Acta Biochimica Polonica

Vol. 53 No. 1/2006, 213–220

on-line at: www.actabp.pl

*Regular paper*

# A biochemical study on the level of lipids and glycoproteins in the serum and platelets of liver cirrhotic bleeders

Sivagurunathan Vijayalakshmi, Arumugam Geetha✉ and Sam Annie Jeyachristy

*P.G. Department of Biochemistry, Bharathi Women's College, North Chennai, Chennai, India;*
*✉e-mail: geethav21@yahoo.co.in; geethabiochem@yahoo.com*

Received: 05 August, 2005; revised: 12 December, 2005; accepted: 11 January, 2006
available on-line: 07 March, 2006

**Bleeding complication and abnormal platelet functions are associated with liver cirrhosis. The aim of the present investigation was to assess the functional integrity of platelets in terms of lipids like cholesterol and phospholipids, glycoproteins and membrane-bound enzymes. Liver cirrhotic patients with bleeding complications were studied. Age and sex matched normal healthy volunteers were also involved in this study as a control group. Levels of cholesterol, phospholipids, glycoproteins and adenosine triphosphatases were assessed in isolated platelet membrane fraction. The level of glycoproteins and the activity of adenosine triphosphatases were found to be decreased significantly in cirrhotic patients. The cholesterol/phospholipid ratio was found to be altered significantly, indicating an alteration in the fluidity of platelet membrane. The results of this study reveal that the functional impairment of platelets in liver cirrhotic patients which is responsible for their bleeding tendency might also be due to altered lipid and enzyme levels in platelet membrane.**

**Keywords:** cirrhosis, platelets, lipids, glycoproteins, adenosine triphosphatases

Cirrhosis is a diffuse process characterized by fibrosis and the conversion of normal liver architecture into structurally abnormal nodules (Blaker *et al*., 2001). It is the final pathologic and clinical expression of a wide variety of chronic liver diseases (Pan *et al*., 2004). The major complications reported to be associated with cirrhosis are jaundice (Gubernick *et al*., 2000), infections, portal hypertension (Bilbao *et al*., 2002), variceal and gastrointestinal bleeding (Odelowo *et al*., 2002; Ito *et al*., 2005). Liver failure in cirrhosis is assessed by features such as ascites (Aalami *et al*., 2000), encephalopathy (Butterworth, 2000), low serum albumin and prothrombin deficiency not corrected by vitamin K (Bustamante *et al*., 1999).

Blood platelets are cells that quite often undergo damage in chronic liver diseases (Panasiuk *et al*., 2001). Abnormalities in platelet count and functions are common in patients with all forms of liver diseases (Goulis *et al*., 1999). Plasma concentrations of thrombopoietin, the key regulator of platelet function, mainly produced by the liver, have been reported to be reduced in liver cirrhotic patients (Schiodt *et al*., 2003). The abnormal bleeding complications in cirrhosis appear to be related to the defective platelet functions (Homoncik *et al*., 2004).

Blood coagulation can be considered as a host defense mechanism aimed at maintaining vascular integrity. Platelets have a normal life span in the circulation, secrete dense granule contents and aggregate normally in response to various stimuli and facilitate hemostasis in injured arterioles (Xu *et al*., 2002; Italiano *et al*., 2003).

The major components of platelet membranes like glycoproteins, lipids and enzymes contribute to the functional integrity and their biological activities like adhesion and aggregation which are important for the formation of primary hemostatic plug (Ramasamy, 2004) and coagulation cascade.

Although many reports have stated that there is variation in the activity and the level of factors which activate platelets, studies regarding the level of lipids, glycoproteins and enzymes of platelet

**Abbreviations:** ALT, alanine transaminase; ALP, alkaline phosphatase; AST, aspartate transaminase; CT scan, computed tomography; γ-GT, γ-glutamyl transferase; PRP, platelet rich plasma.

membrane are limited. Hence, the present work is aimed to find out the level of lipids and glycoproteins in the platelets of cirrhotic bleeders.

## MATERIALS AND METHODS

**Subjects**. Patients registered in the Department of Surgical Gasteroenterology and Proctology, Stanley Government Hospital (Chennai, India) were enrolled in this study. The sample consisted of fifty patients of 30–40 years of both sexes. Age and sex matched healthy volunteers with normal liver functions were used as control subjects. Case histories of patients were obtained which furnished details regarding their habits and symptoms. The patients had their diagnosis confirmed by endoscopy, Doppler use and CT scan. The study was carried out for a period of one year. The patients selected for the study had bleeding in the variceal region or nose or gums etc. The bleeding complication was confirmed by bleeding- and clotting-time determination. The cirrhotic condition was also confirmed by the clinical data such as platelet count, levels of albumin, bilirubin and prothrombin time. The aggregation capacity of platelets was also evaluated. All these analytical procedures were done by using standard kits.

