Prevalence of Hepatitis E Virus among Children Presenting with Diarrhea in Selected Hospitals in Kano Metropolis

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Abstract: Hepatitis E Virus (HEV) is a spherical non-enveloped single-strand positive-sense RNA virus of the *Hepevirus* genus and the family *Hepeviridae*. Its infection is newly recognized serious threat to global public health and Africa has been reported to be among the most severely affected regions in the world, most likely due to poor sanitation and weak public health facilities. The aim of the study was to determine the prevalence of HEV infection among children presented with diarrhea attending Hasiya Bayero Paediatric and Murtala Muhammad Specialist Hospital Kano. The study was a cross sectional studies involving 90 children, selected by simple random sampling. Information was obtained using a questionnaire and stool samples were collected from the subjects for evaluation for detection of HEV immunoglobulin G (IgG) using enzyme linked immunoassay kit. Data were summarized as percentages, charts and frequency tables and results were computed and analyzed using IBM SPSS version 20.0. Out of the 90 participants, 3 (3.3%) were positive while 87 are negative with Hepatitis E virus infection. Age and gender were insignificantly associated with HEV infection among the studied children, while type of toilet use (Pit latrine) is significantly association with HEV infection (P=0.033). The highest prevalence was observed among children aged 10- 16 years.
Keywords: Hepatitis E Virus, children, diarrhea, Kano

INTRODUCTION

Hepatitis E Virus (HEV) is a spherical non-enveloped single-strand positive-sense RNA virus with approximately 32 to 34nm in diameter (Ifeorah *et al.,* 2017). Recognized originally as a member of the *Calicivirus* genus of the *Caliciviridae* family but now reclassified into the *Hepeviridae* family in the *Hepevirus* genus (Teshale and Hu, 2011). Hepatitis E Virus infection is responsible for over 50 % of acute viral hepatitis cases (Nan *et al.,* 2018)

There are four major genotypes of human HEV recognized, HEV genotypes 1 and 2 have been found only in humans and are prevalent in developing countries (Mansuy *et al*., 2016; Wang *et al.,* 2018). They are responsible for both sporadic cases and large out breaks linked to drinking contaminated water (Mansuy *et al.,* 2016).

HEV genotypes 3 and 4 are zoonotic disease maintained in nature by animals and transmissible to humans or vice versa and widespread in developed countries (Mansuy *et al*., 2016).

These HEV genotypes have been detected in a wide range of domestic and wild animals, which are believed to play a major role in the human epidemiology of the virus (Kamar *et al.,* 2012).

HEV has been reported to be transmitted via faecal-oral route (Hoofnagle *et al*., 2012). The first reported cases of Hepatitis E virus was in 1955 during an outbreak in New Delhi, India (Hoofnagle *et al.,* 2012).

HEV has been reported to cause serious sequelae in children resulting from diarrhea with an increase morbidity and mortality (Tan *et al.,* 2019).

Ordinarily, HEV has been known to cause self-limited acute infection in humans, but new reports showed evidence that HEV infection can cause significant morbidity and mortality in certain high risk group and individual with compromised immune systems (Olabode *et al*., 2017). These include patients with pre-existing liver disease (where HEV infection can result in death with mortality rate as high as 60%, or evolve to a chronic state), immune-compromised subjects (Kamar *et al.,* 2012), and transplant recipients (Koenecke *et al.,* 2012). Another indicative clinical feature of HEV is its high frequency and severity in pregnant women in low income countries with mortality rates around 10-20 % (Kamar *et al.,* 2014; Gupta and Agarwala, 2018) specifically in the third trimester (Hoofnagle *et al*., 2012).

In addition to Hepatitis, HEV infection also appears to be associated with some extra-hepatic manifestations; neurological disorders such as Guillain-Barre syndrome and neuralgic amyotrophy due to peripheral nerve involvement (Peter *et al.,* 2017), haematological diseases such as haemolytic anaemia (in people with the hereditary risk factor glucose-6-phosphate dehydrogenase deficiency) and Severe thrombocytopenia, Glomerulonephritis with nephrotic syndrome and Mixed cryoglobulinemia (Hoofnagle *et al*., 2012; Dalton *et al.,* 2018).

