Evaluation of Secretor Status of ABH Antigens in the Saliva of Sokoto Residents in Northern Nigeria: A complementary Evidence of ABO Blood Groups in Forensic Science.

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Abstract

The presence of ABH blood group substances in the saliva of secretors can serve as additional evidence for ABO blood groups especially in the cases of forensic science. The secretor status of these substances have been scarcely examined especially in Northern Nigeria. This study therefore assessed the secretor status of ABH antigens in the saliva of healthy Sokoto residents in Sokoto.A total of 175 Sokoto residents (114 males and 61 females), aged 18-60 years, were recruited between June and October, 2019 at Usmanu Danfodiyo University Teaching Hospital, Sokoto. Standard techniques were employed in the determination of ABO blood groups and secretor status of participants.Out of 175 healthy individuals studied, 144 (82.3%) and 31 (17.7%) were ABH secretors and non-secretors, respectively. Blood group O had the highest frequency in the studied population (39.4%) followed by blood groups B(29.1%), A(24.6%) and AB(6.9%). However, the differences in the frequencies of secretors and non-secretors with respect to A, B, AB and O blood groups were not statistically significant (P>0.05). Age and gender had no significant effects on the secretor status of ABH antigens in the saliva (p>0.05%). In conclusion, 82.3% of the studied population are ABH secretors and their secretion was not significantly influenced by age and gender.

Keywords: Evaluation, ABH antigens, saliva, Sokoto, Northern Nigeria

Introduction

The ABO system consists of antigens found on the outer surfaces of red blood cells and the corresponding antibodies in the serum. A significant proportion of individuals are secretors due to thesecretion of blood type antigens into the body fluids such as saliva, sweat, tears and semen while non-secretors do not secrete their blood antigens in their body fluids.[1-3]

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The expression of ABO blood group antigens are determined by the interaction of three genes: the ABO gene, which controls the expression of the A and B antigens; fucosyltransferase 1 (FUT1) or H gene, which controls the expression of the H antigen and precursor of ABO antigen; and secretor (Se) gene, fucosyltransferase 2 (FUT2) or Se. All of these genes encode glycosyltransferases, which are enzymes that add sugars to precursor substances to create new substances.[4]

The Se gene, which encodes the enzyme α-2-L-fucosyltransferase, controls the formation of the antigen in bodily secretions. In individuals with secretor genotype, the enzyme converts precursor substance found in body fluids to the H antigen, which is then modified by the glycosyltransferases encoded by the ABO gene to produce the antigens corresponding to the person's ABO blood type. However, the non-secretors cannot form H antigen in the body fluids because they cannot express soluble ABO antigens.[4]

Secretors secrete ABH substances according to blood groups. Blood group Oindividual secretes H substance, A blood group secretes A and H substances while B blood group secretes B and H substances in the fluids.[5,6]

The secretor gene is inherited in the autosomal dominant pattern. Se is the dominant form while se is the recessive form and therefore, SeSe or Sese are known as the secretors while sese individuals are referred to as non-secretors.[7]

Expression of the antigens in the Lewis blood group is also affected by secretor status of ABO antigens, as non-secretor status of ABO antigens cannot produce the Le(b) antigen.[8]

In forensic science field, ABO system has been a major focus since A,B and H antigens on erythrocytes are associated with other cells and tissues throughout the body.[9,10] Saliva found on various objects at the scene of crime can be useful in forensic science.[1]

Examination of secretions and tissues may be useful for corresponding findings from blood samples and these may be particularly valuable in the absence of blood. The use of saliva in forensic science is based on the presence of ABH blood group substances found in the saliva of secretors.[1,11]

The overall prevalence of secretors of ABH antigens varies from one locality or country to another as 86.9% of ABH secretors was observed in Calabar,[12]

78.1% was found among Osogbo residents,[13] 79.4% detected in Kadapa city of India,[14] 75.5% was reported in Korea[15] and 64.4% was documented in Karachi city of Pakistan.[16] However, low prevalence rates of 31.8% and 49.5% of ABH secretors were observed amongst the Sudanese and Manipuri population, respectively.[17,18] The importance of the presence of ABH antigens in the saliva as a complementary evidence of ABO blood group in forensic science cannot be overemphasized. Therefore, the scanty knowledge of the secretor status of ABO antigens in the saliva of Sokoto residents in Northern Nigerians necessitated this study on the assessment of secretor status of ABH antigens in the saliva of healthy Sokoto residents.