Blood samples were collected from each patient with sterilised equipment and processed for serum and platelet preparation. The proposed plan of work was approved by the Ethical Committee of Stanley Medical College and Hospital and the blood samples were collected with the consent of each patient.

**Isolation of platelets.** The method of Aster and Jandl (1964) was adopted for the isolation of platelets. Briefly, 10 ml of blood was collected with acid citrate dextrose anticoagulant solution in the ratio of 9:1. The anticoagulated blood was centrifuged at 160 × *g* for 10 min to obtain PRP. The PRP obtained was again centrifuged at 160 × *g* to remove the red blood cells. The PRP was centrifuged at 300 × *g* for 5 min to pellet out platelets. The washing procedure was continued until the suspension was erythrocyte free and the purity was confirmed by microscopic examination. The platelet pellet was suspended in platelet storage buffer containing 0.109 M NaCl, 4.3 mM $K_2HPO_4$, 16 mM $Na_2HPO_4$, 8.3 mM $NaH_2PO_4$ and 5.5 mM glucose, pH 7.5, and stored at 4°C until further analysis.

**Isolation of platelet membrane.** Platelet membrane was isolated based on the method adopted by Lind *et al*. (1982). Briefly, equal volume of platelet suspension and Triton X-100 lysis buffer was taken in microfuge tubes and mixed by inversion. The clarified suspension of platelets was immediately centrifuged at 4°C for 2.5 h at 100000 × *g*. Supernatant was discarded and the translucent platelet membrane pellet was carefully collected from the microfuge tube and used for the estimation of lipids and glycoproteins.

**Assay of liver marker enzymes**. The activities of serum transaminases were assayed by using aspartate (AST) and alanine (ALT) as substrates along with the co-substrate α-ketoglutarate. The activities were expressed in terms of pyruvate liberated (King, 1960).

**Assay of alkaline phosphatase.** The procedure of Bowers and Mc Comb (1975) was adopted for the assay of ALP. The rate of formation of 4-nitrophenol from 4-nitrophenylphosphate is the basis of this analytical procedure.

**Assay of γ-gluatamyl transferase**. The assay method is based on the conversion of L-γ glutamyl *p*-nitroanilide to *p*-nitroanilide which is measured as change in absorbance at 409 nm (Goldberg, 1980).

**Extraction of membrane glycoproteins.** The protein-bound carbohydrate components were precipitated by the method of Glossmann and Newille (1971).

Briefly, 2 ml of membrane suspension was added to 5 ml of 1% phosphotungstic acid solution and centrifuged. The precipitated protein was washed with 3 ml of 5% tichloroacetic acid and sedimented. The pellets obtained were dissolved in 2 ml of 0.1 M NaOH. The extract was suitably hydrolysed and used for the estimation of $\alpha_1$-acid glycoprotein, hexose, hexosamine, fucose and sialic acid.

**Separation of serum glycoproteins.** To 0.1 ml of serum, 2 ml of alcohol was added and centrifuged. The supernatant was decanted. The precipitate was hydrolysed with acid to liberate protein-bound carbohydrate according to the method of Tettamanti *et al*. (1973).

**Assay of glycoproteins.** $\alpha_1$-acid glycoprotein was estimated by the method of Winzler (1955) using Folin–Ciocalteau reagent. After removing heat-coagulable proteins with perchloric acid, the orosomucoid which remains in solution was precipitated by phosphotungstic acid and estimated by determining its tyrosine content using Folin–Ciocalteau reagent.

Hexose was estimated by the method of Niebes (1975) using orcinol-sulphuric acid reagent.

Hexosamine was quantified by the method of Wagner (1979) by using acetyl acetone and Ehrlich's reagent.

Fucose was estimated by the method of Winzler (1955). Briefly, 1.0 ml of precipitated glycoprotein from platelet membrane and 1.0 ml of processed serum were dissolved in 1 ml of 0.1 M NaOH and placed in an ice-bath and 4.5 ml of cold $H_2SO_4$ was added and mixed well. The tubes were heated in a boiling water bath for 3 min and cooled, then 0.1 ml of 3% cysteine was added and mixed immediately.

The tubes were allowed to stand at room temp. for 60–90 min. The absorbance of the solution at 396 and 430 nm was measured in a UV spectrophotometer and the difference in the absorbances was taken for the calculation.

Sialic acid was estimated in terms of thiobarbituric acid reacting substances (Warren, 1959).The same methods were adopted for the estimation of glycoproteins in serum. All the glycoprotein levels were expressed as ng/1 × $10^5$ platelets.

