Asian Journal of Immunology

4(4): 20-35, 2020; Article no.AJI.62481

Association between Secretor Status of ABH Substances and HIV 1& 2 P24 Antigen Screening Status Amongst Eligible Blood Donors with Previously Screened HIV 1& 2 Antibody- Negative Status in Calabar, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. Author FJN gave concept of the study. Authors FJN and IIE performed the study design; Authors FJN, EWO and IIE analyzed the samples, collecting data and written initial draft of the manuscript. Authors FJN and EWO performed statistical analysis. All authors read and approved the final manuscript.

Article Information

Editor(s):
(1) Dr. Cynthia Aracely Alvizo Báez, Autonomous University of Nuevo Leon, Mexico.
Reviewers:
(1) Avvaru Srinivasulu, (GITAM) Gandhi Institute of Technology and Management University, India.
(2) George Gititu, Kenya Methodist University, Kenya.
Complete Peer review History: http://www.sdiarticle4.com/review-history/62481

Original Research Article

Received 01 September 2020
Accepted 06 November 2020
Published 30 November 2020

ABSTRACT

Aims: New hypotheses suggest non-secretors may be less vulnerable to the HIV infection than secretors. Association between secretor status and HIV P24 antigen status was studied in 400 HIV antibody sero-negative prospective blood donors aged above 20 years of both genders recruited within Calabar, Nigeria.
Materials and Methods: About 3 ml of saliva and 5ml of blood samples were analyzed for HIV antibodies using Stat Pak (by Chembio Diagnostic System International), Determine (by Inverness

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Medical Japan Cooperation, Limited), Unigold (by Trinity Biotech, USA) rapid test Kits and antigens using HIV P24 antigens Combi test Kits (by Inverness Medical Japan Cooperation, Limited) respectively. Secretor Status done using Anti-H Lectin reagent by BIOTEC Laboratories Limited, UK.

Results: Out of 400 samples, 80 (20%) were non-secretors and 320(80%) secretors. while 6(8.1%) non-secretor and 9(2.9%) secretor samples reacted to HIV P24 antigen screening test. Chi-Square (X^2=5.655, *P*=0.025) showed significant difference between secretor and non-secretors based on gender. No significant difference exits between HIV p24 antigen status and secretor status (chi squared X^2 =0.007, *P* =0.9) and secretor status and the three HIV Antibody test kits (F -ratio = 1.7997, *P* =0.307)

Conclusion: No significant association found between secretor status and HIV P24 antigen status but detectable P24 antigens were more in secretors than non-secretos.

Keywords: Association; secretor status; ABH substances; P24 antigen screening status; HIV 1& 2 antibody negative; appararently healthy employees; eligible blood donors.

ABBREVIATION

NAT : Nucleic Acid-based Tests
PCR : Polymerase Chain Reaction
RT-PCR : Real time Polymerase Chain Reaction tests

1. INTRODUCTION

The first attempt to correlate the human blood group systems to some pathological conditions was by [1] while [2] established the first successful positive relationship between the human blood group systems and diseases. More new hypotheses are pointing to the fact that non-secretors status are more susceptible to some diseases than secretors [3,4,5,6]. It was the inconsistent relationship between the incidence of blood groups types and the natural defense mechanisms against HIV infection that have stimulated more interest in correlative study of blood groups and different pathological conditions or diseases [7]. Recently, it has been shown that P^k blood group has a direct association with resistance to HIV infection [8]. However [9] made a conclusion in this discovery by saying that P^k blood group variation may be another new genetic mutation and risk factor for HIV infection.

The ideological phenomenon behind the existence of infected screened HIV antibody-negative individuals was first hypothesized by [10] and later by other researchers [11,12,13] who had encountered the same phenomena. Well known examples of this phenomenon include mutations in the C- chemokine receptor type 5 (CCR5) receptor gene or in such markers as human leucocyte antigen (HLA) B57 and Fucosyltransferase 2 gene (FUT2) which have been found to correlate with a reduced ability to become infected with HIV or are associated with a reduced chance of disease progression. The FUT2 gene encodes glycosphingolipids antigens on the surface of a variety of epithelial cells that encode for an enzyme called α (1, 2)-fucosyltransferase that is involved in regulating ABH blood group antigens in saliva and mucosal secretions [14,15,16].