Infected individuals subsequently develop antibody to the HEV which are both of the immunoglobulin M (IgM) and immunoglobulin G (IgG) types (Webb and Dalton, 2019).

The study was aim to determine the prevalence of HEV infection among children attending Hasiya Bayero Paediatric and Murtala Muhammad Specialist Hospital Kano.

MATERIALS AND METHODS

Study Area

This study was conducted in Hasiya Bayero Paediatric and Murtala Muhammad hospital located within Kano metropolis. The state has a total land area of 20,760sq kilometer. It is located on latitude 11^0N and longitude 8E. Kano state borders with Katsina state to the north-west, Jigawa state to the north-east, Bauchi state to the south-east and Kaduna state to the south-west (Kurawa, 2006).

Study Population

Study population comprised of all children attending Hasiya Bayero Paediatrics and Murtala Muhammad Specialist hospitals Kano from July 2019 to September, 2019.

Ethical Approval

Ethical clearance to conduct the research was collected from Ministry of Health Kano State.

Inclusion Criteria and Exclusion Criteria

Children presenting with diarrhea who gave inform consent or whose care givers consented for the study were included in the study. While those children that were not presented with diarrhea and those who did not consent for the study was excluded.

Sample Size Determination

The sample size was calculated using the following formula (Cochran, 1977)

$$n = \frac{z^2pq}{d^2}$$

Where;

n = sample size

z = statistic for level of confidence at 95% (1.96)

p = prevalence of HEV among children (6.5%) = 0.065 (Bugaje *et al.,* 2011)

d = 1 – 0.065 = 0.935

d = allowable error of 5% (0.05)

$$n = \frac{(1.96)^2 \text{ x } 0.065(0.935)}{(0.05)^2} = \frac{3.8416 \text{x} 0.065(0.935)}{0.0025}$$

n = 93 children

Sampling Technique

Simple random sampling was employed

Data Collection

Stool samples were collected and socio-demographic data of children, was obtain using a questionnaire.

Sample Collection, Processing and Storage

Stool sample was collected from each of the participant in a clean screw capped container and was stored at -20 degree until ready for use.

Sample Analysis

Stool sample was assayed for HEV-IgG antibody, using HEV-IgG ELISA assay kit technique (Melsin medical co., Limited).

Principle of Test

This HEV-IgG Elisa employs solid phase, indirect Elisa method for detection of IgG class antibodies to HEV (Anti-HEV) in two step incubation procedure. Polystyrene microwell strips are pre-coated with HEV recombinant. During the first incubation step, HEV specific antibodies if present will be bound to the solid phase pre-coated HEV antigens. The wells are washed to remove unbound proteins and then anti-Human IgG antibodies (Anti-IgG) conjugated to horseradish peroxidase (HRP Conjugate) is added. During the second incubation step, these HRP-conjugated antibodies will be bound to any antigen-antibody (IgG) complexes previously formed and the unbound HRP-Conjugate is then removed by washing chromogen solutions containing Tetramethyl benzidine (TMB) and urea peroxide are added to the wells and in presence of the antigen-antibody-anti-IgG (HRP) immunocomlex. The colorless chromogens are hydrolysed by the bound HRP conjugate to a blue coloured product. The blue colour turns yellow after stopping the reaction with sulfuric acid. The amount of colour intensity can be measured and is proportional to the amount of antibody captured in the wells and to the samples respectively. Wells containing samples negative for HEV-IgG remain colorless.

Procedure

The assay was performed following the manufacturer instruction.

