Serological Evidence and Risk Factors of Hepatitis A Virus among Blood Donors in 3 Tertiary Hospitals in Sokoto, Nigeria

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Background and study aim: Globally, Hepatitis-A Virus (HAV) is a major etiology of acute hepatitis. Though, enterically transmitted, there is evidence HAV being transmitted through blood transfusion. The presence of HAV antibodies may indicate infectious threat to blood transfusion safety. In view of these, the present study was aimed to determine the seroprevalence and associated risk factors of HAV among blood donors in three tertiary Hospitals in Sokoto State, Nigeria.

Materials and Methods: Blood samples were collected from one hundred and sixty eight (168) blood donors >20 years. Sera from these samples were investigated for anti-HAV specific IgG and IgM antibodies using a commercial enzyme immunosorbent assay (ELISA) Kits. Structured questionnaires were used to access socio-demographic variables of subjects.

Results: Seroprevalence of HAV- specific IgG and IgM were 86.3.1% and 0.0%, respectively. Of 168 blood donors, 145 had previous exposure to HAV [IgG (+) IgM (-), and none had recent infection [IgG (-) IgM (+)], 23 were susceptible to HAV [IgG (-) IgM (-). There was no significant association between sero-prevalence and any of the risk factors and sociodemographic variables studied ($p>0.05$). Findings from this study revealed that larger proportion of blood donors are already immune to HAV infection, while none of them had active HAV infection.

Conclusion: The high prevalence of Hepatitis A antibodies among the studied subject reflects a high HAV transmission rate in this area. Hence, determination of HAV should be taken into consideration before blood transfusion.

INTRODUCTION

Hepatitis A virus (HAV), belongs to *Picornaviridae* family. It's a naked single-stranded RNA virus **[1]**. HAV replicates and multplies in liver cells and interferes with liver function, activating an immune response that causes hepatitis. HAV has fecal-oral route of transmission through direct contact with an infectious person and ingestion of contaminated food/water **[1]**.

Hepatitis A virus (HAV) is endemic in many developing countries, where the prevalence can approach 100% in children by 5 years of age **[2]**. It is responsible for all 75% of viral hepatitis in the world. The disease has global distribution and is more common in areas with low levels of socio-economic development. Incidence is elevated in developing countries and the majority of these populations will become infected before the age of 10 years **[3]**.

The seroprevalence of HAV is low in developed countries and the greater part of those populations will remain susceptible to infection even at advanced ages **[4]**. Persons infected shed HAV in their feces 7-10 weeks before the onset of clinical symptoms. HAV

concentration in feces is highest during the prodromal phase. For those who develop symptoms, the concentration is usually very low by the time jaundice appears and undetectable before symptoms abate **[4]**.

Epidemiologically, predictors of HAV infection include poor household condition, low socio-economic status, residence in rural community, poor and inadequate water sources, and limited access to environmental sanitation facilities **[5]**. Several additional high-risk population groups have been identified, including those who travel or emigrate from a non-endemic region to an endemic region and those who work in certain high-risk occupations, such as some daycare employees **[6]**.

The disease spreads most quickly in highly populated areas with poor sanitation infrastructure and a shortage of water. Infections occur most commonly among children under the age of ten, and in most cases, the patients remain asymptomatic **[7]**. In most of the developed world, HAV is more likely to be contracted by older adolescents or adults **[7]**. In some cases, HAV infection has significantly increased the age at contraction. Delay in HAV exposure has made large population of adolescents and adults susceptible and of high risk of HAV and could lead to outbreaks of hepatitis A **[8]**. In view of this, it's important to consider implementing vaccination programs that target to certain populations at higher risk of infection **[9]**.

There is paucity of data on the prevalence of HAV in Sokoto State, North Western Nigeria. This prospective cross-sectional study was designed to determine the seroprevalence and risk factors of HAV among blood donors in three tertiary Hospitals in Sokoto State, Nigeria.

MATERIALS AND METHODS

Study design and Site

This cross-sectional study was carried on one hundred and sixty eight (168) blood donors that attended Maryam Abacha Hospital, Specialist Hospital and Women-children Welfare clinic in Sokoto State, Northwestern Nigeria.

Selection criteria

Inclusion criteria

1. Those who donate blood voluntarily
2. Individuals who were apparently healthy in the last 21 days
3. Those who gave consent to participate in the study

Exclusion criteria

1. Those who do not consent to voluntarily participate in the study
2. Non blood donors
3. Those who are willing but not healthy in the last 21 days

Questionnaire

Structured open ended questionnaires were prepared and administered on the spot to each participant. The participants were asked about their socio-demographic details including identification number, sex, age, ethnicity, educational qualification, occupation and marital status. Information was also asked about the place of residence, availability of toilet facilities, and availability of good drinking water, good waste disposal facility.

