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ASSESSMENT OF ENDOTHELIAL FUNCTION IN PATIENTS WITH PORTAL HYPERTENSION

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Objectives: *To evaluate the role of nitric oxide (No) and endothelin-1 in portal hypertension patients with compensated decompensated liver cirrhosis. Also, the effect of splenectomy and shunt operation on their levels in compensated patients.*
Design: *A randomized, group comparative, single center study.*
Subjects: *The present study included 32 bilharzial patients with portal hypertension (Clinically and radiologically diagnosed), in addition to 10 matched subjects as a control group. The patients were classified into two groups: compensated and decompensated.Setting: Mansoura University.*
Methods: *serum albumin, bilirubin, ALT, AST, and nitric oxide (NO) Were measured by colorimetric assays while plasma endothelin-1 (ET-1) was measured by radio immunoassay after extraction. Prothrombin time was measured by using standard thromboplastin methods.*
Results: *The results of this study revealed significant increase of NO in compensated and decompensated groups in comparison to control. After splenectomy and shunt operation No was significantly decreased than before operation but still higher in comparison to control. Plasma endothelin-l levels were significantly decreased in two groups in comparison to control. After operation endothelin-l was significantly increased in comparison to before operation, however, it is still lower than control. No showed significant negative correlation with endothelin-1 in different studied groups.*
Conclusion: *Portal hypertension is associated with significant increase in NO and significant decrease in endothelin-l levels. These findings may play an important role in hemodynamic changes in cirrhosis and shunt operation may improve liver functions by reducing portal hypertension and modulating the levels of NO and endothelin-1 patients with chronic liver diseases.*

Key words: Nitric oxide. Endothelin-l. Portal hypertension.

INTRODUCTION

An increased portal venous inflow secondary to generalized splanclinic arteriolar vasodilatation plays an important role in increased portal pressure [(1)]. The precise cause of the vasodilatation remains unknown but has traditionally been viewed as an imbalance between endogenous vasoconstrictors (e.g angiotensin II, vasopressin, norepinephrine, and endothelin) and vasodilators (e.g. glucagons, prostacyclin, and nitric oxide (NO).

Nitric oxide (NO) radical is increasingly recognized as an important mediator of physiological and pathological processes [(2)] Endothelin (ET) peptides are potent vasoconstrictor substances released from endothelial cells; the predominant peptide released from the endothelium is ET-1. ET-1 acts at 3 types of ET receptor in vascular tissues. ETA and ETB2 receptors are localized to smooth muscle and mediate contraction[(3)], while ETBI receptors are localized to endothelial cells and mediate release of nitric oxide and vasodilator cyclo-oxygenase products [(4)].

This study was carried out to evaluate the role of NO and endothelin-1 in portal hypertension patients with compensated and decompensated liver cirrhosis. Also, the effect of splenectomy and shunt operation on their levels in compensated patients.

SUBJECTS AND METHODS

Thirty-two patients with hepatic cirrhosis and portal hypertension were selected to participate in this study. The patients were diagnosed by clinical examination (pallor, jaundice, edema, liver and spleen sizes) and abdominal ultrasonography (hepatic and splenic sizes, and ascites). They were adult males, and their ages ranged from 35-55 years. They were admitted to general surgery department and gastroenterology center for treatment. They were classified into two groups:

* Compensated liver cirrhosis group: 17 patients with portal hypertension and splenomegaly undergone splenectomy and shunt operation.

* Decompensated hepatic cirrhosis group: 15 patients with ascites, splenomegaly and severe portal hypertension.

Diabetic patients and those with cardiac, respiratory or renal disorders were excluded from the study.

Statistical analysis was carried out with the SPSS 7.5 for Windows statistical software package. S. albumin, bilirubin, ALT, AST, NO, and endothelin-l were tested by paired t test. The criterion of significance was value of $p<0.05$. Correlation was calculated with Pearson's method.

