Serological and Molecular Detection of Hepatitis A Virus among Women of Childbearing Age Attending Some Health Facilities within Maiduguri Metropolis, Borno State, Nigeria.

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Abstract: Hepatitis A virus (HAV) is the primary cause of acute viral hepatitis globally. It is primarily spread by eating or drinking contaminated food or water, as well as by direct contact (either through sexually or through exchange blood transfusion) with individuals who have been infected. The virus is widespread in low-income nations with unsanitary and poor sociodemographic conditions. In this study a total of 100 blood (plasma) samples consisting of 25 each from women of child bearing age attending clinics in four hospitals (University of Maiduguri Teaching Hospital; General Mamman Shuwa General Hospital, State Specialist Hospital, and Umaru Shehu Ultra-Modern Hospital) all within Maiduguri Metropolis were screened for HAV- IgM and IgG specific antibodies using One Step Palmatec® Rapid test kit (UK). The women were in the age group of 17 – 38 years with Mean ± SD age of 25.5 ± 5.4 years, of the 100 women tested, only one woman was seropositive for HAV-IgM specific antibody in the study, giving an overall seroprevalence of 1.0%. The seropositive woman was pregnant and aged 31 years, an antenatal clinic attendee of General Mamman Shuwa in Maiduguri Metropolitan Council Local Government Area.Using HAV-specific primers and RNA taken from her plasma, a conventional PCR tested revealed a positive reaction at the 175 bp Amplicon location. This study therefore, reports an overall low seroprevalence rate of HAV-IgM specific antibody of 1.0% in the study area. The implication from this study could be that HAV may not be a common infection in the area. Therefore, in order to determine the actual level of virus activity in the study area, a larger serological survey with a wider range of ages, gender, occupational groups, and geographic area may be required.

Key word: Hepatitis A Virus, Immunoglobulin G, Polymerase Chain Reaction, Maiduguri

INTRODUCTION

Hepatitis A infection is caused by the hepatitis A virus (HAV). Humans are the only known reservoir, hence, the virus can successfully be eradicated by employing widespread prevention strategies (Hofmeister *et al.*, 2019). Hepatitis A Virus infection is usually a self-limited illness that does not become chronic, while fulminant hepatic failure cases rarely occurs (Girish *et al*., 2024). Fulminant hepatic failure occurs in less than 1 percent of cases. Infection confers lifelong immunity and is preventable via vaccination. The Hepatitis A virus, or HAV for short, is a member of the Picornaviridae family and Picornavirales order of viruses. It is classified as a genus called Hepatovirus. HAV is a positive single-stranded RNA virus that is non-enveloped. The hepatitis A virus was found and described in 1973 by Stephen Feinstone, Albert Kapikian, and Robert Harry Purcell discovered and characterized hepatitis A virus (Shouval 2020). In Nigeria, HAV is endemic, mostly in communities with poor sanitation and living conditions. The virus is endemic in developing countries of the world including Nigeria, (El-Yuguda *et al*., 2016). The hepatitis A virus has been associated with the etiology of fecally transmitted hepatitis worldwide (Chuffi *et al*., 2022). The transmission of the virus is primarily through fecally contaminated foods and drinks and it has been associated with outbreaks among injection drug users, men who have sex with men and food handlers, (Gholizadeh *et al.*, 2023). The virus causes a milder form of hepatitis which is self limiting with a life-long immunity and absence of chronic form, the dangers of debilitating symptoms and fulminant hepatitis (acute liver failure) by the virus still remains a point of concern. Unlike hepatitis B and hepatitis C infections with

