SERUM THROMBOPOIETIN LEVELS IN PATIENTS WITH LIVER CIRRHOSIS; RELATION TO SEVERITY OF THE DISEASE, SPLEEN SIZE AND PLATELET COUNT

By

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ABSTRACT

Background : Thrombopoietin (TPO) is an important regulator of megakaryocyte maturation and platelet production. The role of TPO (which is mainly produced by the liver) in thrombocytopenic cirrhotic patients is still under investigation. The aim of this study was to measure the serum TPO levels in cirrhotic patients and examine its relationship with circulating platelet count, splenic size and clinical stage of liver cirrhosis.

Study design and methods : This study was conducted on 88 subjects, divided into 2 groups, group I (patient group) included 72 patients with liver cirrhosis (diagnosed by combination of clinical, laboratory, ultrasound and histopathological data), they were fur-

ther divided into 2 subgroups, group IA: included cirrhotic patients with thrombocytopenia (36 patients, 28 males and 8 females with age 50.3 ± 8.5 years), and group IB: included cirrhotic patients with normal platelet count (36 patients, 26 males and 10 females, with age 50.64 ± 6.8 years). Group II comprised 16 healthy persons with matched age and sex, used as a control group. All included persons were subjected to: thorough history taking, full clinical examination, beside the following investigations: complete blood picture, kidney and liver function tests, Hepatitis B and C markers, serum TPO level (by sensitive sandwich ELISA) and abdominal Doppler ultrasound. The following invasive investigations were

done for group I (patients) only: bone marrow aspiration, upper gastrointestinal endoscopy, sigmoidoscopy and liver biopsy (the latter was done for 21 patients only). Patients with pure schistosomal fibrosis were excluded from the study. Patients were classified according to the Child-Pugh score into 3 classes of clinical severity : A, B and C. **Results:** Cirrhotic patients were thrombocytopenic in comparison to control ($P < 0.0001$). Serum TPO levels were lower in cirrhotic patients (130.6 ± 79 Pg/ml) than control group (225.5 ± 36 pg/ml) ($P < 0.0001$) and also in patients with thrombocytopenia (101 ± 77.5 pg/ml) than in patients with normal platelet count (160.2 ± 70.3 pg/ml) ($P < 0.001$). TPO had a significant positive correlation with platelet count ($P = 0.0001$ for subgroup IA & $P = 0.04$ for subgroup IB). However serum TPO did not correlate with spleen size. Splenic size had a significant negative correlation with platelet count in cirrhotic patients ($P = 0.03$ for subgroup IA & $P = 0.004$ for subgroup IB). In cirrhotic patients, serum TPO levels were found to be decreased as the disease progressed [in subgroup IA, 188.25 ± 73.05 pg/ml in patients of Child-Pugh class A, 63.8 ± 23.28 pg/ml in class B and 51 ± 26 pg/ml in class C, while in group

IB, 247.3 ± 40.49 pg/ml in class A, 121.3 ± 29.6 pg/ml in class B and 112 ± 27 pg/ml in class C]. Child-Pugh score has a significant negative correlation with TPO level in both subgroups IA & IB ($P = 0.0001$) and with platelet count ($P = 0.0001$ for subgroup IA and 0.01 for subgroup IB), but no significant correlation with spleen size. In comparing class A, B and C in both subgroups (IA & IB), spleen size was significantly larger in Child class A of subgroup IA when compared to same class of subgroup IB ($P = 0.0001$) with slight significant decrease in TPO in class A of subgroup I than class A of subgroup B ($p = 0.02$).

Conclusion : we concluded that low TPO production may play a role, along with hypersplenism, in the development of thrombocytopenia in patients with liver cirrhosis. In early stage of cirrhosis (Child-Pugh class A), splenomegaly and hypersplenism may be the main pathomechanism of thrombocytopenia. While advanced liver cirrhosis (Child-Pugh class B & C), causing more reduction in TPO production, plays a central role in the pathogenesis of thrombocytopenia.

INTRODUCTION

Thrombocytopenia is a common haematological defect among patients

with cirrhosis^(15,21). The concept of hypersplenism proved to be unable to explain why splenectomy⁽⁵⁾ or porto-systemic shunt^(6,16) failed to augment platelet count in contrast to liver transplantation⁽²⁵⁾. The recent description of thrombopoietin (TPO) as a cytokine that regulates megakaryocyte maturation and platelet production has modified the historical concept of thrombocytopenia in cirrhotic patients⁽¹¹⁾.

The main TPO production site is the liver⁽²⁷⁾, and decreased TPO production by the liver has been proposed as being an important concomitant cause of the reduction in platelet production in cirrhotic patients, although not all studies obtained similar results^(1,8,9,12,20). These conflicting results were attributed to the limited number of observed subjects, the variations in patients populations (cirrhotic or non cirrhotic, cirrhotic patients with or without thrombocytopenia, different severity of liver disease, etc), and the limitations of various assays used^(9,15). Thus, the role of TPO in cirrhosis is under investigation.

