Abstract
One of the major problems in transfusion medical practice in the developing countries is the incidences of transfusion transmissible infections, especially viral infections. Some of these viral infections share similar transmission pathways, making co-infections of these viruses a possibility. We investigated the possible co-infection of two viral infections-human immunodeficiency virus (HIV) and hepatitis C virus (HCV) in 1,490 blood donors in a Teaching Hospital in a south-eastern state of Nigeria. This number was made up of 1384(92.9%) males and 106(7.1%) females. Antibodies to these viruses were detected using ELISA methods. Our results showed that 12(0.81%), 9(0.60%), and 2(0.13%) were positive for HIV, HCV, and both HIV and HCV respectively. Greater percentage of females than males were positive for both HIV and HCV (2.8% and 0.65% for HIV and 2.8% and 0.43% for HCV) while age group 21-30 showed highest frequency (38.5%). We advocate for wider mandatory pre-screening of blood donors, increased public health education and enlightenment on modes of transmission of these viral infections, as well as counseling of donors before screening.

Key words: HIV/HCV co-infection; Blood donors; Transfusion

INTRODUCTION
Blood safety remains one of the major concerns in transfusion medicine, especially in many developing countries where normal blood transfusion services and financial resources are grossly inadequate. In developed countries, it is mandatory that every unit of blood is screened for hepatitis B and C viruses, HIV 1 and 2 and Treponema pallidum before transfusion (Garson et al. 1992; Aubuchon et al. 1997). Also, epidemiological data has shown that the introduction of pre-screening of blood donors has actually reduced the frequency of post-transfusion transmission of hepatitis B virus in developing countries (Abiodun and Umokei, 1990; Schneiberer et al. 1996; Chukwurah el al. 2005). Furthermore, provision of blood screening materials for HIV by international donor agencies has made it possible that many developing countries of the world screen their blood donors for HIV before transfusion. However, most developing countries and some centers in Nigeria do not screen blood donors for hepatitis C virus (Chukwural et al. 2005), probably due to inadequate financial resources for the screening and ignorance or lack of required attention to its ravaging power.

Hepatitis C virus (HCV), which is a known cause of non-A, non-B hepatitis, has a long incubation period of 2 to 26 weeks. Its infection is thought to induce lipid accumulation in hepatocytes, producing lipid droplets that play significant role in liver morphogenesis and efficient viral production (Barth et al. 2008). However, HCV core proteins have been implicated in modification of in vivo expression of cytokines, tumor necrosis factor II and interleukin β which are characteristic features of chronic liver disease and liver dysfunction (Tanizimu and Muyajima, 2007). In addition, core proteins in transgenic mice have been shown to confer resistance to autoimmune liver injury induced by a T-cell mitogen, hence, despite the aggressive immune response, spontaneous resolution to chronic HCV is frequent (Crotta et al2002; Barth et al. 2008). Furthermore, the
development of these infections actually come slowly and as many as 80% of affected individuals later become chronically infected and risk serious long term sequelae (Touzet et al. 2000). With all these, the expression and even suspicion of HCV infection is generally unnoticed, ignored and rarely taken into consideration in resource-limited areas. Yet, this infection has been severally implicated in post-transfusion complications (Nelson et al. 1993; Tamim et al. 2001; Chukwurah et al. 2005).

Human immunodeficiency virus (HIV) is a lentivirus that causes acquired immunodeficiency syndrome in infected individuals (Ogobo et al. 2015). Its infection affects and reduces vital immune cells, including T-helper cells, paving way for opportunistic infections and other malignancies associated with failure of the immune system (Lawn, 2004). In Nigeria, HIV shares many transmission routes with HCV, including unsafe blood transfusion and vertical transmission (Chukwurah et al. 2005; FRN, 2012), hence co-infection of the two is ordinarily expected. Worldwide, about 20% of HIV-infected persons are known to have HCV infection (Soriano et al. 2010) while a study in Ebonyi and Enugu states of Nigeria reported that 3.0% of HIV infected individuals also have HCV (Ogobo et al. 2015). It has been reported that HIV has negative impact on the prognosis of HCV infection, leading to rapid liver disease progression to fibrosis, end-stage liver disease and death (Highleyman, 2010; Opershaski and Kovacs, 2011). Also chronic immune activation noticed in co-infections (Gonzalez et al. 2009; Kovacs et al. 2010) can cause immune dysfunctions and cytokine production, enhancing HIV and HCV replications and further reduction in T-cells count (Kovacs et al. 2008).

From the foregoing therefore, co-infection by these two viruses is an increased risk on those affected. Particularly, co-infected potential blood donors who are usually “apparently healthy” at the time of blood donation, are double danger to the possible recipients of such blood, endangering them to both infections and hastening the progression of any or both of them. In addition, these donors are great risk to the laboratorians and all the health workers that come in contact with both donors and recipients. Though HIV positivity is enough to discard any unit of blood, there is possibility of HCV positivity in HIV-negative blood donors. Hence we had earlier reported the prevalence of HCV alone among blood donors in a neighboring state (Chukwurah et al. 2005). In furtherance of this advocacy for encompassing pre-screening of blood donors for the benefit of blood recipients and health workers, we report the prevalence and co-infection of HIV and HCV among blood donors in another state of south-eastern Nigeria.