Sample Collection

3 mL of blood sample was collected from individual participant using standard venipuncture phlebotomy. The sample was carefully and gently dispensed into a sterile plain sample container. The tubes were labelled appropriately with participants' identification number.

Sera from the blood samples were separated by allowing the blood to clot at room temperature then by centrifugation at 2500rpm for 10 minutes. Thereafter transferred into serum aliquot container and stored at -20'C pending laboratory analysis.

Serum HAV Enzyme Linked Immunosorbent Assay (IgM and IgG ELISA)

The serum samples were tested for the presence of HAV-IgM level using the sandwich Enzyme Linked Immunosorbent Assay (ELISA) kit produced by Melsin Medical Co., Limited. Jilin Province, China. The Melsin HAV-IgM and IgG ELISA kits have Assay sensitivity of 99.75% and Specificity of 99.9%. Analyses were done based on kits manufacture's instruction.

HAV-IgM and IgG ELISA are solid phase, two-step incubation, antibody capture ELISA assay in which, polystyrene microwell strips are pre-coated with antibodies directed to IgM and IgG proteins (anti-ì chain). The subjects' sera were added. During the 1st incubation, any antibodies will be captured in the wells. After washing out

all the other components of the sample, the specific HAV antibody will be captured on the solid phase, and was detected by the addition of HAV antigens conjugated to horseradish peroxidise (HRP-Conjugate). During the second incubation, the HRP-conjugated antigens will specifically react only with the HAV antibodies. After washing to remove unbound HRP-conjugate, a tetramethyl-benzidine (TMB) chromogen solution was added to the wells. In presence of the (anti-ì)-(HAV-antibody)-(antigen-HRP) immunocomplex, the colorless Chromogens were hydrolyzed by the bound HRP conjugate to a blue-colored product. The blue color turns yellow after stopping the reaction with sulfuric acid. The amount of color intensity can be measured and is proportional to the amount of antibody captured in the wells, and to the sample respectively. Wells containing samples negative for HAV-antibodies remain colorless.

Sample size

The sample size was determined using prevalence rate demonstrated by Ramezani *et al* [10]. This was 150. Hence the minimum calculated sample size was N = 150

Attrition rate= (150X0.2)= 30. N= 30 +150= 180

Data collection

Questionnaires were used to collect socio-demographic data, such as gender, age, marital status, occupation, number of Household members, availability of hygienic toilet facility and good water supply.

Sample collection

2 milliliters of blood sample was collected from individual participant using standard venepunture phlebotomy. The sample was carefully and gently dispense into a sterile plain sample container. The tube was labeled appropriately with participants' identification number. Sera from the blood samples were separated by allowing the blood to clot at room temperature then by centrifugation at 2500rpm for 10 minutes. Thereafter transferred into serum aliquot container and stored at -20'C pending laboratory analysis.

Statistical analysis

Data obtained from ELISA and questionnaire were presented in tabular and graphical forms. Pearson Chi square test at a 95% confidence interval and a significance level of 0.05 was used to determine the association between socio-demographic data and seroprevalence rates using Statistical Package for Social Sciences (SPSS version 23, California, USA).

RESULTS

One hundred and sixty-eight (168) blood donors participated in the study. All participants were within the age range of 20-45 years old. The results of the ELISA were categorized into 3 serological responses. The first group was immune to HAV [IgG (+) and IgM (-)], which consisted of 145 Blood donors. In the second group, there was none with only one recent infection [IgG (+) and IgM (-)]. In the third group there were 23 women who were susceptible to HAV infection [IgG (-) but had IgM (-)]. Thus, anti- HAV IgG seropositivity was 86.3% and anti-HAV IgM seropositivity was 0.0% (Table 1). Table 2a shows the distribution of HAV- specific IgG and IgM seropositivity across ages of blood donors. HAV IgG seropositivity was mostly observed among those age range (20-30) years; (31-40) years had 86.2% while above 41 years (76%). Table 3 shows the distribution of Human HAV- specific IgG seropositivity across occupation, number of household members, availability of good toilet facility, and availability of good water supply. There was no significant association between seroprevalence and any of the risk factors and sociodemographic variables studied ($p>0.05$).

Table (1) : Summary of anti-HAV IgM and IgG antibodies among blood donors and their corresponding diagnostic interpretation

S/No	Antibodies reactivity	Number of subjects (%)	Interpretation
1	HAV IgG (+ve) and IgM (-ve)	145 (86.3)	Past infection
2	HAV IgG (-ve) and IgM (+ve)	0 (0.0)	Recent infection
3	HAV IgG (-ve) and IgM (-ve)	23 (13.6)	Susceptible

Table (2) : Distribution of Hepatitis A Virus IgG by sociodemographic variables of subjects

