Original Article

Hepatitis B, Human Immunodeficiency Virus and Hepatitis C Virus Infections among Marriage Intending Couples in Calabar, Nigeria

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Premarital testing for genotypes, blood group, hepatitis B surface antigen (HBsAg) and human immunodeficiency virus (HIV) is compulsory for marriage intending couples in some faith based organizations in South southern Nigeria. **These tests are important for the prevention of genetic disorders and some infectious diseases. Objectives**: **This prospective study was designed to establish the infection rates of HBV, HIV and HCV among prospective spouses attending marriage counseling in churches in Calabar. Methods: The study ran from January 2010 to December 2014. Informed consent was obtained from the 240 intending couples. Data for demography and medical history were collected through oral interview. Sera from patients were tested for HBsAg with the rapid chromatographic immunoassay one step lateral flow kit (Acumen labs and diagnostic centre, Bangalore, India). Anti HCV was tested with one step lateral flow rapid chromatographic immunoassay (ABON Biopharm). Determine kits (Abbot, Japan) were used for screening subjects for HIV 1/2. Results: Out of the 240 subjects screened, 14(5.8%) were positive for HIV, HBsAg and HCV infection. All the subjects positive for HBV 8(3.3%) were HIV negative, while all the HIV positive subjects were HBV negative. There was no co-infection between HIV and HBV ($\chi^2 = 1.7$, $p = 0.19$). Infection rates peaked at age 26-35 years 10(71.4%) with 4.0% of females positive for HBV. Conclusion: Results from this study may provide some epidemiological evidence for policy making for mandatory premarital testing. A break in transmission of hepatitis B virus infection may occur as a result of vaccination of negative partners.**

Key words: HBV, HIV, HCV, infections, Premarital screening

Introduction

Background

Premarital screening is an important examination carried out to detect viral infections and genetic blood diseases (e.g. sickle cell anemia and thalassemia). These tests are important for the control and prevention of genetic disorders, and some infectious diseases. The end result is universal health in families [1]. Mandatory premarital testing is a requirement and a condition for entering into marriage [2]. Although this is beginning to gain acceptance in the Nation, some couples see it as an economic waste because of the cost implication while others are appreciative of the knowledge imparted during the pre and post test counseling.

Premarital testing for genotypes, blood group, hepatitis B surface antigen (HBsAg) and human immunodeficiency virus (HIV) is compulsory for marriage intending couples in some faith based organizations in south southern Nigeria. This is because sexual intercourse is an important route of transmission for hepatitis B virus (HBV), hepatitis C virus (HCV) and HIV infections. The

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determination of a carrier status during premarital testing will create awareness between the couples, lead to the protection of the prospective spouse by early vaccination which is imperative [3].

HIV and HBV infection rates are higher in the developing world especially Africa and Asia. About 25 million people are infected with HIV while another 50 million are HBV positive in sub-Saharan Africa [4]. Despite this alarming statistics, data on the infection rates among the young adults in our locality is sparse.

Nigeria has a high burden of HBV infection. The prevalence in many parts of Nigeria is higher than 8% [5-6]. A declined in HBV infection has been reported over the years in most developed economies due to the implementation of the World Health Assembly resolution of provision of life-saving HBV vaccination [7].

The serological indicator of HBV infection is HBsAg which appears 1-10 weeks after acute exposure to HBV in patient's serum [8]. Chronic HBV infection which is associated with viraemia occurs when HBsAg persist for more than 6 months in the serum.The transmission route for HIV and HBV is similar, this includes; sexual intercourse, vertical and horizontal routes from infected mothers to their children. Therefore premarital testing of intending couples prior to marriage for carrier status is critical before consumation of marriage [9-10].

Several immunological methods are available to detect HBsAg, enzyme immunoassays (EIA) and radioimmunoassay (RIA) are more sensitive methods than immunochromatographic assays (ICA) and Haemaglutination assays. EIA methods are generally used by reference laboratories and blood banks because of its accuracy, low cost and safety when compared to RIA methods [11-12].

Rapid diagnostic tests based on immunochromatographic principles are widely used in most developing countries, for the detection of HBsAg. These methods are considerably cheaper than EIA methods, less time consuming and does not require expert training. However, the ICAs may not be accurate enough to detect acute or chronic HBV infections, as both have serious consequences in HBV infection control [13].