asymptomatic cases, HAV infected person often show symptoms of the virus. The common symptom associated with hepatitis A include: Anorexia, nausea, vomiting, jaundice, dark urine, pale feces and elevated liver transaminase levels among others, (Levinson, 2013). Hepatitis A virus (HAV), classified as hepatovirus (virus of the liver) is a small nonenveloped symmetrical RNA virus which shows many of the characteristics of the Picornaviridae family, and is the cause of infection or epidemic hepatitis transmitted by faecal-oral route. Hepatitis A infection is caused by hepatitis A virus which is found in feces of infected persons and spread and transmitted through ingesting of contaminated food or water. Some can be spread in some form of sexual intercourse between persons or oral intercourse and anal sex spike up risk of contracting hepatitis A (Chuffi *et al.*, 2022). HAV infection are slight most times and majority recover fully and remain immune from subsequent infection. Hepatitis A can be life threatening in cases of epidemic and cause extensive economic loss. Hepatitis A Virus (HAV) is the only representative of genus Hepatovirus in the family Picornaviridae and the etiological agent of acute hepatitis A. It is a linear single-stranded ribonucleic acid virus (RNA). Hepatitis A virus (HAV) is a major cause of water borne hepatitis worldwide especially in tropical and subtropical regions. HAV is found in the stool of the patient contaminated with hepatitis A virus and is usually transmitted from person-to-person through HAV contaminated water and food stuff. Therefore, HAV can spread under bad sanitary conditions and also when there is no good personal hygiene. This virus can be deactivated by heating at 85°C for one minute (Szybala and Szybala, 2020). Adequate and suitable chlorination of water can remove hepatitis A Virus. There are 6 main hepatitis viruses referred to as types A, B, C, D, E and G (Gillcrist, 1999). These types are of greatest concern because of the burden of illness and death they cause and the potential for outbreaks and epidemic spread. In particular type B and C lead to chronic disease in hundreds of millions of people. Hepatitis A and E are typically caused by ingestion of contaminated food or water hepatitis B, C and D usually occurs as a result of parental contact with infected body fluids (WHO, 2023) common modes of transmission for these viruses include receipt of contaminated blood or blood products, invasive medical procedures using contaminated equipment and for hepatitis B transmission from mother to baby at birth, form family member to child, and also by sexual contact (WHO, 2023).

MATERIALS AND METHODS

Study Area: This study was carried out in Nigeria's Borno State, in the Maiduguri Metropolis (Figure 1). Maiduguri has an elevation of 350 meters above sea level, it is situated in the Sahel Savannah region of northeastern Nigeria, between latitudes 11º 50'30" N and 11º 47'45" N and longitudes 13º5'45" E, 13º8'30" E, and 13º11'15" E. It is 50,778 square kilometers in size (mapcarta.com//Maiduguri). Maiduguri Metropolis is bordered to the north by Jere Local Government Area (LGA), to the north-east by Mafa L.G.A., to the south by Konduga L.G.A., and to the north and west by Magumeri. With an average annual temperature of 32°C and 650 mm of rainfall, Maiduguri has a pleasant climate. The warmest months of the year are March and April, when temperatures often range from 30 to 40°C throughout the harmattan, temperatures typically drop to between 10 and 25°C, making November through January the coldest months (Dawurung *et al*., 2014). The weather was typically dry and chilly throughout the dry time season. The population of Maiduguri Metropolitan Area was estimated to be 540,016 (NPC, 2006) and rose to 786,000 in 2020. The Kanuri, Shuwa-Arab, Bura, Marghi, Gwoza, and Mandara are the principal ethnic groupings. Kibaku, Fulani, and Hausa were among the others. The majority of the population in the area makes their living mostly from the cultivation of crops and

livestock, especially poultry (NPC, 2006). The twin settlements of Yerwa and Maiduguri make up modern Maiduguri. Kukawa, which was once the capital of the Bornu kingdom and was located 80 miles (130 km) northeast of Yerwa, was replaced by Yerwa, which was established in 1907 on the site of the hamlet of Kalwa and named by his Royal Highness, Shehu Bukar Garbai as the new traditional capital of the Kanuri people (Kanem-Bornu). In the meantime, the British chose the adjacent market hamlet of Maiduguri to serve as their military headquarters in place of Mafoni, and in 1908 they constructed a residence there that would later serve as the capital of British Bornu. In 1957, the merged metropolis, known locally as Yerwa, was split into the rural Maiduguri district and the urban Yerwa district; nonetheless, both political entities are located outside of Borno State, (Advertorial, 2016).

Ethical Clearance: The ethical clearance was obtained from the Borno State Ministry of Health and Human Services before the commencement of the study with reference number SHREC Number, 53/2023. Informed written or oral consent was obtained from all women of child bearing age according to United Nations Children's Fund (UNICEF, 2023) classification (women within the age group of 15 and 49 years) that were willing to participate in the study.

Study Design: The study is cross-sectional conducted in selected health facilities within Maiduguri Metropolis designed to assess the serological and molecular detection of the HAV among women of reproductive age. This study was conducted between January and September 2023.

