

ABO (H) secretor status of sickle cell disease patients in Zaria, Kaduna State, Nigeria

* Olorunshola, K.V. and Audu, L.

Department of Human Physiology, Faculty of Medicine, Ahmadu Bello University, Zaria, Nigeria

Summary: Certain individuals secrete ABO blood group antigens in body fluids and secretions while others do not. In this study, the presence of water soluble agglutinogens in body fluids such as blood, saliva and urine of 64 sickle cell disease patients and 75 AA genotype subjects who served as control were taken and tested by hem-agglutination inhibition method. Data obtained was expressed in percentages. Results revealed that 84.4% sickle cell patients were secretors while 15.6% were non secretors. Amongst the control, 97.3% were secretors while 3.1% were non secretors. 81.2% SS and 3.2% SS+F patients were secretors while 15.6% SS were non secretors, 68% AA were secretors and 29.3% AS were secretors while 2.7% AA were non secretors. The result showed that a non secretor is more likely to be an SS than a secretor and Secretor status is influenced by hemoglobin genotype.

Keywords: ABH secretor status, Sickle cell disease, Saliva, Urine, Morbidity susceptibility

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*Address for correspondence: sholagreenland@yahoo.co.uk

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INTRODUCTION

A secretor is defined as a person who secretes their blood type antigens into body fluids and secretions like the saliva in the mouth, the mucus in the digestive tract and respiratory cavities, urine, and semen where traces of the water soluble A, B, or O agglutinogens that determine blood group are found (D'Adamo and Kelly, 2011). ABH refers to the "A" and "B" antigens of the ABO blood group system and "H", the heterogenetic substance which is found in persons of all ABO types including type "O" (Cohen *et al.* 1980, D'Adamo and Kelly, 2011). The H antigens are indirect gene products expressed as fucose-containing glycan units, residing on glycoproteins or glycolipids of erythrocyte membranes or on mucin glycoproteins in secretions and are the fucosylated glycans substrates for glycosyl transferases that give rise to the epitopes for the A, B blood group antigens (Prakobphol *et al.*, 1993).

The ability to secrete A, B and O was found to be inherited in a Mendelian manner, genetically independent of ABO. ABH secretion is controlled by two alleles, Se and se. Se is dominant and se is recessive (or amorphic). Approximately 80% of people are secretors (SeSe or Sese). The major difference between the two genes is in their pattern of expression: the FUT1 (H) gene is expressed predominantly in erythroid tissues giving rise to FUT1 (H enzyme) whose products reside on

erythrocytes, whereas the FUT2 (Secretor) gene is expressed predominantly in secretory tissues giving rise to FUT2 (Secretor enzyme) and to products that reside on mucins in secretions. When alleles of both genes fail to express active enzymes, individuals bearing them in homozygous state lack the substrates for the A or B glycosyltransferases and do not express the A and B epitopes (Prakobphol *et al.* 1993).

ABH secretor status has been linked to several pathological conditions. For example, increased *H. pylori* infection with increased duodenal ulcer and peptic ulcer disease and hyperpepsinogenemia (Odeigah *et al.* 1990, Sumii *et al.* 1990, Dickey *et al.* 1993), recurrent urinary tract infections (Kinane *et al.* 1982, May *et al.* 1989), *neisseria meningococcal* disease (Blackwell, 1986,1990., Zorgani *et al.* 1994), recurrent idiopathic hyperplastic *candida* vulvovaginitis and oral candidiasis (Bufford-Mason *et al.* 1988, Thom *et al.* 1989, Lamey *et al.* 1991, Chaim *et al.* 1997) are reported to be associated with non ABH secretor status. Other disease states associated with non ABH secretors are autoimmune diseases (Shinebaum 1989, Shinebaum *et al.* 1987), chronic obstructive pulmonary disease (Cohen *et al.*, 1980., Kauffman *et al.*, 1996), dental caries and abscess (Triadou *et al.*, 1981), myocardial infarction (Hein *et al.* 1992, Ellison *et al.* 1999), rheumatic fever and rheumatic heart disease (Dublin *et al.*, 1964., Robinson *et al.* 1984., Jhinghan *et al.*, 1986.), blood clotting and thrombotic diseases (Orstavik *et al.*, 1989,

Orstavik, 1990), diabetes mellitus (Peter and Gohler 1986, Patrick and Collier 1989), and immunological disorders (Tandon et al 1979, Al-Agidi and Shukri, 1982, Blackwell et al 1988). Athreya *et al* (1967) reported the relationship between ABH secretor status and the lethality of *plasmodium falciparum* malaria.

