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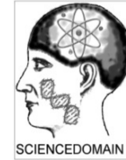

Study of Hepatitis E Virus in Minia Governorate, Upper Egypt

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Authors' contributions

This work was carried out in collaboration between all authors. Author SMAEE designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author MSM managed the analyses of the study and participated in writing the first draft of the manuscript and also the corresponding author. Author MAME managed the literature searches. Author SAEAH collected the samples. All authors read and approved the final manuscript.

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ABSTRACT

Aim: Hepatitis E is a viral liver disease caused by the hepatitis E virus (HEV), which is endemic in many tropical countries. We aimed to study the incidence of HEV infection at Minia governorate through Enzyme Linked Immunosorbent Assay (ELISA) and Polymerase Chain reaction (PCR) tests.

Methodology: The study found that among 129 cases of acute hepatitis, laboratory methods for diagnosis of Hepatitis viruses(A , B and c) were done by using ELISA for detection HBs Ag, HCV antibodies and HAV antibodies and all positive cases of Hepatitis A, B and C were excluded. All Negative cases were screened for HEV Using ELIZA and PCR

Results: Eight cases were positive by PCR and Five cases positive by ELIZA.

Conclusion: The incidence of HEV was low and PCR method was accurate than ELIZA in diagnosis.

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Keywords: Acute hepatitis; HEV infection; ELIZA and PCR.

1. INTRODUCTION

Hepatitis E virus (HEV) continues to represent a public health problem in developing countries. In developed countries, it has emerged as a significant cause of acute hepatitis. HEV infects a range of mammalian species. Genomic sequence similarity between some human HEV genotypes and swine HEV strains identified and known that humans can acquire HEV infection from animals. Although HEV infection is asymptomatic or mild, a significant risk of serious disease occurs in pregnant women and those with chronic liver disease. There are data on the threat of chronic infections in immunocompromised patients [1]. Beyond management of exposure by public health measures, recent studies support that active immunization can prevent hepatitis E, Focusing on the need for vaccination programmes as shown by [1] that the first one that reported that in Asia, 30 years ago, the main cause of non-A, non-B enterically transmitted hepatitis is hepatitis E virus (HEV). In countries with poor sanitation, HEV is endemic and typically causes outbreaks of acute hepatitis, usually associated with fecal contamination of the water supply. The disease is mild, but pregnant women suffer significant morbidity and mortality as reported by [2] due to pregnancy appears to be a potential risk factor for viral replication and an extremely low immune status of pregnant women, and a high level of steroid hormones that influence viral replication/ expression during pregnancy appear to be the major reasons for severity of the disease. In contrast, in countries with high standards of sanitation, hepatitis E occurs sporadically, initially identified as an imported disease in travelers from highly endemic regions, but subsequently diagnosed in patients with no travel history as well; this latter form has been named 'hepatitis E indigenous to developed countries [3].

Genetic analysis of HEV genome from different isolates was divided to four main genotypes, with genotypes 1 and 2 circulating in Africa and Asia, genotype 3 showing a broad distribution worldwide and genotype 4 being restricted to Asia. Genotypes 3 and 4 are zoonotic in a variety of wild and domestic animals, particularly pigs, which gave rise to the answer of whether human HEV infection is a zoonosis? Evidence from Japan [4] and China [5] now confirms that humans can acquire HEV infection from animals.

This study was aimed to assess the incidence of HEV infection at Minia governorate.

2. MATERIALS AND METHODS

In this study, One hundred and twenty - nine serum samples were collected from Minia Fever Hospital in the period from February 2013 to February 2014, after approval from the local ethical committee and obtaining consent about the aim of this study. All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki. Their age ranged from 18-48 year old.

