BIOCHEMICAL PATTERN OF LIVER FUNCTION TESTS IN HEPATITIS E EPIDEMIC

Waqar Azim, Wajiha Farooq, Muhamad Qaisar Alam Khan, Nasira Shaheen, Mobeen Iqbal, Saad Azim

Army Medical College Rawalpindi

ABSTRACT

Objective: To evaluate the biochemical pattern of liver function tests in acute hepatitis E epidemic.

Study Design: Cross sectional descriptive study.

Place and Duration of Study: Department of Pathology Army Medical College, Rawalpindi from June '09 to Dec '09.

Patients and Methods: A total of 81 patients of acute hepatitis of all age groups, both male and female were included in the study. Patients who reported with symptoms of acute hepatitis and tested positive for hepatitis E IgM antibody were included. Detailed history, clinical examination and serial liver function tests (LFTs) were carried out. Weekly data of LFTs was arranged serially up to 4 weeks. The results were analyzed on SPSS version 17.

Results: The patients' age ranged from 17-59 years. Serum Alanine Transaminase (ALT) values were highest in the first 2 weeks reaching 100 times the upper limit of normal (ULN). Similarly serum total bilirubin (TBil) reached up to 8 times ULN in the 1st week. The increase in serum alkaline phosphatase (ALP) was 2 times ULN and then returned to within the reference range in the 3rd and 4th weeks before other variables. Serum albumin levels remained unchanged. Prothrombin time (PT) was found to be prolonged. Two cases ended up fatally. Rest recovered fully and became symptom free.

Conclusion: Hepatitis E is a serious clinical condition. LFTs play an important role in its diagnosis. There is marked derangement of LFTs. Sharp rise in serum ALT up to 100 times ULN is a significant feature and should raise the suspicion of acute Hepatitis E.

Keywords: ALP, ALT, Hepatitis E, Total Bilirubin.

INTRODUCTION

Hepatitis caused by hepatitis E virus (HEV) is a common cause of acute viral hepatitis particularly in the developing world. HEV was discovered in 1980 when the evidence of another non A, non B virus was provided[1]. It is a single stranded RNA virus, having diameter of 32kb and consists of 3 open reading frames (ORF). Four genotypes of HEV have been identified. Genotype 1; comprises Asian and African strains, genotype 2: the prototype Mexican strain and few African strains, genotype 3: strains from sporadic cases in industrialized countries, and genotype 4: strains from China and Africa[2]. Humans are considered to be the natural hosts of HEV but antibodies to this virus have also been detected in animals like swine, rodents and shellfish[3].

Infection by Hepatitis E virus is generally self limiting. The mortality rate is low (1-2%), however among pregnant women it is high (15-20%)[6]. The virus is shed in feces and fecal contamination of drinking water has been implicated as the source of infection in disease endemic parts of the world like Asia and Africa. However in non endemic areas sporadic cases and small outbreaks have been linked to zoonotic transmission of virus. For example, in Japan sporadic cases of acute hepatitis E have been linked to undercooked deer meat[4]. In March 2008, an outbreak occurring in a cruise ship was linked to the use of seafood on board[5]. Travel to disease endemic areas is also a source of spread of infection in non endemic parts of the world[6].

Hepatitis E is endemic in Pakistan and several epidemics have been reported[7]. The rural population of Pakistan does not have access to safe water supply. As they use untreated water from wells, ponds and rivers, the chances of contamination with human waste are quite high. Unfortunately the urban population is not safe either. Defective water

Correspondence: Brig Waqar Azim, Department of Pathology, Army Medical College Rawalpindi

Received: 01 March 2010; Accepted: 28 April 2010

supply lines and inappropriate layout of sewage pipes running parallel to water pipes greatly increases the likelihood of contamination. Keeping these facts in mind the importance of proper knowledge of hepatitis E cannot be undermined. Considering that it is a self limiting disease confirmatory tests for HEV are not done routinely, lack of funds also contributes to this. Early detection of hepatitis E is very important as it can lead to fatal outcome and patient remains disabled for a couple of months. In this scenario pattern of LFTs can be extremely helpful. This study was conducted with the objective of highlighting the importance of pattern of liver function tests during the course of the disease. Thus knowledge of how the LFTs respond to the hepatocellular insult caused by HEV is of fundamental importance in this part of the world where hepatitis E is endemic.

