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ORIGINAL ARTICLE

# Serotonin: Is it a marker for the diagnosis of hepatocellular carcinoma in cirrhotic patients?

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## KEYWORDS

Serotonin;  
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**Abstract** Hepatocellular carcinoma (HCC) is the third most frequent cause of cancer mortality among men worldwide. Serotonin is a biogenic amine, ligand of a family of 5-HT receptors that reflect the diversity of serotonergic actions. Majority of serotonin in body (90%) is synthesized by enterochromaffin cells of the gastrointestinal tract and is exported to various sites. Serotonin regulates blood flow and vascular tone at portal and sinusoidal levels, serotonin acts as a mitogen for hepatocytes and promotes liver regeneration. 5HT emerges as a mediator of different pathological conditions (double edged sword). It contributes to liver fibrosis, mediates oxidative stress in nonalcoholic steatotic hepatitis and aggravates viral hepatitis, these conditions are involved in tumorigenesis of hepatocellular carcinoma (HCC). Impaired metabolic function in liver cirrhosis and slow uptake and storage of serotonin by the platelets is a sequelae of kinetic change of serotonin transport mechanisms or abnormal serotonin release from dense granules of activated platelets is a condition defined as “platelet exhaustion”, contributes to elevated plasma serotonin which may facilitate tumour growth of primary liver hepatocellular carcinoma.

*Aim of this work:* To determine whether serotonin is a marker for the diagnosis of hepatocellular carcinoma in cirrhotic patients.

*Methods:* Patients were classified into two groups; 45 patients with cirrhosis only and 30 patients with cirrhosis and HCC. Ten healthy subjects were taken as controls. Patients underwent; full history taking, clinical examination, and abdominal ultrasonography. Laboratory methods include SGOT, SGPT, GGT, bilirubin, alkaline phosphatase, total proteins, albumin, CBC, prothrombin, INR, APRI score, Child-pugh score, MELD score, AFP and serum serotonin.

*Results:* Plasma serotonin was significantly higher in the patients group with cirrhosis with a median level of 119.4 ng/ml than in the control group which showed a median value of 51.5 ng/ml  $p < 0.001$ .

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A significance difference was also seen between cirrhosis and the HCC group with a median value of 478.35 ng/ml than the control group and a cirrhosis group with  $p < 0.001$  was found.

**Conclusion:** Plasma serotonin level was significantly higher in patients with cirrhosis and HCC than in those with cirrhosis only and it was involved in the tumourigenesis of hepatocellular carcinoma.

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## 1. Introduction

Each year, hepatocellular carcinoma (HCC) is diagnosed in more than half a million people worldwide, including approximately 20,000 new cases in the United States.<sup>1</sup> Liver cancer is the fifth most common cancer in men and the seventh in women. Most of the burden of disease (85%) is borne in developing countries.<sup>2</sup> Incidence of HCC in Egypt is currently increasing, which may be the result of a shift in the relative importance of HBV and HCV as primary risk factors in addition to exposure to aflatoxin as an additional risk factor.<sup>3–5</sup> HCC is the second most frequent cause of cancer incidence and mortality among men in Egypt.<sup>6</sup> Egypt has the highest prevalence of HCV in the world, with estimates ranging from 6% to 28% and a reported average of ~13.8%, also investigations in Egypt have also shown the increasing importance of HCV infection in the aetiology of HCC, accounting for 40–50% of cases.<sup>3,5,7,8</sup>

HCV mostly plays an indirect role in tumour development and appears to increase the risk of HCC by promoting fibrosis and cirrhosis.<sup>5,9</sup> On the other hand, HCV may play a direct role in hepatic carcinogenesis through the involvement of viral gene products in inducing liver cell proliferation. However, it seems that cirrhosis is the common pathway by which several risk factors exert their carcinogenic effects.<sup>9,10</sup>

The diagnosis of HCC is made by liver imaging tests such as abdominal ultrasound, helical CT scan or triple phase CT scan in combination with the measurement of serum markers such as alpha-fetoprotein (AFP) which has been used as a serum marker for HCC for many years but this test had a sensitivity of 39–65%, a specificity of 76–94% in the presence of HCC.<sup>11,12</sup>