MATERIALS AND METHODS

Ethical clearance: Ethical clearance for this study was obtained from the Ethical and Research Committee of Ebonyi State University Teaching Hospital (now Federal Teaching Hospital, Abakaliki). Additional informed consents were sought and obtained from the subjects.

Subjects: Our subjects were apparently healthy individuals who came to the Blood Bank of Ebonyi State University Teaching Hospital, Abakaliki, Nigeria to donate blood as volunteers, patients' relatives/friends or commercial donors. They were 1,490 donors, aged between 18 and 50 years and made up of 1,384 males and 106 females. The study covered all persons who presented for blood donation in the hospital between October, 2009 and August, 2011.

Sample: After counseling and obtaining informed consent, 2.0ml of blood was collected from the cubital vein of each subject into a grease-free glass test tube. This was allowed to clot and retract before centrifuging at 3,000rpm for 5 minutes to obtain the serum. Every sample was analyzed on the day of collection.

Methods: Antibody to HCV (anti-HCV) was tested using a third generation assay (ELISA) kit manufactured by ABON Biopharm (Hangzhou, China) as earlier reported (Eke et al. 2015). This is a rapid immunochromatographic direct binding test that has a sensitivity of 99.0% and specificity of 98.6% (ABON, 2010). Antibody to HIV was tested using Uni-Gold®
This test kit detects antibodies to both HIV 1 and 2 in serum, plasma and whole blood. Positive samples were confirmed using a second kit – Stat-Pak®, manufactured by Chembio Diagnostic System (Medford, NY, USA).

**Statistical analysis:** Data were analyzed using the statistical package for social sciences (SPSS) software Version 16.0 (Chicago, IL, USA) and presented as percentages and frequencies.

### Table 1: Distribution of the positive subjects according to sexes

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>No. positive for HIV</th>
<th>No. positive for HCV</th>
<th>No. positive for both</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>1384</td>
<td>9(0.65)</td>
<td>6(0.43)</td>
<td>2(0.14)</td>
</tr>
<tr>
<td>Female</td>
<td>106</td>
<td>3(2.83)</td>
<td>3(2.83)</td>
<td>0(0.00)</td>
</tr>
<tr>
<td>Total</td>
<td>1490</td>
<td>12(0.81)</td>
<td>9(0.60)</td>
<td>2(0.13)</td>
</tr>
</tbody>
</table>

*percentage in parenthesis

Table 2 is the distribution of the subjects according to age groups. The results show that age group 21 – 30 years has the highest frequency while those less than 20 years of age has the lowest frequency. Also, the table shows that no female above 40 years was involved in blood donation while very few (3) below 20 years of age were involved.

### Table 2: Frequency distribution of the subjects in age groups and sexes

<table>
<thead>
<tr>
<th>Age group (Years)</th>
<th>Male</th>
<th>Female</th>
<th>Total</th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 20</td>
<td>72</td>
<td>3</td>
<td>75</td>
<td>75</td>
<td>5.03</td>
</tr>
<tr>
<td>21 - 30</td>
<td>534</td>
<td>70</td>
<td>604</td>
<td>604</td>
<td>40.54</td>
</tr>
<tr>
<td>31 - 40</td>
<td>425</td>
<td>33</td>
<td>458</td>
<td>458</td>
<td>30.74</td>
</tr>
<tr>
<td>41 - 50</td>
<td>353</td>
<td>0</td>
<td>353</td>
<td>353</td>
<td>23.69</td>
</tr>
<tr>
<td>Total</td>
<td>1384</td>
<td>106</td>
<td>1490</td>
<td>1490</td>
<td>100.0</td>
</tr>
</tbody>
</table>

Table 3 shows the distribution of the positive subjects according to age groups. It shows that all those positive for HIV fall between 21 and 40 years of age. Also only one subject above 40 years was positive for HCV while the remaining HCV-positive subjects were between 21 and 40 years.