The control of HIV/AIDS in Nigeria is carried out by government agencies, non-governmental agencies and religious bodies [14]. Studies conducted on intending couples from religious organizations for HIV infection has showed a relatively high HIV prevalence [15-16]. There is no baseline data on the distribution of HBV, HCV and HIV among intending couples in Calabar. This prospective study was designed to establish the infection rates of HBV, HIV and HCV among prospective spouses attending marriage counselling in churches in Calabar, Cross River State.

Materials and Methods

Cross River state is located in the South southern part of Nigeria with a population of 1.2 million residents and a land mass of about 20,000km^2. The natives of the city are the Efiks and the Quas, but since it is a city with lots of economic and social potential, people from all tribes in Nigeria are found here together with people from all continents of the world. The main occupation of the indigenous people is civil service and fishing. They are predominantly Christians [16-17].

The laboratory diagnosis was conducted in the Microbiology/Parasitology Department of the University of Calabar Teaching Hospital, Calabar. The hospital is a 2000 bed space tertiary institution, attended by people resident in Calabar metropolis, neighboring Local Government areas and States.

The study included 240 subjects, out of which 116 were intending couples alongside 8 female subjects whose male partners were in different locations in the country. All the subjects were attending marriage counseling in churches and were asked to undergo premarital testing. The male partners of the 8 female subjects underwent counseling and premedical testing in their various locations. The study ran from January 2010 to December 2014. All the subjects enrolled gave their informed consent to participate and to have their blood samples tested. Data for demography and medical history was collected through oral interview with the subjects.

Five millilitres of whole blood was collected using a sterile syringe into a plane container. Samples were allowed to clot and centrifuged at 1000rpm for five minutes. The serum was separated and used for the various tests. Sera from patients were tested for HBsAg with the rapid chromatographic immunoassay one step lateral flow kit (Acumen labs and diagnostic centre, Bangalore, India). Anti HCV was tested with one step lateral flow rapid chromatographic immunoassay (ABON Biopharm) and Determine kits (Abbot, Japan) was used for screening subjects for HIV 1/2.

Data analyses were performed using the Epi-info CDC, 2012 Package. Interactions between specific categorical clinical variables were tested for significance using the Chi-square corrected (Yates) with two tailed

p. A p-value ≤ 0.05 was considered statistically significant.

Limitations of the study

The rapid chromatographic immunoassay one step lateral flow kit for HBsAg detection are invitro qualitative methods. The quantitative value nor the rate of increase in the concentration of HBsAg cannot be determined by this test. The test indicates the presence of HBsAg in the specimen and should not be used as the sole criteria for the diagnosis of Hepatitis B viral infection. As with all diagnostic tests, all results must be considered with other clinical information available to the physician. A negative result at any time does not preclude the possibility of Hepatitis B Virus infection.

Results

Males and females were in the age range of 26 to 55 years and 16 to 55 years respectively. The mean age for males and females was 34.29±4.68 and 28.52±4.37 with a minimum age of 25 and 19 years respectively. The mean age for all the subjects was 31.31± 5.35, with a maximum age of 51 and 46 years for males and females respectively (Table 1). The number of females screened were more than that of the males because some of their partners were in another city or state of the Federation and had a test conducted where ever they were domicile.

Table 2 shows the the prevalence of HBV by the HIV status of subjects. All the subjects positive for HBV infection 8(3.3%) were HIV negative, while all the HIV positive subjects were HBV negative. There was no co-infection between HIV and HBV (χ^2 =1.7, p =0.19).

Table 1 Demographic characteristics of subjects

Age (years)	Female (%)	Male (%)	Total
16-25	27(21.7)	0(0.0)	27(11.3)
26-35	93(75.0)	80(69.0)	172(71.7)
36-45	3(2.4)	32(27.6)	35(14.6)
46-55	1(0.8)	4(3.5)	5(2.1)
Mean ± SD	28.52±4.37	34.29±4.68	31.31±5.35
Total	124(51.7)	116(48.3)	240

Table 2 Prevalence of HBsAg by HIV status of subjects

HIV status	HBsAg status No (%) infected		Total	Statistics
	Positive	Negative		
Positive	0(0.0)	3(100)	3(1.3)	χ^2 = 1.7
Negative	8(3.4)	229(96.6)	237(98.7)	P =0.19
Total	8(3.3)	232(96.7)	240	F = 0.90

Out of the 240 subjects screened, 14(5.8%) were positive for at least one of HIV, HBV and HCV infection, 64.3% were females while 35.7% were males. Although all the 3 subjects infected with HIV were males, gender seem not to have influenced HIV infection status (χ^2 = 1.5, p = 0.22). On the other hand, 87.5% of the subjects with hepatitis B virus were females and there was a statistically significant influence of gender on hepatitis infection (Table 3).

