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Identification of Marker Protein In Patient with Chronic Liver Disease

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Abstract: Hepatitis C virus causes acute and chronic hepatitis. It has a high propensity for transition to chronicity. In some cases over 50% patients developed chronic hepatitis. We have undertaken studies on protein/enzyme of serum of both normal and patient with chronic liver disease. Serum was analysed enzymatically and electrophoretically using reducing and non-reducing condition. It is observed that a protein of 14 Kda may be missing or in trace amount in serum of patients with chronic liver disease. Therefore it may be possible that this may be a cause of chronic liver disease. Further research is in progress for identification and characterization of missing protein.

Key words: Chronic liver disease, Protein and electrophoresis

Introduction

Liver diseases are hard to diagnose because of its complexity to find out the exact nature of disease, is quite difficult particularly in asymptomatic patients. Diagnosis with existing procedures turns out to be a dilemma because of several host variables that may alter the interpretations. Liver is infected with Hepatitis virus A, B, C, D etc (Vehida, 1993). Chronic liver disease (CLD) is an on going injury to cells of liver with inflammation that lasts for longer than 6 months. Hepatitis C virus (HCV) cause acute and chronic hepatitis. Chronic carrier state is also linked to causation of hepatocellular carcinoma. Acute hepatitis caused by HCV is mild and fulminant hepatitis is much less frequent. HCV has a high propensity for transition to chronicity. In some cases over 50% patients developed chronic hepatitis. High risk groups include those who receive blood transfusion, patients who receive hemodialysis or renal transplant, drug abusers and medical personal exposed to blood and blood products. Mode of spread in sporadic cases is unclear. Causes of chronic hepatitis are viruses, metabolic or immunologic, medication or chemicals. Signs and symptoms may include fatigue, mild discomfort in upper abdomen, loss of appetite and aching joints (Mukaiya *et al.*, 1998). Wiley *et al.* (1998) proposed an etiologic association of alcohol consumption, cigarette smoking and development of CLD. Another group of workers (Cordoba *et al.*, 1998) observed a diurnal variation of serum alanine transaminase (ALT) activity in CLD. A correlation between alkaline phosphatase and CLD also suggested by Verdau (1974). Study of patients, based on the use of sodium dodecyl polyacrylamide gel electrophoresis has rendered it possible to analyze the specific proteins associated with certain form of pathology. In view of this it is proposed to observe the protein pattern of normal subjects and patient with chronic liver disease by electrophoresis. In addition the activity of ALT by electrophoresis/biochemical assay will be determined as it is related to liver disease.

Materials and Methods

About 5 ml of blood was drawn from 50 patients with CLD and 20 normal subjects. Blood was centrifuged and serum was separated and stored at -20°C until use.

Protein estimation: Concentration of total protein of normal

subjects/patients was estimated by Biuret method (Wotton and Freeman, 1972).

Enzyme assay: Activity of ALT and alkaline phosphatase of both normal/patients was estimated by enzyme and King Armstroms method (Teitz, 1985). Activity of ALT was also found by charge (PAGE) electrophoresis using 10% polyacrylamide gel.

Electrophoresis: Slab gel electrophoresis of serum was carried out using 12% polyacrylamide gel (separation on the basis of molecular weight) using the method of Laemmli (1970). Separation on the basis of charge was done by charge (PAGE) gel electrophoresis on 10% polyacrylamide gel. Calibration was achieved using molecular mass kit.

