Seroprevalence of Hepatitis B surface antigen (HBsAg) and: Hepatitis C virus (HCV) co-infection among Apparently Healthy Students of a Tertiary Institution in North-eastern Nigeria.

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Abstract: This is a cross-sectional laboratory based study in which two hundred blood samples were aseptically and randomly collected from apparently healthy students of Federal Polytechnic Mubi. Participants were screened for Hepatitis B surface antigen and anti HCV antibody using standard procedures. Ethical clearance and participant's informed consent were sought and obtained from appropriate authorities and concerned participants respectively. All data generated from this study were analysed for statistical relevance using Mann-Whitney and Duncan Chi square test. Of the 200 samples screened, 20% were seropositive for HBsAg while 11.5% were seropositive for anti-HCV antibody. The sex related prevalence of 22.1% formales and 15.9% forfemales were obtained for HBV, while 13.7% for males and 7.2% for females were obtained for HCV. There was no significant association between gender and HBsAg (P=0.281) and HCV (P=0.196) infections. All the age brackets showed no HBsAg/HCV co-infection. While HBsAg was found to be more prevalent among age group 25-29yrs (32.8%), HCV was found to be more prevalent among the age group 30-34yrs (20.6%) but with no statistical difference for both HBsAg (P=0.135) and HCV (P=0.199).

Keywords: Seroprevalence, **Hepatitis, Apparently healthy, Co-infection.**

Introduction:

Infection with hepatitis viruses results in the inflammation of the liver. Notably, among the hepatitis viruses of public health concern which are highly endemic in sub-Saharan Africa are hepatitis B virus (HBV) and or hepatitis C virus (HCV) (Madhava *et al*., 2002; Kramvis and Kew 2007). In Nigeria, HBV and HCV are still endemic with high prevalence in blood donor populations. Previous finding reported that blood transfusion especially in places where screening is not practiced may be one of the commonest means of transmitting these infections (Bukbuk *et al*., 2005).

Hepatitis B virus, a DNA virus of the family hepadnaviridae has a circular genome of partially double-stranded DNA of approximately 3.2kb. The virus is transmitted through infected blood, sexually and vertically (mother to child) in the perinatal period. Perinatal transmission is the most common mode of HBV transmission Worldwide (Tran, 2009). The Hepatitis B surface antigen (HBsAg) is the serologic hallmark of HBV infection. It is 10 times more infectious than Hepatitis C virus with many carriers unaware they are infected (Samuel *et al*., 2004).

Hepatitis C virus has a single stranded RNA genome of approximately 9.6kb. The virus belongs to the family Flaviviridae and appears to have humans and chimpazees as the only species susceptible to its infection (Polyak, 2006). It is also transmitted through infected blood, sexually and from mother to child (Wejstal *et al*., 1992). Before the discovery of adequate testing techniques for HCV infection, almost 10% of transfusion recipients acquired the infection. This made it one of the commonest causes of transfusion related hepatitis (Imoru *et al*., 2003; Dodd *et al*., 2002). HCV infection is still endemic in Nigeria with varying prevalence rate in the different geographical states.

The most common causes of chronic liver disease globally have been reported to be hepatitis B and hepatitis C viruses. These viruses shared common routes of transmission and co-infection with HBV and HCV is usually common among individuals resident in HBV endemic areas who also have a high risk of parenteral infections, such as injection drug users (Pennab *et al*., 2010; Dawurung *et al*., 2012), patients on haemodialysis, patients undergoing organ transplantation and HIV-positive individuals. Chronic infection with HBV and HCV are often asymptomatic, and can lead to liver cirrhosis and hepatocellular carcinoma (Schiff, 2002, WHO, 2000). Hence, most infected people are unaware of their HBV or HCV statuses (Mishra and Seeff, 1992; Pondei and Ibrahim, 2013). Co-infection of HBV and HCV seems to result in more severe disease than either infection alone.