Unfortunately, up to 42% of patients with HCC present with serum AFP levels within normal values and also the fibrolamellar type of HCC do not secrete AFP.<sup>13,14</sup> On the other hand, the AFP could also be elevated in pregnancy, other tumours of gonadal origin even in acute or chronic viral hepatitis and liver cirrhosis.<sup>11,15,16</sup>

Serotonin is known as 5-hydroxytryptamine (5-HT), a biogenic amine that functions as a ligand for a large family of 5-HT receptors.<sup>17</sup> The majority of serotonin in the body (90%) is synthesised by enterochromaffin cells of the gastrointestinal (GI) tract, where it regulates intestinal motility.<sup>18</sup>

It plays a major role in neurotransmission within the central nervous system (CNS) and the autonomic nervous system (ANS). In the CNS serotonin is known to control mood, behaviour, learning, sleep and anxiety. Peripherally, serotonin is able to mediate vascular contraction and relaxation, cell proliferation, apoptosis and platelet aggregation.<sup>19</sup>

Serotonin is actively taken up by cells expressing the Na<sup>+</sup>/Cl<sup>-</sup> dependent serotonin transporter (SERT) where it is stored in intracellular vesicles and released in response to

various stimuli. Once bound to target receptors or taken up by the SERT, internalised serotonin can be metabolised by monoamine oxidase (MAO) leading to the generation of 5-hydroxyindoleacetaldehyde (5-HIAA) which is excreted in urine.<sup>19,20</sup>

Platelets are responsible for picking up serotonin from the gut and lungs and provide the main peripheral storehouse of serotonin, platelets release serotonin in sites of injury where it contributes to platelet recruitment and thrombus propagation.<sup>21,22</sup>

The family of receptors that bind serotonin is subdivided into seven subgroups and where appropriate these subgroups are again divided reflecting the diversity of serotonergic actions. All members of the serotonin receptor family are linked to G-proteins, except the 5-HT<sub>3</sub> receptor which is a ligand gated Na<sup>+</sup>/K<sup>+</sup> channel.<sup>19,23</sup>

With respect to the liver, it was found that serotonin has the ability to regulate hepatic blood flow at both the portal and sinusoidal levels.<sup>19</sup>

Intraportal injections of serotonin were found to significantly increase portal pressure, events that were antagonised by the serotonin antagonist (ketanserin) in portal hypertensive rats, this suggests that serotonergic mechanisms may contribute to maintaining portal hypertension in patients with cirrhosis. Serotonin may play a role in hepatic regeneration following partial hepatectomy in rodents.<sup>24–27</sup> One resident of the hepatic sinusoid that is postulated to regulate blood flow is the hepatic stellate cell (HSC). The HSC is known to undergo an activation process acquiring a smooth muscle cell-like phenotype with enhanced contractile capabilities in response to liver injury and are instructed by serotonin to make more scar tissues and switch off the healthy regeneration, has also recently been demonstrated to express functional 5-HT<sub>2A</sub> and 5-HT<sub>2B</sub> receptors and therefore it is possible that HSC is able to regulate sinusoidal blood flow. Sinusoidal endothelial cells (SECs) are also known to respond to serotonin inducing contraction of the fenestrae.<sup>28–30</sup>

Patients with advanced liver disease often present with variceal haemorrhage and a more generalised “bleeding tendency”. These additional symptoms linked to hepatic cirrhosis are thought in part to be due to impaired platelet aggregation. Under normal conditions platelets operating as buffers, maintain low levels of free circulating serotonin, are activated in response to a variety of different stimuli, releasing various aggregating factors including serotonin, and following this they become exhausted.

Patients with cirrhosis are known to have platelet storage pool defects, significantly lower intraplatelet serotonin concentrations when compared to healthy individuals. It is therefore tempting to propose that the reduced platelet serotonin storage ability was in part responsible for the haemorrhagic tendency of cirrhotic patients.<sup>19,31,32</sup>

The liver and platelets display a very intimate, complex interconnection. The liver plays a critical role even during the synthesis of platelets from megakaryocytes through thrombopoietin (TPO) which was the most important growth factor in the regulation of megakaryocyte development and platelet production, is produced mainly in the liver and kidney.

