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### Original Article

# Blood Group Antigens and Secretor Phenotypes among Voluntary Blood Donors and Female Sex Workers in Nairobi, Kenya

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### ABSTRACT

**Background:** Blood group antigens are collectively classified into major and minor blood group systems based on their serological characteristics. These antigens may also be found in mucosal secretions resulting in secretor or non-secretor phenotypes. In this regard, assessing blood group antigen frequency distribution is multipurpose, as besides their importance in blood transfusion, the correlation of these antigens is increasingly being sought in relation to disease. **Aim:** To determine ABO, Duffy and Rhesus (D) blood group antigen frequencies and secretor and non-secretor status among blood donors and female sex workers in Nairobi, Kenya. **Methodology:** Blood and saliva samples were obtained from 142 blood donors and 280 female sex workers. ABO, Rhesus (D), and Duffy blood grouping were done by standard haemagglutination techniques. Secretor and non-secretor phenotypes were determined by the agglutination inhibition method using anti-H lectins specific to salivary H antigen. **Results:** ABO frequency distribution was O>A>B>AB comprising 47.2%, 28.4%, 19.9% and 4.5% respectively with 96.7% Rhesus positive cases. Two (0.47%) of the study participants were Duffy positive and 78.9% were secretors and 21.1% non-secretors. **Conclusion:** This is the first report to document secretor and non-secretor phenotype frequencies in Kenya. The phenotype profiles are similar to previous reports on African populations.

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### 1. Introduction

Landsteiner's discovery of the human blood groups in the 1900s led to the understanding of blood group antigens as patterns of inherited serological reactions [1]. Today, there are more than 20 distinct blood group systems, comprising approximately 400 antigens [2]. Of these, the ABO and Rhesus blood groups systems remain the most important clinically, in transfusion and transplantation medicine [3]. The ABO antigens, comprising A, B and H are carbohydrate moieties expressed on red blood cells depending on the activity and specificity of enzyme glycosyltransferases encoded by the Fucosyltransferase 1 (FUT1) gene [4, 5]. They catalyze the transfer of N-acetyl-D-galactosamine or D-galactose to the non-reducing ends of suitable oligosaccharide chains found on red cell membrane glycoproteins and glycolipids [6, 7]. This results in the expression of A and B blood group antigens respectively while the blood group O phenotype results from inactivity of the glycosyltransferase gene that generates A and B antigens.

Interestingly, it was originally thought ABO blood group antigen expression was only confined to the red blood cells, but in the early 1930s it was discovered that in certain individuals known as 'secretors', these blood group antigens are additionally

expressed on the surface of various cell types as well as in mucosal secretions [8]. In secretors, the ABO antigens are expressed on epithelial cells, body tissues and in body secretions including saliva, gastric fluids, tears, breast milk, semen, vaginal and cervical secretions [9]. While, in non-secretors ABO antigen expression is restricted to the red blood cells - a phenotype determined by the expression of the Fucosyltransferase 2 (FUT2) gene [10]. The secretor phenotype is of particular interest, as there are well-established correlations between secretor status and susceptibility to various bacterial and viral infections. Secretors have been shown to have increased susceptibility to noroviruses [11], campylobacter [12], Helicobacter pylori [13] and the Human Immunodeficiency Virus (HIV) [14]. In fact, following HIV infection non-secretors have been shown to have slower disease progression [15].

The Rhesus (Rh) and Duffy blood group systems additionally, comprise immunologically important blood group antigens. Rh antigens are protein motifs with the ability to mount potent alloimmune reactions in transfusion, pregnancy - haemolytic disease of the newborn and play a key role in sickle cell disease [16]. While, the Duffy antigens are receptors for Plasmodium vivax, Plasmodium knowlesi malarial parasites [17] and have been shown to mediate binding of HIV to red blood cells; and thereby subsequent transfer of the virions to target cells [18].

