Premarital Screening of HIV, Haemoglobin Genotype, ABO and Rhesus Blood Group among Intending Couples in Yenagoa, Nigeria

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Abstract

Background
Premarital screening is fast gaining ground as a prerequisite for the solemnization of holy matrimony by many faith-based organizations in Nigeria, yet there is no literature on the subject in Yenagoa, Bayelsa State.

Methods
One hundred and five (105) premarital heterosexual couples (105 males and 105 females) were screened for the presence of human immunodeficiency virus (HIV), haemoglobin genotypes, and ABO and Rhesus blood groups using standard laboratory procedures.

Results
The prevalence of HIV in this study was found to be 1.0%. The frequencies of the HbAA (74%), HbAS (24%), HbAC (1.5%), HbSS (0.5%) while that of ABO and Rh blood groups were: group A (21%), B (25%), AB (6.0%), 0 (48%), Rh ‘D’ positive (97%) and Rh ‘D’ negative (3.0%). The distribution of these variables between the sexes was statistically significant (χ²=25.68, p<0.01). The frequencies of the haemoglobin genotype combinations of the intending couples were as follows: HbAA/AA (55%), AA/AS (33%), AS/AS (8.0%), AA/AC (2.0%), AS/AC (1.0%) and AS/SS (1.0%). This distribution pattern was also found to be highly statistically significant (χ² = 24.459, p < 0.0001). The mean age of the participants was 31.91 ± 4.80 years for male and 26.105 ± 4.29 years for female (t = 3.272, p < 0.05).

Conclusion
This study observed a low prevalence of HIV among the premarital couples and a relatively high frequency of HbAS/AS couples. The ABO and Rh blood groups were found to be stable and consistent with previous reports. For the prevention of hereditary blood diseases, haemolytic disease of the newborn and transmission of HIV to offspring, premarital testing should be encouraged.

Keywords: Human immunodeficiency Virus(HIV), Haemoglobin Genotype, ABO And Rhesus Blood Group.

Introduction
A premarital test is defined as a test in which intending couples are tested for genetic, infectious and blood-transmitted diseases to prevent any risk of transmitting the disease to their children. It provides the baseline assessment of prospective marriage couples with the aim of reducing the unproductive genetic risk and also reduces the incidence of babies born with common haemoglobinopathies and infectious diseases (Sharaf.,2006). One of the biggest health challenges threatening the human race in recent times is the HIV/AIDS pandemic. The disease has continued to be in the front burner for many years now, despite initial denials and cover-ups by some countries. UNAIDS estimated that globally, there were 33 million [30 million–36 million] people living with HIV in 2007. Overall, 2.0 million [1.8million–2.3 million] people died due to AIDS in 2007, compared with an estimated 1.7 million [1.5million–2.3 million] in 2001. Sub-Saharan Africa with just over 10 percent of the world’s population has the greatest burden of this disease. It is estimated that close to two thirds of all people living with HIV are in sub Saharan Africa with South Africa having about 5.3m million people living with HIV/AIDS-the largest in Africa (UNAIDS, 2008b).

The HIV/AIDS pandemic has significantly interfered with the gains which has been made in the area of health and development in many African countries over the years and has also reduced the quality of life. According to UNAID with AIDS claiming so many people’s lives, Nigeria’s life expectancy has declined (UNAIDS, 2008b). In 1991, the average life expectancy was 53.8 years for women and 52.6 years for men. In 2007, these figures have fallen to 46 for women and 47 for men (Spink, G. 2009). There is therefore a disparity between what ought to be and what is happening presently as regards the situation of HIV/AIDS in Nigeria (FMOH, 2006c. 2005).

The disparity between what should have been and what will exist will worsen further if the HIV prevalence is allowed to rise further and the epidemic is not adequately controlled. A particular response which has attracted a lot of controversies is mandatory pre-marital HIV testing. Mandatory premarital HIV testing...
refers to the requirement of an HIV test as a condition for entering into marriage. The practice of mandatory pre-marital HIV testing which originated from the states of Louisiana and Illinois in the United States has also been documented in Nigeria, Democratic Republic of Congo (DRC), Ghana, Burundi and Uganda (Open Society Institute, 2008).