Materials and Methods

A total of 175 healthy Sokoto residents comprising 114 males and 61 females with age range of 18 to 60 years were recruited for this study between June and October, 2019 in Usmanu Danfodiyo University Teaching Hospital (UDUTH), Sokoto after the collection of ethical clearance for research from the Ministry of Health, Sokoto State with reference number SMH/1560/V.IV and the informed consent obtained from all the participants.

Collection of samples

Ten milliliters of saliva was collected into sterilized disposable plastic containers from all the participants and out of the 5ml of venous blood collected from each subject, 3ml was dispensed into a plain container for serum grouping while 2ml of blood was put in EDTA container for ABO cell grouping.

Preparation of 3% red cells suspension and serum for grouping

3% red cells suspension was prepared from the 2ml of blood collected into EDTA container by centrifuging the sample at 3000 revolutions per minute (rpm) for 3minutes to remove the plasma. After the removal of the plasma, the tube was filled with normal saline and mixed properly before its centrifugation at 3000 rpm for 3 minutes. This washing of cells was done three times. The drop of packed washed cells was diluted with normal saline to give approximately 3% cells suspension (1 in 33 dilution).[16]
However, serum was separated into another plain container after the centrifugation of the clotted blood collected at 3000 rpm for 5 minutes.

Cell grouping

Three clean glass test-tubes were labelled as tube A for anti-A serum, tube B for anti-B serum and tube AB for anti-AB serum and one drop of 3% participant's cell suspension was added to tube A, tube B and tube AB containing the anti-sera.

All the contents were mixed and centrifuged at 3400 rpm for 15 seconds. Each was examined individually for agglutination.

Serum grouping

Three clean glass test-tubes were labelled as tube "a" for A cells, tube "b" for B cells and tube "o" for O cells. Two drops of participant's serum were added to each of the 3 tubes and one drop of known 3% red cells suspension of A, B and O was added to tubes "a", "b" and "o", respectively. All the tubes were mixed and centrifuged at 3400 rpm for 15 seconds. Each tube was examined individually for agglutination.

Preparation of Saliva for testing

Ten milliliters of saliva collected from all subjects into sterilized plastic containers were transferred into sterilized disposable glass tubes and centrifuged at 2000 rpm for 10 minutes. The supernatants from the tubes were transferred into another glass tubes and placed in a water-bath at 56^0C for 30 minutes to inactive salivary amylase. Then, the sample tubes were cooled and centrifuged at 2000rpm for 10 minutes. The supernatants were transferred into another disposable test-tubes and used for the haemagglutination inhibition test.[16]

Principle of secretor status testing by haemagglutination inhibition:

If ABH antigens are present in a soluble form in a fluid such as saliva, they will neutralize their corresponding antibodies and these antibodies will no longer be able to agglutinate red cells possessing the same antigens.

Preparation of reagents

The anti-A, anti-B and anti-H antisera manufactured by Biotech Laboratories, UK were diluted with 0.85% normal saline to give a titre of 1 in 16 before use.

Procedure

One-hundred microliters (100µl) of diluted group specific antiserum (anti-A or anti-B or anti-H antiserum) and 100microliter of undiluted saliva were placed in clean test-tube and mixed. This serves as the test group. However, in the case of the control group, 100µl of normal saline instead of saliva in the test group was used. The test and control tubes were incubated in the water-bath at 37^0C for 30 minutes and after incubation, 100µl 3% group specific suspensions of A, B and O red cells were added to test-tubes containing diluted anti-A antiserum, anti-B antiserum and anti-H antiserum, respectively in the test and control groups. The contents were mixed and incubated at 37^0C for 30 minutes, and then, all the tubes were observed for

agglutination macroscopically under good light source.

