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ELIVATED ALT AS A SURROGATE MARKER IN HAEMOPHILIC SUBJECTS FOR DIAGNOSIS OF HEPATITIS C

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ABSTRACT:

INTRODUCTION: *Hepatitis C is a major cause of non-A, non-B viral liver disease through out the world in haemophilic patients. There is an urgent need to eliminate HCV from clotting factor concentrates by screening and other means; only when this has been achieved would it be possible to determine whether other agents cause chronic liver disease in patients with hemophilia. There have been many studies from Pakistan using prevalence of hepatitis C in chronically transfused patients (thalasemics, dialysis patients, blood donors and general population), but there has been no published study from Pakistan stating hepatitis C prevalence in haemophilic patients.*

OBJECTIVE : *This study was planned to see the prevalence of hepatitis C antibodies in haemophilic patients and its association with raised ALT (Alanine Aminotransferase) as a surrogate marker for hepatitis.*

PATIENTS AND METHODS: *Sera of eighty haemophilic patients registered and treated at JPMC and Fatmid center, Karachi were collected along with 20 control subjects and analyzed for hepatitis C antibody by Abbott HCV EIA 3.0 Kit. ALT was also done on all patients.*

RESULTS: *The prevalence of hepatitis C antibody in haemophilic patients was 80%.*

CONCLUSION: *The study concludes that its incidence is certainly high for which necessary steps are required to be taken but the results of ALT (Alanine Aminotransferase) clearly conclude that ALT can not be used as a surrogate marker for hepatitis C infection. Anti-HCV antibody may relatively be an expensive test but according to this study ALT as a surrogate marker for hepatitis could not replace it, because we could not establish a good correlation between hepatitis C positivity and elevated ALT levels*

KEY WORDS: Haemophilic, Hepatitis C, HCV, ALT.

INTRODUCTION

Viral hepatitis remain a major public health problem in the developed world. Hepatitis is characterized by the inflammation of the liver usually producing swelling and in many cases permanent damage to liver tissue. The most common types are Hepatitis A, B, C, D and E. While A and E are water borne and usually produce a milder form without chronic outcome. Both hepatitis B and C being blood born can lead to liver damage and in many cases death can ensue. All over the world about 75% people are infected with hepatitis C virus (Tanveer et al., 2008)[1].

Hepatitis C is caused by an RNA virus of the flaviviridae family. Hepatitis C is a disease with varying rates of progression. HCV is a blood born pathogen and is predominantly spread via exposure to contaminated blood or blood products. The incubation period on the average ranges from 2 to 7 weeks. Each year 30,000 new cases are being diagnosed. Prevalence is highest among groups with specific risk factors especially drug users by injection and patient with hemophilia or those on long term hemodialysis and also people who have received blood or organ product prior to June 1992 (Communicable disease service manual, 2005)[2].

Hepatitis C virus (HCV) infection appears to be endemic in most parts of the world with prevalence of 3%. Hepatitis B and C is highly endemic in Pakistan and its incidence has been increasing within the last decade. The commonest mode of HCV transmission is by contaminated blood and its products (Chaudhary et al., 2005)[3].

In Canadian post transfusion cohart information that haemophilic patients who are younger and are frequently co-infected with human immunodeficiency virus would have higher risk of cirrhosis (37%), hepatocellular carcinoma (12%) and liver related death (19%) (Thein

et al., 2009)[4].

Hepatitis C virus (HCV) infection has remained a major cause of morbidity and mortality in haemophiliacs who received non virucidally treated large pool clotting factor concentrates before 1986. Haemophiliacs infected patients with HCV have a slow progression of liver disease. In a majority of them hepatitis evolves towards end-stage liver disease and hepatocarcinoma (Franchim et al., 2006)[5].

Thalasemia and haemophilia are both life long crippling genetic disorders, both needing frequent blood or blood product transfusions. These disorders lead to premature death due to complication of disease and transfusion (Majrooh et al., 2003)[6].

About 1870 million or 3% people all over the world were infected with HCV. United Kingdom had lowest prevalence (5%) of HCV (0.1) and Egypt had the highest prevalence of HCV i.e. 28%. In India the HCV prevalence was 1.3% while in Pakistan the prevalence of HCV was 6.21% at the Rawalpindi Islamic Centre (Tanveer et al., 2008)[1].

The natural history of HCV infection is quite variable in immunocompetent patients. There are shared routes of transmission, coinfection with HIV and hepatitis C virus is common. It occurs in up to 60-90% in HIV positive patients who have received blood or its products. Furthermore, end-stage liver disease currently represents the leading cause of death among HIV infected injected drug users and haemophiliacs (Benhamou et al., 2004)[7].

