VARIATIONS OF SERUM SIALIC ACID LEVEL IN LIVER CIRRHOSIS


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Background: Cirrhosis liver claims many lives in our country. However early diagnosis carries good prognosis and prevents complications. Estimation of serum sialic acid level may be helpful in the diagnosis of liver cirrhosis and following the dynamics of the disease especially during treatment and follow up to see the prognosis. Methods: Sialic acid level of 82 confirmed liver cirrhosis patients of age between 18-60 years and admitted in Khyber Teaching Hospital Peshawar was determined and compared with 40 normal controls. The patients were studied in three groups according to the stage of the disease, i.e. the patients in early stages, in advancing stage, and those in terminal stage of liver cirrhosis. Sialic acid level was determined on HPLC (Hitachi) with D-2500 chromatographer. Result: Significantly high levels of sialic acid were recorded in patients as compared to controls. It was normal in early stage of liver cirrhosis (667±8.06nmol/ml), markedly increased in advancing cirrhosis (952±3.29nmol/ml) (P<0.05) and very high levels were observed in terminal stage (1058±7.50nmol/ml). Conclusion: Serum Sialic acid level was high in advancing and terminal stages of disease as compared to early stage and controls that showed normal levels.

Key words: N-Acetyle Neuraminic Acid (NANA), Sialic acid of glycoproteins, Cirrhosis liver.

INTRODUCTION

Altered carbohydrate content of plasma glycoproteins has been described in patients with a variety of liver diseases. N-Acetyl neuraminic acid is the most prominent sialic acid in eukaryotes. The structural diversity of sialic acid is exploited by viruses, bacteria and toxins and by the sialoglycoproteins and sialoglycolipids involved in cell to cell recognition in their highly specific recognition and binding to cellular receptors. Serum Sialic acid is a protein bound carbohydrate considered to be a monosaccharide and occurs in combination with other monosaccharides like galactose, mannose, glucosamine, galactosamines and fucose. Sialic acid is the group name for the acetylated neuraminic acids such as N-acetyl neuraminic acid, N-glycolyl neuraminic acid and D-acetyl neuraminic acid.

Only N-acetyl neuraminic acid has been isolated from human serum. Reports of the research work done in this field since the last few years reveal that the concentration of Sialic acid in the human serum is higher in a number of pathological states where the indulging pathology is either of tissue destruction, tissue proliferation, depolymerization or inflammation. Sialic acid is being studied since the last two decades by the research workers in liver diseases. It is now over twenty years since Martinez et al reported abnormal sialic acid content of the dysfibrinogenemia associated with liver diseases. They reported that sialic acid content of the purified fibrinogen was 12.7% to 71.4% higher in patients when compared to controls.

They suggested that bio-chemical alteration of the functionally abnormal fibrinogen found in patients with cirrhosis liver was due to increased level of sialic acid in this disease. Variation in serum sialic acid level in a variety of inflammatory liver diseases is an important diagnostic and prognostic tool. Matsuzaki et al reported abnormal sialic acid levels in liver cirrhosis, liver cancer, viral hepatitis, fatty liver and hepatoma. In metastatic liver cancer, the level was much higher than the upper range of normal.

The objective of the present study is to compare the levels of serum sialic acid in different stages of liver cirrhosis to determine value of sialic acid in diagnosis and prognosis of the disease.

MATERIAL AND METHODS

In a period of one year (June 1999 to May 2000) 82 cirrhotic patients of ages between 18-60 years, both males and females belonging to different socioeconomic classes were selected from medical D unit, Khyber teaching hospital Peshawar. Their inclusion criterion of age was between 18-60 years. Forty (40) age, sex and socioeconomically matched controls were taken having no history of viral hepatitis and cirrhosis liver. They were selected from the family members of patients, staff of Khyber teaching hospital, Khyber Medical College and Pakistan Medical Research Council (PMRC) KMC. Past history of all patients and controls regarding blood transfusions, injections, jaundice, use of razors and history of dental or surgical procedure or haemodialysis was recorded and patients were clinically examined for signs of liver cirrhosis.

Abdominal ultrasound was done to diagnose liver cirrhosis in the patients. Other specialized tests done to diagnose cirrhosis in patients included ultrasound guided biopsy in selected cases where
there was no contraindication, ascitic fluid (if present) was sent for serology and relevant biochemical tests like LFTs, Hbs Ag and HCV were also done to confirm liver cirrhosis.

About 10 ml of blood was drawn from the antecubital vein of the subjects and serum was extracted for sialic acid determination in addition to other tests. Rest of the blood was used for other relevant biochemical tests. The patients were studied in three groups on the basis of stage of the disease.

Group I (Early Stage): The selection criteria was the patients presenting with non specific symptoms like fatigue, weight loss, upper abdominal discomfort, anorexia, nausea and vomiting which on examination revealed jaundice and other signs like palmer erythema and spider telangiectasia. Their abdominal ultrasound showed cirrhotic liver changes and hepatomegaly in most of the cases, in addition to other biochemical tests which confirmed cirrhosis.

Group II (Advanced Stage): An ultrasound diagnosis of spleenomegaly and ascites in addition to cirrhotic liver changes on ultrasound or presenting with hepatic encephalopathy and hepatorenal syndrome.

