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Pattern of Serum ALT and AST Associated with Hepatitis E Virus (HEV) Infection among Various Populations in Plateau State, Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Author SAJ designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors SAJ and SEA managed the analyses of the study. Authors SAJ and SEA managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aim: This study was undertaken to determine the effect of HEV on liver function enzymes. HEV is endemic in most developing countries, where the prevalence of HEV IgG antibody can be as high as 50%. Acute HEV infection is known to be a cause of decompensated liver cirrhosis. Several studies have suggested that elevated serum ALT and AST may be markers of hepatitis E virus (HEV) infection.

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Study Design: Cross sectional epidemiological survey.
Place and Duration: The study was carried out in three geographical zones of Plateau State, over a six month period from July to December, 2012.
Methodology: A total of 426 human subjects were recruited for the study; categorized into 4 groups: apparently healthy (190), pregnant women (108), HIV positive patients (80), and animal handlers (48). Blood samples were collected and analyzed for HEV antibodies (IgG and IgM) using ELISA technique. HEV seropositive samples were then subjected to measurement of liver enzymes (ALT and AST), using Randox kits (Randox USA). Results obtained were analyzed using SPSS version 17.0 statistical software.
Results: Elevated ALT and AST levels of up to 1.5 folds to 3 folds above normal in seropositive subjects were found. HIV positive subjects and animal handlers recorded the highest elevated ALT level up to twice (2 folds) the upper limit of normal, (21.0±0.0 I.U/L and 19.0±0.0 I.U/L respectively), while apparently healthy subjects had the least elevated ALT level 1.5 folds the upper limit of normal (14.0±0.0 I.U/L). With regards to AST, pregnant women had the highest level of up to 3 folds of the upper limit of normal with mean of 47.7±45.5 I.U/L, followed by apparently healthy subjects with a mean of 36.2±14.2 I.U/L, but least among animal handlers that recorded a mean of 35.0±0.0 I.U/L.
Conclusion: Hepatitis E Virus infection is associated with elevated ALT & AST values. However, this association needs further evaluation by researchers.

Keywords: ALT; AST; hepatitis E virus Plateau State; Nigeria.

1. INTRODUCTION

Hepatitis E virus (HEV) is a causative agent of enterically transmitted acute hepatitis in humans [1,2]. It is a major public health issue in developing countries, where it causes large waterborne epidemics [3,4]. Hepatitis E is an inflammatory liver disease caused by hepatitis E virus (HEV) infection, which is a single-stranded, non-enveloped RNA virus and the only virus within the genus *Hepevirus* and the family *Hepeviridae* [5,6]. The first described cases of acute liver disease caused by an enteric infectious agent that differed from hepatitis A and hepatitis B viruses were reported in India in the 1970s [6].

HEV is the leading or the second leading cause of acute hepatitis in adults in many parts of the developing world, and an increasing number of sporadic autochthonous acute hepatitis E cases have been reported in industrialized countries [4]. Unexpectedly, a new clinical feature has been described in association with autochthonous hepatitis E virus (HEV) infections in developed countries [7,8,9]. Indeed, since February 2008, cases of HEV-related chronic hepatitis have been reported in organ transplant recipients [9].

HEV is endemic in China, India, Nepal, as well as in several Asian and African countries, where the prevalence of HEV IgG antibody can be as high as 50% [5]. It has been recently estimated that its infection causes more than 3 million symptomatic cases of acute hepatitis E each year, resulting in approximately 70,000 deaths worldwide [10]. In non-endemic countries, an increasing number of non-travel associated HEV cases have been reported in recent years, particularly in Europe [11,12].