Lack of adequate surveillance and database on HBV and HCV in Nigeria particularly the North-Eastern part that will help to prognosticate future

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direction of the disease is worrisome. Hence, studies which elucidate on potential carriers and hepatitis patients among apparently healthy individual is paramount in the management, control and prevention of the disease outbreak. This will ultimately minimize morbidity and mortality associated with the infections. Therefore this study was aimed to outline the seroprevalence of HBsAg, HCV and HBsAg/HCV co-infection among apparently healthy students of a tertiary institution in North-Eastern Nigeria.

Materials and Methods

Study Design

This was a cross-sectional study carried out over a period of 6 months spanning from July to December, 2015. 200 apparently healthy students were randomly recruited for this study.

Study Area

The study area was Federal Polytechnic Mubi located in Mubi metropolis, Adamawa State, Nigeria. The institution was established by decree no. 33 of 1979 constitution. Mubi metropolis, a geo-political area comprising of two local government areas; Mubi North and Mubi South. The metropolis is located between latitudes 10° 05' and 10° 30'N of the equator and between longitude 13° 12' and 13◦ 19'E of the Greenwich meridian. The two Local government areas occupy a land area of 192,307 Km^2 and support a total population 260,009 people (National Population Census 2006). The area shares boundary with Maiha L.G.A in the South, Hong L.G.A in the West, Michika L.G.A and Cameroon Republic in the East (Adebayo, 2004).

Ethical Consideration

Verbal informed consent of each participant was obtained prior to testing. Ethical approval was also obtained from the institutions' Research and Seminar Committee Board. The individual laboratory results were kept confidential and given to the participants at the completion of the project. Positive individuals were referred to medical facility within the institution for guidance.

Inclusion Criteria

All subjects who gave informed consent were included in the study.

Exclusion Criteria

Subjects who had once been vaccinated with the required doses of the vaccines and those who declined to offer consent were excluded from the study.

Sample collection and processing

Five millilitres of blood was collected with sterile syringes and needles from each donor into EDTA specimen tubes and centrifuged for 5 min at 2500 rpm. The plasma was separated and stored at refrigerator until ready for use.

Laboratory Assay for detection of HBsAg and HCV

The assay for the detection of HBsAg and HCV was carried out in microbiological laboratory of Federal Polytechnic Mubi, Adamawa State, Nigeria between July and December, 2015. The samples were tested for the presence of HBV infection using adsorption qualitative technique based on the principle of antigen-antibody reaction. DiaSpot® HBsAg Test strips (manufactured by Dia Spot Diagnostics, USA) was employed for this purpose. The screening for HCV antibodies was carried out using Anti-HCV antibody third generation enzyme immunoassay kit (Skytec™). The test and interpretation of the results were carried out according to the manufacturer's specifications (Egah*et al*., 2007; Khattak *et al*., 2008).

Data Analysis

The prevalence of HBsAg and HCV infections were calculated. The data generated was subjected to statistical analysis. Analysis of variance (Duncan multiple range test) was used to test for significant difference among age brackets, while non-parametric Mann-Whitney test was used to test for significant difference among males and females. All statistical analyses were carried using SPSS computer software version 16.0 for Windows to determine any significant relationship between infection rate, age and gender.

Results

Two hundred students participated voluntarily in the study. There were 131(65.5%) males and 69(34.5%) females. Although, results from Table 1 revealed a total prevalence rate of 20% for HBsAg and 11.5% for HCV infection with no HBsAg/HCV co-infection, the results however showed no significance difference (P=0.314).With respect to gender, males were more likelyto be infected with both HBV (22.1%) and HCV (13.7%). Females however, showed a lesser prevalence rate to both HBV (15.9%) and HCV (7.2%) infection. Co-infection was however not recorded in both males and females. However, the results revealed that gender is not a risk factor for both HBsAg (P=0.281) and HCV (P=0.196) infections.