Interpretation

Agglutination in the test group indicates a negative result for secretor status while absence of agglutination indicates a positive test for secretor status.

Statistical analysis

Statistical package for social sciences (SPSS) version 20 was used for the data analysis. Descriptive statistics was used for the calculation of frequencies and percentages while chi-square test was used to compare among the groups. $P < 0.05$ was considered to be statistically significant.

Results

Table 1 shows the prevalence of ABH secretors and non-secretors in the studied population. Out of 175 healthy individuals studied in Sokoto metropolis, 144 (82.3%) were ABH secretors while 31 (17.7%) were non-secretors.

Secretor status of ABH antigens according to gender was summarised in table 2. Out of 175 individuals studied, 144 were males and 61 were females. Among the males, 97 (85.1%) were secretors while 17 (14.9%) were non-secretors compared to females with 47 (77.0%) and 14 (23.0%) for secretors and non-secretors, respectively. However, the differences were not statistically significant ($P > 0.05$)

Distribution of ABH secretors and non-secretors based on ABO blood group system is revealed in table 3. Out of 175 subject, 43 were of blood group A, and among this subjects, 37 (86.0%) were secretors and 6 (14.0%) were non-secretors; 51 were of blood group B, and out of this, 43 (84.3%) were secretors and 8 (15.7%) were non-secretors; 12 were of blood group AB and out of this, 10 (83.3%) were secretors and 2 (16.7%) were non-secretor and finally, 69 were of blood group O, and out of this, 54 (78.3%) were secretors while 15 (21.7%) were non-secretors. However, the differences in the frequencies of secretors

Table1· Prevalence of ABH secretors and non-secretors in the studied population

Status	Number	Frequency (%)
Secretors	144	82.3
Non-secretors	31	17.7

Table 2. Secretor status of ABH antigens according to gender in the studied population.

Gender	Secretor number(%)	Non secretor number(%)	P value
Males	97(85.1%)	17(14.9%)	0.1844
Females	47(77.0%)	17(23.0%)	

Table 3. Distribution of ABH secretors and non-secretors based on ABO blood group system among Sokoto residents.

Blood group	N= 175(%)	Secretors number(%)	Non secretors number(%)	P value
A	43 (24.6)	37 (86.0)	6 (14.0)	0.7204
B	51 (29.1)	43 (84.3)	8 (15.7)	
AB	12 (6.9)	10 (83.3)	2 (16.7)	
O	69 (39.4)	54 (78.3)	15 (21.7)	

Table 4· Prevalence of ABH secretors and non - secretors according to age.

Age (years)	Secretors number (%)	Secretors number (%)	P-value
18 – 28	87 (80.6)	21 (19.4)	0.6603
29 – 39	51 (83.6)	10 (16.4)	
40 – 50	5 (100)	0 (0)	
51 – 61	1 (100)	0 (0)	

and non-secretors with respect to A, B, AB, and O blood groups were not statistically significant (P >0.05). However, blood group O had the highest frequency, followed by blood groups B, A and AB.

Table 4 shows the prevalence of ABH secretors and non-secretors according to age. Out of 144 secretors, 18-28 years age group had 87 (80.6%) as secretors and 21 (19.4%) as non-secretors; 29-39 years age group had 51 (83.6%) as secretors and 10 (16.4%) as non-secretors; 40-50 years age group had 5 (100%) as secretors and none (0%) as non-secretor while 51 – 61 years age group had 1 (100%) as secretor and 0 (0%) as non-secretor. However, the differences in the frequencies with regard to age, were not statistically significant (P > 0.05).