Most infections have a clinically silent course. In symptomatic cases, the incubation period ranges from 2 to 8 weeks, with a mean of 40 days [4]. Initial symptoms of acute hepatitis E are typically unspecific and include flu-like myalgia, arthralgia, weakness and vomiting. However, more severe forms of acute liver disease can occur in pregnant women or patients with underlying chronic liver diseases, sometimes progressing to fulminant hepatic failure [5]. The most frequent clinical and biological signs of HEV infection are jaundice, vomiting, hepatalgia, hepatomegaly, asthenia, distended abdomen, fever and high levels of transaminases (ALT and AST) [13]. Clinical features of HEV infection range from asymptomatic hepatitis to severe, fulminant hepatitis, which can result in liver-related mortality [13]. In immunocompetent patients, HEV is mainly self-limited and causes no chronic evolution. In fact, in these individuals, acute hepatitis E does not usually require therapy [14]. Nevertheless, in immunocompromised patients, HEV can pursue a chronic course [5]. Persistent HEV infection was first reported in 2008 in 8 French solid organ transplant recipients on immunosuppression. Furthermore, one kidney-transplant patient had cirrhosis attributed to

chronic HEV infection [6]. HEV-associated liver cirrhosis or hepatocellular carcinoma in the immunocompetent patient has not been reported, so far; however, acute HEV infection is known to be a cause of decompensated liver cirrhosis [15].

1.1 Justification

Hepatitis E (HE) is a significant public health concern that occurs in both epidemic and sporadic-endemic forms usually associated with contaminated drinking water. Major waterborne epidemics have occurred in Asia, North and East Africa. The potential exists for food-borne transmission as well as from close interactions with animals and poor sanitary condition of environment and inadequate/poor availability of potable water supplies in Nigeria. Accumulating evidence indicated that hepatitis E is a zoonotic disease. Since animals share the same habitat with humans and possibly drink from common source, this may lead to cross contamination. The effect in pregnant women and animals as likely reservoir is well documented. HEV screening is not routinely done in Nigeria, and neither documented prevalence data nor that on the likely effect on Liver enzymes in the current study area exists. HE is documented to have a mortality rate of up to 20% in pregnant women. It is possible that the disease has been thriving unnoticed, among vulnerable groups such as; pregnant women, animal handlers, HIV positive subjects and apparently healthy people in the study area, most likely because an epidemic data has not been documented in Nigeria. There is therefore an urgent need for a research of this nature to provide necessary information for pro-active strategy formulation.

2. MATERIALS AND METHODS

2.1 Study Design

The study was a descriptive cross-sectional epidemiological study. It examined the relationship between HEV seropositivity and Liver function enzymes (ALT and AST) as they exist in the study population.

2.2 Study Area

The research was carried out in Plateau State with its capital as Jos, and located in the North Central Region of Nigeria. Jos is situated on latitude 9.5ºN and longitude 8.5ºE, and is 4000 feet above sea level. Principally, the state experiences two types of seasons (dry and rainy seasons), with modifications resulting from its higher altitude. The sources of water supply in the study area were; stream, well and borehole. General observations were made about the hygiene situation in the households and water sources proximity. Animals faeces, were regularly seen inside the houses, yard and around the water sources. Additionally, there was little care for maintenance of sanitary standards.

2.3 Study Population

The study population included: apparently healthy, pregnant women, HIV positive patients and animal handlers (Animal handled included; pigs, goats, sheep, and cattle). Extensive efforts were made to ensure high participation rates, i.e. through the hospital authorities, village heads and churches by announcements and encouraging the people to participate.

2.4 Inclusion Criteria

Adults and children of both sexes and all age groups that are sick or apparently healthy were chosen as the study population.

2.5 Exclusion Criteria

Individuals with drug history of immuno-suppressive therapy or critically ill were excluded from the study population.

2.6 Ethical Considerations

The study protocol was reviewed and approved by the Ethical Committee of Plateau State Specialist Hospital Jos, with respective protocol number “NHREC/05/01/2010b”. All participants endorsed a written informed consent form.

2.7 Sample Size

The minimum sample size was calculated from the general formular; $n = \frac{(Z)^2(P)(1-P)}{d^2}$ as described by Fisher et al. [16]; Thrustfield [17].