The Open Society Institute (2008) stated that in the late 1990s, Orthodox and Pentecostal churches began to require a mandatory premarital HIV test for those who wish to marry in the church. Mandatory premarital HIV testing, in spite of the opposition has continued to gain ground among intending couples. The 2005 National HIV/AIDS and Reproductive Health Survey showed that the number of people who desired HIV testing to fulfill mandatory marriage requirement rose to 3.0% from 2.4% in the 2003 survey. Furthermore, a study conducted among health care workers in North-central Nigeria revealed the support for the enforcement of pre-marital HIV testing for intending couples (Musa, O. I. 2005). Orthodox churches such as the Anglican and the Baptist Churches as well as Pentecostal Churches such as Deeper Life Bible Church, Watchman Catholic Charismatic and Assemblies of God Church are some of the churches in Nigeria that have instituted mandatory pre-marital HIV testing. While the Baptist Church stated that the intention is to prevent HIV infection, rather than punish those living with the virus, the Anglican Church instituted the test to help couples make more informed choices when choosing marriage partners (Eyoboka, S. 2004). Similarly, a report by the Centre for the Right to Health, 2003 indicated the position of one of the Pentecostal churches where this policy is in place that there was no need to continue marriage with somebody who already has a death sentence. In this case, the HIV infection is the death sentence. The human rights violation inherent in the practice, the limited population it targets as well as the belief that it is not cost-effective. HIV prevention and control measure has generated a lot of concerns particularly among human rights groups, government and nongovernmental organizations who are into HIV/AIDS-related issues. According to Gruskin, Roseman and Ferguson, requiring individuals to submit to a pre-marital HIV test before being allowed to marry raises many of the human rights concerns such as voluntariness, privacy and confidentiality as well as appropriate counseling.

In Nigeria, studies conducted to determine the prevalence of HIV infection among intending couples referred from religious organizations for pre-marital HIV testing showed a relatively high HIV prevalence among intending couples. This may seem to justify the rationale for the institution of mandatory pre-marital HIV testing by some religious organizations. Furthermore, the issue of mandatory pre-marital HIV testing affects them especially the larger proportion who are unmarried.

Non-communicable genetic diseases such as sickle cell disease is a cause of morbidity and mortality. Haemoglobin S (HbS) differs from normal haemoglobin (HbA) because it has a valine in place of a glutamic acid in position number six of the beta chain of the globins’ molecule. When the availability of oxygen is reduced, the erythrocytes containing haemoglobin S change from biconcave to sickle-shaped cells. (Tamarin, 2002). There are several common forms of sickle cell disease. These are called SS (individuals inherit one sickle cell gene from each parent), SC (the child inherits one sickle cell gene and one gene for another abnormal type of haemoglobin called “C”). The clinical course of sickle cell disease is extremely variable (Platt et al., 1991). Some patients have nearly no symptoms. Others are severely incapacitated (Bray et al., 1994).

The ABO and Rh blood groups are among the most important blood groups (Seeley et al., 1998). Karl Landsteiner first described the ABO blood group in 1900 and it served the beginning of blood banking and transfusion medicine (Ali et al., 2005). Even after 100 years, the single most important test performed in blood banking services is determination of ABO blood groups to avoid morbidity and mortality (Honig and Bore, 1980). In the ABO blood group, individuals are divided into four major blood groups, A, B, AB and O, according to the presence of the antigens and agglutinins. Type A blood has type A antigens, type B blood has type B antigens, type AB blood has both types of antigens, and type O blood has neither A nor B antigens. In addition, plasma from type A blood contains type B antibodies, which act against type B antigens, whereas plasma from type B blood contains type A antibodies, which act against type A antigens. Type AB has neither type of antibody and type O blood has both A and B antibodies (Seeley et al., 1998).