The concept of secretor status reveals and explains why some individuals in the population have the ability or the inability to secrete soluble ABH blood group substances into their body secretions [17,18] and its historical account came by [19] who discovered that A and B substances were present in the saliva of most blood group A and B individuals. Four years later [20] suggested that the presence or absence of the blood group substances in the saliva were determined by some special genes, the secreting gene which was designated as *Se* (for secretor), and the non-secreting gene was designated as *se* for non- secretors . In recent times based on the genetic characteristics of the secreting gene , the secretor status of individuals in any population can be determined as secretors or non –secretors with great accuracy and precision [21]. The term 'secretor' is applied to those individuals in the population with genotype (*Se/Se or Se/se)* who are able to secret H-substance with or without A or B substance and does not take into account the presence of Lewis or any other blood group substance in their saliva [17,21]. According to many available researched and published data worldwide, approximately 80 percent of individuals in the population are secretors, (*Se/ se or Se,/se*) and they secret H substances irrespective of their ABO blood group [21]. Thus the saliva of blood group O secretors

contain H and that of group A and B secretors contain A, B and H and AB secretor contain A, B and H [22]. The amount of A substance in saliva of group A secretor follows a log normal distribution .The amount of A in saliva appears to vary independently of one another, although there is a significant correlation between the ratio of A to H amongst siblings [23]. ABH secretion is controlled by two alleles, *Se* and *se*. *Se* is dominant and *se* is recessive (or amorphic).On the other hand individuals who do not secrete their blood type antigen into their secretions are termed 'non-secretors'. They do not secret A, B or H either. About 15-20 percent of the individuals in the population are non-secretors. [17,21]. The Se locus is located on chromosome 19 at 19q13.3 which contains two exons that span about 25 kb of genomic DNA. The Se locus encodes a specific fucosyltransferase that is expressed in the epithelia of secretory tissues, such as salivary glands, the gastrointestinal tract, and the respiratory tract [21]. The enzyme it encodes, catalyzes the production of H antigen in bodily secretions. “Secretors" have at least one copy of the Se gene that encodes a functional enzyme—their genotype is *Se/Se or Se/se*. They secrete H antigen which, depending on their ABO genotype, is then processed into A and / or B antigens. Non-secretors are homozygous for null alleles at this locus (*se/se*). They are unable to produce a soluble form of H antigen and hence do not produce A and B antigens) and the Secretor function is centred on the action of a cluster of genes which control the production of enzymes called '*fucosyltransferases'* [24,25,26].

Records on disease susceptibility among secretors & non- secretors states have been well studied over the pasts decades. The FUT2 gene encodes the enzyme α (1, 2) fucosyltransferase, which determines expression of blood-group antigens on mucosal epithelial cell surfaces and in secretions. Homozygotes for a specific gene stop mutation in FUT2 (non- secretors) and cannot produce this enzyme and thus are unable to express blood group antigens. Non secretor status is therefore associated with a decreased risk of several infections than secretors. Some of these infections include digestive system diseases, oral pathology like throat and oesophageal cancers, duodenal ulcers, peptic ulcers diseases, gastritis, respiratory system diseases like chronic obstructive pulmonary disease (COPD), habitual snoring, bleeding times, diabetes and Syndrome X , heart disease , variety of autoimmune diseases including ankylosing spondylitis, reactive arthritis, psoriatic arthropathy, Sjogren's syndrome, multiple sclerosis, Grave's disease, bacterial urinary tract infections, fungal infections, HIV I & 2 infections and alcoholism [18]. Shift down to start new paragram here.

Recent statistics show that 75.7 million people have been infected with HIV 1 and 2 since the onset of of the epidemic and about 38.0 million people were living with the Human immunodeficiency virus (HIV) infection in 2019 worldwide, with adult prevalence rate of 0.8 percent [27,28]

Human immunodeficiency virus infection, first described in the 1980s in the USA has continued to spread rapidly [29]. HIV is a lentivirus (belonging to the retrovirus family) known to cause Acquired Immunodeficiency Syndrome (AIDS), a condition in humans in which the immune system begins to fail, leading to life-threatening opportunistic infections and some malignancies [30,31]. The adult prevalence rate of HIV in Nigeria was 1.4 percent in 2019 amongst adults aged between 15-49 years [31], while Cross River State current prevalence rate is 7.1% [32,33]. The Routes of HIV transmission are unsafe sex, contaminated needles, breast milk, and transmission from an infected mother to her baby at birth (perinatal transmission) and transfusion of infected blood or blood products. Around 5 percent of the AIDS cases in the world are contracted through blood or blood products [34,35,36,37]. Five to ten percent of all new HIV cases in Africa are caused by contaminated blood used in blood transfusion therapy. This translates to between 250 and 500 patients every single day [38]. HIV transmission through unsafe blood is the second largest source of HIV infection in Nigeria [39].