We studied 72 patients with liver cirrhosis and 16 healthy controls to

determine the association between TPO levels, platelet count and various clinical and laboratory liver function parameters. Furthermore, we examined the possible influence of liver disease aetiology, Child-Pugh class of cirrhosis, and spleen size on TPO levels in patients with liver cirrhosis.

PATIENTS AND METHODS

The present study was conducted on 88 subjects, divided into 2 groups:

Group I (patients group): including 72 patients with liver cirrhosis selected from Hepatology Unit, Medical Department, Mansoura University Hospital in the period between March 2003 to April 2004. Diagnosis of liver cirrhosis was made on histopathological findings (in 21 patients) or on the basis of clinical, biochemical, ultrasonographic and endoscopic findings in the remaining of patients. They were 54 males and 18 females with age ranged between 23 and 62 years with mean of 50.48 ± 8.32 years. This group was further subdivided, according to platelet count, into 2 subgroups: subgroup IA: cirrhotic patients with thrombocytopenia (36 patients, 28 males and 8 females) with age ranged between 23 and 62 years with mean of 50.3 ± 8.5 years, subgroup IB: cirrhotic patients with normal platelet count (36

patients, 26 males and 10 females) with age ranged between 24-61 years with mean of 50.6 ± 6.8 years. Group II (control group): comprised 16 healthy persons (12 males and 4 females) with age ranged between 24-60 years with mean of 50.3 ± 6.8 years.

The exclusion criteria were: Hepatocellular carcinoma, pure bilharzial hepatic affection, genetic and metabolic liver disease, autoimmune hepatitis, portal vein thrombosis, splenectomy, recent (Less than 1 month) episode of gastro-intestinal bleeding, disseminated intravascular coagulation, haemolytic anaemia, aplastic anaemia, leukemia, idiopathic thrombocytopenic purpura, patients received drugs that may affect platelet function, patients treated by interferon or immunosuppressive agents, neoplastic disease and chronic renal failure. Informed consents were obtained from all individuals.

Selected patients were subjected to (1) thorough history taking & full clinical examination, (2) laboratory investigations including: complete blood count, liver function tests (ALT, AST, serum bilirubin, serum albumin), prothrombin time, bleeding time, activated partial thromboplastin time (APTT),

serum creatinine, blood glucose level, Hepatitis B and C markers, quantitative determination of TPO concentration. (3) Bone marrow aspiration (4) Abdominal Doppler ultrasound (5) Upper gastro-intestinal endoscopy & sigmoidoscopy (6) Liver biopsy for histopathological examination (for only 21 patients). Control group patients were subjected to all the above investigations except invasive types: bone marrow aspiration, endoscopy and liver biopsy.

The severity of liver disease was graded according to Child-Pugh score⁽¹⁸⁾ and each subgroup of patients had 12 patients in every class (A, B and C).

METHODS

1) Laboratory investigation : Venous blood was collected from fasting case by clean venipuncture. Blood sample was distributed into 2 tubes as the following:

(1) One ml of blood was delivered into plastic tube containing 50 μ l of the dipotassium salt of EDTA solution. This was used for performing complete blood count using automated count (model Cell-dyne 1700, Abbott Diagnostic, USA). The estimated normal range of platelet was 140-

400x10³/μl and number below 140x10³/μl was considered thrombocytopenia⁽¹⁾. (2) 5 ml blood were allowed to clot in plain container and the serum was taken for the following investigations: (a) Prothrombin time, and APTT were done by manual method (double determination). (b) Bleeding time by Dukes Method. (c) Albumin using quantitative Calorimetric method. (d) Total serum bilirubin using calorimetric method. (e) ALT & AST: using calorimetric method. (f) Serum creatinine using Jaffe deproteinization method. (g) Anti hepatitis C antibodies were detected by a third generation enzyme immunoassay containing HCV antigens from the viral core and from areas of non-structural N53, N54 and N55 regions. Diagnosis of HCV was confirmed by HCV PCR in the serum assessed by means of nested reverse transcription polymerase chain reaction described elsewhere⁽²²⁾. (h) Hepatitis B surface antigen and anti HBc antibodies (IgG & IgM): using kits from Abbott Diagnostic. (i) Quantitative determination of TPO serum levels (in picogram per milliliter): This was done by commercial ELISA (Quantikine-T Human TPO immunoassay, R & D systems, Minneapolis, Minnesota, USA). This assay employs the quantitative sand-

wich enzyme immunoassay technique. According to manufacturer's instructions: the lower limits of serum TPO detection was 15 pg/ml^(7,10).