RESULTS

Serum albumin, total and direct bilirubin, prothrombin time showed no significant difference on the other hand ALT, AST and, NO, were significantly increased while endothelin-1 was significantly decreased in compensated group in comparison to control. In decompensated group serum bilirubin, AST, ALT, prothrombin time (seconds) and No were significantly increased while serum albumin and endothelin-1 were significantly decreased (Table 1).

After splenectomy with shunt operation, serum albumin, bilirubin and prothrombin time showed no significant changes on the other hand ALT, AST and NO were significantly decreased while endothelin-1 was significantly increased after operation in comparison to before operation. In comparison of control group to after operation serum albumin, bilirubin, prothrombin time showed no significant changes while ALT, AST, and NO were still sigificanlty higher. Also plasma endothelin-l was still significantly lower after operation (Table 2).

Serum albumin, bilirubin, AST, ALT and prothrombin time showed no significant correlation with NO while endothelin-l showed significant negative correlation with No in different studied groups (Table 3 and Fig 1).

Table (1): ***Comparison of studied parameters between control and compensated and decompensated groups.***

Group	*Albumin*	*Bilirubin (mg/dl)*		*ALT (U/L)*	*AST (U/L)*	*PT (seconds)*	*NO (µmol/l)*	*Endothelin-1 (ng/ml)*
		T	D					
Control M	4.36	0.63	0.20	24.8	25.2	13.1	5.96	1.01
(n=10) SD	0.38	0.16	0.05	4.3	4.0	1.2	1.85	0.30
__drnone	4.30	0.80	0.25	41.7	59.7	13.4	27.2	0.16
Compensated M								
(n=17) SD	0.22	0.28	0.09	9.0	9.5	1.4	3.00	0.03
P_1	>0.05	>0.05	>0.05	<0.001	<0.001	>0.05	<0.001	<0.001
Decompensated M	2.1	2.8	1.2	29.6	30.5	27.3	85.5	0.08
(n=15) SD	0.35	0.5	0.3	4.8	3.9	3.01	20.3	0.03
P_2	<0.001	<0.001	<0.001	0.01	0.005	<0.001	<0.001	<0.001

P_1 Control versus compensated group
P_2 Control versus decompensated group

Table (2): ***Comparison of studied parameters before and after operation***

Group		*Albumin*	*Bilirubin (mg/dl)*		*ALT (U/L)*	*AST (U/L)*	*PT (Seconds)*	*NO (µmol/l)*	*Endothelin-1 (ng/ml)*
			T	D					
Control	M	4.36	0.63	0.20	24.8	25.2	13.1	5.96	1.01
(n=10)	SD	0.38	0.16	0.05	4.3	4.0	1.2	1.85	0.30
Before		4.30	0.80	0.25	41.7	59.7	13.4	27.2	0.16
Operation	M								
(n=17)	SD	0.22	0.28	0.09	9.0	9.5	1.4	3.00	0.03
	P_1	>0.05	>0.05	>0.05	0.02	<0.001	>0.05	<0.001	<0.001
After		4.38	0.08	0.23	33.4	42.7	13.0	8.61	0.76
Operation	M								
(n=15)	SD	0.18	0.2	0.06	2.49	5.39	1.6	1.07	0.14
P_2		>0.05	>0.05	>0.05	<0.001	<0.001	>0.05	0.01	0.02

P_1 before versus after operation P_2 Control versus after operation

Table (3): ***Correlation between nitric oxide and different studied parameters***

Group		Albumin	Bilirubin Total	ALT	AST	PT	Endothelin-1
Control	r	0.20	0.30	0.32	0.28	0.18	-0.64
(n=10)	p	>0.05	>0.05	>0.05	>0.05	>0.05	0.001
Compensated							
(n=17)	r	0.30	0.28	0.21	0.19	0.21	-0.84
	p	>0.05	>0.05	>0.05	>0.05	>0.05	0.001
Decompensated							
(n=15)	r	0.25	0.27	0.23	0.21	0.24	-0.92
	p	>0.05	>0.05	>0.05	>0.05	>0.05	<0.001