Today in most resource poor countries of the world, the risk of human immunodeficiency virus (HIV) transmission through blood and blood products from infected but HIV antibody - negative blood donors is still an issue of concerned. Blood donated by these individuals remain a major avenue of transmission of HIV in that much of the blood supply is simply not pre-tested with HIV 1 and 2 antigens screening panels [40]. This is in sharp contradiction to most developed countries of the world where the use of fourth generation HIV 1 and 2 kits such as P24 antigen screening, nucleic acid-based tests (NAT), RT-PCR tests have largely reduced the risk of HIV transmission through blood transfusion especially from infected but HIV

antibody-negative blood donors [41,42]. Infected but HIV antibody-negative blood donors are those donors that test negative to antibody tests but positive to the antigen test. There may be some percentage risk of HIV transmission from screened units donated by these donors [43,44, 45,46].

2. SUBJECTS, MATERIALS AND METHODS

The study area is Calabar which is the capital of Cross River State in the south eastern part of Nigeria. Geographically Calabar has a total surface land area of 142 km² while the total local government area population is estimated to be 320,826 of which 166,203 are males and 154,659 females [47]. They are mainly civil servants, subsistent farmers, traders and fishermen. Subject's recruitments sites: Subjects were recruited at the Haematology/Blood Transfusion Department of University of Calabar Teaching Hospital (UCTH) and General Hospital Calabar (GHC), in Calabar, Cross River State, Nigeria

Laboratory analysis of samples was carried out in the Haematology & Blood Transfusion Unit, Department of Medical Lab Science, and University of Calabar Nigeria.

2.1 Study Design

A cross –sectional study was conducted between December 2017 and December 2018.

The formula of Cochran, 1977 for calculating the sample size (S) was adopted in this study and is given by [48]: $S=t^2\ p\ (1-p)/\ e^2$, *Where* t= t value (The alpha level used in determining sample size in most educational research studies is either .05 [49]. In Cochran's formula, t-value for alpha level of .05 is 1.96 for 95% confidential level for sample sizes above 120.p= prevalence rate in percentage (%) of infected HIV antibody-negative prospective blood donors population in Calabar and in this case it was taken to be 0.5 or 50% since nobody had ever worked on this population [50]. While e = tolerance error or confidence interval expressed as decimal and it is taken to be 0.05.Therefore $S =(1.962)^2\ (.5(1-0.5)/\ (0.05)^2 =(1.962)^2\ (0.5)^2/\ (0.05)^2 =384.16= \sim 400$ subjects were –recruited in cases of any loss data or specimen during the study.

Inclusive and exclusive criteria for subject selection: A total of 400 apparently healthy prospective voluntary blood donors of both genders, aged between 20 to 50 years were randomly recruited from Haematology/Blood Transfusion Department of the University of Calabar Teaching Hospital (UCTH) and General Hospital Calabar (GHC) in Calabar, Cross River State, Nigeria. The subjects were divided into six study groups according to their ages and genders and a questionnaire was used for data collection.

Administration of questionnaire was administered to obtain information about the clinical and medical history of donors. After the Pre-blood donation counselling, informed consent forms were filled and signed by these donors for screening to start.

Treatment of Collected blood samples: About 5ml of venous blood were collected from the ante-cubital vein of pre-counseled prospective blood donors of both genders by means of disposable plastic 5mls syringe fitted with 21 SWG needle .The area of the venipuncture was first of all cleansed with 70 percent alcohol and allowed to dry. A tourniquet was tied just for a short time. About 3 mls out of the 5mls of blood withdrawn were dispensed into dried and labeled samples bottles to be used for serum and cell ABO & Rh blood groupings. Samples which were not analyzed immediately were stored in the refrigerator at 4- 6°C.