Sickle-cell disease is an inherited disorder which occurs from a genetic mutation leading to an amino acid substitution of valine for glutamic acid at position 6 of the adult β -globin chain. In the homozygous (SS) form, this substitution results in polymerization of hemoglobin at low oxygen tension (PO_2), leading to deformed (sickle shaped), dense red blood cells and making them to be more fragile and hemolyse in patients with sickle-cell anemia (Fleming and Lehman, 1982). The predominant pathophysiological feature of homozygous SCA is vaso-occlusion, which leads to acute and chronic complications such as painful crises, increased risk of infection, acute chest syndrome, stroke, and severe pain episodes with dramatic hemolysis and anemia which progress to involve multiple organs, including the central nervous system, cardiovascular system, lung, liver, bone, skin, and kidneys (Hiran, 2005).

This research is aimed to answer the questions: is a sickle cell patient likely to be an ABH secretor or non secretor, and do sickle cell patients carry additional risks if they are ABH secretors or non secretors? The findings will be useful in the management of sickle cell patients especially during counseling sessions.

MATERIALS AND METHODS

Study Design

This study was conducted between June and August 2011 in the Laboratory of Department of Human Physiology, Faculty of Medicine, Ahmadu Bello University, Zaria, Nigeria. Questionnaires were given to sickle cell disease patients and control subjects to obtain their medical history and socio-demographic parameters (age, sex, tribe, Hemoglobin genotype and blood group if known, presence of current illness and past blood transfusion and willingness to participate in the study).

Ethical Consideration

Approval for this study was obtained from the Ethical Committee on Human Research of the Faculty of Medicine, Ahmadu Bello University, Zaria. The study recruited sickle cell patients attending hematology clinics in Ahmadu Bello University Teaching Hospital, Shika Zaria and control subjects among students and staff of Faculty of Medicine, Ahmadu Bello University, Zaria after detailed explanation on the nature and benefit of the study had been given and verbal and written consent obtained.

Study Population.

A total of 64 Sickle Cell Disease (Hemoglobin SS) patients and 75 control (Hemoglobin AA) subjects were used. All the subjects were of Hausa tribe from northern Nigeria. The SCD patients used for this research were both children and adults in stable hemodynamic states and with no apparent sickness, with age range of 10-40 years and mean age of 25.8 years. The control subjects were age matched with a mean age of 23years.

Collection of Blood, Saliva and Urine

Following aseptic techniques, two milliliter (2ml) of blood was collected from the cubital vein in all the subjects (sickle cell disease and control) into heparinized bottles. Five to ten (5-10mls) of urine was also collected from all the sickle cell disease and control subjects into clean universal bottles. Also, after proper rinsing of mouth with distilled water and discarding first few drops, 2 ml of saliva was collected into a dry sterile container from each subject

Determination of Hemoglobin Genotype

Blood samples were collected by venopuncture in all subjects into EDTA anti-coagulant bottles. The sickling test was carried out at a slightly acidic pH of 6.8 (Fleming and Lehman, 1982) to observe the presence of sickle cells under reduced oxygen tension. The electrophoretic method described by Fleming and Lehman (1982) and Graham (1988) was used as a confirmatory test. For the study of the haemoglobin electrophoresis, a small quantity of haemolysate of venous blood from each subject was placed on the cellulose membrane and carefully introduced into the electrophoretic tank containing Tris-EDTA-Borate buffer 89 as described by Fleming and Lehman, (1982). The electrophoresis was then allowed to run for 15 to 20 minutes at an *emf* of 160V. The results were read immediately. Haemolysates from blood samples of known haemoglobin were run as controls.

Processing of saliva

The saliva was transferred to a test tube and placed in a boiling water bath for 10 minutes to denature the salivary enzymes. It was then cooled and centrifuged for 5 minutes at 1000g, then supernatant was collected and diluted with an equal volume of normal saline.

Determination of ABO blood group in blood, urine and saliva.

Determination of the ABH secretor status was done by haemagglutination inhibition method. After proper cleaning of the *Adams* slide with saline solution and cotton wool, it was allowed to dry. One drop of blood, urine and processed saliva was dropped on one of three different column of the slide respectively. A

drop of blood antisera A and B was placed on the blood, urine and saliva. This was stirred with a glass rod and the slide rocked for a few minutes for any reaction to occur. The stirring rod was cleaned with water, saline solution and cotton wool before and after each use. The presence or absence of agglutination was confirmed by the presence or absence of thick masses (clumping) of blood, saliva and urine, thus determining the blood group and secretion of antigens in saliva and or urine.