Sample Size Determination: A prevalence of 3.6% based on a previous study in Borno by Dawurung et al. (2014) was used to determine the sample size. The total sample size was estimated using the standard WHO formula (Lwanga and Lemeshow, 1991; Thrusfield, 2005).

$n = Z^2 pq/d^2$

Where, n = sample size; Z = the normal deviation (1.96) corresponding to a confidence interval of 95%; p = prevalence from previous study (3.6% or 0.036) by Dawurung et al in 2014 among residents of Konduga LGA in Borno state; q = (1 – p) 1 - 0.036 = 0.964; d^2 = sampling error, or degree of accuracy, which is taken as 5% (0.05). n = $(1.96)^2$ × 0.036 (1-0.0356) / $(0.005)^2$ = 3.8416 × 0.036(0.964) / 0.0025 = 3.8416 × 13.8816 / 0.0025 = 53.327; ≈ 53. The sample size was rounded up to 100 to increase precision and to allow for an attrition rate of about 10%.

Study Population and sample collection: About 5 ml blood samples were obtained by venepuncture from a total of one hundred participants who gave oral or written consent to participate in the study at four secondary and tertiary health facilities namely State Specialist Hospital (SSH), General Mamman Shuwa Hospital (GMSH), Umaru Shehu Ultra-Modern Hospital (USUMH), and University of Maiduguri Teaching Hospital (UMTH) (Figure 1). An open-ended questionnaire was administered to capture the sociodemographic data such as age, location, and clinical details of the study subjects. A simple random sampling technique was used in selecting the study participants.

Data Collection and Statistical Analysis: After obtaining written or oral consent a self-administered structured questionnaires were given to each participant. The questionnaire had contained questions on the sociodemographic characteristics of respondents.The clinical presentations of the study subjects were obtained and documented. The data generated from the study were collected on Excel Spreadsheet and analysed using GraphPad Prism version 5.00, for windows GraphPad software, San Diego California USA, www.graphPad.com. Categorical variables were presented graphically, and as frequencies compared using Chi-square test descriptive statistics. A P-value less than or equal to 0.05 ($P \leq 0.05$) was considered statistically significant.

Sample Collection and Processing: Five millilitres (5 mL) of venous blood was aseptically taken from each of the

participants by vein puncture or sterile needle and discharged into a labeled plain sterile vacutainer tube. The blood was allowed to clot at room temperature and sera separated by centrifugation at 3,000 rpm for 10 minutes and stored in a freezer at -20ºC until needed for analysis (Figure 2).

Serological Test: A total of 100 plasma samples were screened for HAV IgG and IgM antibodies, using an chromatographic Assay kit (Palmatec® HAV IgG/IgM One Step Test Cassette, Lot number: 2022/08/10 and Expiry date: 2026/08/09) according to the manufacturer's instructions (Palmatec® Diagnostics, UK). This One Step HAV IgG/IgM Rapid Test is a lateral flow immunoassay for the qualitative detection of anti-HAV IgG and IgM in serum, plasma or whole blood. All HAV antibody seropositive sample(s) are stored at minus 20ºC freezer until used in the molecular assays.The Palmatec® HAV-IgM and IgG test kit have assay sensitivity of 94.0% and specificity of 98.0% for IgM, and 95.3% and 94.0% sensitivity and specificity respectively for IgG.

Ribonucleic Acid Extraction: For extraction of viral RNA, a QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany) was used according to the manufacturer's instructions. The extraction was performed from 140 µl of plasma and the extracted RNA was eluted in 60 µl of elution buffer.

Reverse Transcription and Amplification: The HAV RNA was amplified by nested or semi-nested PCR using primers previously described (Nainan *et al.,* 2006). A SuperScript™ III One Step RT-PCR System with a Platinum™ Taq DNA Polymerase (Invitrogen, Carlsbad, CA, USA) kit was used for the reverse transcription and the first round of amplification; the enzyme Platinum™ Taq DNA Polymerase (Invitrogen, Carlsbad, CA, USA) was used for the second round. For subsequent reaction set-up, the templates, specific primers (forward and reverse) and water were added to the premix. HAV68F (F-5'-TCACCGCCGTTTGCCTAG-3') and HAV240R (R 5'-GGAGAGCCCTGGAAGAAAG-3') primers with a 175 bp band size were utilized (Costa-mattioli *et al.,* 2003). RNA was amplified using 35 cycles of denaturation at 94ºC for 20 seconds, annealing at 55 ^{0}C for 20 seconds, extension at 72 ^{0}C for 1 minute, and a final extension at 72 ^{0}C for 5 minutes, following an initial denaturation phase at 95^{0}C for 5 minutes. An amplicon of size 175bp was detected by running a gel electrophoresis.The PCR products were loaded onto a 1.5% agarose gel and stained with ethidium bromide to visualize the bands of an expected length of 175 bp.