Recently an epidemic occurred in Rawalpindi during the summer of 2009. Aims and objective of this study are to analyze the pattern of LFTs including serum ALT, serum TBil, serum ALP, serum albumin and prothrombin time (PT) in patients of acute hepatitis E over a period of 4 weeks.

PATIENTS AND METHODS

This was a retrospective, laboratory based cross sectional observational study. Eighty one patients of all age groups and both sexes were included. The study was conducted in Army Medical College; Rawalpindi. All patients were positive for anti HEV IgM detected by enzyme linked immunosorbant assay (ELISA), which confirmed the diagnosis of acute hepatitis E. Serum ALT, serum TBil and serum ALP were done by colorimetric methods using kits by Pioneer (USA). Serum albumin was done by colorimetric method using Bromocresol Green (Diamate biotechnologies). These biochemical analyses were done on automated clinical chemistry analyzer Selectra E. PT was done on KC4 semi automated coagulometer, based on the principle of plasma activation by thromboplastin. Patient's values were compared with control value. The data was arranged serially into 4 weeks. The results were analyzed on SPSS version 17. Correlation among the variables was determined week vise using Pearson correlation. P value <0.05 was considered significant.

RESULTS

A total 81 patients were included in the study. Out of them 76(93.8%) were male. Mean age was 27.62 years, and age range was 17-56 years. Majority of patients (47/81, 58%) in this study were in the age group 17-27 years. The age distribution is shown in figure 1.

Serial values of LFTs over a period of 4 weeks were studied. Serum ALT was markedly raised. Highest values were seen in the first 2 weeks which then started declining in 3rd and 4th weeks. Mean serum ALT was raised up to 100 times ULN in the 1st and 2nd week. In the 3rd week the values were 12 times the ULN and in 4th week about 7 times the ULN. Mean serum TBil increased up to 8 times ULN in the first 2 weeks, declines to 6 times ULN in the 3rd week and 4-5 times ULN in the 4th week. Mean serum ALP was found to be elevated to less than 2 times the ULN in the first 2 weeks and then returned to baseline levels in the 3rd and 4th week. Serum ALP levels were the first to return to within the reference range as compared to other variables. Figure 2 shows the mean ALT, TBil and ALP values over time (weeks).

In our study we have worked out how the 3 variables correlate with each other during the course of illness over a period of 4 weeks. There was significant positive correlation between TBil and ALT in the 1st week ($r=0.433$, $p<0.01$), 2nd week ($r= 0.284$, $p<0.05$), 3rd week ($r=0.682$, $p<0.01$) and 4th week ($r=0.305$, $p<0.01$). Similarly there was significant positive correlation between TBil and ALP ($r=0.385$, $p<0.01$) in the 2nd week, and 3rd week ($r=0.289$, $p<0.01$). PT was prolonged in all patients when serum ALT values were highest. Serum albumin was found to be normal in all patients.

Incidentally we did not come across any pregnant female in our selected group of patients. There were two mortalities in the

epidemic and the rest recovered fully over

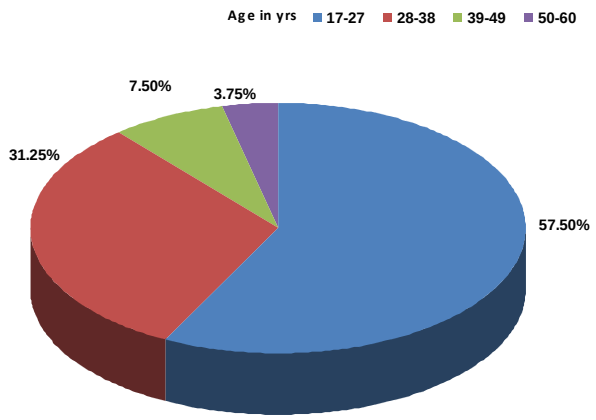

Figure 1: Age distribution in acute hepatitis E.