Furthermore, the presence of Rhesus system was recognized in 1939 and it was confirmed within few years (Landsteiner and Weiner, 1940). Rhesus system emerged as second most important blood group system due to haemolytic disease of newborn and its importance in Rhesus D negative individuals in subsequent transfusions once they develop Rh antibodies (Dennis et al., 1998). People are positive if they have a certain Rh antigen (the D antigen) on the surface of their erythrocytes, and people are Rh–negative if they do not have this Rh antigen. Rhesus incompatibility can pose a major problem in some pregnancies when the mother is Rh–negative and the foetus is Rh–positive (Avent, 1999). If foetal blood leaks through the placenta and mixes with the mother’s blood, the mother becomes sensitized to the Rh antigen. The mother produces Rh antibodies that cross the placenta and cause agglutination and haemolysis of foetal erythrocytes. This disorder is called Haemolytic disease of the newborn (HDN), or erythroblastosis fetalis, and it may be fatal to the foetus (Dennis et al., 1998).

Although premarital screening by faith-based organizations has been going on for about five years, few
published reports on the process are to be found in southern Nigeria (Akani et al., 2005, Umeora et al., 2005 and Jeremiah et al., 2007) and the few publications that are available centre mainly on HIV and premarital couples, with little or no regard for other genetic and infectious diseases. Youths constitute a substantial proportion of the population in Nigeria and have been identified as one of the sub-populations driving the spread of HIV and genetic disease in Nigeria. In this report, we present Premarital Screening of HIV, Haemoglobin Genotype, ABO and Rhesus Blood Group among Intending Couples in Yenagoa, Nigeria on the basis of the faith-based referral approach and also to assess the awareness of mandatory premarital screening among unmarried youths.

MATERIALS AND METHOD

SETTING

The study population consisted of two hundred and ten (210) premarital men and women (i.e. 105 couples) who attended the voluntary premarital counseling of some religious organizations and who were referred to the medical laboratory for premarital testing. Institutional ethical approval was received prior to the commencement of the study. All the subjects were offered confidential pre-test HIV and genetic counseling by the marriage committee, headed by a medical officer, and thereafter informed consent was obtained for blood sample collection. The researchers returned the results of the tests directly to the marriage committee. The marriage committee then disclosed the results of the tests to the intending couples during their counseling sessions. The stages involved in the counseling process fall beyond the scope of this study. All the participants were apparently healthy individuals with no clinical evidence of any disease. The mean age of the males was 26.11± 4.29 years, while that of the females was 26.11± 4.29 years. All laboratory tests were performed at the Laboratory department of Gloryland Medical Centre, Yenagoa, Bayelsa State. Health care services in Bayelsa state are poorly provided and absence of basic primary health care facilities in the rural areas has created a situation where those who could afford, normally seek medical attention at Yenagoa where this hospital is situated. This is the reason for the choice of the hospital as the research base for the study.

COLLECTION OF BLOOD SAMPLES

Approximately four milliliters (4ml) of venous blood were collected using standard venipuncture techniques. Two milliliters was dispensed into potassium ethylenediaminetetracetic acid (EDTA) salt container and used for blood grouping and genotype, while the remaining 2ml was dispensed into a plain container and allowed to clot. It was used for determining the presence of human immunodeficiency virus (HIV) using standard techniques. The institutional Ethical committee approved the study. All the participants gave their consents and willingly presented themselves at the Medical Laboratory for sample collection.

HUMAN IMMUNODEFICIENCY VIRUS (HIV) TESTING

The antibodies to human immunodeficiency virus (HIV) in the serum of the intending couples were tested using a double ELISA test: determine TM HIV 1 & 2 (Abbott, London) and Stat Pak HIV 1 & 2 Bispot (Orgenics, Israel). The tests were performed in accordance with the manufacturers’ standard operating procedures. HIV seropositivity was defined as a reactive result on both ELISA tests, as recommended by WHO/UNAIDS for asymptomatic patients. The positive samples tested for HIV-1 only.

BLOOD HAEMOGLOBIN GENOTYPE TEST

For the study of the blood haemoglobin genotype, cellulose acetate electrophoresis technique was used to determine haemoglobin genotype. The method described by Brown was used for haemoglobin electrophoresis. A small quantity of haemolysate of venous blood from each of the subjects was placed on the cellulose acetate membrane and carefully introduced into the electrophoresis tank containing Tris - EDTA - Borate buffer at pH 8.6. The electrophoresis was then allowed to run for 15 – 20 minutes at an electromotive force (emf) of 160 V. The results were read immediately. Haemolysates from blood samples of known haemoglobin (i.e. AA, AS, AC) were run as controls.