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Determination of prevalence rates of the major and minor blood group phenotypes including ABO, Rhesus and Duffy, and secretor and non-secretor phenotypes in a population is therefore multipurpose. The importance of these well-defined antigens is increasingly being sought in relation to disease, forensic pathology, and population genetics. In this regard, baseline data on the variable expression of blood group antigens in the context of secretor status in the Kenyan population is not available. This study was therefore designed to profile ABO, Rhesus (D) and Duffy blood group antigen phenotype frequencies and secretor and non-secretor phenotypes among a HIV low-risk population of voluntary blood donors and a comparative HIV high-risk population of female sex workers in Nairobi, Kenya.

## MATERIALS AND METHODS

This study enlisted four hundred and twenty two (422) adults, comprising one hundred and forty two (142) voluntary blood donors (106 males and 36 females) and two hundred and eighty (280) female sex workers. The volunteers aged 18-65 years were recruited consecutively from the regional blood transfusion centre and from sex worker outreach program clinics within Nairobi, Kenya respectively. This study was conducted from October 2012 - October 2013.

**Ethics Statement:** Ethical approval for the study was sought from the Kenyatta National Hospital-University of Nairobi Ethics and Research Committee. Informed consent was obtained from all volunteers based on a non-coercive approach from study participants.

**Sample Collection:** A 3 ml sample of venous blood was obtained using standard sterile phlebotomy techniques. A 2 ml saliva sample was obtained by placing a sterile salimetrics oral swab in the mouth for five (5) minutes to absorb saliva directly from the parotid gland or sublingually.

**Sample Analysis:** Analysis was performed at the KAVI-Institute of Clinical Research laboratories, College of Health Sciences, University of Nairobi, Kenya.

**ABO Blood Grouping:** ABO and Rhesus (D) blood grouping was determined using commercial antisera kits: murine monoclonal anti-A, anti-B and anti-D antisera (Plasmatec Laboratory Products Ltd., Bridport, Dorset, UK) by the haemagglutination technique on a 2-3% red cell suspension of the blood sample. 100 µl of anti-A, Anti-B and anti-D was added into clean test tubes labelled A, B and D, containing 100 µl of a 2-3% red cell suspension and incubated at room temperature for 10 minutes. The reaction mix was then centrifuged at 1000 rpm for 5 minutes. Red cell buttons were gently re-suspended and observed for agglutination macroscopically. Agglutination was interpreted as a positive result and absence of agglutination as a negative result. For the ABO blood grouping, negative results were confirmed by reverse typing on the sample serum, and for Rhesus (D) typing, negative results were confirmed using the indirect anti-human globulin test procedure.

**Fya, Fyb Blood Grouping:** Duffy blood grouping was determined using human monoclonal anti-Fya and anti-Fyb blood grouping reagents (Lorne Laboratories Ltd., Reading, Berkshire, UK) by the Indirect Antiglobulin Technique on a 2-3% red cell suspension of the blood sample. An equal volume (100 µl each) of a 2-3% suspension of washed test red cells prepared in saline was mixed with anti-Fya and anti-Fyb reagents, in two separate tubes, and incubated at 37°C for 15-30 minutes.

Samples were centrifuged at 1000 rpm for 5 minutes, and the test cells washed four (4) times in saline. 200 µl of anti-human globulin was added to each 'dry' cell button, mixed and centrifuged at 1000 rpm for 5 minutes. The cell button was re-suspended and agglutination was interpreted as a positive result. Validity of all negative reactions was confirmed with IgG sensitized red cells by adding a 100 µl of Coombs control cells to all negative tubes.

## Secretor Phenotyping:

Secretor status was determined using Ulex Europaeus specific anti-H Lectin (Lorne Laboratories Ltd., Reading, Berkshire, UK) to salivary H antigen by the agglutination inhibition technique. 2 ml whole saliva samples collected using sterile salimetrics oral swab kits were centrifuged at 1500 rpm for 15 minutes. The saliva samples were boiled for 10 minutes to denature salivary enzymes, allowed to cool and centrifuged at 1000 rpm for 5 minutes. Equal volumes of the anti-H lectin and sample supernatants (1 ml each) were incubated for 10 minutes at room temperature. An equal volume of a 2-3% O positive red cell suspension was added to each reaction, and centrifuged at 1000 rpm for 5 minutes. No agglutination was interpreted as the secretor phenotype and agglutination indicated the non-secretor phenotype.