Variables		IgG Positive	IgG Negative	No. of Subjects tested	*P*-value
Gender					0.830
Male		132(86.8)	20(86.8)	152(100)	
Female		13(81)	2(9)	16(100)	
Age					0.5694
20	-30	79 (88.7)	10(11.3)	89(100)	
31	– 40	50(86.2)	8(13.8)	58(100)	
41	>	16(76)	4(24)	20(100)	
Marital status					0.461
Married		117(86.6)	18(13.4)	135	
Single		28(84)	4(16)	33	

Table (3) : Risk factors of Hepatitis A Virus IgG of blood donors

Variables	IgG Positive (%)	IgG Negative (%)	No. of Subjects tested (%)	P-value
Occupation				0.568
Trader	26 (86.6)	11 (13.3)	31	
Farmer	48 (82.7)	4 (17.8)	52	
Civil Servant	22 (84.6)	1 (15.4)	23	
Artisan	13 (92.8)	3 (8.2)	16	
Student	36 (92.3)	23 (8.7)	59	
NHHM				0.639
1-5	49 (87.2)	7 (12.8)	56	
6-10	79 (85.1)	15 (14.9)	94	
11-15	12 (92.3)	1 (7.7)	13	
16-20	5 (100)	0 (0)	5	
AVHTF				0.158
Yes	42 (75.9)	10 (14.1)	52	
No	103 (92)	13 (8)	116	
AVGWS				0.640
Yes	46 (85)	8 (15)	54	
No	99 (87)	15 (13)	114	

AVHTF=Availability of hygienic toilet facility AVGWS= Availability of good water supply, NHHM= Number of Household members

DISCUSSION

The findings of this study revealed that 86.3% of the blood donors had anti- HAV IgG with no corresponding anti-HAV IgM antibodies. Development of IgG antibodies to HAV indicates that these blood donors had previous infection with HAV in their life time. This seroprevalence was higher than that reported by Ikobah et al. **[11]** with 55.5% in Cross River state and Ayoola et al. **[12]** with 42.5% in Oyo state. However it is lower than that reported by David et al. **[13]** with 97.85% in Osun state and Ramezani et al. **[10]** in central Iran with 90% prevalence. The reason for this high prevalence may be as a result low levels of socioeconomic development, no availability of good toilet facility and good water supply in Sokoto metropolis.

Incidence is elevated in developing countries and the majority of these populations will become infected before the age of 10 years **[4]**. This may reflect the ignorance of the disease and low hygienic and cultural practices, which can increase HAV infection transmission in low income countries. However, this study agrees with Hadler **[14]**, who reported that prevalence of HAV infection is not the same in different parts of the world (varies between 15% and 100%), and depends on geographic area, sanitary levels and socioeconomic conditions. This disparity in prevalence could be due to difference in duration of study, the location of the study, level of hygiene, water supply, vaccination, endemicity of the virus, the study subjects and use of different test system with varying sensitivity.

There was an exponential decrease in prevalence HAV based on age distribution. Age group of 20-30 years has a prevalence of 88.7%, while 30-40 years had 8.2%, above 41 years, 76.0%. This may be due to the high activities of this age group and the facts that this age group has the highest frequency. Our finding is in accordance with that of Okara et al. **[15]**, which showed a highest prevalence among age group 20-40 years, but contrary to that of, Jacobsen and Wiersma **[16]** and Colak et al. **[17]** which showed that those positive for anti-HAV antibody were older than those without the infection. Age range between 20-30 years should be giving priority, since this represents greater risk of outbreaks in near future, and analyzing the cost effectiveness of vaccination programs might be worthwhile.

Gender distribution of HAV showed that a prevalence 88.7% amongst male blood donors and 86.6% amongst females. This could be attributed to the number of male to females in the study. This agrees with Ikobah et al. **[11]** and Gomes et al. **[18]** which showed that prevalence is higher in male than female. This could also be ascribed to the fact the female are more hygienic than male counterparts as this infection can be easily transmitted through fecal-oral route, by close contact with infected person, and contaminated food and water and even blood and blood products.

This present study recorded 100% prevalence for those with household size of 16-20, then household size of 11-15 with 92% while those 1-5 members recorded the lowest with 81%. This findings corroborate with that of Jacobsen and Jacobsen and Koopman **[8]**. This shows that larger household size and crowding are part of the predictors of Hepatitis A virus infection.

CONCLUSION

Findings from this study indicated that a large number of the blood donors are already immune to HAV infection, while none of them had active HAV infection. The high prevalence of Hepatitis A antibodies among the studied subject reflects a high HAV transmission rate in this area. Hence, determination of HAV should be taken into consideration before blood transfusion.

Ethical Consideration and Informed Consent

Ethical approval was obtained from the Ethical and Human research committee of the Ministry of Health, Sokoto state. Informed consent was also obtained from all participating subjects in accordance with the standards of human experimentation and with the Helsinki Declaration of 1975, as revised in 2000. This was done via an informed consent forms duly completed by all the subjects.

Conflict of interest

None

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None

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