All reagents were prepared before starting assay procedure. Three wells were marked as Negative control, two wells as positive control and one blank. 100µl of specimen diluents was added into the wells except the blank and separate pipettes was used to add 50µl of positive control, Negative control reagent and 10µl specimen into their respective wells except the blank. And the plate was mixed by tapping gently. The plate was covered with adhesive strip and incubated for 30minutes at 37^{o}C. At the end of the incubation, the plate cover was removed and discarded. The wells were washed five times with diluted wash Buffer. 100µl of HP-Conjugate was added into each well except the blank. The plate was covered with plate cover and incubated for 30minutes at 37^{o}C.At the end of the incubation, the plate cover was removed and discarded. The wells were washed five times with diluted wash Buffer. After the final washing cycle, the plate was turned down onto a blotting paper and was tapped to remove any remainders. 50µl of chromogen A and 50µl of chromogen B was added into each well including the blank. The plate was incubated at 37^{o}C for 15minutes avoiding light.50µl of stop solution was added into each well and mixed gently. The absorbance was read within 10minutes after stopping the reaction. The plate reader was calibrated with the blank and the absorbance was read at 450nm. The cut off value was calculated and the results were evaluated.

Statistical Analysis

Data obtained was summarized as percentages, and frequency tables and results computed and analyzed using statistical package for social sciences version 20.0Chi-square test was performed to analyse the differences for categorical variables. P value of < 0.05 was considered to be statistically significant at 95% confidence interval.

RESULTS

Out of the 90 children that presented with diarrhea, 3 (3.3%) was positive while 87 (96.7%) were negative (Table 1).

Table 1 further shows that out of 51 males screened for HEV 1 (1.1%) was positive, while out of 39 females screened 2 (2.2%) were infected with HEV (P= 0.407). it was also shown that out of the 90 children studied, 33 were aged 3 – 9 years with 1 (3.03%) of them infected with HEV, while 57were aged 10-16 years with 2(3.51%) of them infected with HEV (P=0.903) (Table 1).

Table 1 also shows that there was no significant difference in the prevalence of HEV 1 among the studied children with regards to their educational level where 5% (2) of the 40 children with primary education had HEV 1 compared to 4% (1) out of 25 children with secondary education(P=0.538). Similarly, Table 1 show that there was no significant difference in the prevalence of HEV 1 among the studied children with regards to their ethnic background where 1.60% (1) of the 64 children were Hausa had HEV 1 compared to 8% (2) out of 25 children that were Fulani (P=0.309).

Table 1: Prevalence of Hepatitis E virus infection among studied children and their socio-demographic factors

Variables	Number screened	Number positive	Prevalence (%)	P value
Gender				0.407
Male	51	1	1.10	
Female	39	2	2.20	
Total	**90**	**3**	**3.30**	
Age group (Years)				0.903
3 – 9	33	1	3.03	
10 -16	57	2	3.51	
Total	**90**	**3**	**3.30**	
Educational level				0.538
None	25	0	0	
Primary	40	2	5	
Secondary	25	1	4	
Total	**90**	**3**	**3.30**	
Ethnic group				0.309
Hausa	64	1	1.60	
Fulani	25	2	8	
Yoruba	1	0	0	
Total	**90**	**3**	**3.30**	

Table 2 revealed that 1.1% (1) out of the 10 studied children that use well as their water source were infected with HEV while 2.2% (2) of the remaining 80 children use borehole as their water source (P=0.213). The prevalence of HEV infection was found to be significantly associated with the type of toilet used with 2.3% of the children using Pit latrine and 20% using open field for (P=0.033) (Table 2).

Table 2 also shows that animal rearing was insignificantly associated with the prevalence of HEV infection among the studied children although only 1 (6.25%) out of the 16 children were associated with cattle rearing and 2 (3.77%) out of the 55 children did not rare any of the animals (P=0.882).Blood transfusion was also insignificantly associated with the prevalence of HEV infection among the studied children (P=?) (Table 2).