**Assay of platelet membrane-bound enzymes.** The activity of total ATPase was determined by the method of Evans (1969) by using ATP as the substrate in the presence of $Na^+$, $K^+$, $Mg^{2+}$ and $Ca^{2+}$ ions.

$Na^+$,$K^+$-ATPase activity was measured according to the method of Bonting (1970) in the presence of $Na^+$ and $K^+$ ions. The level of $Mg^{2+}$-dependent ATPase was determined by the method of Ohnishi *et al*. (1982) and $Ca^{2+}$-dependent ATPase activities were quantified by the method of Hjerton and Pan (1983) using ATP as substrate in the presence of $Mg^{2+}$ and $Ca^{2+}$ ions, respectively.

In all the ATPases assays, the activity was expressed in terms of nanomoles of phosphorus liberated/minute per 1 × $10^5$ platelets.

**Estimation of lipids in serum and platelet membrane.** Lipids were extracted from the platelet membrane by the method of Schick *et al*. (1983). The membrane preparation containing 2 mg of protein/ml was mixed with chloroform/methanol mixture (2:1, v/v) in the ratio of 1:9 (v/v). The solution was homogenised at low speed and the organic lipid layer was carefully separated and evaporated to dryness in a conical flask. The lipid was dissolved in a known amount of chloroform/methanol mixture. Total phospholipids were estimated in terms of inorganic phosphorus by the method of Fiske and Subbarow (1925) after Barlett (1959) perchloric acid digestion. Serum and platelet membrane cholesterol were estimated by using ferric acetate/uranyl acetate reagent (Jung & Parekh, 1971).

The method of Roughan and Batt (1968) was adopted for the estimation of glycolipids. It was estimated by determining the hexose content and multiplying it by 4.45.

**Statistical analysis.** The values were subjected to analysis of normal tests of significance. Fischer's null hypothesis (1995) was adopted to find out the significance of variations between the analytical data of normal subjects and cirrhotic patients. For statistical analysis group 1 (normal subjects) were compared with group 2 (cirrhotic patients). Some of the parameters were also analysed by Spearman test and the $r_s$ values were calculated to find the correlation (Sundar Rao & Richard, 1999).

## RESULTS

Table 1 presents the clinical characteristics of the patients selected for the study; about 70% of the cases came under Child-Pugh class C category and the platelet count was found to be significantly low when compared to normal healthy volunteers. Eighty percent of the cirrhotic patients were chronic alcoholics. The albumin level was found to be significantly low in 70% of the cases and 90% of the cases were found to be jaundiced. Prothrombin time was found to be significantly prolonged in 90% of the cases. About 90% of the patients had defective platelet aggregation.

Table 2 shows the activity levels of transaminases, alkaline phosphatase and γ-GT in the serum of liver cirrhotic patients and of normal healthy volunteers. The activity levels of the liver marker enzymes are found to be increased significantly ($P < 0.001$) in liver cirrhotic patients.

The levels of hexose, hexosamine, fucose, sialic acid and $\alpha_1$-acid glycoprotein in the platelet membrane of liver cirrhotic patients and of normal healthy volunteers are presented in Table 3. The levels of hexose, hexosamine, fucose and sialic acid are found to be significantly low ($P < 0.001$) in liver cirrhotic patients. A significant decrease ($P < 0.01$) is observed in the level of $\alpha_1$-acid glycoprotein in the platelet membrane of liver cirrhotic patients. The serum levels of hexose, hexosamine, fucose, sialic acid and $\alpha_1$-acid glycoprotein in liver cirrhotic patients and of normal healthy volunteers are shown in Table 4. There is a significant decrease ($P < 0.001$) in the levels of hexose, hexosamine, fucose, sialic acid and $\alpha_1$-acid glycoprotein in the study group.

Table 5 presents the activity of total ATPases, $Na^+$,$K^+$-ATPase, $Mg^{2+}$-ATPase and $Ca^{2+}$-ATPase in the platelet membrane of liver cirrhotic patients and of normal healthy volunteers. The activities of all these enzymes are significantly reduced ($P < 0.001$) in the platelet membrane of liver cirrhotic patients.

The levels of cholesterol and phospholipid in the serum of liver cirrhotic patients and of normal healthy volunteers are shown in Table 6. Significant decreases in the levels of phospholipid and cholesterol ($P < 0.001$) are found in the serum of liver cirrhotic patients. The platelet lipid profile shows a decrease ($P < 0.001$) in the level of phospholipid and an increase ($P < 0.001$) in the level of cholesterol in liver cirrhotic patients. The cholesterol/phospholipid ratio is also represented in Table 6. The cholesterol/phospholipid ratio is found to be increased significantly in the platelets of liver cirrhotic patients.