Hence platelets are not expected to function properly in diseased liver. Platelets harbour important growth factors for liver regeneration, e.g. Hepatocyte growth factor (HGF). Contrariwise, platelets contain Transforming growth factor  $\alpha$  (TGF- $\alpha$ ), which is required for the termination of liver regeneration. Thus, it is plausible that platelets may participate in orchestrating liver regeneration through the stimulation and inhibition of growth-related signals. The ability of serotonin to modulate all these factors renders it crucial in times of hepatic injury and repair.<sup>33</sup>

Platelet derived serotonin has been shown to be beneficial in terms of stimulating hepatocyte proliferation following hepatic ischaemia in mice.<sup>34</sup> In addition over proliferation of hepatocytes can lead to HCC and this would raise the possibility that serotonin may play a role in HCC pathogenesis.<sup>19</sup>

Serotonin is emerging as a mediator of different pathological conditions. It contributes to liver fibrosis, mediates oxidative stress in nonalcoholic steatotic hepatitis, and aggravates viral hepatitis promoting the progression of steatohepatitis by oxidative stress.<sup>30,35,36</sup> It promotes tumour growth in a mouse model of subcutaneous colon cancer allografts. 5HT deficiency led to decreased vascularity and increased necrosis reflecting cell death of the tumour.<sup>37</sup>

High levels of plasma serotonin in the liver cirrhosis could be due to slow uptake and storage of serotonin by the platelets as could be the sequelae of the kinetic change of serotonin transport mechanisms or abnormal serotonin release from dense granules of activated platelets. Concentration of circulating serotonin in liver cirrhosis can be influenced by other factors, such as altered serotonin catabolism due to an elevated activity of monoamine oxidase and impaired metabolism of tryptophan, as a serotonin precursor. Impaired metabolic function in liver cirrhosis contributes to elevated plasma serotonin.<sup>38</sup>

## 2. Aim

The aim of this work was to evaluate the role of serotonin in the diagnosis of hepatocellular carcinoma in cirrhotic patients.

## 3. Methods

The studied subjects were recruited from the hepatology department in medical research institute, Alexandria university. Written informed consent was obtained from all participants before starting the study.

The patients were subdivided into two groups:

- 1- Forty-five cirrhotic patients (Group I).
- 2- Thirty cirrhotic patients with hepatocellular carcinoma (HCC) (Group II).
- 3- Ten healthy persons were considered as the control group.

All subjects were subjected to full history taking, clinical examination with measurement of mean blood pressure and calculation of body mass index (BMI) using the formula: weight in kg/height in meter<sup>2</sup>.<sup>39</sup> In addition to an abdominal ultrasonography and computed tomography.

Patients are matched for age, gender and body mass index. All had clinically evident portal hypertension, none of them had episodes of bacterial peritonitis and were free from other neoplastic diseases.

A routine biochemical evaluation was performed as follows:

- (a) Liver function tests including serum aspartate and alanine aminotransferase. (AST + ALT), gamma glutamyl transpeptidase (GGT), alkaline phosphatase (ALP), total proteins, albumin, bilirubin (Total and direct).<sup>40</sup>
- (b) Complete blood picture, prothrombin activity and INR.<sup>41</sup>
- (c) Calculation of aspartate aminotransferase-to-platelet ratio index (APRI) score using the formula: (AST/upper limit of normal  $\times$  100)/platelet count. The reference value of AST was considered to be 45 IU, which is the upper normal limit in our laboratory.<sup>42</sup>
- (d) Hepatitis virus markers: Hepatitis B surface antigen done by the Eliza technique.<sup>43</sup> Hepatitis C virus antibodies done by the Eliza technique.<sup>44</sup>
- (e) Child-Pugh<sup>45</sup> and model of end stage liver disease (MELD) scores were evaluated.<sup>46</sup>
- (f) Determination of serum AFP<sup>47</sup> and serotonin<sup>48</sup> by the Eliza technique.