All patients admitted to Minia Fever Hospital complaining of malaise, anorexia, fever and pruritus as a symptoms and icterus and hepatomegaly on examination were subjected to

- Demographic data including name, age, sex and residence were collected.
- Full history was taken with stress on history of elements playing a role in the mode of transmission of Hepatitis viruses as waterborne, food borne, person-to-person transmission and parenteral transmission.
- Liver enzymes assay in the form of AST (Biomed, Germany, reference range up to 37 for male and 40 for female), ALT Biomed, Germany, reference range up to 37 for male and 40 for female), and Total billirubin (Diamond, France, Reference range up to 1.0), All were performed according to manufacturer information.
- laboratory methods for diagnosis of Hepatitis viruses (A, B and C) were done by using ELISA for detection HBs Ag, HCV antibodies and HAV antibodies and all positive cases of Hepatitis A, B and C were excluded.

Five ml serum was taken and transferred in ice packs. The samples were centrifuged and put in the freezer at -80ºC, then all serum samples (non A non B non C) were tested for detection of antibodies to HEV followed by Polymerase chain reaction (PCR).

2.1 Detection of HEV Ab

ELIZA test for qualitative detection of antibodies (IgM) to Hepatitis E virus in human serum were done according to manufacturer's instructions (Diasorinkit, Italy). This kit is two-steps incubation. Solid phase antibody capture ELISA assay in which polystyrene microwell strips were pre-coated with antibodies directed to human immunoglobulin M proteins (anti- µchain).

2.2 Detection of HEV by PCR

2.2.1 RNA extraction

For purification of viral RNA, QIAamp® viral RNA Mini Kit (QIAGEN® INC, Germany -52906) was used.

2.2.2 Detection of viral RNA

90 µl of master mix was added to each sample and the mixture was incubated at: 42℃ for 45 min (one cycle), followed immediately by 35 cycles of the following conditions, 94℃ denaturation for 1 minute , 55℃ annealing for 1 minute, 72℃ extension for 1 minute; A final cycle of 72℃ for 10 minutes . PCR products were visualized by electrophoresis on ethidium bromide- stained 3% agarose gel in 0.5X Tris-borate-EDTA buffer (TBE) and evaluated under UV light. The sizes of PCR products were according to the migration pattern of 100-bp RNA ladder (GIBCO-BRL, Life Technologies, Gaithersburg, MD, USA).

2.3 HEV Primers (Ahmad et al., 2011)

ORF2 (s1) 5' GACAGAATTRATTTCGTCGGCTGG 3'

ORF2 (s2) 5' GTYGTCTCRGCCAATGGCGAGC 3'

2.4 Statistical Methods

The following tests were used:

- **Descriptive analysis** of the results in the form of percentage distribution for qualitative data (minimum, maximum, mean and standard deviation) calculation for quantitative data.
- **Cross tabulation test:** For comparison between percentage values.
- **Student t- test:** For comparison between means of two groups.
- **Fisher's exact test:** Used to calculate an exact P-value for a 2x2 frequency table with a small number of expected frequencies, for which the Chi-squared test is not appropriate.
- **P:** The probability/significance value
  - P value> 0.05 (NS) Not significant.
  - P value<0.05 * Significant at 0.05 level.
  - P value< 0.01 ** Significant at 0.01 Level.

3. RESULTS

The study was conducted in the period from February 2013 to February 2014, we collected 129 blood sample referred to Fever Hospital, Minia, Egypt. Serological tests of HBs Ag, HCV antibodies and HAV antibodies were done in all cases. Results were collected and we found that 43 blood samples were non A non B non C, which tested for detection of HEV IgM by Enzyme-Linked Immunosorbent Assay and nucleic acid detection of HEV by Polymerase Chain Reaction.

The mean age of the studied individual was 31.3 ±8.7 years and it ranged from 18-48 years**.** The frequency of male (69.8%) was more than the frequency of female (30.2%) among the studied individuals**,** the frequency of patients from rural areas (53.5%) was more than the frequency from urban areas (46.5%) among the studied individuals.

