

## The Relation between Serum Serotonin and Esophageal Varices in Egyptian Patients with Viral Hepatitis Related Cirrhosis : A Case-Control Study

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varices.

**CONCLUSION:** serum serotonin level is significantly correlated to the grade of esophageal varices in patients with viral hepatitis related cirrhosis.

**Key words:** Serotonin; Cirrhosis; Esophageal varices; Viral hepatitis; Portal hypertension

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### ABSTRACT

**AIM:** Serotonin is a one of the monoamine neurotransmitters secreted by serotonergic nerve endings in multiple sites in the brain and gastrointestinal tract wall. Serotonin can induce contraction in the smooth muscles of the gut wall enhancing gut motility and in blood vessels wall causing vasoconstriction. The presence of serotonin receptors on hepatic stellate cells can cause contraction of these cells closing the sinusoidal fenestrae and raising the portal vein pressure. The aim of this work is to study the relation between serotonin level and the presence and the severity of esophageal varices in Egyptian patients with viral hepatitis related cirrhosis.

**PATIENTS AND METHODS:** Two hundred patients were included in the study group I: 120 patients with viral hepatitis related cirrhosis and group II: 90 healthy volunteers. Patients as well as healthy subjects performed all routine investigations, upper GI endoscopy and serum serotonin level.

**RESULTS:** serum serotonin level in group I was significantly higher than group II ( $135 \pm 33.7$  vs  $41 \pm 19.6$ ). serum serotonin level was significantly correlated to albumin, bilirubin, platelet, portal vein diameter, spleen size, esophageal varices grade, grade of portal hypertensive gastropathy and Child's score ( $r = -0.35, 0.45, -0.44, 0.5, 0.39, 0.33, 0.36$  and  $0.41$  in succession). It was also clear that serotonin level rises significantly with higher grades of esophageal

### INTRODUCTION

Portal hypertension means elevated blood pressure inside the portal vein and its branches and tributaries. Portal hypertension definition is the elevation of hepatic venous pressure gradient (HVPG) more than 5 mmHg, it's considered clinically significant when it exceeds 12 mmHg<sup>[1]</sup>.

The presence and development of oesophageal varices is a clinical manifestation of portal hypertension, with a prevalence that range from 40% to 80% in patients with cirrhosis<sup>[2]</sup>. The development of esophageal varices in patients with cirrhosis occurs when the hepatic venous pressure gradient (HVPG) is greater than 10 mmHg<sup>[3]</sup> with an incidence of approximately 5% per year, and a yearly rate of progression to larger varices of 5% to 15%. The clinical relevance of esophageal varices is linked to the risk of bleeding that occurs when HVPG is greater than 12 mmHg<sup>[4]</sup>.

Serotonin (5-hydroxy tryptamine 5HT) is a one of the monoamine neurotransmitters secreted by serotonergic nerve endings in multiple sites in the brain as well as gastrointestinal tract wall. Serotonin acts as a hormone in many tissues where it mediated vari-

ous functions such as modulation of proliferation and differentiation of muscles, neurons and mammary glands<sup>[5,6]</sup>. Serotonin mediates its action by binding to 5HT receptors on the target cell membrane. The reaction between serotonin and its receptor leads to increased level of intracellular calcium by release of calcium from endoplasmic reticulum. These changes mediate series of calcium dependent intracellular reaction. Through this mechanism serotonin can induce contraction in the smooth muscles of the gut wall enhancing gut motility and in blood vessels wall causing vasoconstriction. Excess serotonin in the serum is metabolized to 5 hydroxy indol acetic acid before being excreted in urine<sup>[7]</sup>.

Serotonin is released from platelets at the site of injury in the liver to regulate the process of hepatic regeneration and fibrosis<sup>[8,9]</sup>. In the pathogenesis of cirrhosis the hepatic stellate cells (HSC) are transformed into myofibroblasts under the influence of the inflammatory mediators secreted by the damaged liver cells<sup>[10]</sup>. There's also increased number of 5HT receptors on the HSC cell membrane enhancing the cell response to serotonin. This can cause contraction of these cells closing the sinusoidal fenestrae and raising the portal vein pressure<sup>[11]</sup>.

**Aim of the work**

Study the relation between serum serotonin level and severity of portal hypertension and the presence and severity of esophageal varices in Egyptian patients with viral hepatitis related cirrhosis.