Group III (Terminal Stage): Patients presenting with bleeding tendencies or giving such a history or hepatic coma, in addition to other signs and symptoms of liver cirrhosis and an ultrasound diagnosis of shrunken liver were included in third stage of cirrhosis. Sialic acid determination was done on HPLC. The hydrolyzing solution was 0.1M H$_2$SO$_4$ (95-98%) and for mobile phase 0.006 M H$_2$SO$_4$ was used. Standard NANA solution used in the procedure had a strength of 1μmol/ml (Sigma, N-acetyl neuraminic acid 80 mg). 0.1ml of sample was added to 1.9 ml of 0.1 M H$_2$SO$_4$ used for hydrolyzing.

The mixture was heated at 80°C for one hour, cooled for 15 minutes in water bath and diluted (1.1 v/v) with distilled water. For each series of determinations a standard sialic acid solution was treated similarly. After ultra centrifugation of hydrolysate supernatant was used for NANA estimation. 10 μl of standard solution was injected in HPLC by universal column spherisorb ODS 150 mm x 4.6 mm. The concentration of sialic acid in the sample was calculated by D-2500 chromatographer and results obtained as print outs.

The data was expressed as mean values ± standard deviation. Statistical difference in the mean values was evaluated by students ‘t’ test. P value <0.05 was considered significant.

**RESULTS**

There were variations in sialic acid level in the patients of cirrhosis liver (shown in Table-1). However the level remained with in normal range in controls (739.6±5.88 nmol/ml).

An elevated sialic acid level was observed in 72 (87.8%) patients with a value of 953.2±7.59 nmol/ml while it was with in normal range in 12.19 % patients (n=10). This shows a highly significant increase in serum sialic acid level in the patients as compared to controls (P < 0.001).

**DISCUSSION**

Biochemical alteration of sialic acid in various liver diseases has been studied from time to time. Reports of Jose Martinez et al$^{10}$ suggest that patients with many liver diseases exhibit a disturbance of the carbohydrate content of several of the plasma glycoporoteins synthesized by this organ and this alteration of the carbohydrate moiety may in some
cases be responsible for a functional defect of the protein. The most common sialic acids are N-acetyl neuraminic acid (NANA) (NEU 5AC) and N-glycosy neuraminic acid (Neu 5 GC). Although NANA is a major sialic acid in mammals including humans it is thought to be absent in healthy humans. The present study was carried out to ascertain levels of sialic acid in the patients of cirrhosis liver and the results were nearly consistent with those of previous research workers depicting a marked increase in blood sialic acid level. Serum sialic acid in this work is increased in advanced and terminal stages of cirrhosis liver. These were the patients who had developed complications of the disease. The level was normal in early stage of disease in the patients who had no complications. Alarming high levels were seen in some patients ranging form 1001-1098 nml/ml. This range correlated with the clinical condition of these patients.

Previous research studies in hepatobiliary diseases have indicated that an increase in sialic acid level may occur in the patients suffering from viral hepatitis liver cirrhosis, inflammation of biliary tract and malignant neoplasms of liver. Matsuzaki et al have also reported variations in serum sialic acid level in liver cirrhosis, liver cancer, viral hepatitis, fatty liver and hepatoma and they believe that the determination of sialic acid can be clinically useful for the diagnosis of cirrhosis and liver cancer.

The elevation of sialic acid content in our patients suggest that abnormality is a consequence of liver damage resulting in abnormal carbohydrate composition of the fibrinogen in this disease. Fibrinogen contains 0.6 % sialic acid. Fibrinogen and sialic acid are both acute phase reactants. Kaniak et al have studied sialic acid contents of the glycoproteins and seromucoid in viral hepatitis, liver cirrhosis, inflammations of the biliary tract and malignant neoplasms of liver. In liver cirrhosis and viral hepatitis a decrease in sialoprotein was found initially which varies with the course of disease. Very high levels are reported in malignancy of the liver. They have reported a rise of seromucoid level and fluctuations in sialic acid content in these diseases. Recent research studies in the field of sialic acid in chronic liver diseases and liver cirrhosis report that its level is elevated after massive tissue destruction. Increased levels may reflect generalized endothelial cell dysfunction or macro vascular disease either through loss of sialic acid containing glycoproteins from vascular cells into blood stream or through an acute phase response. In liver destruction its level rises proportionally to hepatic damage because much of the circulating sialic acid is covalently attached to glycoproteins and more than 50% of total sialic acid comes from acute phase proteins such as alpha acid glycoproteins, alpha anti trypsin and fibrinogen, factor VII antigen and activation markers of coagulation.

Kongtawelert et al have recently reported a high sialic acid concentration in cholangiocarcinoma, hepatocellular carcinoma and liver cirrhosis.

**CONCLUSION**

It is concluded that variation in sialic acid level in the patients of cirrhosis liver is an important diagnostic tool in addition to its value in prognosis. Patients under going treatment for cirrhosis liver may benefit more in future from this non invasive test. Further investigations into the nature of alterations in the sialic acid content of plasma glycoproteins may provide a basis for better understanding of pathogenesis and mechanism responsible for it in the patients of liver cirrhosis.

**REFERENCES**


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