The results in Table 2 showed seroprevalence of HBsAg, HCV and HBsAg/HCV co-infection in relation to age group. All the age brackets showed no HBsAg/HCV co-infection. While HBsAg was found to be more prevalent among Age group 25-29yrs (32.8%). HCV was found to be more prevalent among the age group 30-34yrs (20.6%) but with no statistical difference for both HBsAg (P=0.135) and HCV (P=0.199). Among females however, the highest prevalence rate of HCV occur in the age bracket 25-29yrs (Table 3).

Table 1:Seroprevalence of HBsAg, HCV and HBsAg/HCV co-infection based on Gender

		HBsAg		HCV		No. with co-infection
Gender	No. tested(%)	No. positive (%)	No. negative (%)	No. positive (%)	No. negative (%)	No. Positive
Male	131(65.5)	29(22.1)	102(77.9)	18(13.7)	113(86.3)	0
Female	69(34.5)	11(15.9)	58(84.1	5(7.2)	64(92.8)	0
Total	200(100)	40(20)	160(80)	23(11.5)	177(88.5)	0

Table 2: Seroprevalence of HBsAg, HCV and HBsAg/HCV co-infection based on age group

		HBsAg		HCV		No. with co-infection
Age group	No. Tested	No. positive (%)	No. negative (%)	No. positive (%)	No. negative (%)	No. positive
15-19	26(13)	3(11.5)	23(88.5)	2(7.7)	24(92.3)	0
20-24	77(38.5)	12(15.6)	65(84.4)	6(7.8)	71(92.2)	0
25-29	58(29)	19(32.8)	39(67.2)	8(13.8)	50(86.2)	0
30-34	34(17)	6(17.6)	28(82.4)	7(20.6)	27(79.4)	0
35-39	5(2.5)	0(0)	5(100)	0(0)	5(100)	0
Total	200(100)	40(20)	160(80)	23(11.5)	177(88.5)	0

Table 3: Seroprevalence ofHBsAg, HCV and HBsAg/HCV co-infection based on Gender and age group

	Male					Female				
	No.	HBsAg		HCV		No.	HBsAg		HCV	
Age Group	Tested	No.+ve (%)	No.-ve (%)	No.+ve (%)	No. -ve (%)	Tested	No.+ve (%)	No. -ve (%)	No.+ve (%)	No.-ve (%)
15-19	15	3(20)	12(80)	2(13.3)	13(86.7)	11	0(0)	11(100)	0(0)	11(100)
20-24	44	9(20.5)	35(79.5)	5(11.4)	39(88.6)	33	3(10)	30(90)	1(3.0)	32(97)
25-29	42	11(26.2)	31(73.8)	4(9.5)	38(90.5)	16	8(50)	8(50)	4(25)	12(75)
30-34	25	6(24)	19(76)	7(28)	18(72)	9	0(0)	9(100)	0(0)	9(100)
35-39	5	0(0)	5(100)	0(0)	5(100)	0	0	0	0	0

Discussion

Screening for both HBsAg and HCV in apparently healthy individuals is importantin early diagnosis which will go a long way in determining strategic intervention programme for outbreak prevention. This is so important because infection with hepatitis viruses can be asymptomatic for quite a long time. Consequently, asymptomatic infection with hepatitis viruses is of grave health consequences in both males and females.

The results obtained from this study showed a prevalence rate of 20% for HBsAg among apparently healthy students of Federal Polytechnic Mubi, Adamawa State, Nigeria. This finding is comparable to a previous report (20.6%) among blood donors in Jos south LGA of Plateau State (Adekeye *et al*., 2013) and 19.9% prevalence rate reported among blood donors in Osogbo, Osun State (Oladele *et al*., 2013). Contrary to the findings of this study, previous report from the same study area showed a higher prevalence rate of 31.5% (Tula and Iyoha, 2015).The differences may be attributed to differences in the time of sample collection. Otherstudies within Nigeria in which prevalent rate higher than the one we have reported includes 44.7% reported among apparently healthy pupils in Borno state (Bukbuk *et al*., 2005), 23.9% reported in Jos among blood donors (Uneke *et al*., 2005), 22% among antenatal clinic attenders in Onitsha (Mbamara and Obiechina, 2010) and 21.3% also reported in Ibadan among blood donors (Otegbayo *et al*., 2003). Other studies around the world that reported prevalent rate higher than that of our studies includes prevalent rate of 42.7% that was reported among Afro-descendent community of Brazil (Motta-Castro *et al*.,

2003, and 42.3% prevalence rate that was also reported among cohort of students in Bangui, Central Africa Republic (Komas *et al.*, 2010).