II) Bone Marrow aspiration : It was performed using Salah needle from anterior superior iliac spine.

III) Doppler Ultrasonic evaluation : using ultrasound equipment Sono-ACC-Digital-GAIA to inspect liver morphology and data interpreted according to the standards for using ultrasonography in schistosomiasis proposed by Cairo Working Group⁽⁴⁾. Maximal longitudinal spleen size was estimated and was used because it correlates with spleen surface area⁽¹⁾. Portal and splenic vein diameter, portal and splenic blood flow and velocity were assessed by Doppler sonography.

IV) Liver biopsy (by percutaneous, ultrasound-guided) for histopathological examination, was performed for 21 patients (with platelet counts greater than 70x10³/μl and a prothrombin activity of ≥70%). The pathological assessment was made on section from formalin-fixed and paraffin-embedded liver biopsy, stained with haematoxylin-eosin, Masson's tri-

chrome and Prussian blue reaction. HBsAg and HBc Ag immunohistochemistry were applied routinely to all liver biopsy specimens^(22,13). Any positive cases for pure schistosomal hepatic affection were excluded.

V) Endoscopy : (1) oesophago-gastroduodenal endoscopy to reveal the presence of gastro-oesophageal varices and/or portal hypertensive gastropathy (2) sigmoidoscopy using rigid sigmoidoscope with rectal and lower colonic biopsies which were examined by transparency technique for schistosoma mansoni ova (viable or non viable), such patients were excluded from the study.

Statistical analysis : Statistical analysis was done using the SPSS Base 10.0 program for Windows, 1999 (SPSS, Chicago, IL). The data were parametric by using kolmagrov survival test. Quantitative data were summarized as means ($\pm$ SD) and qualitative data were summarized as percentages. Chi square test with Yates' correction was used for qualitative data (absolute frequencies) and Student's t-test was used to compare continuous variables between 2 groups and one way ANOVA f-test for comparing more than 2 groups. The

correlation between the parameters was obtained through Spearman's rank-order correlation coefficient test(r). A P-value less than 0.05 was considered statistically significant.

RESULTS

Patient population: The present study population consisted of 72 liver cirrhosis patients and 16 healthy subjects with matched age and sex. Post hepatitis B & C cirrhosis, either alone or mixed with schistosomal hepatic affection, were the most common diagnosis of aetiology of hepatic cirrhosis (fig. 1 & Table 1). The stage of liver disease varied with a predominance of mild to moderate disease (66% of patient populations are of Child-Pugh class A & B). Comparing of patients (and its subgroups) with controls showed that cirrhotic patients displayed significant abnormal liver functions in comparison to controls (table 1). Also cirrhotic patients had significant lower haemoglobin, RBC and Platelet count, with prolonged bleeding, prothrombin and activated partial thromboplastin times when compared with controls. On the other hand, thrombocytopenic cirrhotic patients (group IA) had lower platelet count and prolonged bleeding time when compared to cirrhotic patients with

normal platelet count (group IB). No significant statistical difference was observed regarding other parameters (Table 2).

Serum thrombopoietin (TPO) levels: Serum TPO levels were significantly lower in cirrhotic patients (130.6 ± 79 pg/ml in group I, 160.2 ± 70.3 pg/ml in subgroup IB and 101 ± 77.5 pg/ml in subgroup IA) when compared with control group (225.5 ± 36 pg/ml) ($P=0.0001$).

Thrombocytopenic cirrhotic patients (group IA) had significant lower TPO serum levels when compared to cirrhotic patients with normal platelet count (group IB) ($P=0.001$) (Table 3). Serum TPO levels were compared among Child-Pugh classes of cirrhotic patients and analysis showed progressive decrease of TPO levels with increased severity of liver disease, with still significant lower TPO levels in the 3 classes of subgroup IA when compared to those of subgroup IB (Table 6). Moreover, a negative correlation was found between Child-Pugh score and TPO levels in both subgroups of cirrhotic patients (Table 8), ($r = -$

0.7 , $P = 0.0001$). This indicates a gradual decrease of serum TPO levels with advanced stage of cirrhosis.

Platelet count: Cirrhotic patients had lower platelet count [129.6 ± 52 ($\times 10^3 \mu\text{l}$) in group I, 85.5 ± 26.9 ($\times 10^3 \mu\text{l}$) in group IA and 176.5 ± 20.1 ($\times 10^3 \mu\text{l}$) in group IB] when compared with controls [311.5 ± 56 ($\times 10^3 \mu\text{l}$)], [Table (2), $p=0.0001$]. A progressive decrease in platelet count was recorded with the increase of severity of cirrhosis (from Child A to Child C) (Table 6).