## Statistical Analysis:

Chi-square 2 tests using the software SPSS Version 20.0 were used to determine the significance of the influence of individual ABO blood group phenotypes, Rhesus (D) type, Duffy (Fya, Fyb) and age on the frequency of secretor status, age and sex among the study population. P values <0.05 were regarded as statistically significant.

## RESULTS

ABO, Rhesus (D) and Duffy (Fya, Fyb) erythrocyte phenotype profiles and secretor and non-secretor status were determined for 422 adults in Nairobi, Kenya. The study population comprised 142 voluntary blood donors (106 males and 36 females) and 280 female sex workers.

## Age Stratification among Study Participants

The age range for all study volunteers was 18-65 years with a mean age of 33.01 years (SD, 9.3 years), as outlined in Table 1.

Majority of the blood donors were under the age of 30 (69.7%), while the female sex workers were mostly above the age of 30 years (72.5%) as shown in Figure 1 below. This difference was statistically significant ( $p=0.0001$ ).

## Blood Group Phenotypes

ABO blood group phenotype distribution was O>A>B>AB among both the blood donors and female sex workers. Collectively, blood group O were a majority comprising 199 (47.2%), A 120 (28.4%), B 84 (19.9%) and AB 19 (4.5%) respectively with 408 (96.7%) Rhesus (D) positive cases. Duffy positive phenotypes were reported in 2 (0.47%) of the study participants, both female sex workers. The frequencies of the respective blood group phenotypes are shown in the Table 2.

Further stratification, based on the ABO and Rhesus (D) phenotypes confirmed > 95% of the blood donors were Rh positive as outlined in Table 3 below.

## Regional and Ethnic Distribution

The study participants were all recruited from Kenya,

however, there was further stratification based on individual ancestral descent. 392 (92.9%) were from Kenya and of these, study participants from Central region represented a 209 (49.53%) majority, while study cases from North-Eastern region represented a 3 (0.71%) minority. The non-Kenyan study participants represented a 2.8% minority from East and West Africa (Figure 2). In Central, Eastern, Nyanza, and Coastal regions of Kenya the blood group phenotype distribution patterns were O>A>B>AB, while in Western and Rift valley regions, the distribution patterns were O>B>A>AB. 18 (4.27%) of the study participants declined to disclose their nationality and/or tribe.

#### Secretor and Non-secretor Status

Saliva testing showed that among the blood donors, 121 (85%) were secretors and 21 (15%) were non-secretors, while among the female sex workers, 212 (76%) were secretors and 68 (24%) non-secretors (Figure 3).

#### Secretor Status and Gender

The study participants comprised 142 blood donors (106 male and 36 female), and 280 female sex workers. Of the 106 male study subjects 87.7% were secretors and 12.2% were non-secretors, while among the 316 female study participants 77.5% were secretors and 22.5% were non-secretors. Statistical analysis of the proportions indicated the secretor phenotype was more frequent among the male study subjects (87.7%) in comparison to the female study cases (75.9%). This difference was statistically significant ( $p=0.027$ ) (Table 4).

#### Secretor Status and ABO Phenotypes

Based on the ABO blood group phenotypes, blood group O secretors were a majority:  $n=163$  (81.9%) and blood group AB secretors the least frequent:  $n=14$  (73.7%) as shown in Table 5.

However, when comparing the individual phenotypes, there was no significant difference in the incidence of secretors per blood group phenotype as shown in Figure 4 below:

#### Blood Group Antigens and Secretor Phenotypes among Voluntary Blood Donors and Female Sex Workers in Nairobi, Kenya

**Table 1: Age Distribution among The Study Participants.**

These were 142 voluntary blood donors and 280 female sex workers ( $n=422$ ). \*The percentage was calculated based on the group totals i.e. ( $\text{No.}/142 \times 100$ ) for blood donors, ( $\text{No.}/280 \times 100$ ) for female sex workers and ( $\text{No.}/422 \times 100$ ) for the totals respectively.