Blood samples for HIV antibody and antigen screening assays: The remaining 2mls of blood samples were dispensed into dried, labelled plain tubes and centrifuged for 10 minutes at 4000 revolutions per minutes after being allowed to retract for two hours. Finally, the supernatant was removed from the retracted, centrifuged samples, dispensed into another cleaned, labelled dried tubes for HIV antibody and antigen screening assays.

Collection and treatment of saliva for determining secretor status (ABH substances): About 2mls of saliva were collected from each subjects into sterilized universal containers before transferring into clean, dried and labelled 16 x 100mm centrifuge Pyrex tubes. Commercially bottled sterilized clean water was used to stimulate the secretions. The collected saliva samples were placed in water bath for 10 minutes to inactivate enzymes that might otherwise destroy blood group substances. The saliva samples in the boiled test tubes were cooled. After cooling the saliva samples were centrifuged for about

5minutes at 4000 revolution per minute and the supernatant was collected.

3. METHODOLOGY

Materials and Methods for the Determination of ABH secretor status: The Method of Haemagglutination Inhibition was used for the determination of ABH secretor status – using the saliva test using Anti-H Lectin (Ulex Europaeus) reagent produced by BIOTECT laboratories limited BIOTEC Laboratories Limited, Ipswich, Suffolk, UK,

Materials & methods for HIV 1 AND 2 antibody assay: Three different types of HIV & Antibody rapid test kit methods were used for HIV 1 & 2 Antibody assay as approved by (UNSAID, 2011). These included; Determine HIV1& 2 Antibody rapid test kit (Produced by Inverness Medical Japan Cooperation, Limited).Stat-Pak HIV 1& 2 Antibody Rapid Screening test kit (Produced by Chembio Diagnostic System International ,). HIV Uni-gold rapid test kit (Produced and supplied by Trinity Biotech United State of America).The procedure of each test was strictly followed as outlined in the Manufacturer Technical Manual. A commercial Determine HIV-1 & 2 Ag/ Ab Combo test kit produced by Inverness medical Japan Cooperation, Limited was used for HIV 1 & 2 antigen determination. This is based on an immunochromatographic test for the qualitative detection of p24 antigen and antibodies to HIV-1 and HIV-2. The procedure of each test was strictly followed as outlined in the Manufacturer Technical Manual.

3.1 Statistical Analysis

The statistical analysis was computed using SPSS Statistics software version 20 (SPSS Inc., Chicago, United States of America). The results of this study were analyzed with the aid of cross tabulations to explore proportional associations between variables. Data were represented with frequency and percentages while continuous data were expressed as mean and two standard deviations and Chi Square (X^2)test was used to explore proportional association between groups. While comparison among various age groups were analyzed using analysis of variance (ANOVA), independent t-test and the prevalence rate formulae were used to calculate the prevalence rate .The level of statistical significance was set at $p \leq 0.05$ (95% confidence interval).

4. RESULTS AND DISCUSSION

The fundamental reasons of implementing mandatory blood donor screening for transfusion transmissible infections (TTIs) are to protect donors as well as to hinder the transmission of blood pathogens to recipients via blood and/or blood products [51]. However, HIV remains a threat to this practice, most especially in developing countries of Africa where there is a high demand of blood for blood transfusion therapy [52]. The discovery of blood transfusion as a factor for HIV transmission made it imperative to screen all blood donors to reveal their HIV status and this served as one of the critical criteria for eligibility to qualify and certify a prospective blood donor as fit to donate blood or not. However, this practice have never taken into consideration the secretor status of the prospective blood donor concerned.

The results in Table 1 of this study show demographic distributions of 400 voluntary apparently healthy prospective blood donors according to age group, gender, and turn out rate in Calabar, Nigeria as follows: more female subjects that turned out fall between the age range of 20-25 years (45%), more male subjects that turned out fall between the age range of 26-30 years (44.7%). The mean age and age range was 33.33±7.13) and 20-50 years respectively. Using t-test there was statistically significant difference between male and female subjects (Calculated t- test =3.0, Degree of freedom (df) =6, alpha value =0.05, t-test critical value =1.943, Right-tail p-value is 0.012, $P<0.05$) and using ANOVA there was statistically significant difference between age range and number of groups F-ratio=5.125, Degree of freedom (df) =6, 12, alpha value =0.05, F-test critical value =3.00, Right-tail p-value was 0.008 ($P<0.05$). These results mean that more male subjects were willing to participate in the study than females in the various age groups and these gender imbalanced distribution is in line with the study of [53] and is similar in many countries, with Italy being an exception in that women account for only 30% of donors [54]. This gender disparity and inequality is also in the results of this study are global and are in line with the observations of [55] who has published a work on the gender disparity in epidemiological trend of HIV/AIDS infection and treatment in Ethiopia. This is also in line with work of [56] who noticed a sharp variation of gender disparity in HIV infection across many countries in sub-Saharan Africa and in USA by the observation of [57,58]. This means that more male subjects were willing to