Statistical Analysis

Results were expressed in %. The difference between secretors and non secretors were compared for sickle cell disease patients and control subjects..

RESULTS

Sixty four (64) SCD patients (23 males and 41 females) and 75 control (37 males and 38 females) subjects participated in this study. A total of 54(84.4%) SCD (HbSS) patients and 73(97.3%) control subjects (HbAA) were secretors while 10(15.6%) SCD patients and 2(2.7%) subjects were non-secretors (see figure 1). Both SCD secretors and control secretors secreted ABO antigens in saliva more than in urine, a few subjects secreted ABO antigens in both saliva and urine (see figure 2).

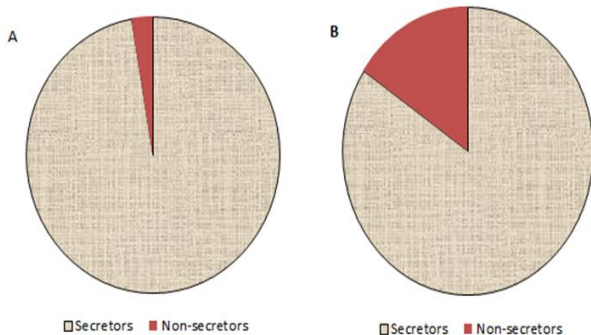


Figure 1. Secretor status of (A) Control and (B) Sickle Cell Disease – SCD subjects

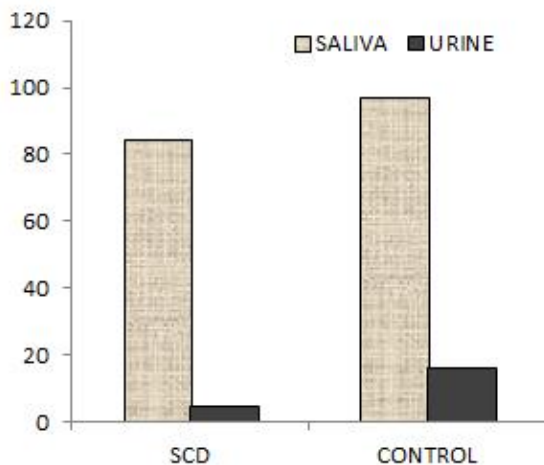


Figure 2. Secretors in saliva and urine

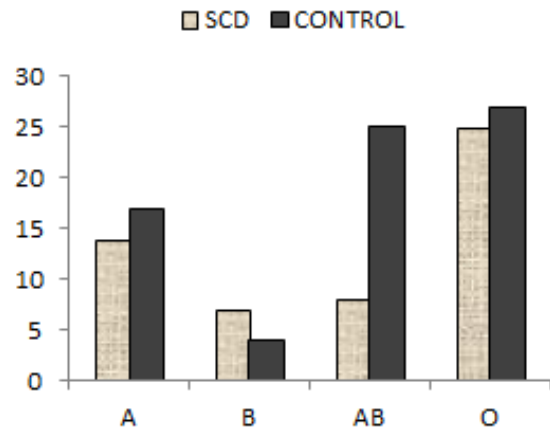


Figure 3. Blood group of secretors

Table1. Sex distribution and secretor status of Control and SCD subjects.

Sex	Control (n=75)		SCD (n= 64)	
	S (%)	NS (%)	S (%)	NS (%)
Male	37 (49.3)	-	20 (31.3)	3 (4.7)
Female	36 (48.0)	2 (2.7)	34 (53.4)	7 (10.9)

S=secretor, NS=Non secretor

Table2. Blood group and secretor status of Control and SCD subjects.

Group	Control (n=75)		SCD (n= 64)	
	S (%)	NS (%)	S (%)	NS (%)
A	17 (22.7)	-	14 (21.9)	3 (4.7)
B	4 (5.3)	-	7 (10.9)	-
AB	25 (33.3)	-	8 (12.5)	3 (4.7)
O	27 (38.7)	2 (2.7)	25 (39.1)	6 (9.4)
Total	73 (97.3)	2 (2.7)	54 (84.4)	10 (15.6)

S=secretor, NS=Non secretor

The sex distribution of non secretors showed that in the control subjects, all the non secretors were females (2.7%) while in the SCD non secretors, 10.9% were females as compared to 4.7% males (see table 1).