### 3.1. Statistical analysis

Data were fed to the computer using the predictive Analytics Software (PASW Statistics 18).

Qualitative data were described using number and percent. Association between categorical variables was tested using Chi-square test. When more than 20% of the cells have an expected count less than 5, correction for Chi-square was conducted using the Fisher's exact test or the Monte Carlo correction.

Quantitative data were described using median, minimum and maximum as well as mean and standard deviation.

The distributions of quantitative variables were tested for normality using the Kolmogorov–Smirnov test and the Shapiro–Wilk test. D'Agostino test was used if there was a conflict between the two previous tests. If it reveals normal data distribution, parametric tests were applied. If the data were abnormally distributed, non-parametric tests were used.

For normally distributed data, comparison between two independent populations were done using the independent *t*-test while when more than two populations were analysed the *F*-test (ANOVA) was to be used and the post Hoc test (LSD). Correlations between two quantitative variables were assessed using the Pearson's coefficient.

For abnormally distributed data, Mann–Whitney Test (for data distribution that was significantly deviated from normal) were used to analyse two independent population. If more than two population were analysed Kruskal Wallis test to be used. Correlations between two quantitative variables were assessed using Spearman coefficient.

Agreement of the different predictives with the outcome was used and was expressed in sensitivity, specificity, positive predictive value, negative predictive value and accuracy. Receiver operating characteristic curve (ROC) was plotted to analyse a recommended cutoff, the area under the ROC curve denotes the diagnostic performance of the test. Area of more than 50% gives an acceptable performance and an area of about 100% is the best performance for the test.

Significance test results were quoted as two-tailed probabilities. Significance of the obtained results was judged at the 5% level.

#### 4. Results

Patients enrolled in this study were subdivided into two groups:

Group I included 33(73.3%) cirrhotic males and 12 (26.7%) cirrhotic females their mean age was  $53.98 \pm 9.07$  years.

Group II included 17(56.7%) males and 13(43.3%) females with liver cirrhosis and HCC, their mean age was  $55.60 \pm 9.95$  years.

**Table 1** Clinical data of patient groups.

|                               | Group I (n = 45)  | Group II (n = 30) | Test of sig.      |
|-------------------------------|-------------------|-------------------|-------------------|
| <i>Sex</i>                    |                   |                   |                   |
| Male                          | 33(73.3%)         | 17(56.7%)         | <i>p</i> = 0.134  |
| Female                        | 12(26.7%)         | 13(43.3%)         |                   |
| <i>Age (years)</i>            |                   |                   |                   |
| Min–Max                       | 38.0–75.0         | 34.0–74.0         | <i>p</i> = 0.466  |
| Mean $\pm$ SD                 | 53.98 $\pm$ 9.07  | 55.60 $\pm$ 9.95  |                   |
| Median                        | 52.0              | 54.0              |                   |
| <i>BMI (kg/m<sup>2</sup>)</i> |                   |                   |                   |
| Min–Max                       | 16.40–37.70       | 17.10–41.60       | <i>p</i> = 0.200  |
| Mean $\pm$ SD                 | 26.56 $\pm$ 3.75  | 27.90 $\pm$ 5.17  |                   |
| Median                        | 26.50             | 27.65             |                   |
| <i>MBp (mmHg)</i>             |                   |                   |                   |
| Min–Max                       | 70.0–120.0        | 70.0–120.0        | <i>p</i> = 0.663  |
| Mean $\pm$ SD                 | 87.57 $\pm$ 10.87 | 89.70 $\pm$ 12.79 |                   |
| Median                        | 83.30             | 83.30             |                   |
| <i>Child class</i>            |                   |                   |                   |
| A                             | 8 (17.8%)         | 1 (3.3%)          | <i>p</i> = 0.011* |
| B                             | 27 (60.0%)        | 13 (43.3%)        |                   |
| C                             | 10 (22.2%)        | 16 (53.3%)        |                   |
| <i>Child class score</i>      |                   |                   |                   |
| Min–Max                       | 5.0–13.0          | 6.0–14.0          | <i>p</i> = 0.015* |
| Mean $\pm$ SD                 | 8.58 $\pm$ 1.90   | 9.63 $\pm$ 1.83   |                   |
| Median                        | 9.0               | 10.0              |                   |
| <i>MELD score</i>             |                   |                   |                   |
| Min–Max                       | 7.0–27.0          | 7.0–25.0          | <i>p</i> = 0.235  |
| Mean $\pm$ SD                 | 13.60 $\pm$ 4.91  | 14.47 $\pm$ 4.19  |                   |
| Median                        | 13.0              | 14.50             |                   |

Group I = patients with liver cirrhosis.