Results and Discussion

Table 1 shows mean age of male patient with CLD was 33.2 years whereas mean age of female patient was 30.6 years with an age range of 20-70. Leclercq *et al.* (1998) also observed that most of male/female patients were in a age range of 18-70 years. 50% male were smokers. Mean serum protein concentration of male patients was found to be 6.3 gm/dl and 6.5 gm/dl in female patients. In normal male/female patients mean serum protein level was found to be approximately 6.9 gm/dl. This shows no significant difference. This report is in contradiction to the study of Preedy *et al.* (1999) who observed changes in protein level of patients. Mean level of ALT in male/female patients was

Table 1: Physical findings in Male/ Female patients

| Parameters | Male (25) | Female (25) |
|------------|-----------|-------------|
| Age (yrs) | 33.2 | 30.6 |
| S.E status | Lower | Lower |
| Smoking | 50% | Non Smoking |

S.E = Socio-economic

31.0 units/ml and 26.0 units/ml (Table 2). This shows a highly significant difference ($p < 0.005$). It was reported by number of studies (Sengupta, 1979; David *et al.*, 1979) that ALT level is the most common screening test as part of a routine evaluation of liver damage. Alkaline phosphatase in male patient was 15.6 KAU and 15.8 KAU whereas in normal male/female subject, it was approximately 9.0

Table 2: Biochemical parameters of male/female patients with chronic liver disease and normal subjects

| Parameters | Male subjects | Male patients | Female subjects | Female patients |
|----------------|---------------|---------------|-----------------|-----------------|
| ALT (UM) | 4.0 ± 3.0 | 31.0 ± 4.1 ** | 4.6 ± 4.0 | 26.0 ± 1.7 ** |
| Alk.phos (KAU) | 9.04 ± 1.1 | 15.6 ± 1.2 | 9.0 ± 1.3 | 15.8 ± 1.46 |
| Protein (gm/l) | 6.85 ± 0.1 | 6.3 ± 0.12 | 6.9 ± 0.11 | 6.5 ± 0.1 |

**P<0.005 = Highly significant difference

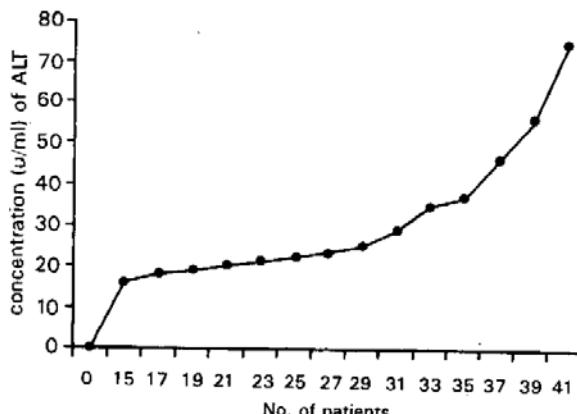


Fig. 1: Activity of ALT (units/ml) in patients with chronic liver disease

KAU. Although the level of Alk. Phosphatase was increased in both sexes but it shows no significant difference. As ALT is a significant parameter of chronic liver disease, its individual value was shown by a graph (Fig. 1). Graph shows that most of the patients have a range of ALT is 20-25 units/ml. Whereas some of patients have a high level of ALT i.e. 55-75 units/ml. This increase level may be due to severity of disease.

Electrophoretic assay of ALT shows that enzyme is basic in nature (Fig. 2). Electrophoresis of serum of both normal subjects and patient shows protein of wide range molecular weight i.e. from 70-4 Kda (Fig. 3). It was observed both by spectrophotometry/electrophoresis (SDS/PAGE) that the content of protein was low in patients as compare to normal subjects. It is observed that a protein of 14 Kda may be missing or in trace amount in serum of patients with chronic liver disease. Therefore it may be possible that this may be a cause of chronic liver disease.

It is suggested that electrophoresis may prove to be more helpful for an early diagnosis, for timely remedial measures and better management in effected subjects. Further research is in progress for identification and characterization of missing protein (Fig. 4).

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Fig. 2: Electrophoresis of ALT to shows the basic nature of enzyme



Fig. 3: Electrophoresis (on the basis of molecular weight) of serum of normal subjects/patients with CLD. A (normal subjects), B (patients) and C (standard marker molecular weight of 120, 67, 50, 30, 16, 14 and 8 Kda)



Fig. 4: Charged electrophoresis of serum of normal subject/patients A (normal subject) B (patients)