Table 4 shows the distribution of infections by age of couples. Infection rates peaked at age 26-35 years (7/14, 50.0%). Two, (2/172, 1.2%) couples were both infected with HBV. One male (1/79, 1.3%) had HBV/HCV co-infection.

Discussion

In many developing countries, including Nigeria, immunochromatic assay (ICA) based rapid diagnostic tests are widely used to detect HBsAg for both diagnosis and screening of acute and chronic HBV infections. Ideally, screening should be done using more advanced and accurate methods such as enzyme immunoassays (EIAs). Although this study was carried out in the hospital laboratory, ICA was used instead of EIAs because ELISA machine was not available for diagnostic purposes in our laboratory.

Table 3 Distribution of HIV, HBsAg and HCV among subjects by gender

Viral antigens/antibodies	Females (n=124)	Males (n=116)	Total (n=240)	Statistics
HIV	0(0.0)	3(100.0)	3(1.25)	$\chi^2 = 1.5$, p = 0.22
HBsAg	7(87.5)	1(12.5)	8(3.33)	$\chi^2 = 2.9$, p = 0.08
HCV	2(66.7)	1(33.3)	3(1.25)	$\chi^2 = 0.003$, p = 0.95
Total	9(64.3)	5(35.7)	14(5.8)	

Table 4 Distribution of infections among couples by age and gender

Age (years)	*Types of infection/couple affected (n=240) No. (%) infected*									
	No examined		*Both couple with HBV*	*Female with HBV*	*Female with HCV*	*Male with HCV*	*Male with HBV & HCV*	*Male with HIV*	*Male with HBV*	*Total*	*Statistics*
	M	*F*									
16-25	*1*	*27*	*0(0.0)*	*0(0.0)*	*1(3.7)*	*0(0.0)*	*0(0.0)*	*0(0.0)*	*0(0.0)*	*1(7.1)*	
26-35	*79*	*93*	*1(1.2)*	*5(5.4)*	*0(0.0)*	*1(1.3)*	*1(1.3)*	*1(1.3)*	*0(0.0)*	*10(71.4)*	$\chi^2 = 31.0$
36-45	*32*	*3*	*0(0.0)*	*0(0.0)*	*0(0.0)*	*0(0.0)*	*0(0.0)*	*0(0.0)*	*1(3.1)*	*1(7.1)*	*p = 0.03*
46-55	*4*	*1*	*0(0.0)*	*0(0.0)*	*0(0.0)*	*0(0.0)*	*0(0.0)*	*2(50)*	*0(0.0)*	*2(14.3)*	
Total	*116*	*124*	*1(0.4)*	*5(4.0)*	*1(0.8)*	*1(0.9)*	*1(0.9)*	*3(2.6)*	*1(0.9)*	*14*	

In Nigeria, the prevalence of HBV increases as one migrates from the South to the Northern part of the country, the reason for this is not yet documented [18]. In this study HBV infection rates among subjects who were both HIV-infected and uninfected was 3.3%. This is close to the 4.98% recorded in Port Harcourt, South-south Nigeria [19] but less than the 10.7% reported in North Central Nigeria among apparently healthy male blood donors in Kano by Imoru *et al* [20].

There was no burden of HIV and HBV co-infection among our subjects. All the HBsAg positive subjects (8/240, 3.0%) were HIV negative. Statistically, there was no significant relationship between HIV and Hepatitis B infection among the subjects (χ^2 =1.7, p = 0.19). Nigeria is endemic for both HBV and HIV and the route of transmission for HBV in endemic areas is through horizontal and vertical transmission during childhood. This may be the reason for a higher HBV infectivity rate compared to the HIV infection rates (3/240. 1.3%) among our subjects. The countries' HIV prevalence rate has also reduced due to National campaign on HIV prevention and control, proper funding from both internal and external agencies and implementation of government policies [21]. On the other hand, HBV infection has not received a similar attention as it reflects on the higher prevalence among the subjects.