Group II = patients with liver cirrhosis and hepatocellular carcinoma.

BMI = body mass index.

MBp = mean blood pressure.

MELD = model of end stage liver disease.

The healthy control group included 6(60%) males and 4(40%) females with matched age, gender and body mass index (BMI), their mean age was  $48.40 \pm 9.17$  years.

Group I, 25 patients (55.6%) were bleeders; 32 patients (71.1%) had ascites, 16 patients (35.6%) presented with hepatic encephalopathy.

Group II, 13 patients (43.3%) were bleeders; 25 (83.3%) had ascites and 10 patients (33.3%) presented with hepatic encephalopathy with no significant difference between both groups.

Five patients (11.1%) of group I were hypertensive while 7 patients (23.3%) of group II were hypertensive and 4 patients

**Table 2** Laboratory investigations in both patient groups.

|                           | Group I (n = 45)   | Group II (n = 30)  | Test of sig.      |
|---------------------------|--------------------|--------------------|-------------------|
| <i>Creatinine (mg/dl)</i> |                    |                    |                   |
| Min–Max                   | 0.50–2.80          | 0.60–2.20          | <i>p</i> = 0.229  |
| Mean $\pm$ SD             | 1.04 $\pm$ 0.42    | 1.10 $\pm$ 0.36    |                   |
| Median                    | 0.90               | 1.10               |                   |
| <i>AST</i>                |                    |                    |                   |
| Min–Max                   | 10.0–186.0         | 23.0–309.0         | <i>p</i> = 0.001* |
| Mean $\pm$ SD             | 53.40 $\pm$ 42.56  | 88.93 $\pm$ 64.90  |                   |
| Median                    | 34.0               | 78.50              |                   |
| <i>ALT</i>                |                    |                    |                   |
| Min–Max                   | 7.0–97.0           | 6.0–140.0          | <i>p</i> = 0.001* |
| Mean $\pm$ SD             | 27.36 $\pm$ 20.21  | 44.37 $\pm$ 30.88  |                   |
| Median                    | 20.0               | 33.50              |                   |
| <i>Total Bilirubin</i>    |                    |                    |                   |
| Min–Max                   | 0.40–14.0          | 0.50–25.0          | <i>p</i> = 0.058  |
| Mean $\pm$ SD             | 2.56 $\pm$ 3.04    | 4.22 $\pm$ 5.16    |                   |
| Median                    | 1.60               | 2.0                |                   |
| <i>Direct Bilirubin</i>   |                    |                    |                   |
| Min–Max                   | 0.10–10.50         | 0.30–16.30         | <i>p</i> = 0.026* |
| Mean $\pm$ SD             | 1.40 $\pm$ 2.15    | 2.63 $\pm$ 3.54    |                   |
| Median                    | 0.70               | 1.05               |                   |
| <i>Total proteins</i>     |                    |                    |                   |
| Min–Max                   | 4.40–8.60          | 5.40–8.80          | <i>p</i> = 0.021* |
| Mean $\pm$ SD             | 6.73 $\pm$ 0.88    | 7.24 $\pm$ 0.96    |                   |
| Median                    | 6.90               | 7.15               |                   |
| <i>Albumin</i>            |                    |                    |                   |
| Min–Max                   | 1.60–4.50          | 1.50–3.50          | <i>p</i> = 0.392  |
| Mean $\pm$ SD             | 2.61 $\pm$ 0.64    | 2.42 $\pm$ 0.42    |                   |
| Median                    | 2.50               | 2.45               |                   |
| <i>GGT</i>                |                    |                    |                   |
| Min–Max                   | 6.30–208.0         | 14.0–479.0         | <i>p</i> = 0.043* |
| Mean $\pm$ SD             | 43.69 $\pm$ 37.44  | 76.77 $\pm$ 90.83  |                   |
| Median                    | 33.0               | 37.0               |                   |
| <i>ALP</i>                |                    |                    |                   |
| Min–Max                   | 47.0–522.0         | 52.0–333.0         | <i>p</i> = 0.041* |
| Mean $\pm$ SD             | 110.91 $\pm$ 81.84 | 145.30 $\pm$ 82.21 |                   |
| Median                    | 87.0               | 128.0              |                   |