Table 7 presents the associations between variables as determined by Spearman's correlation coefficient. The results clearly show that the degree of liver damage is closely correlated with the level

of platelet dysfunction assessed by means of platelet count, cholesterol/phospholipid ratio and total AT-Pases.

**Table 1. Clinical characteristics of patients**

| Characteristics | Number of patients |
|---|---|
| Total number | 50 |
| Male / female | 32/18 |
| Age in years (range) | 30–40 |
| Grade (according to Child–Pugh class) | |
| A | 5 |
| B | 10 |
| C | 35 |
| Platelet count | |
| Below 1 × $10^5$ cells/μl | 38 |
| Above 2 × $10^5$ cells/μl | 12 |
| WBC count | |
| 4.0 to 11.0 × $10^3$ cells/μl | 35 |
| More than 11.0 × $10^3$ cells/μl | 15 |
| Cause of cirrhosis | |
| Alcoholic | 40 |
| Viral and metabolic | 10 |
| Albumin level | |
| More than 3.5 g/dl | 5 |
| 2.8 to 3.5 g/dl | 18 |
| Less than 2.8 g/dl | 27 |
| Bilirubin | |
| Less than 2 mg/dl | 5 |
| 2 to 3 mg/dl | 15 |
| More than 3 mg/dl | 30 |
| Prothrombin time | |
| Less than 4 seconds | 5 |
| 4 to 6 seconds | 15 |
| More than 6 seconds | 30 |
| Ascites | 35 |
| Percentage platelet aggregation when compared to age and sex matched normal volunteers | |
| 80% | 5 |
| Less than 50% | 45 |

**Table 2. Activity of transaminases, ALP and γ-GT in the serum of liver cirrhotic patients and normal healthy volunteers.**

Activities are expressed as (IU/L). Values are expressed as mean ± S.D. for 25 individuals in each group.

| | Normal subjects | Liver cirrhotic patients |
|---|---|---|
| AST | 10.86 ± 1.06 | 75 ± 5.56* |
| ALT | 12.46 ± 1.4 | 63 ± 3.27* |
| ALP | 53 ± 4.91 | 107.9 ± 16* |
| γ-GT | 45.6 ± 5.9 | 66 ± 7.5* |

*$P$<0.001 when compared to normal healthy volunteers.

**Table 3. Levels of hexose, hexosamine, fucose, sialic acid and $\alpha_1$-acid glycoprotein in the platelet membrane of liver cirrhotic patients and normal healthy volunteers.**

Levels are expressed as ng/1 × $10^5$ platelets. Values are expressed as mean ± S.D. for 25 individuals in each group.

| | Normal subjects | Liver cirrhotic patients |
|---|---|---|
| Hexose | 0.263 ± 0.017 | 0.206 ± 0.013** |
| Hexosamine | 0.53 ± 0.005 | 0.467 ± 0.069** |
| Fucose | 6.75 ± 1.01 | 4.39 ± 0.69** |
| Sialic acid | 15.0 ± 2.16 | 10.06 ± 1.59** |
| $\alpha_1$-Acid glycoprotein | 0.03 ± 0.0025 | 0.028 ± 0.002* |

**$P$<0.001, *$P$<0.01 when compared to normal healthy volunteers.

**Table 4. Levels of hexose, hexosamine, fucose, sialic acid and $\alpha_1$-acid glycoprotein in the serum of liver cirrhotic patients and normal healthy volunteers.**

Levels are expressed as mg/dl. Values are expressed as mean ± S.D. for 25 individuals in each group.

| | Normal subjects | Liver cirrhotic patients |
|---|---|---|
| Hexose | 65.66 ± 2.12 | 48 ± 2* |
| Hexosamine | 127 ± 2.53 | 80 ± 1* |
| Fucose | 11.1 ± 1.5 | 7.4 ± 0.9* |
| Sialic acid | 61.5 ± 7.2 | 43.6 ± 5.1* |
| $\alpha_1$-Acid glycoprotein | 5 ± 0.75 | 2.6 ± 0.2* |

*$P$<0.001 when compared to normal healthy volunteers.