In Pakistan each type of viral hepatitis is endemic producing its own pattern of illness and disease sequelae. Hepatitis B and C are the major public health problems because of overall higher prevalence and an adverse outcome in a significant number of people. The prevalence is particularly high in those on transfusion (Zuberi, 1996; Shamsi et al., 1998 and Tanveer et al., 2008)[1,8,9].

In the 1970s the treatment of haemophiliacs was revolutionized by the introduction of lyophilized large donar pool clotting factor concentrates. However, the early concentrates were not subjected to a viral inactivation. Procedure during preparation and their use was associated with a high prevalence of non-A, non-B (NANB) hepatitis (Cederbaum et al., 1982)[10].

Treatment of hemophiliacs with human factor VIII concentrate similarly carries a risk of hepatitis. There is a higher incidence of hepatitis in patients receiving transfusions of blood obtained from paid donors compared with patients whose transfusions were obtained from volunteer donors. The commonest form of hepatitis following factor VIII transfusion is reported to be HCV infection (Watson et al., 1992)[11].

FIGURE 1
HEPATITIS C POSITIVITY IN RELATION TO SEVERITY OF DISEASE

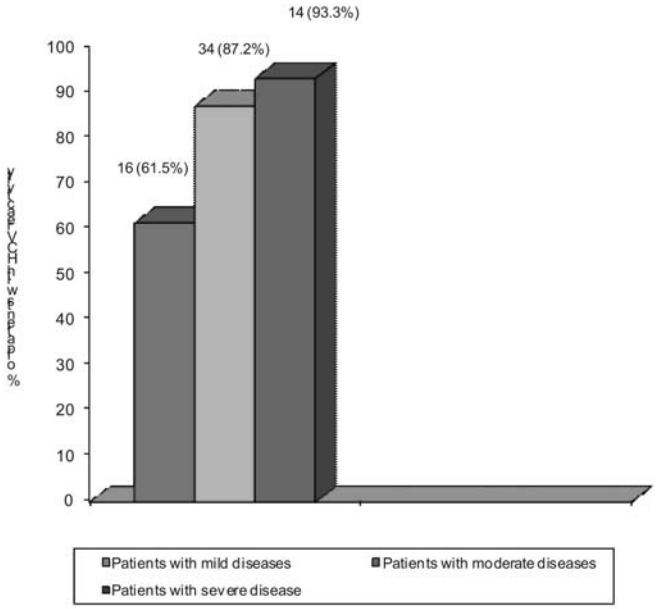


TABLE 1
HEPATITIS C POSITIVITY AND SEVERITY OF HAEMOPHILIA

Group	Hepatitis C reactivity	Anti-HCV negativity
Mild (n=26)	16 (61.5%)	1 (12.5%)
Moderate (n=39)	34 (87.2%)	05 (6.25%)
Severe (n=15)	14 (93.3)	01 (1.25%)
Haemophilic patients (n=80)	64 (80%)	16 (20%)
Control (n=20)	01 (5%)	19 (95%)
P value	<0.001	

MATERIALS AND METHODS

The study was done at the Department of Microbiology, BMSI, JPMC, Karachi during March 1998 to March 1999.

Eighty (80) Hemophilic patients aged between 0-19 years registered with Fatmid Thelasemia Centre and JPMC were included in this study. All patients had documented evidence of hemophilia. Controls were obtained from sera of 20 clinically healthy subjects selected from the staff and students of BMSI, JPMC. From each subject 5 ml of blood was collected by venepuncture. Serum was separated, divided into two equal aliquots that was properly labeled and stored at -20°C till its analysis.

Qualitative anti-HCV determination was done by using Abbott HCV EIA kit. Tests were read on Quantum II Abbott by using Module A, mode 1.24 (492.600 nm filter). Negative and positive controls were included.

INTERPRETATION OF RESULTS

As per criteria of Abbott HCV EIA third generation kit specimens giving absorbance values less than the cut off value were considered negative. Specimens with absorbance value greater or equal to cut off value were considered reactive

Data of patients regarding age, sex and degree

of hemophilia, frequency of transfusions per year etc. were recorded on a specific proforma.

RESULTS

A total of 80 haemophilic patients and 20 healthy subjects (all males) were included in this study. These cases were collected from Fatmid Foundation Blood Bank and Jinnah Postgraduate Medical Centre, Karachi for a prospective study. The study period was from March 1998 to March 1999. Of the 80 patients 26 (32.5%) had mild disease, 39 (48.75%) had moderate and 15 (18.75%) were severe haemophilic. This categorization of the disease was done on the basis of factor VIII assay (functional assay) in the plasma of patients (Figure 1). Hepatitis C antibody on Abbott HCV EIA 3.0 kit was positive in 64 patients (80%). Out of 20 control subjects one was found positive for anti HCV. The prevalence of anti HCV was significantly higher for the patients who had severe to moderate disease (Table 1). The results are statistically significant with P<0.001 when tested by Chi Square test.