participate in the study than females in the various age groups. It also means that more awareness about HIV infection would have to be targeted towards this female age group [59]. The lowest percentage turn out rate and frequency distribution fall in age range between 41 to 50 years (group four to group six) which is about (04.3-4.63)% which is in line with work of [60].

The results in Table 2 shows the frequency distribution of voluntary apparently healthy subjects samples collected from recruitment sites who were willing and ready to be screened as prospective blood donors according to their genders in Calabar, Nigeria. It was observed that the University of Calabar Teaching Hospital Calabar (UCTH) recruitment site had the highest number of subjects and samples which were 224 (56%) than the General Hospital Calabar (GHC) which 176 (44%) and total number of 400 registered male and female apparently healthy subjects respectively .These results were expected because there is a high frequency of activities and workload in UCTH Calabar being a tertiary institution than GHC which is a secondary institution. It was also observed that the frequency distribution and percentage of male group was higher than that of the female group and these results are in line with that of [53] and reasons for this could be attributed to the frequency of some of the male donor's blood groups that are universally needed more than the females and the unbalanced distribution of the blood group genes in the population as have been reported in Nigeria by the study of [61] in Ibadan and [62] in five different zones in Nigeria (South West) (Yoruba)--Zone A, North West (Hausa-Fulani)--Zone B, Plateau (Birom)--Zone C, South East (Igbo)--Zone D and North East (Kanuri)--Zone E). This could also means that more awareness about HIV infection would have to be targeted towards this female age group who are reluctant to come out because of their status.

Table 3 shows the results of Haemagglutination Inhibition test method used for the determination of ABH secretor status (or the saliva typing test results for ABH secretor status) for the 400 saliva samples collected from apparently healthy prospective voluntary blood donors according to their genders. About 41(10.25%) samples from male non-secretor and 39 (9.75%) were female non-secretors and about 183(45.75%) samples from male secretors and 137(34.25%) samples from female secretors. This gives an overall total of 80(20%) samples that were non-secretors and 320(80%) for secretors. The calculated Chi Squared (x^2) test value was 5.655 at degree of freedom (df) of 1, with 400 as the total number of samples or sample (N) and level of alpha of 0.05. This gives Chi Squared (x^2) critical value of 3.84.This gives a *P*- value of 0.025. This means that there was a significant difference between the frequency distribution and prevalence rate of secretors and non-secretors based on gender ($P<0.05$).These results agree with those of other studies [18,21,63,64] who observed in different cities with the same or similar methods and at different times and concluded that approximately 80 % of secretors are in any population but most especially Caucasian population have the secretor (Se) gene or the FUT2 genes which are glycosphingolipids antigens on the surface of a variety of epithelial cells capable of encoding for an enzyme called α (1, 2)-fucosyltransferase that is involved in regulating ABH blood group antigens in saliva and mucosal secretions [14,15,16]. These population of people secrete water-soluble blood group substances in their saliva and other body fluids. Group A secretes A-substances and a small amount of H, group B secretes B (and H)- substances, group O secretes H- substances only, and group AB secretes A, B, and a small amount of H-substances. Recent data from a study by Ali Suleman, et al. [26] indicates that absence of this enzyme resulting in a non-secretor status and is associated with a reduced likelihood of infection after exposure and a greater chance of long-term non-progression [14,15,16,26]. Similarly about 20% of the Caucasian population has a stop mutation in the FUT2 gene (428G→A) that results in the absence of this enzyme and people who do not have this enzyme are referred to as non-secretors, as they cannot express these antigens on their cells or in their secretions and this is in line with the work of [65] carried out in Gwalior, Northern Madhya Pradesh State Central India. Because various bacteria and viruses use attachment to cells as a means of causing infections, it has been determined that some non-secretors have been found to have increased bacterial infections, but a decreased risk in respiratory and gastrointestinal viral infections.