ABO AND RH BLOOD GROUPS TESTS

Red cell phenotyping was carried out with standard tube techniques as described by Judd and Brecher. For ABO blood grouping, a drop of anti-A, anti-B, and anti-AB (Atlas Medical, Cambridge, UK) each was placed in clean test tubes labeled 1,2,3. To each tube was added a drop of 5% red blood cell suspension in saline. The contents were gently mixed together and centrifuged for 30 seconds at 1000g. The cell buttons were resuspended and observed for agglutination. Agglutination of tested red cells constituted positive results. A smooth cell suspension after resuspension followed by a microscopic confirmation constituted negative test results.

For Rhesus D typing, a drop of anti-D serum (Atlas Medical, Cambridge, UK) was placed in a clean labeled test tube and a drop of control placed in a second tube. 1 drop of 5% RBC suspension in saline was then added and incubated at 37°C for 30mins. At the end of the incubation period, the contents of the tube were
mixed gently and centrifuged for 30 seconds at 1000g. Agglutination was read macroscopically and microscopically in doubtful cases. All negative results were confirmed using the indirect anti globulin test (IAT) procedure (also for confirmation of weak D).

Statistical analysis
The data were analyzed using the Statistical Package for Social Sciences (SPSS) for Windows (version 17.0). The frequency distributions of the ABO, Rh and haemoglobin genotypes and HIV prevalence were analyzed using the chi-square ($\chi^2$) test, while the mean age of the study participants was computed using the one-tailed t-test. The statistically significant level was set at alpha 0.05 ($p < 0.05$).

RESULTS
Premarital screening tests were performed on 105 couples (105 males and 105 females) as directed by the marriage committee of the referral churches and with the advice of a medical officer. The frequencies of the variables are shown in Table I. The following results were obtained: group A (21%), B (25%), AB (6%), O(48%), Rh ‘D’ positive (97%) and Rh ‘D’ negative (3.0%) . HbAA (74%), HbAS (24%), HbAC (1.5%) and HbSS(0.5). The prevalence of HIV in this study was found to be 1.0%. The distribution of the variables between the sexes was subjected to a chi-square ($\chi^2$) test and found to be significant ($\chi^2 = 25.68, p< 0.01$).

Table II shows the distribution of the genotype combinations among the intending couples. The following results were obtained: AA/AA (55%), AA/AS (33%), AS/AS (8.0%), AA/AC (2.0%), AS/AC (1.0%) and AS/SS(1.0%). This distribution pattern was also found to be highly statistically significant ($\chi^2 = 24.459, p < 0.0001$). The mean age of the premarital couples was shown in Table III. The mean age of the male participants was 31.91 ± 4.80 years and female was 26.12±4.29. This difference was found to be statistically significant when subjected to a t-test ($t = 3.2719, p < 0.05$). The difference between the observed and expected values of the haemoglobin genotypes among premarital male and female participants was subjected to chi-square ($\chi^2$) analysis, as shown in Table IV. There was a statistically significant difference in values in relation to the sex of the participants ($\chi^2 = 25.68, p < 0.01$).
TABLE III: MEAN AGE OF STUDY POPULATION

<table>
<thead>
<tr>
<th>t- test</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n =105</td>
<td>n=105</td>
</tr>
<tr>
<td>MEAN (YEARS)</td>
<td>31.91</td>
<td>26.105</td>
</tr>
<tr>
<td>SD</td>
<td>4.80</td>
<td>4.29</td>
</tr>
<tr>
<td>t-stat</td>
<td>3.2719*</td>
<td></td>
</tr>
<tr>
<td>t-critical (one tail)</td>
<td>1.96</td>
<td></td>
</tr>
</tbody>
</table>

* = P ≤ 0.05 (significant)

n = number of samples.

TABLE IV: SEX DIFFERENCE IN THE OBSERVED AND EXPECTED COUNTS OF THE HAEMOGLOBIN GENOTYPE STUDY POPULATION.