Discussion

The secretor status of an individual can be used to resolve ABO discrepancies, which may occur in certain conditions like leukaemia, Hodgkin disease and acquired B antigen.[19]

Our study has shown an overall prevalence of 82.3% for ABH secretors in saliva. This is in agreement with the previous findings in Nigeria, as Emeribeet al.[12]reported 86.9% in Calabar municipal residents and Igbenedghu et al.[13] reported 78.1% among the Osogbo residents. However, Saboor et al.[16]established 64.4% of ABH secretors in Karachi, Pakistan; Muddathir et al.[17] documented frequency of 31.8% of ABH secretors among the Sudanese population; Shaik and Sekhar[14] observed 79.4% among healthy blood donors in Kadapa city of India; Moon et al.[15]reported frequency of 75.5% for ABH secretors in Korean adults while Lindel et al.[20] observed 80% among the Caucasians as ABH secretors and 20% as non-secretors. However, the frequency of the non-secretors of ABH antigens in this study was 17.7%. This finding is at variance with the frequencies of ABH non-secretors in Karachi, Pakistan of 35.6% [16], Manipuri, India of 50.5%[18] and Korea of 24.5%[15] but in conformity with the findings in Nigeria of 21.9% by Igbeneghu, et al.[13] and 13.1% by Emeribe et al.[12] These different frequencies of ABH secretors and non-secretors in saliva of the studied populations could be due to geographical and/or racial factors as well as variation in sample sizes.

The secretion of antigens into the saliva and mucus has been associated withthe increase in the protection against bacterial fimbriae lectins[21] while non-secretors of ABH substances have observed to be more prone to peptic ulcer, oral submucous fibrosis auto-immune diseases such as ankylosingspondilitis, reactive arthritis, psoriatic arthropathy, Sjogren's syndrome, multiple sclerosis and Grave's disease.[22-25]

This study revealed that 85.1% of males and 77.0% of females were ABH secretors, however, there was no significant difference with regards to gender. These findings are in line with the report of Igbeneghuet al.[13] which showed 78.7% and 77.5% for male female secretors, respectively. The study therefore shows that gender has no influence on the ability to secrete ABH substances in the saliva.

Our study further confirmed the earlier reports[13, 14] on the frequency distribution of ABO blood group system, as blood group O had the highest frequency, followed by blood group B, blood group A and blood group AB.

Divergent views have been expressed on the distribution of ABH secretors and non-secretors based on the ABO blood group. The study showed that 86.0% of blood group A were secretors while 84.3%, 83.3%, and 78.3% were secretors for blood group B, AB and O, respectively. The different frequencies of secretor status of the blood groups A, B, AB, and O were not statistically significant. However, Igbeneghu et al.[13] showed frequencies of 64.9%, 71.8%, 66.7% and 86.8% for blood groups A, B, AB and O secretors, respectively in Osogboo but Onwuka et al.[26] reported frequencies of 61.9%, 85.33%, 83.33% and 64.18% for blood groups A, B, AB and O secretors, respectively in Kano while Saboor et al.[16] in Karachi revealed frequencies of 71.4%, 79.5%, 45.5% and 61.5% for blood groups A, B, AB and O secretors, respectively. Furthermore, Metgud et al.[1]in Southern Rajasthan documented frequencies of 100%, 95%, 95% and 100% for blood groups A, B, AB, and O secretors, respectively. Variation in the frequencies of secretors with respect to blood groups from the studied populations could be associated with racial factor and different sample sizes. However, the ability to secrete ABH substances contributes to innate defences, irrespective of ABO blood group and this protective effect of the secretor gene is observed particularly among groups of individuals who are immunocompromised while high proportion of non-secretors has been linked to patients with invasive disesase due to *Neisseria meningitidis, Haemophilusinfluenzae* and *Streptococcus pneumonia.*[27-29]

This study has shown that the different frequencies of ABH secretors with age were not statistically significant. These findings are in conformity with the previous report in Calabar Municipal.[12] However, the different frequencies observed in this study could be associated with uneven distribution of sample numbers for the age groups.

In conclusion, 82.3% of the studied population are ABH secretors. Gender and age had no effects on the ability to secrete the ABH antigens. However, this significant number of the secretors in this locality is a very important factor in supporting the investigation of crimes (medico-legal cases) as saliva can be used when there is absence of blood at the scene of crime or alternatively used to substantiate antigens detected on the suspects' red cells.

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