## DISCUSSION

The activities of transaminases, ALP and γ-GT have been used as markers for hepatobiliary diseases. The level of ALT in the blood is increased in conditions in which hepatocytes are damaged or dead. As cells are damaged, ALT leaks out into the blood stream. All types of hepatitis and cirrhosis have been reported to cause liver damage that can lead to

**Table 5. Activity of total, $Na^+$,$K^+$, $Mg^{2+}$ and $Ca^{2+}$ ATPases in the platelet membrane of liver cirrhotic patients and in normal healthy volunteers.**

Activities are expressed as nmoles of phosphorus liberated/min per 1 × $10^5$ platelets. Values are expressed as mean ±S.D. for 25 individuals in each group.

| | Normal subjects | Liver cirrhotic patients |
|---|---|---|
| Total ATPase | 100.5 ± 21.6 | 64.5 ± 13.5* |
| $Na^+$,$K^+$-ATPase | 27.4 ± 6.1 | 21.4 ± 6.0* |
| $Mg^{2+}$-ATPase | 46.6 ± 9.2 | 35.2 ± 8.4* |
| $Ca^{2+}$-ATPase | 41.7 ± 11 | 25.6 ± 8.2* |

*$P$<0.001 when compared to normal healthy volunteers.

elevations in the serum ALT activity (Cohen & Kaplan, 1979). ALT is a cytosolic enzyme of the liver which can be increased in cases of liver cell death resulting also from other causes such as shock and drug toxicity. The level of ALT may also be correlated roughly with the degree of cell death or inflammation. AST is an enzyme similar to ALT which is a mitochondrial enzyme present in large quantities in heart, liver, skeletal muscle and kidney. In many cases of liver inflammation, AST and ALT activities are elevated roughly in the ratio of 1:1 but in some conditions like alcoholic hepatitis and chronic hepatitis C infection, the serum AST levels have been reported to be elevated higher than the serum ALT levels (Khokhar, 2003). In our study, AST level is found to be elevated more than that of ALT which is confined to the liver. The level of ALP is found to be significantly elevated in cirrhotic cases when compared to the activity level of transaminases. It has also been reported to be raised in cholestasis (Whitfield *et al*., 1972). So, the elevated liver marker enzyme levels in cirrhotic patients clearly proved that they had significant liver abnormality.

Primary platelet adhesion to vascular collagen initiates complex intracellular reactions resulting in platelet interaction with other platelets forming aggregates. Furthermore, in addition to releasing activating substances, the collagen-adherent platelets enhance the generation of thrombin, the culmination of the coagulation cascade leading to the formation of a fibrin-stabilized plug of aggregated platelets. This step is critically dependent on the transforma-

**Table 6. Levels of phospholipid and cholesterol in the serum and platelets and cholesterol/phospholipid ratio in the platelets of liver cirrhotic patients and in normal healthy volunteers.**

Values are expressed as mean ± S.D. for 25 individuals in each group.

| | | Normal subjects | Liver cirrhotic patients |
|---|---|---|---|
| Serum (mg/dl) | Cholesterol | 143.3 ± 17.6 | 36.3 ± 3.0* |
| | Phospholipid | 151.3 ± 8.5 | 82 ± 12* |
| Platelets (ng/1 × $10^5$ platelets) | Cholesterol | 0.18 ± 0.01 | 0.25 ± 0.01* |
| | Phospholipid | 0.21 ± 0.01 | 0.099 ± 0.006* |
| Platelets | Cholesterol/Phospholipid ratio | 0.88 ± 0.05 | 2.53 ± 0.28* |

*$P$<0.001 when compared to normal healthy volunteers.

**Table 7. Rank correlation between liver enzyme level and platelet components in liver cirrhotic patients.**

Values are presented as mean.

| Subjects | ALT (IU/L) | Platelet count (cells/μl) | Cholesterol/ Phospholipid ratio | Total ATPases (nmoles of phosphorus liberated/min per 1 × $10^5$ platelets) |
|---|---|---|---|---|
| Normal | 12.46 | 280000 | 0.875 | 100.5 |
| Class A (n=5) | 23.0 | 210000 ($r_s$-0.3, $P$<0.05) | 1.0 ($r_s$-0.4, $P$<0.05) | 90.5 ($r_s$-0.52, $P$<0.05) |
| Class B (n=10) | 35.0 | 175000 ($r_s$-0.7, $P$<0.02) | 1.5 ($r_s$-0.72, $P$<0.02) | 70.65 ($r_s$-0.69, $P$<0.02) |
| Class C (n=35) | 60.0 | 40000 ($r_s$-0.57, $P$<0.01) | 2.3 ($r_s$-0.54, $P$<0.01) | 62.5 ($r_s$-0.56, $P$<0.01) |

Based on the critical values of the rank correlation coefficient (Spearman's rho-$r_s$) null hypothesis of no correlation is rejected and it is concluded that liver enzyme change and the levels of platelet components are correlated (n = number of pairs).

tion of the platelet membrane into a catalytic surface and shedding of microparticles, which influence blood coagulation (Ruggeri & Savage, 1998).