**PATIENTS AND METHODS**

This study was conducted in outpatient clinics, endoscopy units and hepato-gastroenterology inpatient wards, in Departments of Internal medicine and Tropical Medicine in collaboration with clinical pathology department, Zagazig University Hospitals, Egypt in the period between august 2014 and august 2015 the study included 120 patients with viral hepatitis related cirrhosis.

**Inclusion criteria**

Patients with liver cirrhosis evidenced by combination of multiple clinical, sonographic and laboratory data due to chronic hepatitis B and/or C virus infection with well compensated to mild to moderately decompensated liver cirrhosis Child grade A and B.

**Exclusion Criteria:** (1) Patients age < 18 and > 60 years old; (2) Patients who didn't give consent to be included in the study; (3) Patients with cirrhosis due to another cause rather than chronic viral hepatitis B and/or C e.g. metabolic diseases, autoimmune hepatitis, alcoholism; (4) Patients with severely decompensated liver disease (Child C); (5) Patients with portal vein thrombosis; (6) Patients with intra or extra hepatic malignancy; (7) Patients admitted with acute upper GI bleeding (variceal or other cause); (8) Patients with history of depression and/or selective serotonin reuptake inhibitors use.

**The patients were allocated into two groups: Group I (tests group):** 120 patients with chronic viral hepatitis related cirrhosis; **Group II (control group):** 90 healthy volunteers.

**All patients of the study were subjected to the following**

- Full history taking: history of surgery, blood transfusion and significant alcohol intake (> 60 g/day)
  - Thorough clinical examination paying specific attention to the manifestations of liver cirrhosis e.g. palmer erythema, jaundice, ascites, lower limb edema, encephalopathy, splenomegally and abdominal collaterals.
  - The following laboratory investigations:

- Blood samples were drawn from all subjects into vacutainer tubes and sent to the lab and sera were separated immediately.
- Liver function tests: albumin, bilirubin, liver enzymes and Kidney function tests: serum creatinine and blood urea nitrogen were done on Cobas 6000 autoanalyzer Roche diagnostic USA.
- Viral markers: anti-HCV Ab and HBVsAg were done on Cobas e 601 immunoassay Roche diagnostic, USA. Anti-bilharzial antibodies were done using Kits of FUMOUEZ diagnostic, France. The principle based on indirect hemoagglutination. Separated sera were frozen to -70 for serum serotonin blood samples were drawn from all subjects into vacutainer tubes. Sera were separated immediately and stored at -20°C. Routine liver and kidney function tests were done on Cobas 6000 analyzer (Roche diagnostics-Switzerland. Serum serotonin was determined by Abcam's Serotonin in vitro competitive ELISA (Enzyme-Linked Immunosorbent Assay) kit.
- Complete blood count and coagulation profile: prothrombin time and INR were done by sysmex XS (cell counter) and CA1500 respectively.
- The patients liver functions were evaluated according to the (Child-pugh classification) as follows table 1<sup>[12]</sup>.

Table 1 Child-Pugh classification<sup>[12]</sup>.

	1	2	3
<b>Bilirubin Total</b>	<2 mg/dL	2-3 mg/dL	>3 mg/dL
<b>Serum albumin</b>	>3.5 g/dL	2.8-3.5 g/dL	<2.8 g/dL
<b>INR</b>	<1.7	1.71-2.20	> 2.20
<b>Ascites</b>	None	Suppressed with medication	Refractory
<b>Hepatic encephalopathy</b>	None	Grade I-II (or suppressed with medication)	Grade III-IV (or refractory)

  

Points	Class
5-6	A
7-9	B
10-15	C

- **Pelvi-abdominal ultrasonography:** to confirm presence of cirrhosis periportal thickening, exclude focal lesions to rule out patients with primary and secondary liver tumors from the study. Determine spleen size and to detect portal vein diameter and ascites.
- **Upper GI endoscopy:** was performed to patients in group I (test group) in the left lateral position using midazolam i.v. patients were assessed as regards the grade of esophageal varices (EV), the presence or absence of gastric varices (GV) and the grade of portal hypertensive gastropathy (PHG).