The results in Table 4 shows a frequency distribution of HIV 1 & 2 antibody screening test results amongst secretors & non-secretors. Out of 400 samples 12(3%), 10(2.5%) and 9(2.25%) sera samples tested positive to HIV 1& 2 Determine, Stat-Pak, and Unigold antibody

Table 1. shows frequency distribution of demographic parameters of 400 voluntary apparently healthy prospective blood donors according to turn out rate, level of awareness of HIV infection and gender in Calabar, Nigeria

Groups number	Age Range (Years)	Number of female subjects that turned out		Number of male subjects that turned out		Total number of subjects that turned out		F-ratio	*p*-value
		(f)	(%)	Frequency (f)	(%)	(f)	(%)		*P*=0.05
1	20-25	79	44.98	36	13.59	115	28.75		
2	26-30	51	29.97	100	44.69	151	37.75		
3	31-35	36	20.45	60	26.78	96	24.00		
4	36-40	10	5.68	13	5.80	23	5.75		
5	41-45	00	00	10	4.64	10	2.50		
6	46-50	00	00	5	4.03	5	1.25		
Total (N)		176	44	224	56	400	100	5.125	** .008
Calculated t-value			3.0						
P-value			.012*						

N =total number of samples, frequency =f and percentage =%

**Using t-test there was statistically significant difference between male and female subjects (Calculated t- test =3.0, Degree of freedom (df) =6, alpha value =0.05, t-test critical value =1.943, Right-tail p-value is 0.012, P<0.05) and*

***Using ANOVA there was statistically significant difference between age range and number of groups F-ratio=5.125, Degree of freedom (df) =6, 12, alpha value =0.05, F-test critical value =3.00, Right-tail p-value was 0.008, P<0.05)*

Table 2. shows frequency distribution of voluntary apparently healthy prospective blood donors and their blood samples collected from two recruitment centres according to their gender in Calabar, Nigeria

Gender		*f*	(%)	*f*	(%)	*f*	(%)	X^2	
	Female	160	(39.6)	16	(4.4)	176	(44)		
	Male	200	(50)	24	(6)	224	(56)		
Total (N)		360	(89.6)	40	(10.4)	400	(100)	3.10	**0.013
Calculated Chi Squared X^2		5.825							
***p*-value**		* 0.0158							

**Using Chi Square X^2 test there was statistically significant difference between the numbers of samples collected from the two centers. Calculated Chi Square test (X^2) was =5.825, at degree of freedom (df) =1, Total of sample collected (N) =400, alpha value =0.05, and Chi Square test (X^2) critical value or Table value =3.84. The obtained Chi Square test X^2 value (5.825) was greater than the critical value (3.84) or (X^2 calculated value > X^2 table value) and right-tail p-value is 0.0158 (P<0.05)*

***Using Chi Square X^2 test there was statistically significant difference between the numbers of female samples collected from female and male subject . Calculated Chi Square test (X^2) was =, at degree of freedom (df) =1, Total of sample collected (N) =400, alpha value =0.05, and Chi Square test (X^2) critical value or Table value =3.84. The obtained Chi Square test x2 value (5.825) was greater than the critical value (3.84) or (X^2 calculated value > X^2 table value) and right-tail p-value is 0.0158 (P<0.05)*

****University of Calabar Teaching Hospital (UCTH) Calabar had the highest number of samples*

Table 3. Shows the frequency distribution of secretor status of abh substances typing with prevalence rate results done on the 400 saliva samples collected from apparently healthy prospective voluntary blood donors per gender in Calabar Nigeria

	Number of female subjects		Number of male subjects		Total number of male + female		X^2	*p*-value
Secretor status	***f***	**(%)**	***f***	**(%)**	***f***	**(%)**	5.655	P=0.05
ABH substance non-secretor	39	(9.75%)	41	(10.25%)	80	(20%)		*P*<0.05*
ABH substance secretor	137	(34.25%)	183	(45.75%)	320	(80%)		*P*=0.025
Total recruited at site (N)	176	(44%)	224	(56%)	400	(100%)		

**Significant difference, N= total number of samples collected, f=frequency, %= percentages*

The calculated Chi square ($x^{2)}$ test value was 5.655 at degree of freedom of 1, with 400 as the total number of samples or sample (N) and level of alpha of 0.05. This gives chi square (x^2) critical value of 3.84. The calculated Chi square ($x^{2)}$ test value of 5.655 is less than the chi square (x^2) critical value of 3.84.This gives a P- value of 0.025 at the level of alpha =0.05. There was different significant difference between secretors of ABH substances and non-secretors of ABH substances according gender