Glycoproteins present in the platelet are highly important for the platelet adhesion and activation in response to blood vessel damage and crucial for normal hemostasis and these events are also thought to be primary in arterial thrombosis (Hiramatsu *et al*., 1976). Many receptors present in the platelets are glycoproteins and a wide variety of studies have been undertaken to confirm the mode of action of receptors (Nomura *et al*., 1996; Reverter *et al*., 1996). Platelet glycoprotein polymorphism has been intensively studied as a genetic risk factor. We have studied the levels of glycoproteins which were found to be significantly low in both serum and platelets. This would have resulted in the abnormality of platelets which in turn reflected in the defective platelet adhesion and activation properties.

The enzymes associated with the platelet membrane, like ATPases and nucleotidases, are essential for the membrane-related functions like adhesion, aggregation and secretion of granules. $Na^+,K^+$-ATPase belongs to a family of ATPases which are present in virtually all mammalian cell membranes. Other members of this family include enzymes dependent upon magnesium ($Mg^{2+}$-ATPase) and calcium ($Ca^{2+}$-ATPase). Because $Na^+,K^+$-ATPase transports $Na^+$ ions extracellularly and $K^+$ ions intracellularly, it plays an important role in maintaining the level of membrane polarization (Powell & Cantley, 1980). Consequently, modulation of $Na^+,K^+$-ATPase activity may lead to alterations in the function of various cell types, including peripheral blood mononuclear cells. In our study, it is observed that there is a significant reduction in the activity of $Na^+,K^+$-ATPase which might result in reduced cation exchange through the membrane and thereby its reduced energy-dependent secretory functions. Biochemical characterization of the calcium ATPases isolated from human platelet intracellular and plasma membranes has been reported (Enouf *et al*., 1989). This enzyme catalyses $Ca^{2+}$-dependent exchange of hydride ions to calcium ions which influence the rate of signal transduction which is highly essential for the timely release of secretory granules and formation of actomyosin complex in the platelet membrane during adhesion. So, the observed low level of calcium ATPase activity would have also influenced the defective platelet activities such as adhesion, aggregation and secretion.

The liver is the central organ for cholesterol, phospholipid, triglyceride and lipoprotein metabolism. The functional impairment of liver would result in the reduced capacity to synthesize many important biomolecules, including lipids. It has been shown that in cirrhosis with hepatocellular carcinoma, plasma phospholipid levels show a significant negative correlation with total bilirubin and ALT (Ahaneku *et al*., 1992). Also, in our study we have observed increased levels of ALT and decreased levels of phospholipids. It was observed in the present investigation that most of the patients involved in the study have significant hypocholesterolaemia. Hepatitis C virus infection is reported to be associated with hypocholesterolaemia (Polgreen *et al*., 2004).

Platelet dysfunction in liver diseases is associated with an abnormality in lipid composition. Results of our study show an increase in the cholesterol/phospholipid ratio which might lead to a decrease in membrane fluidity. This decrease in membrane fluidity might affect receptor functions by changing the lateral mobility or clustering of receptors in their vertical orientation (Brulet & McConnell, 1976). Phospholipids have been reported to influence membrane fluidity. Our study suggests that the increase in the cholesterol/phospholipid ratio might have affected the membrane fluidity. If the membrane fluidity decreases, the secretory function of the platelet will be severely affected, the procoagulants will not be secreted at a normal level, and thereby aggregation will be affected. As a result of this mechanism, liver cirrhotic patients might have excessive bleeding.

Generally, the fluidity of biomembranes is responsible for their functional integrity, which is largely determined by the levels of protein and cholesterol/phospholipid ratio. A significant alteration in the level of lipids in platelet membrane would contribute to the secretory functions and thereby to the defective adhesion and aggregation properties.

The results were analysed by using Spearman test and we found that the degree of functional disturbance in liver is correlated with the platelet dysfunction.

From the results of this investigation, it could be concluded that the alterations observed in the levels of ATPases, lipids and glycoproteins also contribute to the defective functions of platelets in liver cirrhotic patients with bleeding complications.

**Acknowledgement**

A. Geetha thanks University Grants Commission (New Delhi, India) for the financial support.

## REFERENCES

Aalami OO, Allen DB, Organ CH Jr (2000) Chylous ascites: a collective review. *Surgery* **128**: 761–778.

Ahaneku JE, Taylor GO, Olubuyide IO, Agbedana EO (1992) Abnormal lipid and lipoprotein patterns in liver cirrhosis with and without hepatocellular carcinoma. *J Pak Med Assoc* **42**: 260–263.

Aster RH, Jandl JH (1964) Platelet sequestration in man. I. Methods. *J Clin Invest* **43**: 843–855.

Barlett GR (1959) Phosphorus assay in column chromatography. *J Biol Chem* **234**: 466–468.