### Table 3: Distribution of positive subjects among the age groups

<table>
<thead>
<tr>
<th>Age group</th>
<th>n</th>
<th>No. positive for HIV</th>
<th>No. positive for HCV</th>
<th>No. positive for both</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;20</td>
<td>75</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>21 - 30</td>
<td>604</td>
<td>8</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>31 - 40</td>
<td>458</td>
<td>4</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>41 - 50</td>
<td>353</td>
<td>Nil</td>
<td>1</td>
<td>Nil</td>
</tr>
<tr>
<td>Total</td>
<td>1490</td>
<td>12</td>
<td>9</td>
<td>2</td>
</tr>
</tbody>
</table>
DISCUSSION

The number of potentially transfusion transmissible infections in developing countries is enormous. This has made blood transfusion a nightmare to laboratotians, clinicians, patients and patients' relations. However, with increasing awareness of the dangers of these infections and the help of donor agencies that facilitated increased screening for the infections, these problems are becoming a thing of the past. Hence, 0.60% prevalence of HCV recorded in the current study is quite lower than the values recorded by other researchers within the same region barely a decade ago (Chukwurah et al. 2005; Kaote et al. 2005; Ezeani et al. 2006). Though HCV infection is one of the leading public health challenges globally, accounting for about 115 million infections (Eke et al. 2016), increased screening facilities and health education will definitely reduce its rate of transmission. Furthermore, 0.81% prevalence of HIV in this study is also lower than the reported overall prevalence of 5.1% in the state where this study was done (FGN, 2012). Though the difference between the two prevalence rates may be due to the difference in the study groups, the overall awareness may have reduced the number of positive subjects that present themselves for blood donation either as commercial donors or voluntary donors. Again, 0.13% co-infection of HIV and HCV recorded in the present study is lower than 3.0% HIV patients reported to have been co-infected with HCV in the same area of study (Ogbodo et al. 2015). Therefore, the result of this present study is in agreement with an earlier study in the same region (Ugwuoru et al. 2006) which reported higher HIV seroprevalence in females than in males among prospecting couples.

During this study, it was observed that the majority of the subjects were commercial blood donors, followed by patients' relations. Contributions by voluntary donors were minimal, mainly during anniversary or other activities of their voluntary organizations. The commercial blood donors were mainly between 20 and 40 years of age, hence, the age group 21 – 30 years had the highest frequency followed by 31 – 40 year group. Incidentally, these commercial blood donors accounted for 100% of those positive for HIV and 88.89% of those positive for HCV. Only one subject positive for HCV was a patient's relation and belonged to 41 – 50 year age group. Most commercial blood donors in developing and resource-limited countries are sexually active young men, who exercise little or no precaution in doing so (Ogbodo et al. 2015) and sexual intercourse is one of the principal routes of transmission of these two viral infections in Nigeria (FGN, 2012; Ogboro et al. 2015). Therefore, low socio-economic situation – poverty, is the major driving force for this unwholesome “trade” – commercial blood donation, making some of them who already know their status – carriers, to pretend as if nothing is wrong. Thus, if screening misses such case, the recipient becomes the victim. This is different from what happens in financially buoyant to pay commercial donors or that the family was not disposed to accept another person's blood out of fear of contacting transfusion transmissible infections. Other females who participated in this study and who fell within the age group of 31 – 40 years were voluntary donors that belonged to voluntary organizations. Though the number of subjects positive for both HIV and HCV were smaller in females than males, the percentage positivity was higher in females in each case. This can be interpreted to mean that if female subjects were as numerous as the male subjects, the numbers that were positive might have been more than the male ones. In addition, it was observed during the study that most of the female donors who were not mothers were voluntary donors and none of the mothers were positive for HIV. Therefore, the result of this present study is in agreement with an earlier study in the same region (Ugwuoru et al. 2006) which reported higher HIV seroprevalence in females than in males among prospecting couples.

The result of this study shows that only 106(0.7%) of the total blood donors involved in this study were females. This agrees with the results of earlier study in the same region that recorded 99.2% male donors (Chukwurah et al. 2005). In most developing countries, females are rarely involved in blood donation except when their close relations, especially children, are the recipients. Hence, throughout this present study, only females whose husbands were either unavailable or not fit to donate were part of the study as they donate for their loved ones. In such cases, the family would not be
developed countries where most potential donors who are at high risk of transmitting infectious agents have voluntarily stopped giving blood (Touzet et al. 2000; Chukwurah et al. 2005). Moreover, every blood donor in the developed countries is carefully screened for the four principal transfusion transmissible infections – HBV, HCV, HIV and Treponema pallidum (syphilis), so that absolute numbers of infection complications resulting from blood transfusion are minimal (Ouzen et al. 2000).

CONCLUSION

The need for pre-screening of blood donors cannot be overemphasized. Though the prevalence of each of the viral infections in the present study was low, no stone should be left unturned to ensure 100% safety of blood before transfusion. Therefore, there must be renewed vigor towards pre-screening of blood donors, general health education on these transfusion transmissible infections and counseling before screening of prospective donors. While pre-screening eliminates possible post-transfusion infection transmissions, health education and counseling will ensure that those who are found positive to any of the infections do not move to other centers to donate, or even engage in other activities that may endanger uninfected ones. In addition, various governments, government agencies and donor agencies should increase funding of the national blood bank projects to make safe blood available for patients in need. Voluntary organizations should also increase their response to the plight of patients, especially accident victims, by donating at regular intervals to national blood banks, not necessarily when they have sensitization programmes.

REFERENCES