2.8 Sampling Technique

A non probability sampling technique by Purposive Selection was used to select the study subjects as described by Thrustfield [17]. Those that did not fit the inclusion criteria were eliminated and the next on the list simply

replaced. Subjects were recruited from; hospitals, churches, farms, schools and community halls.

2.9 Collection and Processing of Samples

Ten milliliter (10 ml) of blood samples were collected aseptically from each of the 426 consenting participants in plain tubes. The samples were centrifuged and the sera separated and kept frozen at -80℃ until analyzed.

2.10 Detection of HEV Antibodies

The serum samples were screened for the presence of Hepatitis E virus IgM and IgG antibodies. The test was carried out using Enzyme - linked immumosorbent assay (ELISA) kits for the qualitative detection of IgG and IgM class of antibodies to hepatitis E virus in human serum. The ELISA kits were manufactured by Diagnostic Automation, Inc, craftsman roads, Calabasas, CA91302. USA.

2.11 Assay Principles Scheme: Indirect Elisa

Ag(p) + Ab(s)→[Ag(p) - Ab(s) + ENZ]→[Ag(p) - Ab(s) - ENZ→blue→yellow (+)
Ag (p) +→[Ag(p) + ENZ]→[Ag(p)] →no colour (-)

2.12 Measurement of Liver Enzymes

2.12.1 Alanine aminotransaminase (ALT) and aspartate aminotransaminase (AST)

A total of 200 serum samples (out of the overall total 426) of study subjects that tested positive for HEV IgG and IgM antibodies were obtained and analysed.

The liver enzymes (ALT, AST) were measured in anti-HEV positive human serum based on optical density (OD) of EIA assay (2.0 and above), using Randox kits (Randox USA) for ALT and AST respectively. The Randox kits were based on colorimetric methods as described by Reitman and Frankel [18].

2.12.2 Principle of ALT

The pyruvate produced by transamination activities of alanine transaminase reacts with 2,4-dinitrophyl hydrazine (DNPH) to give a brown coloured hydrazone which is measured colorimetrically at 510nm.

2.12.3 Principle of AST

Oxaloacetic acid reacts with aspartate transaminase which decarboxylates it spontaneously to pyruvate, which then reacts with 2,4-dinitrophenyl to form brown-coloured hydrazone. The hydrazon formed is measured colorimetrically at 510nm.

2.12.4 Statistical analysis (data management and analysis)

Data recorded during sampling and laboratory findings were entered and stored in MS-Excel. The data were thoroughly screened for errors and properly coded before subjected to statistical analysis by means of Statistical Package for Social Sciences (SPSS) version 17.0 statistical software (SPSS, Inc., Chicago, IL, USA).

Descriptive statistics were prepared from the study samples, and results were presented as means ± SD or percentage. The Pearson Chi square (χ^2) test was used to compare categorical data, and to evaluate the statistical significance between relevant variables. All P values were based on a two sided test of statistical significance. Significance was accepted at the level of $P<0.05$.

3. RESULTS

3.1 Liver Enzymes

Out of the total 426 sample screened; Two hundred (200) human sera samples that were anti – HEV positive were selected for the assay of liver enzymes; ALT and AST.

Table 1 represents the distribution of HEV antibody status among study subjects. Results indicated the following seropositivity rate; pregnant women 45/108, HIV-positive 34/80, apparently healthy 91/190, animal handlers 30/48.

3.2 ALT

Among individuals who tested positive for anti-HEV antibodies, 188(94.0%) had normal ALT levels with a mean value of 3.9±2.8 I.U/L, while 9(4.5%) were one and a half times (1.5 folds) the upper limit of normal value (12 I.U/L), with a mean value of 16.2±1.3 I.U/L, and 3(1.5%) had values twice the upper limit of normal with a mean of 19.7±1.2 I.U/L. HIV positive subjects

and animal handlers recorded the highest elevated ALT level up to twice the upper limit of normal. HIV positive subjects recorded a mean of 4.1±2.8 I.U/L, 17.0±1.0 I.U/L and 21.0±0.0 I.U/L, for normal, 1.5 folds, and 2 folds respectively, while animal handlers recorded a mean of 3.6±1.6 I.U/L, 15.0±0.0 I.U/L, 19.0±0.0 I.U/L for normal, 1.5 folds, and 2 folds respectively. Pregnant women and apparently healthy subjects had only up to 1.5 folds above the upper limit of normal ALT level. Pregnant women had mean of 6.0±2.8 I.U/L and 16.5±1.0 I.U/L for normal and 1.5 folds respectively, while apparently healthy subjects recorded mean of 3.0±2.6 I.U/L and 14.0±0.0 I.U/L for normal and 1.5 folds respectively (Table 2).