ALT levels in patients and controls are shown in table 2. Of the 80 haemophilic patients, 39 (Group 1) had normal ALT and 41 (Group 2) had raised ALT. The mean value of first group of patients with normal ALT was 25.97±1.40 IU/L (Mean±SEM), and mean value of raised ALT among haemophilic patients was 90.80±3.29 (Mean±SEM). The mean difference of ALT among group 1 and group 2 is statistically significant with P<0.001 when tested by student's't' test.

The difference in mean ALT amongst both groups i.e. haemophilic patients having normal ALT compared to normal ALT of the control was found statistically significant with P<0.01 when tested by student's 't' test.

The final outcome was that out of 80 patients 64 were positive for HCV antibody with raised ALT. On the other hand 29 were those who were positive for HCV antibody but did not show raised ALT. Of the group of 41 patients with raised ALT levels, 35 were reactive for hepatitis C antibody and six were negative for antibody to hepatitis C virus (Table 3). The results were statistically non-significant when P>0.28 and when tested by Chi Square test.

TABLE 2
ALT LEVEL OF HAEMOPHILIC AND HEALTHY SUBJECT

ALT level	Haemophilic patients		Control n=20
	Group 1 (ALT<40IU/L)	Group 2 (ALT>40 IU/L)	
Range	15-40 IU/L	54-126 IU/L	9-37 IU/L
Mean±S.E.M.	25.97±1.40	90.80±3.29	19.5±1.81

G1 Vs G2 = P<0.001
G1 Vs Control = P<0.01
G2 Vs Control = P<0.001

TABLE 3
CORRELATION OF ALT LEVELS AND ANTI-HCV IN HAEMOPHILIC PATIENTS
N=80

	HCV positive	HCV negative	Total
Normal ALT (<40 IU/L)	29 (36.25%)	10 (12.41%)	39
Raised ALT (>40IU/L)	35 (46.24%)	06 (7.5%)	41
Total:	64 (80%)	16 (20%)	80

Chi square = 1.51 P < 0.28

DISCUSSION

Viral hepatitis is a major public health problem in Pakistan. Each type of virus is endemic, producing its own pattern of illness and disease sequelae. Hepatitis A virus (HAV) and hepatitis E (HEV) are transmitted via water and generally produce a non lethal clinical syndrome without chronic sequelae, while hepatitis B, C and D (HBV, HCV and HDV) are transmitted via blood/ blood products, use of contaminated syringes. Sexual, perinatal and household HCV transmission has also been reported (Zuberi, 1996)[9].

HCV infection is prevalent all over the country but appears to be more frequent in urban areas (Zuberi unpublished data) (Zuberi, 1996)[9]. Titres of HCV in blood are not as high as HBV. Recent data indicate that HCV exists as a family of distinct virus strains and at least six major HCV genotypes have been identified (Simmonds et al., 1993)[12] and there is evidence that these have a variable geographical distribution (Dusheiko et al., 1994; Tisminetzky et al., 1994; Pontisso et al., 1995)[13-15]. It has been also suggested that the activity and prognosis of HCV related liver disease may be dependent on the genotype of the infecting HCV (Pozzato et al., 1991)16. Furthermore several reports agree that the response to inferferon-g (INF-a) therapy is also influenced by the HCV genotype (Tagariello et al., 1995)[17]. The main route of transmission of HCV infection is parenteral and most HCV infected individuals are either intravenous drug users or recipients of blood. Intrafamilial spread is not proven, but mother to infant transmission occurs in about 10% of HCV RNA positive mothers, mainly when maternal virus titres are high. Sexual transmission of HCV does not occur or is rare (Mujeeb et al., 1997)[18].

This study was designed to see the prevalence of hepatitis C antibodies in haemophilic patients and its association with ALT as a surrogate marker for hepatitis. The findings in this study show hepatitis C to be in very high percentage of patients with haemophilia and reasonably a major cause of NANB hepatitis in haemophilic patients. Prevalence was particularly high in haemophilic patients with severe to moderate disease. Clearly this means that chances of hepatitis C positivity increases with increased number of units of FFP/CP transfused. There have been many studies from Pakistan stating prevalence of hepatitis C in chronically transfused patients [thalassemics (Mujeeb et al., 1997)[18], dialysis (Saeed, 1994)[19] patients, blood donors (Lindholm, 1994)20 and general population (Malik et al., 1996)21].