Prevalent rate of HBsAg lower than the one of our study have been variously reported in the country. These includes 3.6% (Pondei and Ibrahim, 2013) and 7.9% (Ibrahim and Pondei, 2014) reported in Bayelsa State, 6% (Adoga *et al.*, 2010), 12.5% (Ugbebor *et al.*, 2011) and 16.5% (Kolawale *et al.*, 2012) all reported in Federal Capital Territory, Abuja, 13.3% (Pennap *et al.*, 2010), 6.6% (Pennap *et al.*, 2011) and 8.7% (Pennap *et al.*, 2015) all reported in Keffi, Nassarawa State, 2.2% (Oladeinde *et al.*, 2013) and 8.3% (Babatope *et al.*, 2015) reported respectively in Benin City and Ekpoma all in Edo State. Also, 6.8% was reported in Ado-Ekiti (Esan *et al.*, 2014), 7.3% (Dawaki and Kawo, 2006) and 7.9% were reported in Kano (Yakassai *et al.*, 2013).

The HCV prevalence rate of 11.5% reported in this study was similar to previous study from Kaduna where 11.09% (Strickland 2002; Pennap, 2010) prevalence rate was reported. Contrary to this findings, higher HCV prevalence rate have been reported previously. These includes 13.3% (Pennap *et al.*, 2010) reported among Asymptomatic individuals in Keffi and 14.9% (Ebie and Pela, 2006) in Enugu, Nigeria. Furthermore, the finding of this study was however higher than various studies reported across the country. These includes 3% prevalence rate among apparently healthy individuals in Ekpoma (Babatope *et al.*, 2015), 4.9% (Adekeye *et al.*, 2013) and 4.3% (Egah *et al.*, 2007) in Jos among blood donors and presumed low risk group respectively. Also, low prevalent rate of 4% (Dawurung *et al.*, 2012) and 1.6% (Ajayi *et al.*, 2013) were reported in Maiduguri, 4.5% (Sheyin *et al.*, 2012) was also reported in Kaduna state.

When compared to some African countries, higher prevalence rate of 20% was reported in Egypt (Frank *et al.*, 2002), while low prevalence rate of 2.1% (Ndong-Afoma *et al.*, 2008) and 1.03% (Kumar *et al.*, 2007) was reported in Gabon and India respectively.

The differences observed may be due to regional differences in risk factors and cultural practices (Ibrahim and Pondei, 2014), differences in the sensitivity of detection methods, sample size, geographical endemicity (Pennap *et al.*, 2015; Tula and Iyoha, 2015), lack of awareness on the risk factors associated with both HBV and HCV. Previous report showed that viral hepatitis is lowest in countries or areas with high standards of living and highest in countries or areas where socioeconomic level is lower (Dienstang *et al.*, 1998; Babatope *et al.*, 2015). Meanwhile, in Nigeria, studies have shown that HCV infection is less prevalent compared to HBV (Jesse *et al.* 2008; Ojo *et al.*1990). In this study, although the prevalence rate of HBsAg is higher than HCV, there is however no significant difference between the duo. This imply that the rate of infection by HBV and HCV in our study area occur at the same rate.

Our finding also supports the WHO report (WHO 1999) which classifies Nigeria as a highly endemic country. Endemicity may be defined as HBsAg burden greater than 7% in an adult population. The 20% and 11.5% HBsAg and HCV seroprevalence rate respectively recorded in Federal Polytechnic Mubi may be explained partly by the sub-urban nature of Mubi and partly by the fact that it is an institution of higher learning where disease prevalence practices which favour transmission of viral hepatitis abound.