Table 4. Shows negative and positive results of the three HIV I & 2 antibody and p24 core antigen screening test kits according to secretor status of ABH substances and gender of study subject in Calabar, Nigeria

Parameters		HIV determine antibody test kit				HIV stat-pak antibody test kit				HIV Uni-gold antibody test Kit				Total			
Secretor status of Abh Substances	Gender	Number of subjects tested Positive		number of subjects tested negative		number of subjects tested positive		number of subjects tested negative		number of subjects tested positive		number of subjects tested negative		tested reactive to both		tested non -reactive to both	
		f	%	f	%	f	%	f	%	f	%	f	%	f	%	f	%
Secretor	Total N=320 (80%)	6	(1.5)	170	(42.5)	7	(1.75)	169	(42.25)	4	(1)	172	(43)	17	(04.25)	303	(75.75)
	Female N=137 (34.25 %)	2	(0.5)	57	14.25	2	0.5	48	(12)	3	(0.75)	129	32.25	7	(1.75)	130	(32.5)
	Male 183 (45.75 %)	4	(1)	113	28.25	5	1.25	121	(30.25)	1	(0.25)	43	10.75	10	(2.5)	173	(43.25)
Non-secretor	Total N=80 (20%)	6	(1.5)	218	(54.5)	3	(.75)	221	(55.25)	5	(1.25)	219	(54.75)	14	(03.25)	66	(17.8)
	Female N=39 (9.75 %)	2	(0.5)	73	(18.25)	2	(0.5)	147	(36.75)	2	(0.50)	88	22	6	(1.39)	33	(8.9)
	Male N=41 (10.25%)	4	(1)	145	(36.25)	1	(0.25)	74	(18.5)	3	(0.75)	131	32.75	8	(1.86)	33	(8.9)
Total (N)	400 (100%)	12	(3)	388	(97)	10	(2.5)	390	(97.5)	9	(2.25)	391	(97.75)	31	(7.50)	369	(92..25)
HIV P24 antigen test	Female	3	.75			2	0.5			1	0.25			6	8.1		
	male	4	1			3	.75			2	0.50			9	2.9		
F -ratio				1.7997													
P-value				0.307													

**There was no statistically significant different between the positive results of the three HIV 1 & 2 antibody screening test kits according to gender despite the disparity in the percentage positivity. (F -ratio = 1.7997, df1 = 2, df2 = 3, F-critical value = 9.55, at alpha value of 0.05, Right-tail p-value is 0.307) (p>0.05)*

***Using t-test there was no statistically significant different between the mean positive results of the three HIV 1 & 2 antibody screening test kits in female and male subjects (Calculated t- test =2.5, Degree of freedom (df) =2, alpha value =0.05, t-test critical value =1.943, Right-tail p-value is s 0.06481, P>0.05)*

Table 5. Shows frequency distribution of 15 reactive samples to HIV P24 core antigen screening test results among secretors & non-secretors of ABH substances of apparently healthy prospective blood donors samples in Calabar, Nigeria

		HIV 1& 2 P24 antigen screening test result							
Parameters		**Non-reactive (Negative)**		**Reactive**	**(Positive)**	**Total**		**X^2**	***P-value***
Secretor status	**Gender**	**f**	**% of P24 in SS**	**f**	**% of P24 in SS**	**f**	**% of P24 in SS**		
Non-secretors of ABH substances	**Total**	76	94.7%	6	8.1%	80	20%	0.007	*P*=0.9
	Female	35	89.7%	4	11.42%	39	9.75%		
	Male	39	95.12%	2	5.1%	41	10.25%		*P*<0.05*
secretor of ABH Substances	**Total**	311	97.2%	9	2.9%	320	80%		
	female	133	97.1%	4	3.0%	137	34.25%		
	male	178	97.26%	5	5.1%	183	45.75%		
Total (N)		387	96.75%	15	3.25%	400	100.0%		