Blood group O had the highest frequency in both control and SCD secretors (38.7% and 39.1% respectively). The least blood group frequency in both control and SCD secretors was blood group B (5.3% and 10.9% respectively). The non secretors among the control subjects (2.7%) were only of blood group O, but among the SCD non secretor, 9.4%, 4.7% and 1.6% were blood group O, AB and A respectively (see table 2 and figure 3). There is a statistically significant correlation (p<0.001) between ABO blood group phenotypes and secretor status.

DISCUSSION

ABO blood group and secretor status are important in clinical and forensic medicine and in relation to some diseases. In this study, the frequency of ABH secretor

status in the control subject was 97.3% and 2.7% non secretors whereas the frequency of secretors in the sickle cell disease (SCD) subjects was 84.4% while the frequency for non secretors was 15.6%. The frequency of ABH secretor status reported in the control subjects in this study is generally higher than frequencies reported by other authors. Emeribe et al (1992) reported a frequency of 86.9% secretors and 13.1% non secretors in Calabar in Southern Nigeria while Jaff (2010) reported a frequency of 76% secretors and 23.9% non secretors in Iraq. Akhter et al (2011) found a frequency of ABH secretor status of 60% and non secretor of 40% in Dhakar. In Caucasians, approximately 80% are secretors and 20% non secretors. Our subjects are all Hausas from Northern Nigeria.

In this study, more sickle cell disease (SCD) subject are non secretors (15.6%) as compared to 2.7% in the control subjects. The conversion of A and B antigens of ABO blood group system from their precursor substance H starts at about 5 to 6 weeks of intrauterine life. It has been reported that when alleles of both FUT1 and FUT2 genes fail to express active enzymes, individuals bearing them in homozygous state lack the substrates for the A and B glycosyltransferases and do not express the A and B epitopes (Prakobpol et al 1993). It is therefore possible that the presence of sickle haemoglobin gene in the homozygous state (HbSS) may down regulate the expression of FUT2 (secretor) gene and secretor enzymes, leading to the higher percentage of non secretors in the SCD subjects. Is there a possible interaction between sickle haemoglobin gene and FUT1/2 gene?

The frequencies of ABO blood grouping in the control subjects for groups O, A, B and AB were 41.4%, 22.7%, 5.3% and 33% and for the SCD were 48.6%, 23.5%, 10.7 and 17.2% respectively. There is no major difference in the ABO blood groups of the controls and SCD subjects except for the AB group that had a higher frequency in the control subjects (33%) as compared with the SCD subjects (17.2%). In our study, the least blood group frequency for both control and SCD subject is blood group B (5.3% and 10.7% respectively). This result is in contrast to the finding of blood group AB being the least frequency among the people of Calabar in southern Nigeria (Emeribe et al 1992) and the reports of Ralman (1997) and Akhter et al (2011). This may be due to racial and geographic variations.

We also reported significant correlation between ABO blood group phenotypes and secretor status frequency which agrees with the findings of Emeribe et al (1992) and Jaff (2010).

The results in this study also revealed that females are more likely to be non secretors. The only non secretors in the control subjects (2.7%) were all

females, in the SCD subjects, females non secretors were 10.9% as compared to 4.7% of males. This is also different from the findings of Emeribe et al (1992) where there was no age or sex correlation with secretor status.

Sickle cell disease patients are known to have serious and peculiar health complications including severe recurrent bone pain (vasculo-occlusive) crisis, sequestration and haemolytic anaemias, chest syndromes and recurrent infections (Hiran, 2005). In tropical Africa, SCD patients have additional health challenge of recurrent malaria infestation owing to the prevalence of anopheles mosquitoes. ABO(H) non secretor status has been linked to several disease conditions such as duodenal ulcers (Dickey et al. 1993), recurrent urinary tract infections (May et al. 1989), oral candidiasis and oral precancerous lesions (Chaim et al. 1997, Compagni et al. 2007) and thrombotic and heart diseases (D'Adamo and Kelly, 2001) amongst others. Increased lethality of plasmodium malaria was also reported by Athreya et al (1967). We are reporting probably for the first time, increased frequency of ABO non secretor status among sickle cell disease patients of Hausa ethnic tribe in northern Nigeria especially females and those with blood group O phenotypes.

We conclude that the ABO secretors status of sickle cell disease patients may be a factor in the occurrence of severe malaria, recurrent infections and multiple organ syndromes frequently encountered by sickle cell disease patients. A possible relationship and or interaction between sickle haemoglobin gene and FUT1/2 gene requires further investigations.

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