<table>
<thead>
<tr>
<th>HAEMOGLOBIN GENOTYPE</th>
<th>MALE (%)</th>
<th>FEMALE(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OBSERVED</td>
<td>EXPECTED</td>
</tr>
<tr>
<td>HbAA</td>
<td>39</td>
<td>19.7</td>
</tr>
<tr>
<td>HbAS</td>
<td>11</td>
<td>5.6</td>
</tr>
<tr>
<td>HbAC</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>HbSS</td>
<td>0.5</td>
<td>0.25</td>
</tr>
</tbody>
</table>

Chi-square ($\chi^2$) test = 25.68*

* = p < 0.01 (significant)

Discussion

Since the first case of AIDS was reported in Nigeria in 1986, over 3 million people have been infected with the virus. The following trend in the prevalence of HIV in Nigeria has been reported: 1.8% in 1991, 3.8% in 1993, 4.5% in 1996, 5.4% in 1999, 5.8% in 2001 and 5.0% in 2003 (FMOH, 2003). Similarly, an HIV prevalence of 6.6% and 4.1% were reported among antenatal women in Rivers State and Bayelsa State respectively (FMOH, 2003 and Buseri et al., 2010). However, no reports on premarital HIV testing demanded by faith-based organizations could be found in Bayelsa State, Nigeria. The earliest report was given in Southeast, Nigeria with the following prevalence among intending couples by faith-based organization: 4.6% in 2000, 3.7% in 2001, 6.5% in 2002, 9.3% in 2003, 5.2% in 2004 (Umeora et al., 2005). An alarming rate of 27.4% HIV prevalence was recorded in Port Harcourt, River State (Akani et al., 2005), followed by another report of 2.0% prevalence after two(2) years later in Port Harcourt, River State (Jeremiah et al., 2007). Contrastingly, an HIV prevalence of 1.0% was recorded among premarital couples in this study, and this 1.0% prevalence was only in a male. The high prevalence recorded by Akani et al., 2005, is comparable only to reports from some parts of South Africa, where greater than 30% was observed among pregnant women. A similarly high prevalence of 25.6% was recorded for 621 South Malawian pregnant women (Verhoeff et al., 1999). A study recorded a prevalence of as low as 3.6% among unemployed females in Port Harcourt (Ejele et al., 2005). The present study is in agreement with a study by Peterson and White, in which the HIV prevalence among female and male premarital couples in the United States of America was found to be 0.0 to 0.4% and 0.0 to 1.1% respectively (Peterson et al., 1990).

This study also confirms the report by UNAIDS that HIV infection is much more common among men in 1997 (estimated at 60% versus 20% in women).

Sickle cell anaemia is a common phenomenon in Africa. Earlier reports on haemoglobinopathies in Nigeria centred mostly on pregnant women, children and the general population (Nwafor et al., 2001, Bakare et al., 2004; Jeremiah et al., 2005), but there is a few information on the haemoglobinopathies among premarital couples. In this study, the overall frequencies of the haemoglobin genotypes were as follows: HbAA (74%), HbAS (24%), HbAC (1.5%), HbSS (0.5%). This report was similar to earlier reports by Nwafor et al., 2001 and Jeremiah et al., 2005 in which HbAA accounted for 73% and 72% respectively while HbAS, HbAC accounted...
for 26% and 2% respectively (Jeremiah et al., 2005). These values, except those for HbSS, were found to be consistent with the values obtained in this study.

Inherited haemoglobin disorder (haemoglobinopathy) is predominantly single-gene autosomal recessive disorder that results in production of structurally abnormal haemoglobin variants (sickle-cell disease) (Milunsky et al., 2010). Sickle-cell disease affect as much as 5% of the world's population, constituting a major public health problem in certain parts of the world including Africa (WHO. Fact sheet, 2006). Sickle cell disease (SCD) has remained an unresolved health problem in Nigeria, as in most of sub-Saharan Africa. Despite the large and increasing burden of the disease, little has been done to reduce it, perhaps because of a lack of political will, or a lack of sufficient funds due to the deteriorating economy. In spite of this, the number of HbSS patients who survive to adulthood in Nigeria is increasing, especially among the elites, who have access to good health care. This is not surprising, as research has shown that socioeconomic status has a modifying influence on morbidity in sickle cell disease (Okany et al., 1993). With more HbSS patients surviving into adulthood, the chances of transferring the gene to their offspring through marriage becomes greater. This is even more reason why premartial screening for haemoglobinopathies should be encouraged in order to offer the intending couples the opportunities to know their haemoglobin genotype status and also to weigh the disadvantages before marriage consumation.