Bone marrow cellularity: Only 3 patients in cirrhotic group (4.17%) showed hypocellular bone marrow. Hypercellular bone-marrow was recorded in 6 patients (8.3 %), while 63 (87.5%) had normocellular bone marrow. There is no significant difference between subgroups of cirrhotic patients (IA vs IB) as regards data of bone marrow cellularity. Patients with hypocellular bone marrow had significant lower TPO levels and platelet count when compared with patients with normo- or hypercellular bone marrow (Table 9).

Data of Doppler ultrasound : Portal vein (PV) diameter, cross sectional area (CSA), blood flow volume (BFV), splenic vein (SV) diameter and spleen size were statistically significant higher, while V. max and V. mean of PV and SV were statistically significantly lower in group IA in comparison to controls ($P=0.0001$ for each finding), and also in group IB in comparison to controls ($P=0.0001$ for each finding), but no significant difference was found when comparing group IA VS group IB (Table 4). When comparing spleen size in each Child-Pugh class, only Child class A of group IA had significant larger spleen size than that of Child class A of group IB ($P=0.0001$, Table 6).

Upper Gastro-intestinal endoscopic findings: Only 6 patients in each subgroup (IA & IB) had no varices, the other 66 patients had oesophageal varices. Fundal varices and portal hypertensive gastropathy were also recorded. No statistically significant differences were found when comparing both subgroups IA & IB as regards endoscopic findings (Table 5).

Correlation studies:

Serum TPO levels were highly

significantly positively correlated with platelet count in thrombocytopenic cirrhotic patients (group IA, $r=0.807$, $P=0.0001$) and slightly positively correlated with platelet count in group IB ($r=0.38$, $P=0.04$) but were not correlated with platelet count in controls (Table 7). However, TPO levels were not correlated to spleen size.

Spleen size was inversely correlated with platelet count in both subgroup IA ($r=-0.31$, $P=0.036$) and subgroup IB ($r=-0.46$ & $P=0.004$) (Table 10)

Child-Pugh score was inversely correlated with TPO levels in both group IA ($r=0.786$, $P=0.001$) and group IB ($r=-0.757$, $P=0.0001$). Also, Child-Pugh score was inversely correlated with platelet count in both group IA ($r=-0.675$, $P=0.0001$) and group IB ($r=-0.418$, $P=0.01$), but not correlated to spleen size (Table 8).

Data of Doppler ultrasound were inversely correlated with platelet count but not to TPO levels (Table 11).

Grades of oesophageal varices

were not correlated neither to serum TPO levels nor to platelet count in cirrhotic patients.

Finally, we evaluated the results

in cirrhotic patients according to aetiology and we found no significant difference in TPO levels and platelet count according to aetiology of liver cirrhosis.

Table (1): Characteristics of 88 subjects included in the study

Variable	Patients (Group I)		Control (group II)
	IA	IB	
Study subjects (N):	36	36	16
Sex (Male: female):	28:8	26:10	12:4
Age (year):			
Range	23-62	24-61	24-60
Mean $\pm$ SD	50.31 $\pm$ 8.59	50.64 $\pm$ 6.8	50.31 $\pm$ 6.85
Liver size (N & %):			
Average	9 (25)	12 (33.3%)	16 (100%)
Shrunken	27 (75)	24 (66.7)	
Spleen size (N & %):			
Impalpable	5 (13.9)	5 (13.9)	16 (100%)
Enlarged	31 (86.1)	31 (86.1)	
Ascites (N & %):			
Absent	8 (22.2)	13 (36.1)	16 (100%)
Present	28 (77.8)	23 (63.9)	
Liver function tests (mean $\pm$ SD)			
ALT (IU/L)	47.8 $\pm$ 18*	44.5 $\pm$ 17.7*	22.3 $\pm$ 6.2
AST (IU/L)	47.7 $\pm$ 20.3*	40.9 $\pm$ 16.5*	22 $\pm$ 4.9
S. Albumin (gm/dl)	2.7 $\pm$ 0.4*	2.8 $\pm$ 0.9*	4.2 $\pm$ 0.4
S. bilirubin (mg/dl)	1.8 $\pm$ 0.47*	2.1 $\pm$ 0.9*	0.9 $\pm$ 0.4
Serological markers of HCV & HBV (N & %):			
HCV positive	24 (66.7)	27 (75)	-
HBV positive	8 (22.2)	7 (19.4)	-
HCV & HBV positive	3 (8.3)	-	-

*P=0.0001 when compared to control group (Other data shows no significant statistical difference)

Table (2): Data of blood picture and haemostatic tests in studied subjects.