Bilbao JI, Quiroga J, Herrero JI, Benito A (2002) Transjugular intrahepatic portosystemic shunt (TIPS): current status and future possibilities. *Cardiovasc Intervent Radiol* **25**: 251–269.

Blaker H, Theuer D, Otto HF (2001) Pathology of liver cirrhosis and portal hypertension. *Radiologe* **41**: 833–839.

Bonting SL (1970) In *Membranes and Ion Transport* (Bitter E, ed) vol 1, pp 257–363, Elsevier, Amsterdam.

Bowers GN Jr, Mc Comb RB (1975) Measurement of total alkaline phosphatase activity in human serum. *Clin Chem* **21**: 1988–1995.

Brulet P, McConnell HM (1976) Lateral hapten mobility and immunochemistry of model membranes. *Proc Natl Acad Sci USA* **73**: 2977–2981.

Bustamante J, Rimola A, Ventura PJ, Navasa M, Cirera I, Reggiardo V, Rodes J (1999) Prognostic significance of hepatic encephalophathy in patients with cirrhosis. *J Hepatol* **30**: 890–895.

Butterworth RF (2000) Complications of cirrhosis III – Hepatic encephalopathy. *J Hepatol* **32**: (suppl 1) 171–180.

Cohen JA, Kaplan MM (1979) The SGOT/AGPT ratio — an indicator of alcoholic liver disease. *Dig Dis Sci* **24**: 835–838.

Enouf J, Bredoux R, Bourdeau N, Sarkadi B, Levy-Toledano S (1989) Further characterization of the plasma membrane and intracellular membrane-associated platelet $Ca^{2+}$ transport systems. *Biochem J* **263**: 547–552.

Evans DJ Jr (1969) Membrane adenosine triphosphatase of *Escherichia coli*: activation by calcium ion and inhibition by monovalent cations. *J Bacteriol* **100**: 914–922.

Fischer RA (1995) Analysis of larger samples. In *Elements of Mathematical Statistics* (Gupta SC, Kapoor VK, eds) pp 307, Sultan-Chand & Sons Publishers, New Delhi.

Fiske CH, Subbarow Y (1925) The colorimetric determination of phosphorus. *J Biol Chem* **66**: 375.

Glossmann H, Newille DM Jr (1971) Glycoproteins of cell surfaces. A comparative study of three different cell surfaces of the rat. *J Biol Chem* **246**: 6339–6346.

Goldberg DM (1980) Structural, functional and clinical aspects of gamma glutamyl transferase. *Crit Rev Clin Lab Sci* **12**: 1–58.

Goulis J, Chau TN, Jordan S, Mehta AB, Watkinson A, Rolles K, Burroughs AK (1999) Thrombopoietin concentrations are low in patients with cirrhosis and thrombocytopenia and are restored after orthotopic liver transplantation. *Gut* **44**: 754–758.

Gubernick JA, Rosenberg HK, Ilaslan H, Kessler A (2000) US approach to jaundice in infants and children. *Radiographics* **20**: 173–195.

Hjerton S, Pan H (1983) Purification and characterization of two forms of a low affinity $Ca^{2+}$-ATPase from erythrocyte membranes. *Biochim Biophys Acta* **728**: 281–288.

Hiramatsu S, Kojima J, Okada TT, Inai S, Ohmori K (1976) The serum protein profile in chronic hepatitis, cirrhosis, and liver cancer. *Acta Hepatogastroenterol (Stuttg.)* **23**: 177–182.

Homoncik M, Jilma-Stohlawetz P, Schmid M, Ferlitsch A, Peck-Radosavljevic M (2004) Erythropoietin increases platelet reactivity and platelet counts in patients with alcoholic liver cirrhosis: a randomized double-blind, placebo-controlled study. *Aliment Pharmacol Ther* **20**: 437–443.

Italiano JE Jr, Bergmeier W, Tiwari S, Falet H, Hartwig JH, Hoffmeister KM, Andre P, Wagner DD, Shivdasani RA (2003) Mechanisms and implications of platelet discoid shape. *Blood* **101**: 4789–4796.

Ito K, Shiraki K, Sakai T, Yoshimura H, Nakano T (2005) Portal hypertensive colopathy in patients with liver cirrhosis. *World J Gasteroenterol* **11**: 3127–3130.

Jung DH, Parekh AC (1970) A new color reaction for cholesterol assay. *Clin Chim Acta* **35**: 73–78.

Khokhar N (2003) Serum aminotransferase levels and platelet count as predictive factor of fibrosis and cirrhosis in patients with chronic hepatitis C infection. *J Pak Med Assoc* **53**: 101–104.