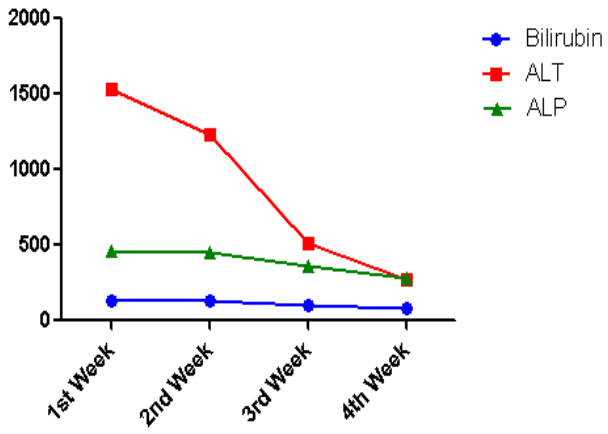

Figure 2: Mean ALT, TBil and ALP over time (weeks)

variable period of time.

DISCUSSION

Epidemics of hepatitis E have occurred across the globe, especially in the developing countries. The largest epidemic is reported to have occurred in Uganda in October 2007, which involved 10,196 people and caused 16 deaths[8]. Several epidemics have been reported in India. In Pakistan also several epidemics have occurred.

In 1987 an outbreak involving 133 people was reported in Sargodha[9]. In 1988 an epidemic was reported in a military unit in Abbottabad[10]. From December' 93 through March' 94 an epidemic occurred in Islamabad in which 3,827 cases were reported[11]. Six hundred cases have been reported in an epidemic affecting Lahore garrison in December 94[9]. In all epidemics the source was traced to be fecal contamination of drinking water caused by sewage mixing into drinking water pipelines.

HEV mostly affects young adults[9,12] as is the case in our study. Mostly males were affected (93.8%). This male dominant pattern of infection is consistent with the study of Bengu Said *et al*[5], but contrary to the findings of Teshale EH[8] where females were affected more.

In our study there is correlation among variables ALT, TBil and ALP. TBil peaks in the 1st two weeks. During the 1st week highly significant correlation between TBil and ALT shows that impaired conjugation by the injured hepatocytes is elevating TBil predominantly. In the 2nd and 3rd week TBil correlates significantly with both ALT and ALP. This possibly shows that both impaired conjugation and intrahepatic cholestasis are causing rise in TBil. In 4th week TBil correlates with ALT, again the probable cause of elevated TBil is impaired conjugation.

This pattern of response to the viral insult indicates that virus typically targets the hepatocytes and that the hepatocellular damage is maximum in the first 2 weeks. Changes in the ALP levels are due to intrahepatic cholestasis which subsides within 4 weeks. The hepatocyte injury (possibly due to viral replication in the hepatocytes) takes several weeks to recover. This is shown by the fact that ALT levels return to normal after several weeks. As in this study ALT levels persisted beyond 4 weeks. Serum albumin levels were not found to be deranged. This is due to the long half life of albumin (approximately 3 weeks). PT, a marker of liver synthetic function was found to be prolonged in all cases. Maximum prolongation of PT was 21 seconds.

These findings are consistent with the guidelines given by National Academy of Clinical Biochemistry on the use of laboratory tests in the diagnosis and monitoring of Hepatic Injury[13]. According to their recommendations, acute hepatic injury can be diagnosed by ALT

>10 times the appropriate upper reference limits and ALP < 3 times the appropriate upper reference limit. We see in our study that in acute hepatitis E serum ALT can be elevated to up to 100 times ULN whereas ALP elevates to < 2 times the ULN. Another recommendation is that 10-12% of patients have TBil >257µmol/L. In this study 8.75% of patients had TBil > 257µmol/L.