During the counseling session, most of the couples admitted that they had undergone the tests privately before the church's mandatory testing. Out of the 1.25% HIV infection rates among the subjects, males had 100% infectivity, but there was no statically significat influence of gender on the infection rates (χ^2 = 1.5, p =0.22). The reason may be due to the fact that females were more passionate to their partners and wish to continue with the marriage even after the knowledge of HIV positivity in these partners. Some of the female subjects said that they knew about their male counterpart's condition before undergoing the church's mandatory tests. On the other hand females were not found with HIV infection probably because those infected were rejected by their male partners after their private medical test.

Females were more infected with HBV (7/8, 87.5%) than males (1/8, 12.5%) and there was a statistically significant influence of gender on HBV infection rates (χ^2 = 42.9, p =0.08). This is different from the work of Okocha *et al* [22] in Nnewi, Nigeria, who reported a higher prevalence of HBV-HIV co-infection among males (8.7%) than females (4.2%). Otegbayo *et al* [23] in South Western Nigeria gave a similar report of higher rates of HBV co-infection among males (17.9%) than females (10.7%). Okocha *et al* [22] attributed the higher HBV infectivity rate among males to aggressive sporting activities among young males which lead to injury and bleeding, thus predisposing them to horizontal HBV transmission. Although we did not investigate the cause of HBV among our subjects, the higher infectivity rate among the females may be due to native customary practices in our locality such as female circumcision and ear hole piercing which are exclusively performed on females. Some of the equipments used for this opera-

tions may have been non-sterile or improperly sterilized thus predisposing the females to HBV infection alongside the vertical transmission during birth. Johnson *et al* [24] reported an onset of viral hepatitis after ear piercing with nonsterile instruments.

Females (2/3, 66.7%) were also more infected with hepatitis C virus (HCV) than males (1/3, 33.3%) but there was no statistically significant effect of gender on HCV infection.

Couples aged 26-35 years had the highest burden (71.4%) of viral infections. The infection of two couples with HBV in the same age group points at the possibility of sexual activity before marriage. The effect of age on the three viral infections was statistically significant ($\chi^2 = 31.0$, $p = 0.03$). This points to the fact that transmission may be through sexual activity as subjects in this age bracket are more sexually active.

Conclusion: Results from this study may provide some epidemiological evidence for policy making for mandatory premarital testing. A break in transmission of HBV infection may occur as a result of vaccination of negative partners. The test may also prevent future transmission and spread of the viruses to the negative spouse and their children as HIV and HCV positive partners have the choice to break the engagement.