The mean HEV IgM concentration was 1.2±3.6 Abs/COV and it ranged from 0- 13.144 Abs/COV. The mean total billirubin was 3.6 ± 0.1 mg/dL and it ranged from 3.5- 3.8 mg/dL. The mean AST was 183.4 ± 0.8 IU/L and it ranged from 183-185 IU/L**.** The mean ALT was 128.1 ± 1.1 IU/L and it ranged from 126-130 IU/L of non A non B non C as shown in Table 1.

As shown in Table 2, the studied individuals were 11.6% reactive to HEV IgM while 88.4% were non reactive. Comparison between negative and positive HEV-IgM cases showed significant difference as regarding age and no statistically significant difference between negative and positive HEV-IgM as regarding sex and residences, HEV IgM concentration, total billirubin, AST and ALT as shown in Table 2.

As shown in Table 3. The studied individuals were 18.6% positive HEV conventional PCR while 81.4% were negative. The comparison between negative and positive HEV-PCR showed a significant difference as regarding age

and HEV IgM concentration, while no statistically significant difference between negative and positive HEV-IgM as regarding sex and residences, total billirubin, AST and ALT

Fig. 1. HEV PCR gel photo

HEV virion RNA was	*343 bp*
M RNA ladder	*100-1000 bp*
Positive samples	*No. 1, 3, 5, 7*
Negative samples	*No. 2, 4, 6*
Negative control	*No. 8*
Positive control	*No. 9*

As shown in Table 4**,** a comparison between positive HEV-PCR and positive HEV-IgM. Regarding the mean HEV-IgM concentration was statistically significant. While the mean of age, sex, residence, total billirubin, AST and ALT, were not statistically significant difference.

4. DISCUSSION

All patients non A non B non C admitted to Minia Fever Hospital complaining of malaise, anorexia, fever and pruritus as symptoms and icterus and hepatomegaly as signs were subjected to study. Only 43 non A non B non C patients with acute hepatitis for detection of HEV antibodies positive samples**.** These 43 non B non C patients were subjected to lab investigations including ELISA test and PCR**.** Five cases proved to be HEV IgM positive samples with an overall infection rate (11.6%) as shown in Table 2 which is lower than another reported author who reported that IgM anti-HEV were detected in 11 (13%) of 87 patients with acute hepatitis of unknown etiology treated between 1992 and 2001[6]. And higher than Fukai et al. [7] who reported that anti-HEV IgM was found in 5.4% of 126 patients. This can be explained by the change in the demographic area.

As shown in Table 2, a comparison between negative and positive HEV-PCR regarding The mean of age and the mean of HEV-IgM concentration were statistically significant difference while there were no statistically significant difference regarding sex, residence**,** total billirubin, AST and ALT.

Table 1. Descriptive analysis of age, HEV IgM concentration, Total Billirubin mg/dL, AST and ALT of non A non B non C

	N	Minimum	Maximum	Mean	SD
Age (Year)	43	18	48	31.3	8.7
HEV IgM Concentration	43	0	13.144	1.2	3.6
Total Billirubin mg/dL	43	3.5	3.8	3.6	0.1
AST IU/L	43	183	185	183.4	0.8
ALT IU/L	43	126	130	128.1	1.1

Table 2. Comparison between negative and positive HEV IgM

		HEV-IgM		P value	Sig.
		Negative (n=38) (88.4%)	**Positive (n=5) (11.6).**		
Age (Mean ± SD)		32.6±8.3	21.2±4.1	0.004	HS
Sex	Male	27(71.1%)	3(60%)	0.613	NS
	Female	11(28.9%)	2(40%)		
Residence	Urban	18(47.4%)	2(40%)	0.756	NS
Concentration (Mean ± SD)		0.1±0.14	10.2±5.1	0.001	HS
Total Billirubin mg/dL (Mean ± SD)		3.6±0.1	3.8±0.04	0.423	NS
AST IU/L (Mean ± SD)		183.5±0.86	185±0.0	0.231	NS
ALT IU/L L (Mean ± SD)		127.6±0.9	128.2±1.1	0.276	NS