	Blood Donors		Female Sex Workers		Total	
Age (Years)	No.	%*	No.	%*	No.	%*
18-24	60	42.2	29	10.4	89	21.1
25-29	39	27.5	48	17.1	87	20.6
30-34	17	11.97	55	19.6	72	17.1
35-39	10	7.04	58	20.7	68	16.1
40-44	10	7.04	28	10.0	38	9.0
45-49	3	2.1	34	12.1	37	8.8
50+	3	2.1	28	10.0	31	7.3
Totals	142		280		422	
Means	27.97 (SD, 7.7 years)		36.1 (SD, 9.3 years)		33.01 (SD, 9.3 years)	

**Table 2: ABO, Rhesus (D) and Duffy Phenotype distribution among the study participants. Blood group O, Rhesus positive and the Duffy null phenotypes were predominant among the study participants.**

	Blood Donors		Female Sex Workers		Total	
Blood Group	No.	%	No.	%	No.	%
ABO						
O	58	40.8	141	50.4	199	47.2
A	47	33.1	73	26.1	120	28.4
B	30	21.1	54	19.2	84	19.9
AB	7	4.9	12	4.3	19	4.5
Rhesus (D)						
Positive	135	95.1	273	97.5	408	96.7
Negative	7	4.9	7	2.5	14	3.3
Duffy (Fya, Fyb)						
Fya <sup>+</sup> Fyb <sup>-</sup>	0	0	1	0.3	1	0.2
Fya <sup>-</sup> Fyb <sup>+</sup>	0	0	1	0.3	1	0.2
Fya <sup>-</sup> Fyb <sup>-</sup>	142	100	278	99.3	420	99.5

**Table 3: Distribution of Rhesus (D) phenotypes between ABO phenotypes among the study participants**

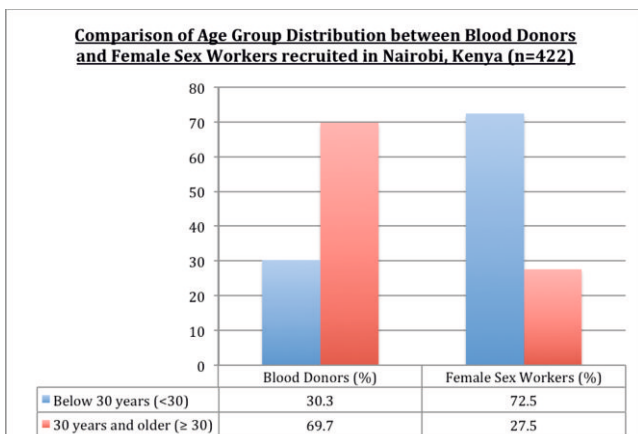
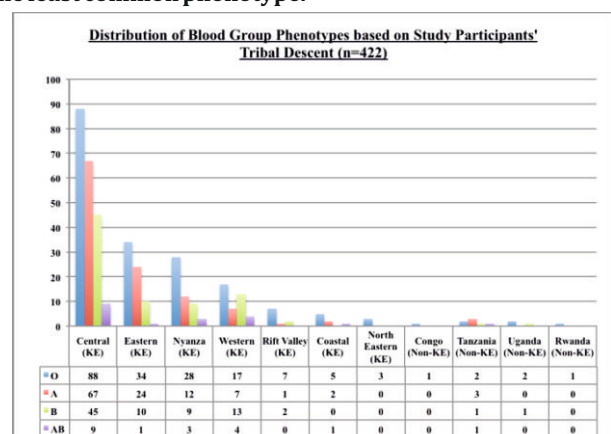
	Blood Donors		Female Sex Workers		Total	
Blood Group	No.	%	No.	%	No.	%
O						
Positive	55	94.8	137	97.2	192	96.5
Negative	3	5.2	4	2.8	7	3.5
A						
Positive	45	95.7	71	97.3	116	96.7
Negative	2	4.3	2	2.7	4	3.3
B						
Positive	28	93.3	54	100	82	97.6
Negative	2	6.7	0	0.0	2	2.4
AB						
Positive	7	100	11	91.7	18	94.7
Negative	0	0.0	1	8.3	1	5.3