Parameter (mean $\pm$ S.D)	Patients (group I)		Control (group II)
	IA	IB	
Hb (gm/dl)	10.19 $\pm$ 1.73*	10.95 $\pm$ 1.71*	13.28 $\pm$ 1.47
RBC ($\times 10^6/\mu\text{l}$)	3.64 $\pm$ 0.611*	3.95 $\pm$ 0.66*	4.86 $\pm$ 0.27
WBC ($\times 10^3/\mu\text{l}$)	3.29 $\pm$ 1.25	3.71 $\pm$ 1.13	4.25 $\pm$ 0.52
Platelet ($\times 10^3/\mu\text{l}$)	85.5 $\pm$ 26.9*1	176.5 $\pm$ 20.1*	311.56 $\pm$ 56.2
Bleeding time (minute)	5.13 $\pm$ 0.8*1	4.27 $\pm$ 0.44	4.06 $\pm$ 0.145
Prothrombin time (seconds)	15.78 $\pm$ 7.9*	15.16 $\pm$ 2.5*	12.3 $\pm$ 0.47
APTT (seconds)	39.4 $\pm$ 7*	39.8 $\pm$ 2.7*	37.6 $\pm$ 2.3

* p=0.0001 when compared to control group.

1 p=0.001 when comparing group IA VS group IB.

Table (3): Serum TPO levels in studied subjects (picogram / milliliter).

	Patients (group I)			Control (group II)
	IA	IB	Total (I)	
mean	101	160.2	130.6	225.5
S.D.	77.5	70.3	79	36

I vs II P=0.0001 (t=4.6)

IA vs II p=0.0001 (t=6.9).

IB vs II P=0.001 (t=3.4).

IA vs IB P=0.001 (t=3.39).

Table (4): Data of Doppler ultrasound finding on the portal and splenic veins in group IA, group IB and group II.

	IA		IB		II		F	P
	Mean	SD	Mean	SD	Mean	SD		
Portal vein (PV):								
PV diameter (mm)	14.8	2.1	14.1	1.8	11	1.6	22.8	0.001
PV CSA (cm ²)	1.91	0.301	1.76	0.109	1.4	0.2	29.8	0.001
V. max (cm/sec)	10.5	3.03	10.94	2.27	16	2.5	34.0	0.001
V. mean (cm/sec)	8.06	2.02	7.84	1.21	13	1.7	61.3	0.001
BFV (ml/min)	931.13	171.2	880.9	165	810	120	3.2	0.045
Splenic vein (SV):								
SV diameter (mm)	9.52	3.9	9.60	2.0	7	1	5.3	0.007
V. max (cm/sec)	13.1	4.2	12.3	3.8	17	3.9	7.98	0.0007
V. mean (cm/sec)	10.32	3.0	9.55	3.0	13	3.6	6.9	0.0017
Splenic size (mm)	137	32.8	126.5	24.29	103	18	8.0	0.004

CSA = Cross sectional area.

Vmax= Maximum velocity

BFV= blood flow volume

V mean= mean velocity.

Table (5): Upper gastrointestinal endoscopic findings in cirrhotic patients.

	IA		IB		X ²	P
	No	%	No	%		
No. of varices						
No	6	16.7	6	16.7	0.753	0.95
One	5	13.9	3	8.3		
Two	9	25.0	10	27.8		
Three	14	38.9	14	38.9		
Four	2	5.6	3	8.3		
Grade of varices						
Grade I	15	41.7	13	36.1	0.024	0.82
Grade II	16	44.4	17	47.2	0.080	0.77
Grade III	18	50	17	47.2	0.733	0.932
Grade IV	9	25	13	36.1	0.026	0.871
Other endoscopic findings						
Red spots	5	13.9	3	8.3	0.710	0.355
PHG	8	22.2	7	19.4	0.721	0.500
Fundal varices	6	16.6	3	8.3	0.478	0.240

Table (6): Comparison between platelet number, TPO level and splenic size in each Child-Pugh class (A, B & C) in both subgroups (IA and IB).

	Group IA		Group IB		t	P
	mean	SD	mean	SD		
Child class A:						
Platelet count (X10 ³ /μl)	107.33	22.03	194.08	33.35	7.518	0.001
TPO level (Pg/ml)	188.25	73.05	247.3	40.49	2.45	0.02
Splenic size (mm)	148.8	39.4	121.25	15.3	5.32	0.0001
Child class B:						
Platelet count (X10 ³ /μl)	85.16	19.42	172.16	17.69	11.969	0.0001
TPO level (Pg/ml)	63.83	23.28	121.33	29.66	5.294	0.0001
Splenic size (mm)	132.41	29.92	128.83	30.49	0.290	0.77
Child class C:						
Platelet count (X10 ³ /μl)	64	20.58	163.33	13.45	15.77	0.0001
TPO level (Pg/ml)	51	26	112	27	5.52	0.0001
Splenic size (mm)	129.0	27.62	129.58	25.0	0.015	0.098

Table (7): Correlation of TPO level with platelet count and splenic size in group IA, IB and II.