Data Analysis: The data generated from the study were collected on Excel Spreadsheet and analysed using GraphPad Prism version 5.00, for windows GraphPad software, San Diego California USA, *www.graphPad.com*. Categorical variables were presented graphically, and as frequencies compared using Chi-square test descriptive statistics. A P-value less than or equal to 0.05 ($P \leq 0.05$) was considered statistically significant.

RESULTS

Table 1 and Figure 3, shows the seroprevalence of HAV of the women studied according age group. Only women of childbearing age were included in the study. Overall, only one sample was seropositive for IgM out of the 100 tested, indicating a prevalence of 1 (1.0%) among the 100 blood samples examined using the Palmatec® One Step HAV IgG/IgM test Kit. The serological prevalence of 4.8% (1 out of 21) HAV IgM specific antibody was found only among women in the age group of 31 to 40 years. The overall seroprevalence however, was not statistically significant according to the different age groups ($p = 0.1496$) of the women in the study population. The distribution of HAV specific antibodies according to the clinical presentation of the study population is as shown in Table 2 and Figure 4. The PCR results of the HAV IgM antibody seropositive sample (MS-17) was reported from General Mamman Shuwa Hospital

having a positive specific reaction with an Amplicon size of 175bp as shown in Plate 1.

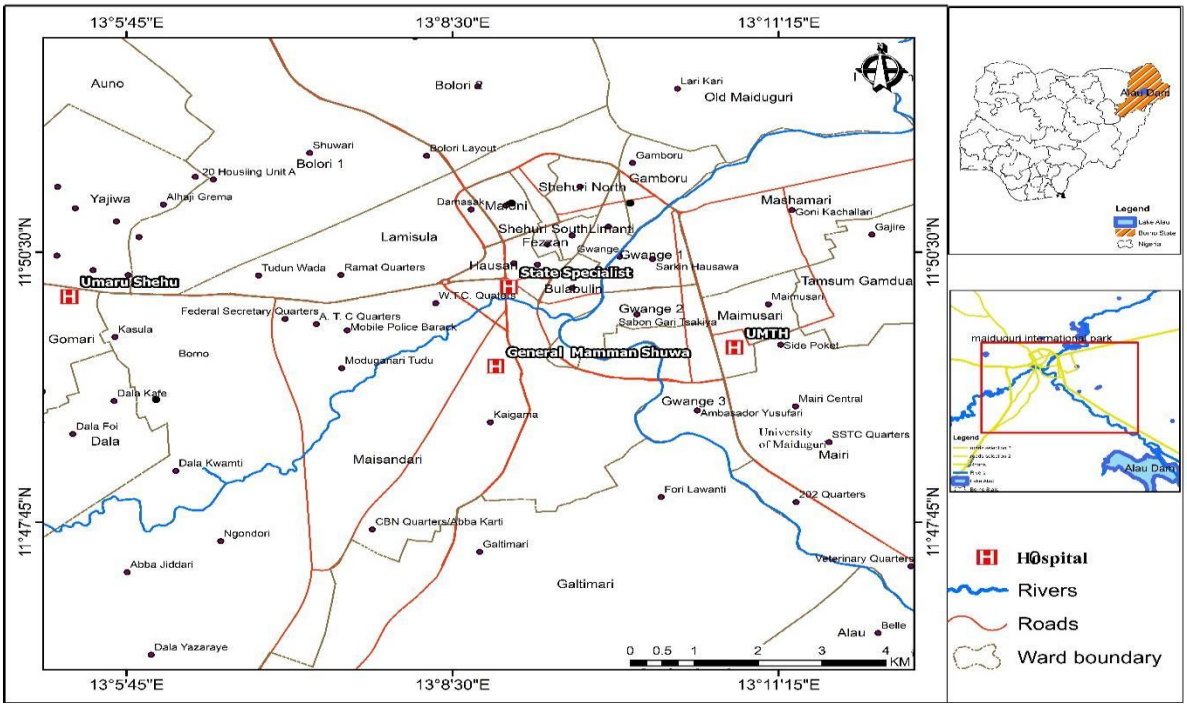

Figure 1: Map of Maiduguri Metropolis with locations of sampling sites Marked H-Hospitals (Umaru Shehu Ultra-Modern Hospital, State Specialist Hospital, General Mamman Shuwa and University of Maiduguri Teaching Hospital (UMTH), (Source: GIS LAB UNIMAD)

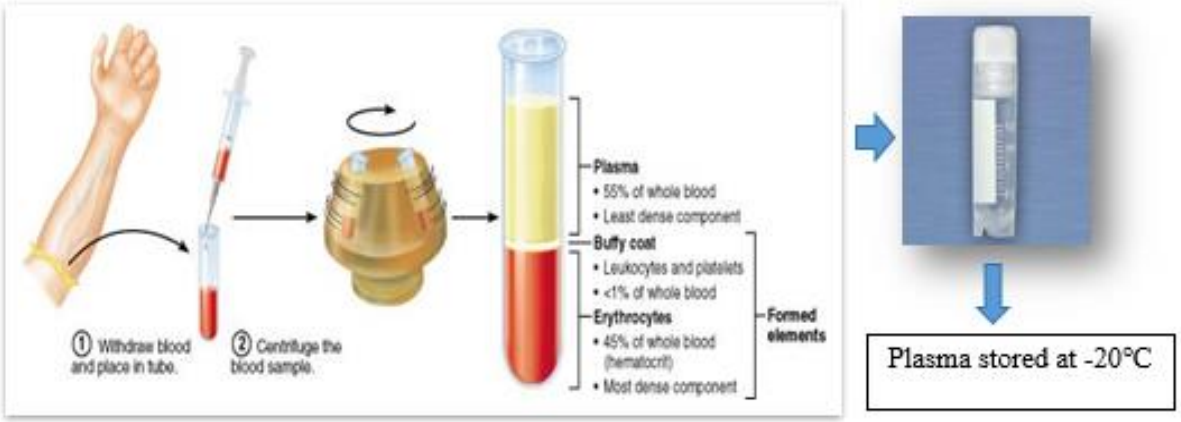

Figure 2. Procedure of blood collection, separation, aliquoting of plasma, and storage at minus 20ºC freezer until needed

Table 1: Seroprevalence of HAV Antibodies Among Women of Childbearing Age

Age group (Years)	Number Tested	Number Seropositive (%)		Overall Seropositive (%)
		IgG	IgM	
10 – 20	20	0 (0.0)	0 (0.0)	0 (0.0)
21 – 30	59	0 (0.0)	0 (0.0)	0 (0.0)
31 – 40	21	0 (0.0)	1 (4.8)	1 (4.8)
Total	100	0 (0.0)	1 (1.0)	1 (1.0)

Key: Chi square = 3.800, df= 2, p=0.1496

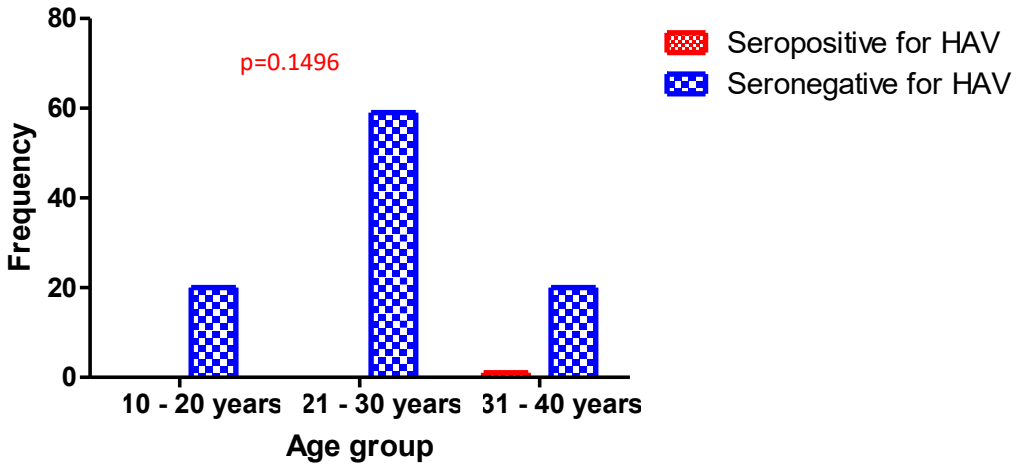

Figure 3: Seroprevalence of HAV Antibodies Among Women of Childbearing Age According to Age