**The grade of EV was assessed according to Paquet system:<sup>[13]</sup>**

- Grade 0: No Varices.
- Grade I: Varices disappearing with insufflations.
- Grade II: Varices is larger, clearly visible, usually straight, not disappearing with insufflations.
- Grade III: Varices is more prominent, locally coil-shaped, partly occupying the lumen.
- Grade IV: Varices is tortuous, or grape-like shape, occupying the lumen.

**Grading of PHG:<sup>[14]</sup>**

- Grade 0: No PHG.
- Grade I: Mild reddening, Congestive mucosa.
- Grade II: Severe redness and a fine reticular pattern separating the areas of raised edematous mucosa.
- Grade III: Grade II + Point bleeding.

**Statistical analysis**

Data were analyzed using SPSS (Statistical Package for the Social Sciences) version 15 for data processing and statistics. Numbers and percentages were used for qualitative data while mean ± standard deviation (SD) was used for quantitative ones. Chi square test  $\chi^2$ , ANOVA and *t*-test were used when appropriate. Spearman's rank correlation test is used to evaluate the correlation. *P* value <0.05 was considered significant.

**Ethics**

The ethical aspects in this study is revised and approved by the institutional review board in Faculty of Medicine, Zagazig University.

**RESULTS**

There were no significant differences between the test and the control groups as regards age, gender distribution as well as rate of positive antibilharzial antibody as shown in table 2. The comparison between the test group and control group as regards all the laboratory data listed in table 3 show significant differences between the two groups except in creatinine level. There's also highly significant difference between the two groups as regards serum serotonin level. Table 3 show also that there are highly significant differences between the studied groups as regards spleen size and portal vein diameter.

Table 4 shows that 66.6 % of patients in group I had child grade A well compensated liver disease and 33.3% of them had child grade B moderately decompensated liver disease. Table 4 also shows the incidence of different grades of EV's, GV's and PHG among patients in the test group.

Table 5 shows that there is highly significant correlation between serotonin level and platelet count, spleen size, portal vv diameter, Child's grade, grade of EV's and PHG as well as incidence of GV's. Table 6 confirms this correlation between serotonin level and grade of EV's by showing that the mean serum serotonin level rise significantly with the increase grade of EV's.

**DISCUSSION**

Portal hypertension is a serious condition with various severe life threatening consequences most important of which is esophageal varices development and bleeding. This study aims at exploring the correlation between serum serotonin level as a vasoactive amine and the severity and the grade of esophageal varices and portal hypertensive gastropathy. The study included two groups of patients. The test group which included patients with post viral hepatitis cirrhosis compared to the control group of healthy subjects. The comparison between both groups as regards serum serotonin level revealed a significantly higher serotonin level in the sera of patients in the test group this finding agrees with Baudry *et al*, 1994 and Culafic *et al*, 2007 who found that the active unconjugated form of 5-hydroxytryptamine (serotonin), were significantly higher in patients with cirrhosis than in controls<sup>[15,16]</sup>.

In our study there was a significant negative correlation between serotonin level and albumin as well as a significant positive correlation with bilirubin and hence a significant positive correlation to the Child's score indicating that the serum serotonin level is correlated to the severity of liver function decompensation in patients with cirrhosis. This also agrees with Baudry *et al*, 1994 who found that free unconjugated form of 5-hydroxytryptamine is significantly higher in patients with Child's grade C. However, that study claims also that the total free and conjugated serotonin level was not significantly correlated to the severity of liver disease

**Table 2** Demographic data.

	Group I No=120	Group II No=90	Test value	P	Signif.
Age (years) mean±SD	52.8±6.3	54.05±4.9	<i>t</i> = 1.56	0.12	NS
Gender	Males	91(75.8%)	72(80%)	$\chi^2$ = 0.51	0.47 NS
	Females	29(24.2%)	18(20%)		
Viral hepatitis	HCV	92(76.6%)			
	HBV	13(10.8%)			
	Both	15(12.5%)			
Anti-bilharzial Ab positive	78(65%)	56(62.2%)	0.17	0.67	NS

Signif.: significance; NS: non-significant.

**Table 3** Comparison between the studied groups as regards routine laboratory parameters, serum serotonin level, sonographic data.