**Table 4: Distribution of secretor status prevalence in men and women. There was a significantly higher proportion of secretors among the male study participants**

Gender	Male	Female	P Value
Secretors	93 (87.7%)	240 (75.9%)	P=0.027
Non-secretors	13 (12.2%)	76 (24.1%)	
Total	106	316	

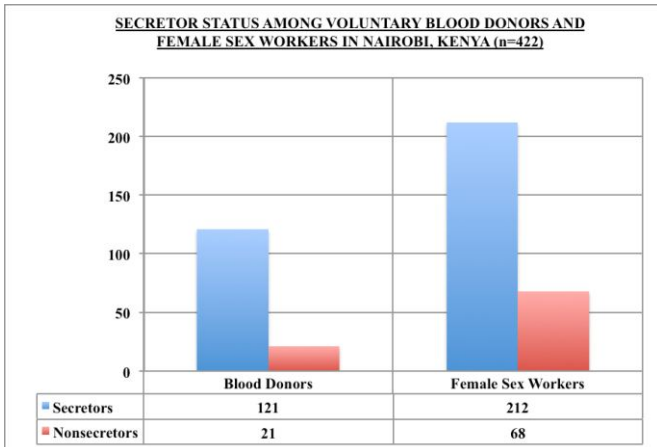
**Table 5: Frequency of secretor status in A, B, AB and O blood group individuals. Collectively, the proportion of secretors versus non-secretors in each blood group ranged from 73% - 82% for the secretors and 18% - 26% for non-secretors**

	Blood Donors		Female Sex Workers		Total	
Blood Group	No.	%	No.	%	No.	%
O						
Secretors	51	87.9%	112	79.4%	163	81.9%
Non-secretors	7	12.1%	29	20.6%	36	18.1%
A						
Secretors	40	85.1%	52	71.2%	92	76.7%
Non-secretors	7	14.9%	21	28.8%	28	23.3%
B						
Secretors	25	83.3%	39	72.2%	64	76.2%
Non-secretors	5	16.7%	15	27.8%	20	23.8%
AB						
Secretors	5	71.4%	9	75.0%	14	73.7%
Non-secretors	2	28.6%	3	25.0%	5	26.3%

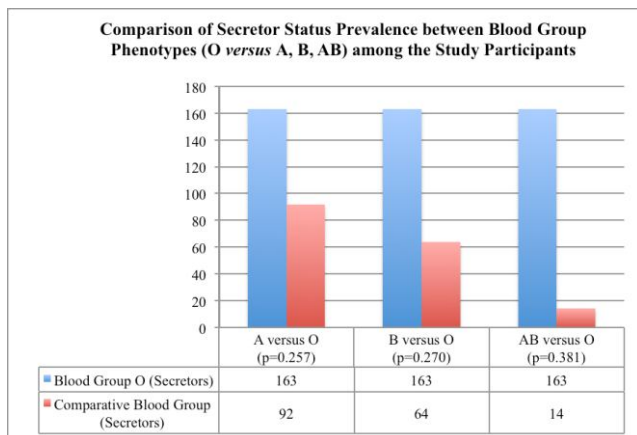
**Figure 1: Age group distribution among the study participants. The blood donors were significantly younger than the female sex workers.**

**Figure 2: Regional distribution of blood group phenotypes. Majority of the study participants were from Central region in Kenya (n=88; 49.53%) and blood group O was primarily predominant in most communities, and AB the least common phenotype.**




**Figure 3: Secretor status among voluntary blood donors (n=142) and female sex workers (n=280) in Nairobi, Kenya. In total, 333 (78.9%) of the study population were secretors and 89 (21.1%) were non-secretors.**