3.3 AST

A total of 137 (68.5%) had normal AST level with a mean of 5.2±3.6 I.U/L, 31(15.5%) had elevated AST of 1.5 folds with a mean of 14.7±1.8 I.U/L, 14 (7.0%) had elevated level of 2 folds with a mean of 20.4±1.6 I.U/L, while 18(9.0%) had elevated AST level of 3 folds with a mean of 40.5±28.8 I.U/L. Among Pregnant women, 22(48.9%) had a normal AST level with a mean of 6.5±3.1 I.U/L, 13(28.9%) had 1.5 folds with a mean of 14.9±2.1 I.U/L, 3(6.7%) had 2 folds with a mean of 21.3±2.1 I.U/L, while 7 (15.6%) had elevated AST level thrice the upper limit of normal with a mean AST of 47.7±45.5 I.U/L. HIV positive subjects recorded AST level of 23(67.6%) with a mean of 5.7±3.7 I.U/L, 5 (14.7%) with a mean of 14.6±2.1 I.U/L, 2 (5.9%) with a mean of 19.5±0.7 I.U/L, 4(11.8%) with a mean of 35.8±4.1 I.U/L for normal, 1.5 folds, 2 folds and 3 folds respectively. Apparently healthy subjects recorded AST level of 64 (70.3%) with a mean of 5.1±4.1 I.U/L, 13(14.3%) with a mean of 14.4±1.5 I.U/L, 8(8.8%) with a mean of 20.3±1.8 I.U/L, 6(6.6%) with a mean of 36.2±14.2 I.U/L, for normal, 1.5 folds, 2 folds and 3 folds respectively. Animal handlers recorded AST value of 28(93.3%) with a mean of 4.2±1.9 I.U/L, 1(3.3%) with a mean of 20.0±0.0 I.U/L, 1(3.3%) with a mean of 35.0±0.0 I.U/L, for normal, 2 folds and 3 folds respectively (Table 3).

4. DISCUSSION

Biochemical determination of hepatic cytolysis by the detection of high levels of transaminases is an important parameter as indicator of and in the control of liver disease. In the current study, the level of transaminases (ALT and AST) was one and half (1.5 folds) to three times (3 folds) higher than normal in seropositive subjects. Animal handlers and HIV positive subjects recorded the highest elevated ALT level up to 2 folds above normal, while apparently healthy had the highest normal ALT level. Pregnant women had the highest elevated AST level of up to 3 folds above normal. This is similar to the finding of Adjei et al. [19] who in their study found significantly high ALT and AST levels >3 folds the expected maximum among persons occupationally exposed to pigs, and suggested the possibility of subclinical infections in Ghana. In a study in Iran, an association between HEV positivity and elevated liver enzymes was established [20].

Table 1. Seroprevalence status of HEV among study subjects

Antibody status	Variable				
	A/ Healthy n=190	P/women n=108	HIV/Positive n=80	A/ Handler n=48	Total N=426
HEV +ve	91	45	34	30	200
HEV-ve	99	63	46	18	226

Key: +ve = positive; A/healthy = Apparently Healthy; A/Handler= Animal Handler; -ve = Negative; P/Women = Pregnant women

Table 2. Distribution of mean values of ALT levels among anti-HEV seropositive subjects