Table 2: HAV Antibody Distribution according to Clinical Presentation of Subjects

Clinical Presentation	Number Tested	Number Positive (%)		Overall Number Seropositive (%)
		IgG	IgM	
Pregnancy (Antenatal)	59	0 (0.0)	1 (1.7)	1 (1.7)
Fever	38	0 (0.0)	0 (0.0)	0 (0.0)
Fever in Pregnancy	2	0 (0.0)	0 (0.0)	0 (0.0)
Fever & Abdominal Pain	1	0 (0.0)	0 (0.0)	0 (0.0)
Total	100	0 (0.0)	1 (1.0)	1 (1.0)

Key: Chi square = 0.7019, df=3, p=0.8727

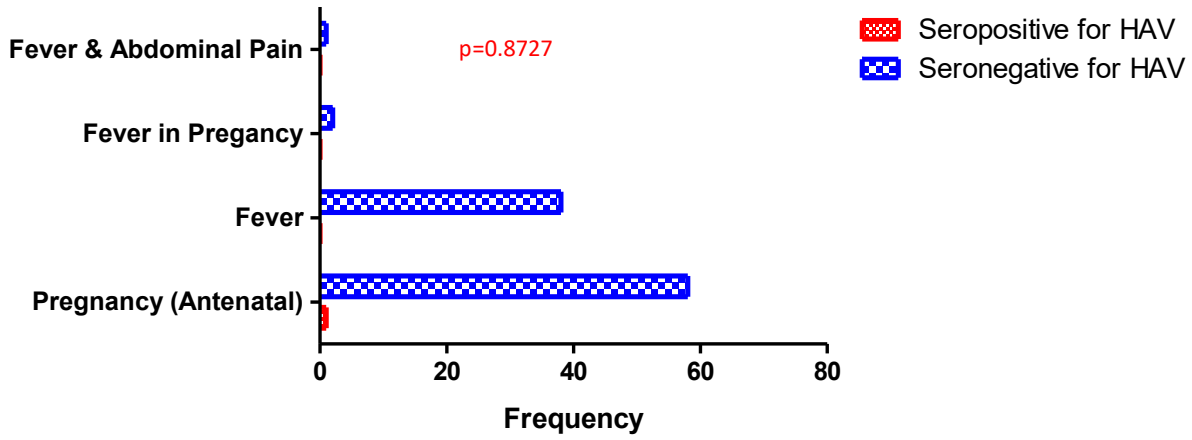

Figure 4: HAV Antibody Distribution according to Clinical Presentation of Study Population