An analysis of the haemoglobin genotype combination among intending couples revealed a highly statistically significant pattern ($\chi^2 = 24.459$, $p < 0.0001$). Fifty-five per cent (55%) of the couples were AA/AA, 33% were AA/AS, while AS/AS, AA/AC, AS/AC and AS/SS accounted for 8.0%, 2.0% and 1.0% respectively. For a single AS/AS combination, there is a one in four chance of having a sickle cell anemia. This implies that 8.0% of the couples in this study population will produce about four homozygous HbSS children (ratio of 1:25). This ratio is compared with the general population, then the frequency of Hb AS/AS couples obtained in this study could be regarded as high. When subjected to chi-square analysis, the observed and expected count of the inherited genes between sexes showed a statistically significant pattern ($\chi^2 = 25.68$, $p < 0.01$). This implies that, in future, there will be a modulation of genes, with a tendency for HbAS to increase in the female population. However, gene frequencies were not calculated using the Hardy-Weinberg equation, so it is difficult to state categorically how the shift in the Hardy-Weinberg equilibrium will affect the gene flow in the population. A detailed study with a larger sample size will be needed to offer explanations for this observation.

The distribution of the ABO and Rh blood groups among the premartial couples in this study was as follows: group A (21%), group B (25%), group AB (6%) and Group O (48%). Rh ‘D’ positive accounted for 97%, while 3% of the study population was Rh ‘D’ negative. The distribution of these variables between the sexes was statistically significant ($\chi^2 = 25.68$, $p < 0.01$). These results are not in agreement with previous reports in the literature that occurred in this order O> A >B (Jeremiah et al., 2007, Iyiola et al 2011) rather it follow such order O>B>A. The clinical significance of testing for blood groups is maternal alloimmunisation, which occurs when a woman’s immune system is sensitized to foreign erythrocyte surface antigens, stimulating the production of antibodies the most common route being blood transfusion, feotal maternal haemorrhage associated with delivery, trauma, spontaneous or induced abortion, ectopic pregnancy or invasive obstetrical procedures. In the event of alloimmunization during pregnancy, these antibodies can cross the placenta and, if the foetus is positive for erythrocyte surface antigens, lead to haemolytic of feotal erythrocytes and anaemia, a condition known as haemolytic disease of the newborn (HDN) (Belmar et al., 2005). HDN can also be caused by maternal anti-A, anti-B, anti-AB IgG isoagglutinins, which are mostly present in type O mothers. Although a type O mother of a type A or B infant occurs in 15% of pregnancies, the frequency of HDN due to ABO isoagglutinins is only one in 150 births (Belmar et al., 2005).

Premarital screening can potentially reduce the burden of inherited haemoglobin diseases by reducing the number of high-risk marriages (Alswardi et al., 2009, Meyer et al., 2005). This was proven to be beneficial in nearby countries with similar endemicity levels of haemoglobinopathies (Karimi et al., 2007 Keskin., 2000 Al-Arrayed, 1997). Because of the burden on the healthcare system and effect on the quality of life in patients with sickle-cell disease, premartial genetic screening was mandated in Saudi Arabia in 2004 (El-Hazmi, 2006). All couples with marriage proposals have to be tested for both diseases and get the appropriate counseling (if required) before completing their marriage proposal.

CONCLUSION

The prevention of hereditary blood diseases and the spread of the overwhelming AIDS pandemic in sub-Saharan Africa are possible by following the same steps of Saudi Arabian government by legalizing premartial screening in all the African countries thereby making it compulsory irrespective of the religious background. Increasing the number of specialized health care reception clinics to cover all the rural and Urban areas. Ministry of health in African countries may enhance the effects of counseling by encouraging couples to seek testing earlier in the marriage process, engage religious figures in counseling, include program information in high school curriculum, allow singles to voluntarily seek genetic testing, and finally augment the help of community figures.
to publicize the program in media and religious gatherings. However, it involves collective effort of individuals and organizations, including churches and government.

References


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