Subjects	Normal		1.5 folds		2 folds		3 folds		
	Mean	N(%)	Mean	N(%)	Mean	N(%)	Mean	N(%)	Total subjects
Pregnant women	6.0±2.8	41(91.1)	16.5±1.0	4(8.9)	-	-	-	-	45
HIV	4.1±2.8	30(88.2)	17.0±1.0	3(8.8)	21.0±0.0	1(2.9)	-	-	34
Apparently Healthy	3.0±2.6	90(98.9)	14.0±0.0	1(1.1)	-	-	-	-	91
Animal handlers	3.6±1.6	27(90.0)	15.0±0.0	1(3.3)	19.0±0.0	2(6.7)	-	-	30
Total	3.9±2.8	188(94.0)	16.2±1.3	9(4.5)	19.7±1.2	3(1.5)	-	-	200

Normal ALT level – up to 12 I.U/L; Values are expressed as Mean ± SEM

Table 3. Distribution of mean values of AST levels among anti-HEV seropositive subjects

Subjects	Normal		1.5 folds		2 folds		3 folds		Total
	Mean	N(%)	Mean	N(%)	Mean	N(%)	Mean	N(%)	subjects
Pregnant women	6.5±3.1	22(48.9)	14.9±2.1	13(28.9)	21.3±2.1	3(6.7)	47.7±45.5	7(15.6)	45
HIV	5.7±3.7	23(67.6)	14.6±2.1	5(14.7)	19.5±0.7	2(5.9)	35.8±4.1	4(11.8)	34
Apparently Healthy	5.1±4.1	64(70.3)	14.4±1.5	13(14.3)	20.3±1.8	8(8.8)	36.2±14.2	6(6.6)	91
Animal handlers	4.2±1.9	28(93.3)	-	0(0.0)	20.0±0.0	1(3.3)	35.0±0.0	1(3.3)	30
Total	5.2±3.6	137(68.5)	14.7±1.8	31(15.5)	20.4±1.6	14(7.0)	40.5±28.8	18(9.0)	200

Normal AST level – up to 12 I.U/L; Values are expressed as Mean ± SEM

Several other studies [21,22,23] had suggested that elevated serum ALT and AST may be markers of HEV infection and that individuals with elevated ALT and AST may have ongoing subclinical infection of HEV. This may confirm the presence of hepatic cytolysis, which usually accompanies infection with hepatitis viruses [24]. Specific changes in the morphology of liver tissue have been observed in HE patients [25].

HEV infection accompanied by chronic hepatitis and liver cirrhosis has been documented in immunocompromised hosts, such as organ transplantation recipients (liver, kidney, or pancreas) and HIV infected patients [26,27]. The HEV affects the hepatocytes resulting in the abnormal function of the liver [28]. The values of alkaline phosphatase (ALP) were increased in positive patients as compared with the control group.

Galiana et al. [29] reported from their work that the fact that the values of transaminases were similar between positive and negative individuals suggests that HEV might be responsible for subclinical infections, because none of the participants reported any past clinical signs of acute hepatitis. Growing evidence suggest that elevated ALT and AST levels are associated with recent acute HEV infection [21,22,30,31]. Similar results were also obtained in the current study and thus provided a unique opportunity to diagnose asymptomatic and symptomatic HEV infection in an occupationally exposed group. The presence of seropositive IgM/IgG anti-HEV and increased levels of ALT and AST usually indicate recent HEV infection [31] but data from current study (ALT levels are usually not as high in chronic hepatitis, ALT and AST often less than 4 times normal) also suggest that the presence of anti-HEV is associated with chronic liver disease, although the specific role, if any, of HEV infection on the severity of chronic liver disease and/or the meaning of enhanced HEV antibody production needs further clarification.

5. CONCLUSION

This is the first study in Nigeria to the best of our knowledge, reporting elevated ALT and AST levels associated with HEV seropositive subject among various groups including those occupationally exposed to animals. Consistent with similar studies worldwide, the results of our studies suggest an association of HEV infection, with elevated ALT and AST values. It is recommended that further studies be done on the association of HEV seropositivity and liver enzymes elevation, alongside HEV RNA testing.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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