VARIATIONS OF SERUM SIALIC ACID LEVEL IN LIVER CIRRHOSIS

Saatea Arif, Najeeb-ul-Haq*, Ruhila Hanif, A. Sattar Khan***, Jamil-ur-Rehman, Taufeeq Ahmed Mufti**

Department of Biochemistry and *Medicine, Khyber Medical College, Peshawar, ** Department of Biochemistry, Ayub Medical College, Abbottabad and ***Division of Biochemistry, Department of Chemistry. University of Peshawar

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.[1] 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.[2] 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.[3] Sialic acid is being studied since the last two decades by the research workers in liver diseases.[4-7] It is now over twenty yeas since Martinez et al[1] 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[8] 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 ascities 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.[9] The hydrolyzing solution was 0.1M H_2SO_4 (95-98%) and for mobile phase 0.006 M H_2SO_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_2SO_4 used for hydrolyzing.

The mixture was heated at 80ºC 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) .

Table-1: Levels of Serum Sialic acid Determined in Patients and Controls with liver cirrhosis

Group	Serum Total Sialic Acid nmol/ml		Serum Sialic Acid with in normal rang		Serum Sialic Acid raised above normal	
	No. & % subject	Mean ± SD	No. & % subject	Mean ± SD	No. & % subject	Mean ± SD
A. Patients (n=82)	(n=82) 100	967.63 ±9.85	(n=10) 12.19	667 ±80.69	(n=72) 87.80	953.2 ±7.59*
B. Control (n=40)	(n=40) 100%	739.6± 5.88	(n=38) 95	721.4± 7.23	(n=2) 05	860.6 ±6.3

Out of total 82 patients, 12 % were in the early stage of cirrhosis liver who had not developed the complications of the disease. The results show (Table 2) that their sialic acid was with in normal range. 70 % patients were in advanced stage and their sialic acid was significantly higher (P<0.05) as compared to controls. The patients in very advanced i.e terminal stage of the disease (n = 15) i.e 18 % showed very high levels of serum sialic acid as compared to controls. (1058±7.50 nmol / ml) (P<0.001). These patients had developed multiple complications of the disease.

Table-2: Serum sialic acid level in different stages of liver cirrhosis.

Stage of Cirrhosis	No.	%	Serum Sialic acid mean±SD(nmol/ml)
Early stage	10	12.19	667±8.06
Advance stage	57	69.51	952±3.29*
Terminal stage	15	18.29	1058±7.50**

- *P < 0.05: markedly significant,*
- *** P < 0.001 highly significant.*

DISCUSSION

Biochemical alteration of sialic acid in various liver diseases has been studied from time to time. Reports of Jose Martinz 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-glycosly 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.[11] 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. Alarmingly 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 form viral hepatitis liver cirrhosis, inflammation of biliary tract and malignant neoplasms of liver.[12,13] 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[14] 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.[15] 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.[16]

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

1. Martinz J, Palascak JE, Kwasniak D. Abnormal sialic acid content of dysfibrinogenemia associated with liver disease. J Clin Investigation 1978;61:535-8.
2. Oliver TK, Riidiger H, Michael P, Carolin S . Biochemical engineering of the N-acyl side chain of Sialic acid: biological implications. Glycobiology 2001;11(2):11-8.
3. Hangloo VK, Kaul I, Zargar HU. Serum Sialic acid levels in healthy individuals. J Postgrad Med 1990;36:140-2.
4. Lindberg G, Iso H, Rastam L, Lundblad A, Folsom AR. Serum Sialic Acid and its correlates in community samples from Akita, Japan and Minnaepolis. USA. Int J Epidemiol 1997;26(1):58-63.
5. Okude M, Yamanaka A, Akihama S. The effects of pH on the generation of turbidity and elasticity associated with fibrinogen fibrin conversion by thrombin are remarkably influenced by sialic acid in fibrinogen. Bio-pharm-Bull 1995;18(2):203-7.
6. Okude M, Yamanka A, Moriomoto Y, Akihama S. Sialic acid in fibrinogen. Effects of Sialic acid on fibrinogen-fibrin conversion by thrombin and properties of asialofibrin clot. Biol-Pharm-Bull 1993: 16(5) : 448-52.
7. O'Kennedy R, Berns G, Moran E, Smyth H, Carroll K, Thornes RD etal. A critical analysis of the use of sialic acid determination in the diagnosis of malignancy. Cancer Lett 1991;58(1-2):91-100.
8. Matsuzaki S, Itakura M, Iwamura K, Kamiguchi H. Serum Sialic acid levels in liver cirrhosis and liver cancer. Nippon-Shoka-Kibyo-Gakkari-Zasshi 1981;78(12):2395-401.
9. Hoon DBS, Ngz Sk, Ramshaw IA. Analysis of mammary tumour cell metastasis and release of bound N-acetyl neuraminic acid. Br J Cancer 1985;51:775-881.
10. Palascak JE, Martinz J. Dysfibrinogenemia associated with liver diseases. J-clin-Invest 1977;60(1):89-95.
11. Hangvoranuntakul P. Human uptake and incorporation of an immunogenic non human dietary SA. Proc Natl Acad Sci USA 2003;14:100(21):12045-50.
12. Narvaiza MJ, Fernandez J, Cuesta B, Paramo JA, Rocha E. Role of Sialic acid in acquired dysfibrinogenemia associated with liver cirrhosis. Ricerca in clinica e in laboratorio 1986;16(4):563-8.
13. Carlson J. α antitrypsin and other acute phase reactants in liver-disease. Acta Med Scand 1980; 207:79.

14. Kaniak J, Mejbaum KBW, Jelewska KZ, Kudreweiz HZ, Kowal GZ. Sialic acid contents of glyproteins and seromuciod in liver diseases. Polish Medical Journal 1980;1(5):1076-81.
15. Luchansky SJ, Goon S, Bertozzi CR. Expanding the diversity of unnatural cell surface S acids. Chem bioch 2004;5(3):371-9.
16. Kongtawelert P, Tangkijvanich P, ong-chi S, Poovorawan Y. Role of SA in differentiating cholangiocarcinoma from hepatocellular carcinoma. World Gastroenterol 2003;9(10):2178-81.

Address For Correspondence:
Dr. Saatea Arif, 26 - Khushal Khan Khattak Road, University Town, Peshawar **Tel:** 091-5825861,091-5844100
Email: drsaark@hotmail.com.