**Figure 4: Comparison of Secretor status Prevalence between Blood Group Phenotypes (O versus A, B, AB) among the Study Participants. There was no significant difference in the incidence of secretors between blood groups.**



## DISCUSSION

This is the first study to date on the frequency of the secretor phenotypes: secretors versus non-secretors in the Kenyan population. The study cases were all recruited from Kenya, and reveal the cosmopolitan flair characteristic of Nairobi as a capital city. There was a representation from each geographical region in Kenya, as well as a proportion from East and Central Africa. Age stratification, revealed majority of the blood donors (69.7%) were aged less than 30 years with a mean age of 27.97 years. Furthermore, there was a male dominance among the blood donors, with the females representing a 25%, minority. This confirms findings by a number of studies within the African context describing the characteristic of African blood donors [ ]. The female sex workers were predominantly aged above 30 years probably because in sub Saharan Africa, owing to the persistent gender and economic imbalance within the societies, lack of education and resources, most women resort to female sex work often after establishment of a family unit.

The fucosyltransferase 2 (FUT2) gene, encodes for the enzyme alpha 1,2 fucosyltransferase which results in the expression of the H antigen and thereby A and B antigens, expressed in epithelial cells, body tissues and in mucosal secretions [ ]. Determination of secretor status was therefore, determined by screening for the variable expression of the H antigen in saliva samples; hence the frequency of the H antigen expression in the saliva (secretors): 78.9% and lack of H antigen expression in the saliva (non-secretors): 21.1% among our target population. Globally, frequency distribution patterns of secretors vary markedly; in Nigeria, one study found 84.4% secretors and 15.6% non-secretors among sickle cell disease patients [ ], 64.4% secretors, 35.6% non-secretors, in Pakistan [ ], 60% secretors, 40% non-secretors in Dhaka [ ], 73% secretors, 26.9% non-secretors in Hungary [ ], and 78.8% secretors, 21.2% non-secretors in Burkina Faso [ ]. The mechanism underlying the variable distribution of both the ABO blood groups and secretor phenotypes are not well understood.

Furthermore, this study profiled the frequencies of the ABO, Rhesus (Rh) and Duffy blood group systems in the Kenyan population. The prevalence patterns were similar to findings from previous studies in African populations. Notably, 1) predominance of blood group O witnessed in similar study populations including an earlier study in Kenya [ ], 2) high prevalence of the Rhesus (D) positive phenotype (95.1%) similar to findings from an earlier study in Kenya 94% [ ], Nigeria ranging from 93%-98% [ ], and 3) high prevalence of the erythroid Duffy null phenotype (Fy a-b-) [ ].

Human populations globally share the same blood groups systems and secretor and non-secretor phenotypes; the difference only comes in, in the frequencies of the specific phenotypes. It is important to know the frequencies of various blood group antigen phenotypes in a population. This information is necessary to predict the availability and for the management of blood units, managing cases of alloimmunization especially in multiply transfused patients such as cancer and sickle cell anaemia patients as well as to determine the prevalence of the individual antigens in a given population. Moreover, in the context of secretor status, questions have been raised on the physiological significance of blood group antigens, aside from their well-described role in blood transfusion and compatibility testing. Indeed, functional relevance of a number of these moieties has been experimented and documented [ ]. In this regard, the blood group O phenotype has been associated with a higher risk of peptic ulcers [ ]. Blood group A with increased Plasmodium falciparum malaria disease severity [ ]. Blood group B, associated with Chagas disease [ ] and type AB individuals have been shown to have an increased incidence of pancreatic cancer [ ]. It is therefore recommended to screen for both red cell and tissular distribution of the ABH antigens in humans. This data may provide additional insight into the correlation of the different secretor and non-secretor phenotypes, in the context of existing inter-individual variations in relation to health and disease susceptibilities. Pathogens including HIV are highly selective agents; therefore for microorganisms that preferentially bind to carbohydrate moieties, host cell surface molecules such as the ABO antigens can reveal patterns of selection in a given population.

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## CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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