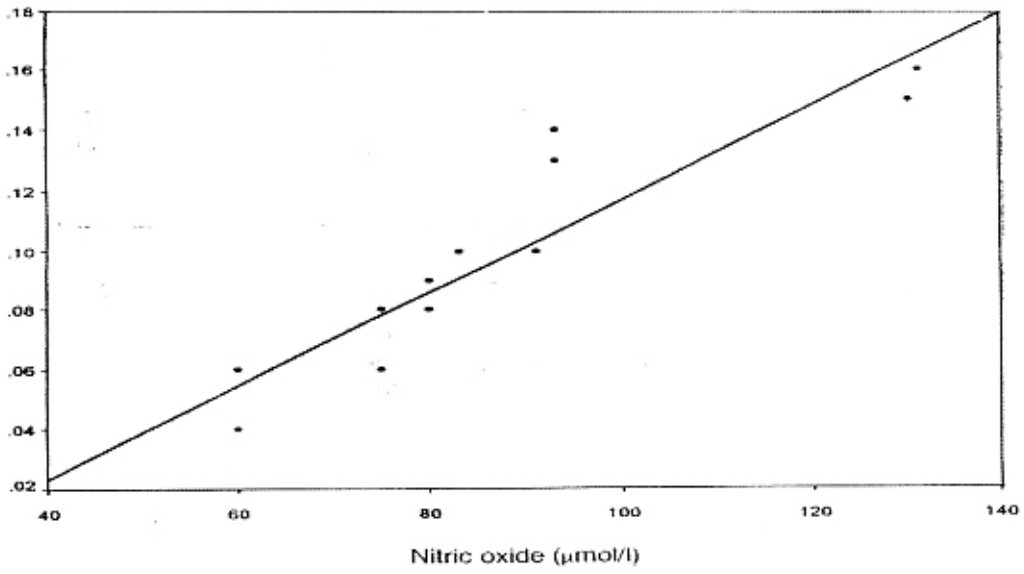

Fig (1): Correlation between nitric oxide and endothelin -1 in decompensated group.

DISCUSSION

A control group consisted of 10 healthy subjects none was suffering from schistosomiasis, hepartitis, or any other medical condition.

Dietary intake was standardized to control possible effects of the diet on NO_2 and NO_3 level. An informed consent was taken from each patient. Fasting blood samples were obtained and divided into:

* 3 ml in plain tube for liver functions (albumin, bilirubin, ALT and AST) and nitric oxide.

* 1.8 ml with 0.2 ml citrate for prothrombin time.

* 5 ml into tube with lmg/ml EDTA and 1500 kalikrin (trasylol) per 1 ml were centrifuged in refrigerated cintrifuge and plasma kept at-70°C for endothelin 1.

* For the group undergone splenectomy and shunt operation the samples were obtained before and one month after operation.

Conventional hepatic function tests were performed on technicon RA-XT clinical chemistry autoanalyzer (Bayer corporation, Diagnostics, NY, USA). Prothrombin time was assessed using standard thromboplastin. Serum total nitrate nitrite concentration was used as an index for No production and was determined by a colorimetric assay using reagent kit (Oxis Innternational Inc, Protland, OR, USA). The method was based on 2 step process. The first step is the conversion of nitrate to nitrite utilizing nitrate reductase. The second step is the addition of Griess reagent which convert nitrite into a deep purple azo compound where photometric measurement of the absorbance at 540 nm using plate reader. Endothelin was measured by competitive radioimmunoassay after extraction of plasma using SEP-column containing 200 mg C_{18} [15].

Portal hypertension (PHT) is characterized by a hyperdynamic circulatory state that is commonly observed in patients with chronic liver disease and in experimental models of PHT with extensive collateral circulation [6]. The main pathophysiological change in the hyperdnamic circulation is a generalized reduced vascular resistance [7].

