24	B	–	++	++	++	+++	++++

TABLE 4: Individual variations in secretory group AB antigen

Sample no.	ABO Group		0	2	4	8	16	32
25		A	-	+	++	+++	++++	++++
	AB	B	-	+	++	+++	++++	++++

Blood group factors in body fluids are of great importance in medical felid and have direct relationship to oral diseases especially the soluble secretory antigen that present in saliva. This study revealed that the secretory individuals have 83% and 13% non- secretory. This result agrees with Pawan et al [12]. Who also show the same ratio for the secretory ABO group. While this results not completely agree with Olorunshola and Audu [13] who show 97.3% from control subject were secretor and this may belong to racial variations among people. The variations between individuals in amount of secretory antigen that present in saliva have not been studied before therefore there are no studies for comparison with them.

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