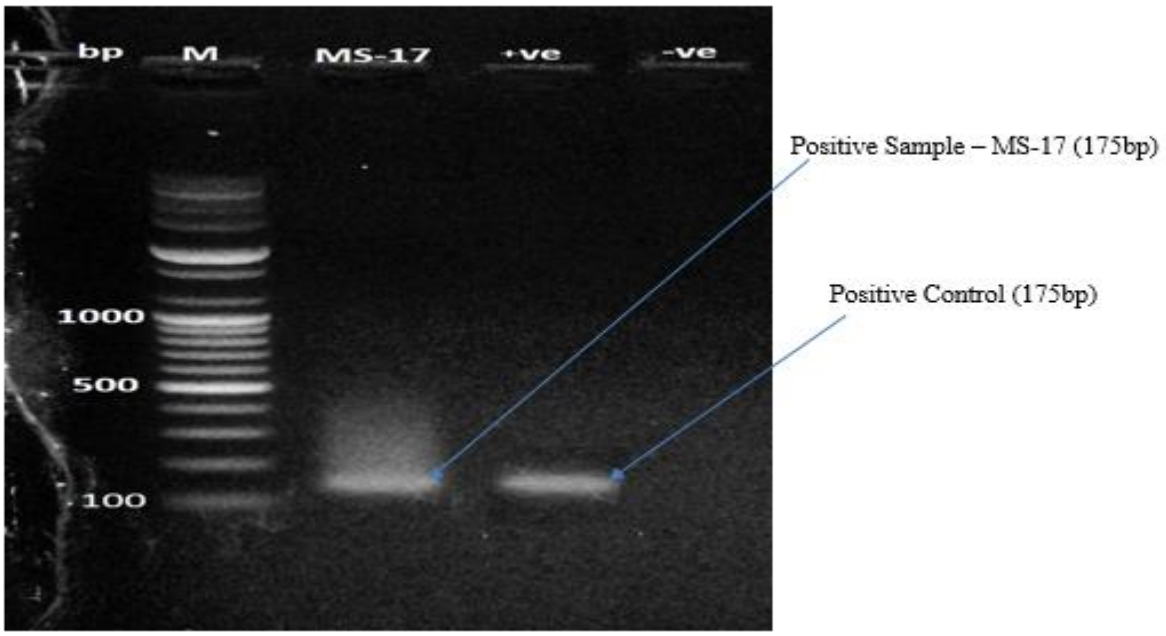

Plate 1: Electrophoresis of RT-PCR amplified HAV amplicon in 1.5% agarose gel and a ladder starting from 100 - 1000bp. Sample MS-17 was positive with a specific band size of 175bp. The positive control is represented as +ve Control (175bp), and negative control as -ve. M: M lane/ladder or Molecular marker; bp: base pair, MS-17: (General Mamman Shuwa Hospital Sample with Lab. Number 17), +ve: positive control, -ve: negative control.

DISCUSSION

This research focused on evaluating HAV seroprevalence and molecular detection among women of childbearing age attending some selected health facilities in Maiduguri Metropolis. The study aimed to determine the proportion of women who had antibodies against HAV indicating recent or acute infection. One serum sample 1 (1.0%) was seropositive for IgM antibodies out of the 100 women tested using the Palmatec® One Step HAV IgG/IgM test Kit, None (0.0%) of the pregnant women tested positive for IgG antibodies, which suggests no past infection. In the same study area, Dawurung *et al*. (2014) reported a higher prevalence of 3.6% and 2.0% HAV IgM among females and residents of Konduga Local Government Area of Borno state, Nigeria, respectively than 1.0% reported in the current study. Also, El-Yuguda *et al*. (2016), reported a much higher prevalence of 18.7% HAV IgG antibodies among females, and 30% in males with an overall seroprevalences of 23.5% among residents of Maiduguri metropolis. Hence, these two seroprevalences reported much higher rates than the 1.0% obtained in this study in Borno state. The differences in the seroprevalences might be due to the sensitivities and specificities of the different assay techniques employed by the three authors. The competitive ELISA technique used by El-Yuguda et al. (2016) possibly demonstrated it's superiority over the immunochromatophic assay technniques by Dawurung et al. (2014) and the current study. Also, variability in the detection power of assays that uses the same test principle might account for the observed differences as is the case with Dawurung et al. (2014) and current study both using same rapid detection methods but from two different manufacturers. The rapid test kit (Palmatec®) utilised in the current assay have been shown by the manufacturers to have a higher sensitivity (94.0%) and specificity (98.0%) relative to ELISA for IgM with a sensitivity and specificity also of 95.3% and (94.0%) for IgG respectively.