Table 3. Comparison between negative and positive HEV PCR

		HEV-PCR		P value	Sig.
		Negative (n=35) (81.4%) Mean± SD	**Positive (n=8) (18.6%) Mean± SD**		
Age (Mean ± SD)		33.5±7.9	21.4±3.2	0.001	HS
Sex	Male	25(71.4%)	5(62.5%)	0.620	NS
	Female	10(28.6%)	3(37.5%)		
Residence	Urban	18(51.4%)	2(25%)	0.176	NS
	Rural	17(48.6%)	6(75%)		
Concentration (Mean ± SD)		0.8±3.0	5.1±5.4	0.002	HS
Total Billirubin mg/dL (Mean ± SD)		3.6±0.1	3.9±0.1	0.463	NS
AST IU/L (Mean ± SD)		183.4±0.8	184.5±0.9	0.761	NS
ALT IU/L (Mean ± SD)		128.1±0.9	130.3±1.7	0.650	NS

Table 4. Comparison between positive HEV-PCR and positive HEV-IgM

		HEV-PCR (n=8)	HEV-IgM (n=5)	P value	Sig.
Age (Mean± SD)		21.4±3.2	21.2±4.1	0.933	NS
Sex	Male	5(62.5%)	3(60%)	0.928	NS
	Female	3(37.5%)	2(40%)		
Residence	Urban	2(25%)	2(40%)	0.569	NS
	Rural	6(75%)	3(60%)		
HEV-IgM Concentration (Mean± SD)		3.1±5.4	10.2±5.2	0.041	S
Total Billirubin mg/dL (Mean± SD)		3.6±0.1	3.6±0.04	0.878	NS
AST IU/L (Mean± SD)		183.5±0.9	183±0.0	0.260	NS
ALT IU/L (Mean± SD)		128.3±1.7	127.6±0.9	0.444	NS

This was in agreement with other studies which detected that regarding the total billirubin, AST and ALT, no statistically significant difference between negative and positive HEV-PCR as reported by [8-13].

Our results were in agreement with [11] who reported that HEV infections in Egypt occur with more frequency in young adults [14]. Our results are in agreement with other paper who reported that in a study of 202 patients with acute sporadic viral hepatitis in Egypt the highest level in the 20-29 years age group.

Authors reported that series of 10 hepatitis E cases, most patients were middle-aged males, with history of consumption of alcohol and undercooked meat [15]. Others reported that hepatitis E is a disease of the middle – aged as average age = 50 years [16]. Regarding sex, this is a different pattern than previously reported by [17] who published that acute hepatitis E was more common in males. This is may be due to the difference in the community.

As regard the percentage of positive HEV-PCR and positive HEV-IgM**,** we can suggest that serological diagnosis alone did not document HEV as HEV PCR was positive in 18.6% of cases.

In our study, 2 patients were found positive with both tests, 3 positive for IgM but negative for viral RNA, and 6 positive for RNA but negative for IgM. So, even though 2 patients were tested positive in both tests, another 3 were only tested positive in HEV IgM, and 6 positive for RNA but negative for IgM. This difference due to the infection was in the initial phase in which no antibodies have been produced. The 3 positive for IgM but negative for viral RNA may be dueto failure of replication of RNA. Our results were similar to another publisher who reported the more accuracy of HEV-PCR than the serological diagnosis [16].

5. CONCLUSION

From the previously mentioned results of this study**,** we can conclude that Hepatitis E virus

(HEV) continues to represent a major public health problem in developing countries. The more accuracy of PCR results in diagnosis of HEV viral infection.

We recommended that more studies for early diagnosis of patients recently infected with HEV and more studies about diagnosis of HEV infection in pregnant women.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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