Table 2: Distribution of Hepatitis E virus in relation to risk factor among studied children

Risk factors	Number screened	Number positive	Prevalence (%)	P value
Source of water				0.213
Well	10	1	1.1	
Borehole	80	2	2.2	
Total	**90**	**3**	**3.30**	
Type of toilet used				0.033**
Open field	5	1	20	
Pit latrine	85	2	2.3	
Total	**90**	**3**	**3.30**	
Animal rearing				0.882
None	53	2	3.77	
Birds	6	0	0	
Cats	13	0	0	
Chicken	2	0	0	
Cattle	16	1	6.25	
Total	**90**	**3**	**3.30**	
Blood transfusion history				0.409
Yes	64	1	1.7	
No	25	2	8	
Total	**90**	**3**	**3.30**	

**Statistically significant

DISCUSSION

The findings of this study revealed the presence of HEV among children that reported to Hasiya Bayero Pediatrics Hospital and Murtala Muhammad Specialist Hospital in Kano metropolis. This observation support earlier documented studies that Hepatitis E virus (HEV) infection remains endemic in developing countries, particularly in regions where there is poor sanitation coupled with contaminated water supply (Teshale and Hu, 2011).The prevalence rate of 3.3% for HEV IgG antibodies obtained in this study is however, much lower compared to the 22.2% reported by Adesina *et al*. (2009) in Ekiti, South Western Nigeria. This variation may be due to differences in the age of the study populations as our studied population were children that most of them were yet to be infected with virus which may have accounted for the observed difference in prevalence, while over 90% of the study population in Adesina's report were adults (>18years) (Tan *et al.,* 2019).

The prevalence rate of 3.3 % in this study was however slightly comparable to that documented by Martinson *et al.* (1999) in rural Ghana where they found a prevalence of 4.4% among children. It is however, lower than that of Bugaje *et al.* (2011) in North Central part of Nigeria, with a prevalence of 6.5% documented among school children.

In Southwest Iran a higher prevalence of 8.5% was documented among school children aged 6 - 15 years (Shamsizadeh *et al.,* 2010). In India Mathur *et al.* (2001) found a much higher prevalence of 26.3% among children. Unlike in this study, Mathur *et al*. (2001), included children aged 6 months to 10 years in their study. Another possible explanation for the observed difference in prevalence could be due to the large and overcrowded population in India compared to Nigeria (Bugaje *et al.* 2011).

In this study, the prevalence of HEV was found to be significantly higher in females than in males. Other studies revealed that, in adults HEV infection is predominantly reported in men with a male: female ratio ranging from 1:1 to 3:1.5 (Dalton *et al*., 2008; Bricks *et al.,* 2019). However, gender bias in relation to hepatitis E virus infection remains bizarre.

This study showed that children aged 10- 16 years had the highest HEV IgG prevalence (66.6%). This finding may be due to the accumulation of infected individuals within this age group resulting in the presence of IgG for up to 14 years (Nicolette and Daniel, 2001) following infection in summation with new or recent infections. It is also likely that new infections possibly increase with age as mobility and risk of exposure to contaminants and high risk institutions becomes increased (Mathur *et al.,* 2001). The finding was however at variance with the findings of Mahrt *et al*. (2018) in southern Germany who found that the prevalence of HEV positivity lightly declined with age and was not statistically significant.

Poor hygienic condition was also found to be significantly associated with prevalence of HEV infection among the study subjects where the prevalence was highest among those who used pit latrine (P= 0.033). These observations were similar to those reported by Bugaje *et al.* (2011) in their study.

The current finding coupled with the prevalence of HEV positivity which was found to be higher, though not statistically significant, among users of Borehole water, suggest poor water supply and or contamination as a contributing risk factor to HEV infection.

CONCLUSION

It was concluded that the prevalence of HEV infection (anti-HEV IgG antibodies) among children within Kano metropolis, Nigeria was found to be 3.3%. The prevalence of HEV among the children was found to be insignificantly higher in females, in those aged 10-16 years, in those with primary education and those that use well water. The prevalence was however significantly associated with type of toilet use (open field).

RECOMMENDATIONS

The study recommends public health education on personal hygiene and promotion of sanitary conditions especially in primary and secondary schools. There is also need for further studies involving large number of samples size.

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