The reasons for the decreased vascular resistance include exaggerated endothelial function with enhanced synthesis and/or release of vasoactive substance (such as prostacyclin [8,9] and nitric oxide [10] and altered vascular responsiveness to vasoconstrictor substances (such as vasopressin, angiotensin, norepinephrine, and methoxamine) [11,12,13]

Nitric oxide (NO), potent endothelium derived relaxing factor, is one of the major vasoactive substances implicated in the pathogenesis of the reduced vascular resistance in PHT [13,14] .The substrate for No formation is l-arginine, from which a terminal guanido nitrogen atom is removed by the enzyme nitric acid synthase to yield one molecule of L-citrulline and one molecule of No [15,16,17]. NO is produced by No synthase (NOS), an enzyme that exists in three isoforms (neuronal type I, NoS), endothelial type III, e NOS), and in ducible (type II, INOS), encoded by distinct genes [18,19].

In the present study NO was significantly increased in compensated and decompensated groups in comparison to control group (Table 1). After operation NO was significantly decreased than before operation but still higher in comparison to control (Table 2).

The altered vascular responsiveness and circulatory abnormalities of PHT can be partially correlated following the inhibition of NO synthase (NOS) with specific inhibitors[20,21].

Heinemann et al [22], has been suggested that excessive production of No underlie both the vascular hyproteactivity to vasoconstrictors such as arginine vasopressin and endothelin-1 and the splauchnic vasodilation seen in portal hypertension.

Although the exact etiology of the enhanced NO response in PHT remains unclear, it is possible that an overproduction of NO is not a primary but is a secondary event to enhanced endothelial shear stress, pressure, or endotoxemia[23], however, most studies have been unable to detect endotoxemia induced NOS expression in PHT which do not involve any hepatic parencymal dysfunction [24,25], however induced NOS expression has been demonstrated in cirrhotic models of PHT, particularly with ascites [26,27]. Hou et al. [28], suggested that enhanced Gi-protein expression may represent an important mechanism for exaggerated NO-dependent relaxation in PHT vasculature.

In the present study endothelin-1 levels were significantly decreased in compensated and decompensated groups (Table 1). After shunt operation endothelin-1 was significantly increased in comparison to before operation, however, it is still lower than control (Table 2).

It has been shown that endothelin-l and endothelin-3 perfused into the portal system results in elevation of portal pressure and an increase m glycogenolysis [29] The precise mechanism by which endothelin-l and endothelin-3 cause vasoconstriction in liver is unknown, but it has been proposed that they act on hepatic stellate cells (also known as lipocytes, Ito cells, perisinusoidal cells, or fat storing cells)[30] .Stellate cells have been shown to express ETA/ETB receptors in far greater abundance than other resident liver cells, including other putate vasoregulartoy elements such

as sinusoidal endothelial cells [31]. Furthermore, the subendothelial location and histological appearance of stellate cells are reminiscent of tissue pericytes, smooth muscle-like cells found in the peripheral microvasculature and thought to regulate capillary blood flow (32,33) . Rocky and Weisiger [34], reported that stellate cell contractility increases with progressive liver injury and that endothelin stimulated contraction of stellate cells in cirrhotic liver may contribute to increased intrahepatic resistance and portal pressure. These findings suggest the role of stellate cells in the pathophysiology of portal hypertension.

In the present study nitric oxide shows significant negative correlation with endothelin-l in compensated and decompensated groups of cirrhosis (Table 3 and Fig 1). Luo et al [35], reported correlation of plasma endothelin-l with both pulmonary endothelial nitric oxide synthase levels and alveolar-arterial oxygen gradients in hepatopulmonary syndrome.

In cirrhosis, hyporeactivity of vasoconstrictors such as angiotensin II or endothelin is related to the overproduction of endogenous substances with vasodilator properties such as nitric oxide [10], as well as to vasoconstrictor receptor down regulartion [36] and post receptor signaling defect in vascular smooth muscle cells (VSMCs0 [37,38]

This study suggests that cirrhosis alters VSMCs responsiveness to vasoconstrictor. These findings may be an important factor to hemodynamic changes in cirrhosis. The cause (s) for vasoconstrictors hyporeactivity merit further investigation.

Because stellate cell contraction might be blocked with appropriate antagonists, treatment by these blockers may prove valuable in improving liver function and reducing portal hypertension in patients with chronic liver disease.

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