Thalassemia is a transfusion dependent

disorder. A recent study from Karachi showed 50% prevalence of HCV infection among beta thalassemia major patients (Mujeeb et al., 1997)[18]. Taking account of an estimated 36000 cases of beta Thalassemia major patients in the country, it appears, there may be 18000 cases of HCV infection among them, indicating that present blood transfusion services are not only adding to the agony of Thalassemia major patients by causing hepatitis, but also increasing the pool of HCV infected persons in the country. This may result in the spread of infection in the general population through the widespread use of unsterilized needles and syringes in many health care institutions and is also a source of interfamilial transmission (Mujeeb et al., 1997)[18]. Same implications can be deduced from our study. Another study from Pakistan reported a high prevalence of HCV infections in hepatocellular carcinoma cases (Mujeeb et al., 1997)[18]. In another study of CLD, the anti-HCV was seen in about 25% of patients, whereas HBV was the main etiological agent in at least half of these CLD cases (Malik and Tariq, 1995)[22].

The prevalence of HCV in blood donors varies from country to country and population to population. Western countries have a prevalence rate of 0.4% or less (Lindholm, 1994; Contreras et al., 1991)20,23, it is relatively higher in Japan at 1.4% (Choo et al., 1990)[24] and significantly higher in third world countries. For example, it was found to be 19.2% in Egyptian workers in Saudi Arabia (Saeed et al., 1991)25. A study on healthy blood donors from AKUH reported that 1.18% of donors were positive for anti HCV (Kekepoto et al., 1996)26. Another study in this city conducted on professional donors showed a very high rate of seropositivity i.e. 20.7% (Ahmed et al., 1995)[27].

Hepatitis C antibody is relatively an expensive test. Alternatively we tried to establish whether raised ALT can serve as a surrogate marker for hepatitis in haemophilic patients or not. It was found that 41 of our patients, had raised ALT, of which 35 were those who along with raised ALT were also HCV antibody positive. It is possible that the remaining 29 who were the HCV antibody positive had hepatitis but with normal ALT. similar findings were also reported by kakepoto et al. (1996)[26]. It was also noticed that raised ALT was particularly seen in patients who had severe and moderate degree of haemophilia and so high transfusion requirements were to be met with, in accordance to the severity.

ALT has been used as a surrogate marker for viral hepatitis to prevent post transfusion hepatitis before anti HCV assays were available (Aach, 1981)28. A study from AKUH indicates that about 64% of donors with antibodies to HCV did not have an elevated serum ALT (Kakepoto, 1996)26, a finding also reported by other workers (Kuperan et al., 1993)[29].

This study too is in line with that of the others, and shows that ALT cannot be used as an effective and cost competitive marker for hepatitis C associated liver disease. Hepatitis C virus (HCV) infection is the major cause of chronic liver disease in individuals with haemophilia. A wide spectrum of disease severity is found in this group, ranging from mild hepatitis to cirrhosis to hepatoma (Hanley et al., 1996)[30]. In 1989 hepatitis C virus was identified and has subsequently been shown to be the major cuse of NANBH in haemophiliacs (Ludlam et al., 1989; Tedder et al., 1991)[31,32]. Almost all haemophiacs treated with non virus inactivated factor concentrates have anti-HCV antibodies and most show evidence of persistent viraemia and elevated ALT is found in many (Watson et al., 1992)[11]. With the development of the polymerase chain reaction (PCR), and its application to the study of HCV, the means to detect and quantify serum HCV RNA and identify the circulating HCV genotype became available (Hanely et al., 1996)[30].

Although anti-HCV antibody tests are good screening assays, yet a positive response does not distinguish between diseased patients, healthy carriers and those with a past HCV infection. The determination of HCV-RNA by PCR is most helpful for diagnostic, therapeutic assessment and follow up of chronic HCV infection (Alberti, 1991)[32].

In Pakistan, apart from few blood bank services who have incorporated anti-HCV screening in 1992-1994 (JPMC, Fatmid, Hussaini, Liquat National hospital, AKUH, Patient Welfare Association and Ziauddin Hospital) majority of blood banks and transfusion services do not routinely perform pre-transfusion testing on all donations whether they are voluntary unpaid directed donations or paid professional blood donations (Ahmed et al., 1995)[27]. Using disposable syringes and adherence to strict disinfection protocols are other measures to check the spread of hepatitis C. Govt. commitment and support is needed to check the spread, but a lot can be achieved through education/awareness and concerted efforts by health care personal.

CONCLUSION

Eighty percent of haemophilics in a given population showed prevalence to hepatitis C, but did not serve as a surrogate marker in patients with hepatitis C associated liver disease because a significant number of patients could be missed if ALT were to be used solely as a marker for liver disease in haemophilic patients.

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CORRENGENDUM

Due to technically mistakes the designations and degrees of principal and coauthors of below papers Volume 16, No 2 2010 published uncorrect.

The correct version read as under.

1. HEPATITIS B & C SHARE OF YOUNG RURAL POPULACE OF SINDH

(Volume 16, No 2 2010, page no 215)

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2. HELICOBACTER PYLORI, FINDING IN PATIENTS OF ACID PEPTIC DISEASE

(Volume 16, No. 2 2010, page No. 237)

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