Thus study highlights the importance of LFTs as adjunct to diagnosis and management of acute hepatitis E. Correct knowledge and interpretation of these tests is valuable. Measurement of serum albumin plays no role in diagnosis and management of acute hepatitis E therefore is not mandatory.

Currently no vaccine is available for HEV. Trials for a successful vaccine are under way; a trial of recombinant HEV in Nepalese army seems promising[14]. The development of an effective vaccine that could prevent possible life threatening epidemics would be a breakthrough.

CONCLUSION

Hepatitis E is a serious clinical problem in Pakistan resulting in both morbidity and mortality in the affected population. LFTs remain the most efficient markers in diagnosis of the disease, as their serial values correlate well with the course of the disease. Sharp rise in serum ALT (up to 100 times ULN) is a significant finding. This along with relevant history can help to differentiate it from other causes of hepatitis. Multicentric studies are required for further evolving an effective strategy for diagnosis, management and prevention of the disease.

REFERENCES

1. Arankalle VA, Chadha MS, Tsarev SA, Emerson SU, Risbud AR, Banerjee K et al. Seroepidemiology of water-borne hepatitis in India and evidence for a third enterically-transmitted hepatitis agent. Proc Natl Acad Sci U S A 1994; 91(8): 3428-3432.
2. Zhao C, Li L, Harrison TJ, Wang Q, Song A, Fan J, et al. Relationship among viral diagnostic markers and markers of liver function in acute hepatitis E. Journal of Gastroenterology, 2009; 44(2): 139-145.
3. World Health Organization. Hepatitis E. fact sheet no 280. available from: http://www.who.intl/mediacentre/factsheet/fs280/en/.
4. Tei S, Kitajima N, Takahashi K, Mishiro S. Zoonotic transmission of hepatitis E virus from deer to human beings. Lancet 2003;362(9381):371-3.
5. Said B, Ijaz S, Kafatos G, Booth LH, Thomas HL, Walsh A et al. Hepatitis E Outbreak on Cruise Ship. EID 2009; 15(11).
6. Harris KR, Dighe AS. Laboratory testing for viral hepatitis. Am J Clin Pathol 2002;118(supp 1):18-25.
7. Alavian SM, Fallahian F, Lankarani KB. Epidemiology of hepatitis E in Iran and Pakistan. Hepatitis monthly 2009; 9(1): 60-65
8. Teshale EH, Howard CM, Grytdal SP, Handzel TR, Barry V, Kamili S et al. Hepatitis E Epidemic, Uganda. EID 2010;16(1):126-129.
9. Malik IA, Tariq WUZ. Hepatitis E in Pakistan. Eastern Mediterranean Health Journal 1996; 2(1): 121-128.
10. Bryan JP, Iqbal M, Tsarev S, Malik IA, Duncan F, Ahmed A et al. Epidemic of Hepatitis E in a military unit in Abbottabad, Pakistan. Am J Trop Med Hyg 2002; 67(6): 662-668
11. Rab MA, Bile MK, Mubarik MM, Asghar H, Sami Z, Siddiqi S et al. Water-borne hepatitis E virus epidemic in Islamabad, Pakistan: A common source outbreak traced to the malfunction of a modern water treatment plant. Am J Trop Med Hyg 1997; 57(2): 151-157.
12. Bashir K, Hussain N, Husnain S, Elahi S. Seroprevalence of hepatitis E virus immunoglobulin G and M antibodies in adults: A hospital-based study. Indian Journal of Medical Microbiology 2009; 27(2):139-141.
13. Dufour DR, Lott JA, Nolte FS, Gretch DR, Koff RS, Seeff LB. Diagnosis and monitoring of hepatic injury.II.Recommendations for use of laboratory tests in screening, diagnosis and monotoring. Clinical Chemistry 2000;46(12):2050-2068.
14. Shrestha MP. Safety and efficacy of a recombinant Hepatitis E vaccine. NEJM 2007; 356(9): 895-903