This study revealed that males were more likely to be infected with both HBV (22.1%) and HCV (13.7%) than their females' counterpart with 15.9% and 7.2% for HBV and HCV prevalence rate respectively. Although this observation did not reach statistical significance, this simply implies that gender is not a risk factor for viral hepatitis and the findings might be due to chance. Our finding is consistent with previous studies reported in Jos, Nigeria (Dawurung *et al.*, 2012; Adekeye *et al.*, 2013). Similar to our findings also, another studies showed higher HBV and HCV among males than females (Martina *et al.*, 2014), but the same study went contrary to our study because according to them, there was significant association between gender and HBsAg infection rate. A study from Keffi (Pennap *et al.*, 2010) showed that more males were infected with HBV (24.1%) than female (9.5%) which is consistent with our study. Contrariwise, the same study showed that more females were infected with HCV (16.6%) than males (3.4%).

Contrary to our findings however, higher HBV and HCV seropositivity among females than males was reported among apparently healthy individuals in Ekpoma (Babatope *et al.*, 2015). According to them, females had prevalence rate of 4.7% and 2.3% for HBV and HCV respectively, while males had 3.6% and 0.7% HBV and HCV respectively. Another report showed that during unprotected vaginal intercourse, a woman's risk of becoming infected with both HBV and HCV may go up to 4 times higher than the risk of man (Royce *et al.*, 1997).

In our study, the age group 35-39 recorded 0% prevalence rate for both HBsAg and HCV. This is contrary to previous studies which revealed that the prevalence of HBsAg and HCV were highest among 30-39 years of old (Baba *et al.*,1998; Adewole *et al.*, 2009; Babatope *et al.*, 2015). In this study however, although HBsAg was prevalent among age group 25-29, while HCV was found more among 30-34years, but with no statistical significant association. This is consistent with previous report on HCV, but contrary to the same report on HBsAg (Dawurung *et al.*, 2012). The lack of significant association between age and viral infection as seen in our study is consistent with previous studies reported among apparently healthy individuals in Maiduguri (Dawurung *et al.*, 2012), in Keffi (Pennap *et al.*, 2010) and among patients accessing health care in Nassarawa State (Akyal *et al.*, 2013).

Contrary to the finding of this study, a study among voluntary blood donors from Enugu showed a significant association between age group and HBsAg and HCV infection (Martina *et al.*, 2014).

The conspicuous absence of HBsAg/HCV co-infection in this study despite the peculiar nature of the study area is a unique feature of this study. Thisfinding is comparable to previous report in apparently healthy individuals from Ekpoma (Babatope *et al.*, 2015). No reason could be attributed to lack of co-infection in our study area. Contrary to this study however, variable co-infection rates were reported in various studies across the country. These includes 0.5% HBsAg/HCV co-infection rate among apparently healthy students of University of Maiduguri (Dawurung *et al.*, 2012), 8.8% among apparently healthy people of a local community in Keffi (Pennap *et al.*, 2010), 14.9% was reported in Enugu (Ebie and Pela, 2006), 12.9% was reported in Lagos (Agwale *et al.*, 2004), 1.0% in Keffi (Pennab *et al.*,2015), 0.5% from Benin (Ugbebor *et al.*, 2011), 0.4% from Calabar (Mboto *et al.*, 2010), 1.3% from Ado-Ekiti (Esan *et al.*, 2014) and 1.2% from Jos South LGA, Plateau State (Adekeye *et al.*, 2013).

Conclusion

Detecting marker for hepatitis virus in apparently healthy individuals is of great significance to health care personnel, policy makers and the general public. This is because of the attendant risk of transmitting the viruses unknowingly couple with the morbidity and consequent mortality associated with the viruses. Consequently, the need for public awareness campaigns on risk factors, preventivemeasure and compulsory introduction of routine screening of all intended blood donors for both viruses is paramount.

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