**Significant difference, N= total number of samples collected, f=frequency, %= percentages*

The calculated Chi square (x^2) test value =0.007), at degree of freedom (df) =1, total number of sample or sample size (N) =400), alpha value=0.05, the Chi square critical value =3.84. The obtained Chi square test (x^2) value (0.007) is less than the Chi square critical value (3.84) and P=0.9. The result of Chi square test between Antibody screening test and secretor status shows that there was no significant difference between the antibody screening test and secretor status (P>0.05). Despite the fact that the distribution shows that almost all the positive cases of the antibody screening test

screening test kits respectively. This results are in line with the recommendations of [58]. There were 7 female secretors that tested reactive to both tests , that is 2 from HIV 1& 2 Determine, 2 for Stat-Pak, and 3 for Unigold antibody screening test kits respectively and 10 male secretors that tested reactive to both tests (that is 4 from HIV 1& 2 Determine, 5 from Stat-Pak, and 1 from Unigold antibody screening test kits respectively. On the other hand there were 6 females non-secretors that tested reactive to both test kits that is 2 from HIV 1& 2 Determine, 2 from Stat-Pak, and 2 from Unigold antibody screening test kits respectively and 8 male non-secretors that tested reactive to both tests, that is 4 from HIV 1& 2 Determine, 1 from Stat-Pak, and 3 from Unigold antibody screening test kits respectively). Among the 31 positive antibody tests, 14 were non -secretors and 17 were secretors. The prevalence rate of HIV by the antibody test was almost the same in both secretors 17 (4.25 percent) and non- secretors 14(3.25percent) and consequently the general prevalence rate of 31(7.5 percent). Using ANOVA statistical test there was no statistically significant difference between the positive results of the three HIV 1 & 2 antibody screening test kits according to gender despite the disparity in the percentage positivity (calculated F -ratio = 1.7997, df1 = 2, df2 = 3, F-critical value = 9.55, at alpha value of 0.05, right-tail p-value is 0.307. p>0.05). So, the table 4 shows a similarity of HIV antibody test whatever the secretor status despite the fact that the distribution shows that almost all the positive cases of the antibody screening test are secretors. This agrees with work of [66] carried in Osogbo, Southwestern Nigeria.

The results in Table 5 shows the frequency distribution of P24 antigen screening test results amongst secretors & non secretors of ABH substances. Out of 400 samples , 80 (20%) were non-secretors and 320(80%) secretors, while 6(8.1%) non secretor (that is 4 female non-secretor and 2male non-secretor respectively) and 9(2.9%) secretor samples (that is 4 female secretors and 5male secretors respectively) tested reactive to HIV P24 antigen screening test. The overall prevalence rate of HIV p24 antigen among secretors and non-secretors that were qualified and certified as HIV seronegative apparently healthy prospective blood donors by antibody-based test who were enrolled in our study sites at the University of Calabar Teaching Hospital and General Hospital Calabar was 15(3.25%). Chi-Squared (X^2) showed significant difference between secretor status and gender. (The calculated Chi square (x^2) test value =0.007, at degree of freedom (df) =1, total number of sample or sample size (N) =400), alpha value=0.05, the Chi Squared critical value =3.84, p=0.9). There was no statistically significant difference between HIV p24 antigen status and secretor status (P>0.05). In this case, secretor status doesn't influence the result of P24 test as shown by the data. These results agree with that of [67,68] who carried out a study in Sokoto, North Western Nigeria. The HIV p24 antigen test has become a vital assay used for the investigation of viral antigen in persons declared HIV seronegative by the HIV antibody-based assay [69,70].

5. CONCLUSION

Based on the findings of this study there was no statistically significant association between the secretor status of ABH substances and HIV 1 &2 P24 antigen screening status amongst apparently healthy prospective blood donors screened as HIV 1 and 2 antibody sero-negative status in Calabar. However HIV 1&2 P24 antigens were detectable more in secretors than non-secretors.

6. AVAILABILITY OF DATA AND MATERIALS

Datasets generated and analyzed in this study are available from the corresponding author on request.

CONSENT AND ETHICS APPROVAL

This study was approved by Health Research Ethical Committee (HREC) of the University of Calabar Teaching Hospital. Oral informed consent was obtained from the participants and same was approved by the ethics committee. These were sought and obtained from the management of the Ethical Committee, University of Calabar Teaching Hospital, Calabar, Cross Rivers State Nigeria and the Research Ethical Committee, Centre for Clinical Governance, Research & Training Ministry of Health Calabar, and Cross Rivers State, Nigeria. These were also sought and obtained from each donor before inclusion in the study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/62481