TPO	Platelet count		Splenic size	
	r	P	r	p
IA	0.807	0.0001	0.103	0.549
IB	0.38	0.041	-0.1	0.56
II	-0.12	0.69	0.04	0.87

Table (8): Correlation of Child Pugh score in group IA and group IB with TPO, Splenic size and platelet count.

Child score	TPO		Splenic size		Platelet count	
	r	p	r	p	r	p
Group IA	-0.786	0.0001	-0.175	0.31	-0.675	0.0001
Group IB	-0.757	0.0001	0.028	0.87	-0.418	0.01

Table (9): TPO level (pg/ml) and platelet count ($\times 10^3/\mu\text{l}$) according to bone marrow cellularity.

Bone marrow	TPO (pg/ml)		Platelet count ($\times 10^3/\mu\text{l}$)	
	Mean	SD	Mean	SD
Hypocellular.	63.2	14	39.3	11.3
Normocellular.	136.4	74	141.23	48.4
Hypercellular	127	99	87.3	21
F	3.24		12	
P	0.045		0.0001	

Table (10): Correlation of splenic size with platelet count in group IA, IB and II.

Splenic size	Platelet count	
	r	p
IA	-0.31	0.036
IB	-0.46	0.004
II	-0.23	0.38

Table (11): Correlation between Doppler finding on the portal vein and on the splenic vein with TPO level and platelet count in cirrhotic patients.

	TPO level		Platelet count			TPO level		Platelet count	
	r	p	r	p		r	P	r	p
PV diameter	-0.14	0.23	-0.25	0.03	SV diameter	0.96	0.42	-0.36	0.04
PV CSA	-0.04	0.73	-0.32	0.006	V max	0.22	0.06	-0.4	0.014
V max	-0.005	0.97	-0.39	0.018	V mean	0.22	0.6	-0.34	0.034
V mean	-0.99	0.45	-0.12	0.03					
BFV	-0.07	0.55	-0.3	0.009

Fig. (1) Aetiology of liver cirrhosis .

DISCUSSION

Thrombopoietin (TPO), also known as C-MPL ligand or megakaryocyte growth and developmental factor, has been isolated and purified by several groups and regarded as the main regulator of thrombopoiesis^(10,11). TPO is mainly synthesized by the liver and its serum levels are regulated, to some extent, by the receptor-mediated uptake of TPO by circulating platelet^(19,27). The development of a sensitive sandwich ELISA for measuring TPO concentration in human serum was employed to study the role of TPO in thrombocytopenia in liver diseases including cirrhosis^(1,8,12,20).

In the present study, we addressed the relationship between serum TPO levels, platelet count, spleen size and severity of liver cirrhosis. Our study population was well characterized by the extent, stage and aetiology of liver disease. The relatively high number of cirrhotic patients with various degrees of severity allowed detailed subgroup analysis. Other previously reported studies involved smaller groups of liver transplant recipients with presumably more advanced stages of cirrhosis, and TPO levels were measured with less sensitive and reli-

able immunoassay^(9,15).

In the present study, we demonstrated that patients with liver cirrhosis had lower serum TPO levels than healthy controls. Moreover, we recorded that cirrhotic patients with thrombocytopenia had lower levels of TPO than cirrhotic patients with normal platelet count. These data, together with the previously reported studies^(1,8,9,12,15), suggest that TPO level directly affects platelet count. Low levels of TPO in cirrhotic patients can be explained by the impairment of TPO production due to decreased hepatocellular activity resulting from decreased hepatic functioning mass⁽⁸⁾.

In particular, we have shown a highly positive correlation of TPO serum levels with platelet counts in cirrhotic patients, the same finding was reported previously^(17,26) indicating the role of TPO in thrombocytopenia and that the recombinant TPO could possibly be an effective drug to treat patients with cirrhosis and severe thrombocytopenia during bleeding episodes⁽²⁶⁾.

Our data for the subgroup of patients with splenomegaly and normal

platelet count (subgroup IB) seem to confirm the fundamental role played by TPO in maintaining platelet homeostasis. In fact, this group showed significantly higher level of TPO (in comparison to subgroup IA), which probably maintained the normal platelet count despite splenomegaly, the same finding reported by Adenolfi et al.⁽¹⁾.

The data from our study showed that both serum TPO level and thrombocytopenia were inversely correlated with Child Pugh score. This suggests that an advanced liver disease impairing the production of TPO may be responsible, in part, for inducing thrombocytopenia. Supporting this, is the fact that liver transplantation restores an adequate TPO production and normal platelet count^(15,9). This finding is in agreement with that reported by Kawasaki et al. ⁽¹²⁾; Adenolfi et al.⁽¹⁾; Koruk et al. ⁽¹⁴⁾ and Giannini et al.⁽⁸⁾ who concluded that in patients with more severe liver disease, there is an inappropriate TPO response to decreased platelet levels which correlates with decreased liver synthetic function. Also Kato et al.⁽¹⁰⁾ explained the lower level of serum TPO in liver cirrhosis than chronic hepatitis by the low

content of total liver TPO mRNA.