	Group I No=120	Group II No=90	<i>t</i>	P	Signif.
Albumin (g/dl) mean ± SD	2.3±0.4	4.5±0.5	13.23	<0.001	HS
Bilirubin (mg/dl) mean ± SD	4.5±1.2	1.3±0.4	22.6	<0.001	HS
WBC's count (cell × 10 <sup>3</sup> /mL) mean ± SD	4.7±1.8	5.8±3.1	2.61	0.009	S
HB (g/dL) mean ± SD	11.5±3.5	13±4.2	2.45	0.015	S
Platelet count (cell × 10 <sup>3</sup> /dL) mean ± SD	78±18	207±22	40.59	<0.001	HS
Serum creatinine (mg/dL) mean ± SD	1.2±0.5	1.1±0.4	1.39	0.16	NS
PT (sec) mean±SD	16±1.5	11±1.3	22.5	<0.001	HS
Serum serotonin (ng/mL) mean ± SD	135±33.7	41±19.6	21.56	<0.001	HS
Spleen size (long axis) (cm) mean ± SD	22±5.3	11±1.5	17.86	<0.001	HS
Portal vv diameter (mm) mean ± SD	17.1±4.2	8.2±2.9	15.59	<0.001	HS

Signif.: significance; NS; non-significant; S: significant; HS: highly significant.

**Table 4** Child score and grade, endoscopic data in group I.

	Group I (No=120)	%
Child's score (points) mean±SD	7±1.4	
Child's grade	A	80 (66.76%)
	B	40 (33.3%)
	0	14 (11.66%)
EV's grade	I	18 (15%)
	II	36 (30%)
	III	24 (20%)
	IV	28 (23.3%)
	GV's	28 (23.3%)
PHG	0	63 (52.5%)
	I	31 (25.8%)
	II	8 (6.7%)
	III	18 (15%)

EV's: esophageal varices; GV's: gastric varices; PHG: portal hypertensive gastropathy.

decompensation<sup>[15]</sup>. This also is agreed with by Culafic *et al*, 2007 who found that serotonin level is correlated to the disease severity in liver cirrhosis and said also that serotonin level in serum is more indicative of liver disease severity than platelet serotonin level<sup>[16]</sup>.

In our study the serum serotonin level was negatively correlated to platelet count, and positively correlated to portal vein diameter, spleen size, grade of EV as well as grade of PHG. This finding means that seotonin level is strongly correlated to the severity of portal hypertension. This comes in agreement with Vorobioff *et al*, 1989 who indirectly investigated this correlation by using 5-HT receptor antagonists (ketanserine) at a mean dose of 51 mg/day over mean of 32 days and noted significant decrease of hepatic venous pressure gradient with the use of serotonin antagonists<sup>[17]</sup>. The correlation

between serum serotonin level and the severity of portal hypertension was confirmed in our study by finding that the serum serotonin level is significantly higher in patients with high grades of EV's and patients with gastric varices.

## CONCLUSION

We conclude from this study that serum serotonin level is higher in patients with cirrhosis than in healthy controls and that the serum level of serotonin is significantly correlated to the severity of decompensation in liver functions. It's also clear that serum serotonin level is correlated to the grade of EV's.

## CONFLICT OF INTEREST

All authors declared no potential conflicts of interest.

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**Table 5** Correlation of serotonin level to all the demographic, laboratory and sonographic parameters in group I (test ggroup).

	r	P	Signif.
Age (years)	0.15	>0.05	NS
Albumin (g/dL)	-0.35	<0.05	S
Bilirubin (mg/dL)	0.45	<0.001	HS
WBC's count (cell/mL)	0.2	>0.05	NS
HB (g/dL)	-0.19	>0.05	NS
Platelet count (cell/dL)	-0.44	<0.001	HS
Serum creatinine (mg/dL)	0.13	>0.05	NS
PT (sec)	0.11	>0.05	NS
Spleen size (long axis) (cm)	0.39	<0.05	S
Portal vv diameter (mm)	0.5	<0.001	HS
Child's score	0.41	<0.001	HS
EV's grade	0.33	<0.05	S
GV's	0.45	<0.001	HS
PHG grade	0.36	<0.05	S

NS; non-significant; S: significant;HS: highly significant.

**Table 6** Comparison between patients with different grades of EV as regards their serum serotonin level.

	Serum serotonin level (ng/mL) mean±SD	F	P	Signif.
Gastric varices	192.25±17.54			
EV grade 0	102.43±10.30			
EV grade I	117.61±18.35	120.09	<0.001	HS
EV grade II	112.16±12.83			
EV grade III	115.25±9.58			
EV grade IV	121.22±19.23			

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