Conflict of Interest: None

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References

1. Fetohy-Khalil EM, Abdelkader SM, Alsaeed MD & Alshahrany NM. Knowledge, Beliefs and Behavior Intention about Premarital Screening among King Saud University Female Students in Riyadh. Scholars Journal of Applied Medical Sciences (SJAMS). Sch. J. App. Med. Sci., 2014; 2(5E):1797-1805.
2. Arulogun OS & Adefioye OA. Attitude Towards Mandatory Pre-Marital HIV Testing Among Unmarried Youths in Ibadan Northwest Local Government Area, Nigeria. Mandatory Pre-Marital HIV Testing. *African Journal of Reproductive Health* Mar 2010; 14(1): 83
3. Lok ASF and McMahon BJ. "Chronic hepatitis B. AASLD Practice Guidelines," *Hepatology*, 2007; 45 (2): 507–539.
4. Ocama P, Opio CK & Lee WM. Hepatitis B virus infection: Current status. Am J Med. 2005; 118:1413.
5. Williams, R. Global challenges in liver disease. Hepatology 2006; 44(3): 521-526.
6. Kramvis A, Kew MC. Epidemiology of hepatitis B virus in Africa, its genotypes and clinical associations of genotypes. Hepatol Res. 2007; 37(1):S9-S19.
7. Olokoba AB, Salawu FK, Danburam A, Desalu OO, Olokoba LB, Wahab KW, Badung LH, Tidi SK, Midala J, Aderibigbe S, Abdulrahman MB, Babalola OM, Abdukkarim A. Viral Hepatitis in Voluntary Blood Donors in Yola, Nigeria. Euro. J. Scientific Res 2009; 31 (3): 329-334.
8. Ndako JA, Nwankiti OO, Echeonwu GON, Junaid SA, Anaele O, Anthony TJ. Studies on Prevalence and Risk Factors for Hepatitis B Surface Antigen among Secondary School Students in North-central, Nigeria. Sierra Leone J. Biomedical Res 2011; 3 (3): 163-168.
9. Ugwuja EI, Ugwu NC. Seroprevalence of Hepatitis B Surface Antigen and Liver Function Tests among Adolescents in Abakaliki, South Eastern Nigeria. The Internet J. Trop. Med 2010; 6 (2): 1-6.
10. World Health Assembly (27th:1974). The expanded programme on immunization: the 1974 resolution by the World Health Assembly. Assignment Child 1985; 69-72:87-88.
11. Sato K, Ichiyama S, Iinuma Y, Nada T, Shimokata K, Nakashima N. Evaluation of immunochromatographic assay systems for rapid detection of hepatitis B surface antigen and antibody, Dainascreen HBsAg and DainascreenAusab. *Journal of Clinical Microbiology* 1996; 34:1420-22.
12. Ansari MHK, Omrani MD, MovahediV. Comparative evaluation of immunochromato- graphic rapid diagnostic tests and PCR methods for detection of human hepatitis B surface antigen. *Hepatitis monthly* 2007; 7:87-91.
13. Chameera EWS, Noordeen F, Pandithasundara H, Abeykoon AMS.Diagnostic efficacy of rapid assays used for the detection of hepatitis B virus surface antigen Sri Lankan Journal of Infectious Diseases 2013; 3(2):21-27 *DOI: http://dx.doi.org/10.4038/sljid.v3i2.5172*
14. Ifemeje S. Mandatory premarital HIV testing policy in Nigeria: a gross violation of the rights of people living with HIV/AIDS. The International Journal of Human Rights 2011.
15. Uneke CJ, Alo M, and Ogbu, O. Mandatory premarital HIV testing in Nigeria: The public health and social implications. AIDS Care 2007;19(1):116-21.
16. Akani, CI, Erhabor, O, and Babatunde, S. Premarital HIV testing in couples from faith-based organizations: experience in Port Harcourt, Nigeria. Niger J Med. 2005 Jan-Mar;14(1):39-44.
17. National Population Commission. *National census, Cross River State*2006; B.183. Lagos: NPC.
18. Nigerian Airport Authority (1991). *Weather reports*. Lagos: Meteorological Division.
19. Nwaba AE, Nwankwo NC. Clinical profile of hepatocellular carcinoma at the University of Port Harcourt Teaching Hospital, Port Harcourt. Trop J Med Res. 2003;7:26–8.
20. Imoru M, Eke C and Adegoke A. Prevalence of Hepatitis-B Surface Antigen (HbsAg), Hepatitis C Virus (HCV) and Human Immunodeficiency Virus (HIV) among Blood Donors in Kano State, Nigeria. J Medical Laboratory Sci.

2003;12:59–63.
21. Ogba, OM**,** Abia-Bassey, LN and Epoke, J. The relationship between opportunistic pulmonary fungal infections and CD4 count levels among Human Immunodeficiency Virus seropositive patients in Calabar, Nigeria. *Transactions of the Royal Society of Tropical Medicine and Hygiene*2013; 107(3): 170-5. doi: 10.1093/trstmh/trs025.
22. Okocha EC, Oguejiofor OC, Odenigbo CU, Okonkwo UC, & Asomugha L. of hepatitis B surface antigen seropositivity among HIV-infected and non-infected individuals in Nnewi, Nigeria. Nigeran Medical Journal 2012 Oct-Dec; 53(4): 249–253.
doi: 10.4103/0300-1652.107605
23. Otebayo JA, Taiwo BO, Akingbola TS, Odaibo GN, Adedapo KS, Penugonda S, et al. Prevalence of hepatitis B and C seropositivity in a Nigerian Cohort of HIV-infected patients. Ann Hepatol. 2008;7:152–6.
24. Johnson CJ, Anderson H, Spearman J, Madson J. (1974). Ear piercing and hepatitis. Nonsterile instruments for ear piercing and subsequent onset of viral hepatitis. *JAMA.* March 11, 227(10): 1165