The comparison of seroprevalence rates of HAV IgM (an indicator of current or acute infection) and IgG antibodies (an indicator of past infection) between different studies conducted in the same geographical area has provided valuable insights into the prevalence and trends of hepatitis A virus infection within the same study population. In the overall seroprevalence of HAV IgM was reported as 1.0%. This indicates the proportion of individuals with acute or recent hepatitis A virus infections among women of childbearing age attending selected health facilities in Maiduguri Metropolis. The variations in the reported seroprevalences between all these studies, likely focused on different population groups in the same geographical area within Borno state, which may have different epidemiological characteristics and risk factors for hepatitis A virus transmission compared to Maiduguri Metropolitan area in this study. Also, El-Yuguda et al. (2016), reported a higher prevalence of 23.5% for HAV IgG antibodies in Maiduguri, IgG antibodies indicate past exposure to hepatitis A virus and provide immunity against future infections. The higher prevalence of IgG antibodies suggests that a larger proportion of the population had been exposed to hepatitis A virus in the past. Several other factors could contribute to the differences in seroprevalence rates between studies, which includes among others variations in study sites, populations, sample sizes, sampling methods, laboratory techniques, and time periods (seasonality) during which the studies were conducted. Additionally, differences in socioeconomic status, hygiene practices, sanitation infrastructure, vaccination status, and access to healthcare services may influence the transmission dynamics of hepatitis A virus within communities. The number, type and localization of toilets in homes as indicators of hygienic conditions. As reported before, there was a statistically significant difference between seropositivity and these variables, (Halicioglu *et al.,* 2012). These data shows the importance of hygienic status, especially hand washing which could contribute to reducing fecal-oral transmission route of HAV. Higher seropositivity rate among subjects who slept in crowded rooms (with more than one person) was determined as an important risk factor associated with hepatitis A. Because close indoor contact and existence of many susceptible subjects may result in person-to-person transmission (Lima *et al.,* 2014) which could result in association with HAV (Halicioglu *et al.,* 2012; Taghavi *et al.,* 2013). Crowded family with low income might be a cause of collective usage of personal belongings such as toothbrush and towels in home. Hepatitis A infection is also a waterborne disease. The main source of water used by subjects was a significant variable in our study. In their extensive epidemiological survey, Jacobsen and Koopman in 2005 reported that water coverage is a significant independent predictor of HAV infection rate, especially in developing countries. The increased quantity of water source results in good washing and cleaning of both hands and kitchenware as well as decreased need for water storage that facilitates transmission of virus. On the other hand, increased quality of water causes diminished ingestion of the pathogen via oral route, (Jacobsen and Koopman, 2005). These variations underscore the importance of continued surveillance and research to monitor trends in hepatitis A virus infection and inform public health interventions aimed at preventing and controlling the spread of the virus within affected populations. Therefore, it might be suggested that it is possible the virus is not extensively distributed in the study area due to its current low seroprevalence. Although HAV infection is acquired early in life in endemic places, the one seropositive woman described in this study is older and aged 31years. Due to the cumulative effect, the prevalence in the older age group is probably larger than in the younger age group. The absence of IgG and IgM in other age groups may suggest that those individuals could be susceptible to

HAV infection in the study area. The single seropositive woman in this study is in her third decade of life; Mathur and Arora, (2008) previously documented a shift in the peak age of seroprevalence from the first to the second or third decades of life. Even though anybody can contract the virus, but the lack of HAV antibodies in participants over 35 years old found in this study suggests that these people are therefore not protected (susceptible). This however is contrary with the findings of Alkhalidi et al. (2009), who found that people between the ages of 41 and 60 years were HAV seropositive. This study population was chosen because of the importance of understanding the activity of HAV among women who may potentially transmit the virus to their offspring during pregnancy or in utero. In addition to seroprevalence assessment, this study, involved molecular technique (conventional PCR) to identify the presence of HAV genetic material (RNA) in blood of the participants. This molecular detection method (PCR) has been known to provide more sensitive and specific detection of the viral genome, especially in individuals who may have acute or recent infections. The findings of this research provided a valuable insight into the prevalence and transmission dynamics of HAV among women of childbearing age in Maiduguri Metropolis. This information can guide public health interventions, including targeted vaccination programs and health education initiatives aimed at preventing HAV transmission and protecting maternal and child health in the study area.

CONCLUSION

This study reported a low seroprevalence of HAV among women of child-bearing age who are often at an higher risk of being infected with HAV or any other opportunistic infections due to their depressed immune status. Although perinatal transmission of HAV is very rare, hence considerations during pregnancy should be primarily to support the mother (Chilaka and Konje, 2021). Their offsprings may also stand a higher risk of being infected in utero (vertical transmission). There is therefore the need to carry out a wider sero-survey and molecular study including different ages, gender, occupational groups and a wider geographic area to reveal the activity of the virus in the study area. We recommend the inclusion of routine screening of pregnant women attending antenatal clinics and women of childbearing age and also blood donors that includes the male gender in the study area. There is need for governments at federal, state and local levels to intensify high quality surveillance and epidemiological investigations and to identify environmental risk factors associated with HAV infection. Since HAV is a vaccine-preventable disease, government should introduce routine HAV vaccination into our current National Programme for Immunisation (NPI).

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