In contrast to our data, Schoffski et al.⁽²⁰⁾ reported that TPO concentrations were not related to platelet counts in 36 cirrhotic Child class B/C patients with thrombocytopenia and reported that TPO serum concentrations were independent of the Child's class. They explained these contradictory findings by stating that the circulating levels of TPO may not be representative in states of altered platelet turnover, as it is unknown how much of the cytokine is bound to the MPL receptor on these platelets. Serum TPO levels could be determined longitudinally which may prove more valuable than the static evaluations of the current series. They recommended further studies to address the positive correlation between TPO and its receptors on platelets. This controversy can be explained also by varied study populations.

The data from our study showed that there was a negative correlation between the spleen size and platelet count. This finding supports the idea that hypersplenism and increased platelet sequestration is a relevant pathomechanism for thrombocytope-

nia. This finding is inconsistent with previous reports (1,12,20,26), that explained this finding by the fact that the increase in the resistance to the flow in the portal territory causes the blood to deflect the spleen and consequently, platelet pooling and clearance by macrophages are promoted.

The role of splenomegaly in inducing thrombocytopenia is also supported by our finding that, in the subgroup of patients with low platelet count (subgroup IA), those with huge splenomegaly (in Child Pugh class A) showed highly significant lower levels of platelets than those of same class of subgroup IB (cirrhotic patients with normal platelet count) ($P=0.0001$ for spleen size, $P=0.001$ for platelet count and $p=0.02$ for TPO level). While in both class B & C, comparing spleen size between both subgroups (IA & IB) showed insignificant difference, on the other hand, TPO levels showed highly significant decrease in patients with thrombocytopenia ($P=0.0001$), indicating that TPO plays the pivotal role of thrombocytopenia in advanced stages of cirrhosis (Child class B and C).

In summary, the comparison of the

parameters evaluated in the subgroups of patients of liver cirrhosis showed that reduced TPO levels in cirrhotic patients may be a consequence of its impaired hepatic production and thus contributes, along with platelet destruction and hypersplenism, to the development of thrombocytopenia in cirrhotic patients. In early stages of cirrhosis (Child-Pugh class A), splenomegaly and hypersplenism may be the main pathomechanism of thrombocytopenia. While advanced liver cirrhosis (class B and C), causing more reduction in TPO production, plays a central role in the pathogenesis of thrombocytopenia. Furthermore, these informations might aid clinicians to optimize methods of management of thrombocytopenia including possibility for usage of recombinant TPO to treat patients with cirrhosis and severe thrombocytopenia during bleeding episodes. In addition, we can not exclude the possibility that other factors may play a minor role in platelet reduction in cirrhotic patients such as antiplatelet antibodies.

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مستوى الثرومبوبويتين فى مرضى تشمع الكبد وعلاقته بشدة المرض وحجم الطحال والصفائح الدموية

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خلفية البحث : يعانى مرضى تليف الكبد من قابلية عالية للنزف، وهذا قد يعكس خلافاً فى عوامل تجلط الدم أو فى وظائف أو نقص عدد الصفائح الدموية، وهذا الأخير قد ينتج من فرط الطحالية (زيادة تكسير الصفائح الدموية بواسطة الطحال) أو من عوامل أخرى مثل نقص إفراز مادة الثرومبوبويتين من الكبد (المسئولة عن زيادة تكوين وفو الصفائح الدموية).

هدف البحث : يهدف هذا البحث إلى دراسة الثرومبوبويتين فى مصل مرضى تشمع الكبد وعلاقته مع شدة مرض التشمع - حجم الطحال وعدد الصفائح الدموية فى هؤلاء المرضى ..

مادة البحث : أجرى هذا البحث بوحدة الجهاز الهضمى والكبد بقسم الأمراض الباطنة بمستشفى المنصورة الجامعى على ٧٢ مريضاً بتليف الكبد (مجموعة ١) وتشمل ٥٤ رجلاً و ١٨ أنثى ، ويتراوح أعمارهم من ٢٣ حتى ٦٢ سنة بمتوسط 50.48 ± 8.32 سنة، هذا بالإضافة إلى ١٦ شخص سليم كمجموعة ضابطة (مجموعة ٢).