King J (1960) A study of human serum transaminases. *J Med Lab Technol* **17**: 1–21.

Lind SE, Yin HL, Stossel TP (1982) Human platelets contain gelsolin. A regulator of actin filament lengths. *J Clin Invest* **69**: 1384–1387.

Niebes P (1975) Micromethod for the determination of glycosaminoglycans in the serum. Results from the serum of healthy and varicose subjects. *Clin Chim Acta* **62**: 195–202.

Nomura S, Komiyama Y, Matsuura E, Xie GL, Katsura K, Miyake T, Miyazaki Y, Kagawa H, Koike T, Fukuhara S (1996) Participation of $\alpha$IIb $\beta$3 in platelet microparticle generation by collagen and thrombin. *Haemostasis* **26**: 31–37.

Odelowo OO, Smoot DT, Kim K (2002) Upper gastrointestinal bleeding in patients with liver cirrhosis. *J Natl Med Assoc* **94**: 712–715.

Ohnishi T, Suzuki T, Suzuki Y, Ozawa K (1982) A comparative study of plasma membrane $Mg^{2+}$-ATPase activities in normal, regenerating and malignant cells. *Biochim Biophys Acta* **684**: 67–74.

Pan Z, Wu XJ, Li JS, Liu FN, Li WS, Han JM (2004) Functional hepatic flow in patients with liver cirrhosis. *World J Gasteroenterol* **10**: 915–918.

Panasiuk A, Prokopowicz D, Zak J, Matowicka-Karna J, Oscada J, Wysocka J (2001) Activation of blood platelets in chronic hepatitis and liver cirrhosis P-selectin expression on blood platelets and secretory activity of beta thromboglobulin and platelet factor-4. *Hepatogastroenterology* **48**: 818–822.

Polgreen PM, Fultz SL, Justice AC, Wagner JH, Diekema DJ, Rabeneck L, Weissman S, Stapleton JT (2004) Association of hypocholesterolaemia with hepatitis C virus infection in HIV-infected people. *HIV Med* **5**: 144–150.

Powell LD, Cantley LC (1980) Structural changes in Na+K+ ATPase acompanying detergent inactivation. *Biochim Biophys Acta* **599**: 436–447.

Ramasamy I (2004) Inherited bleeding disorders: disorders of platelet adhesion and aggregation. *Crit Rev Oncol Hematol* **49**: 1–35.

Reverter JC, Beguin S, Kessels H, Kumar R, Hemker HC, Coller BS (1996) Inhibition of platelet-mediated, tissue factor-induced thrombin generation by the mouse/chimeric 7E3 antibody. *J Clin Invest* **98**: 863–874.

Roughan PG, Batt RD (1968) Quantitative analysis of sulfolipid (sulfoquinovosyl diglyceride) and galactolipids (monogalactosyl and digalactosyl diglycerides) in plant tissues. *Anal Biochem* **22**: 74–88.

Ruggeri ZM, Savage B (1998) Biological functions of von Willebrand factor. In *Von Willebrand Factor and the Mechanisms of Platelet Function* (Ruggeri ZM, ed) pp 79, Springer-Verlag Publishers, Berlin.

Schick PK, Tuszynski GP, Vander Voort PW (1983) Human platelet cytoskeleton: specific content of glycolipids and phospholipids. *Blood* **61**: 163–166.

Schiodt FV, Balko J, Schilsky M, Harrison ME, Thomton A, Lee WM; Acute liver failure study group (2003) Thrombopoietin in acute liver failure. *Hepatology* **37**: 558–561.

Sundar Rao PSS, Richard J (1999) In *An Introduction to Biostatistics – a Manual for Students in Health Sciences.*

3rd edn, pp 112–113, Asoke K Gosh Publishers, New Delhi.

Tettamanti G, Bonali F, Marchesini, Zambotti V (1973) A new procedure for the extraction, purification and fractionation of brain gangliosides. *Biochim Biophys Acta* **296**: 160–70.

Wagner WD (1979) A more sensitive assay discriminating galactosamine and glucosamine in mixtures. *Anal Biochem* **94**: 394–396.

Warren L (1959) The thiobarbituric acid assay of sialic acids. *J Biol Chem* **234**: 1971–1975.

Whitfield JB, Pounder RE, Neale G, Moss DW (1972) Serum gamma-glutamyl transpeptidase activity in liver disease. *Gut* **13**: 702–708.

Winzler RJ (1955) Determination of serum glycoproteins. *Methods Biochem Anal* **2**: 279–311.

Xu CQ, Zeng YJ, Gregersen H (2002) Dynamic model of the role of platelets in the blood coagulation system. *Med Eng Phys* **24**: 587–593.