تم تقسيم مجموعة المرضى إلى قسمين (حسب عدد الصفائح الدموية) ، يشمل ٦٦ منهما ٣٦ مريضاً : (أ) مرضى تليف الكبد مصحوب بنقص الصفائح الدموية ويشمل ٢٨ رجلاً و ٨ إناث، ويتراوح أعمارهم من ٢٣ حتى ٦٢ سنة بمتوسط 50.31 ± 8.59 سنة (ب) مرضى تليف الكبد مصحوب بعدد طبيعى من الصفائح الدموية ويشمل ٢٦ رجلاً و ١٠ إناث، ويتراوح أعمارهم من ٢٤ حتى ٦١ سنة بمتوسط 50.64 ± 8.78 سنة.

تم استبعاد المرضى المصابين بتليف الكبد إذا كانوا يعانون من : سرطان الكبد - استئصال الطحال جراحياً - أمراض مثل اللوكيميا أو تحلل الصفائح الدموية التلقائى أو فقر الدم

اللاتنسجى - إستعمال أدوية تؤثر على الصفائح الدموية - الإصابة بالبلهارسيا منفردة (بدون فيروسات كبدية) - أمراض الكبد الإستقلابية (الأبيضية) - نزيف حديث (أقل من شهر) من دوالى المرئ - الفشل الكلوى المزمن .

خطة البحث : تم إستقصاء التاريخ المرضى الشخصى بعناية وتفصيل مع فحص إكلينيكى شامل ودقيق بالإضافة إلى الفحوصات والأبحاث التالية :-

(أ) فحوصات عامة :

١- بول وبراز وصورة دم كاملة، خاصة عدد الصفائح الدموية (مع إعتبار نقص عددها هو الرقم أقل من ١٤٠ ألف / ميكروليتر) .

٢- وظائف كبد كاملة .

٣- زمن النزف وزمن البروثرومين .

٤- سرعة الترسيب .

٥- مستوى الكرياتينين بالدم .

٦- دلالات الفيروسات الكبدية ب ، سى .

٧- أشعة موجات فوق صوتية على البطن مع دوبلر ملون على الوريد البابى والوريد الطحالى .

(ب) فحوصات خاصة :

١- تعيين مستوى الثرومبوبويتين بالدم.

٢- منظار للمرئ والمعدة وكذلك منظار للشرح والمستقيم (مع أخذ لتشخيص البلهارسيا) وذلك لمجموعة المرضى فقط.

٣- بزل وتحليل نخاع العظام (لمجموعة المرضى فقط).

٤- عينة من الكبد لفحصها باثولوجيا (لعدد ٢١ مريضاً فقط).

تم تقسيم مجموعة المرضى (طبقاً لتقسيم تشايلد) إلى ٣ مستويات أ.ب.ج وذلك حسب شدة المرض.

نتائج البحث :

١- ينخفض مستوى الثرومبوبويتين وعدد وظائف الصفائح الدموية إنخفاضاً ذا دلالة إحصائية فى مرضى تليف الكبد اذا قورنت بالمجموعة الضابطة.

٢- لقد وجد أن مرضى تليف الكبد المصحوب بنقص الصفائح الدموية ينخفض لديهم مستوى الثرومبوبويتين إنخفاضاً ذا دلالة إحصائية، عن مرضى تليف الكبد غير المصحوب بنقص الصفائح

الدموية.

- ٣- يعتبر فرط الطحالية من أسباب نقص الصفائح الدموية خاصة فى حالات تليف الكبد غير المتقدم، حيث يتناسب حجم الطحال تناسباً عكسياً مع عدد الصفائح الدموية
- ٤- كما يعتبر إنخفاض نسبة الثرومبوبويتين من أسباب نقص عدد الصفائح الدموية فى حالات تليف الكبد المتقدم، حيث يتناسب مستوى الثرومبوبويتين تناسباً طردياً مع عدد الصفائح الدموية
- ٥- يتناسب مستوى الثرومبوبويتين تناسباً عكسياً مع شدة التليف الكبدى طبقاً لتقسيم تشايلد .
- ٦- قلة خلوية النخاع العظمى فى ٣ حالات فقط (١٤٪ من المرضى).

خلاصة البحث :

- ١- المرضى المصابون بتليف الكبد يعانون من نقص فى الصفائح الدموية، ويحدث هذا نتيجة لفرط الطحالية (خصوصاً فى مستوى تشايلد أ) أو نقص مادة الثرومبوبويتين (خصوصاً فى مستوى تشايلد ب ، ج) أو الإثنين معاً.
- ٢- كلما زادت شدة تليف الكبد، كلما قل إفراز مادة الثرومبوبويتين ومستواها بالدم .
- ٣- لقد لوحظ أن قلة خلوية النخاع العظمى لا توجد بصورة شائعة فى مرضى تليف الكبد، مما قد يعنى أنها غير مسئولة بصورة مباشرة عن نقص الصفائح الدموية فى مرضى تليف الكبد.