Group I = patients with liver cirrhosis.

Group II = patients with liver cirrhosis and hepatocellular carcinoma.

ALT = alanine aminotransferase.

AST = aspartate aminotransferase.

GGT = gamma glutamyl transpeptidase.

ALP = alkaline phosphatase.

(8.9%) of group I had IHD while 5 patients (16.7%) of group II had IHD.

In group I, 8 patients (17.8%) were of child class A, 27 patients (60.0%) were of child class B and 10 patients (22.2%) were of child class C. While in group II, 1 patient (3.3%) was of child class A, 13 patients (43.3%) were of child class B and 16 patients (53.3%) were of child class C with significant difference between both groups ( $p = 0.011^*$ ) (Table 1).

**Table 3** Haematological investigations in both patient groups.

|                                   | Group I (n = 45) | Group II (n = 30) | Test of sig.  |
|-----------------------------------|------------------|-------------------|---------------|
| <i>HB (g/dl)</i>                  |                  |                   |               |
| Min–Max                           | 5.80–13.20       | 6.90–14.50        | $p = 0.685$   |
| Mean ± SD                         | 10.07 ± 1.79     | 9.89 ± 1.91       |               |
| Median                            | 9.80             | 9.55              |               |
| <i>WBCs × 10<sup>3</sup></i>      |                  |                   |               |
| Min–Max                           | 1.16–15.80       | 1.90–13.27        | $p = 0.516$   |
| Mean ± SD                         | 5.51 ± 2.92      | 5.29 ± 2.90       |               |
| Median                            | 4.76             | 4.11              |               |
| <i>Platelets × 10<sup>3</sup></i> |                  |                   |               |
| Min–Max                           | 35.0–255.0       | 35.0–209.0        | $p = 0.036^*$ |
| Mean ± SD                         | 130.80 ± 60.1    | 100.07 ± 47.08    |               |
| Median                            | 131.0            | 92.0              |               |
| <i>APRI score</i>                 |                  |                   |               |
| Min–Max                           | 0.17–2.83        | 0.32–5.29         | $p < 0.001^*$ |
| Mean ± SD                         | 1.08 ± 0.77      | 2.29 ± 1.48       |               |
| Median                            | 0.82             | 2.07              |               |
| <i>Proth activity</i>             |                  |                   |               |
| Min–Max                           | 26.10–92.30      | 23.30–91.30       | $p = 0.665$   |
| Mean ± SD                         | 52.17 ± 15.05    | 53.51 ± 16.30     |               |
| Median                            | 49.10            | 50.60             |               |
| <i>INR</i>                        |                  |                   |               |
| Min–Max                           | 1.04–2.50        | 1.06–3.20         | $p = 0.664$   |
| Mean ± SD                         | 1.62 ± 0.38      | 1.62 ± 0.46       |               |
| Median                            | 1.60             | 1.54              |               |

Group I = patients with liver cirrhosis.  
 Group II = patients with liver cirrhosis and hepatocellular carcinoma.  
 Hb = haemoglobin level.  
 NR = international normalised ratio.  
 APRI = aspartate aminotransferase-to-platelet ratio index.  
 \* Statistically significant at  $p \leq 0.05$ .

The mean value of child class score in group I was  $8.58 \pm 1.90$  while in group II, it was  $9.63 \pm 1.83$  with significant difference between the two groups ( $p = 0.015^*$ ) (Table 1).

The mean value of MELD score in group I was  $13.60 \pm 4.91$  while in group II it was  $14.47 \pm 4.19$  with no significant difference between both groups ( $p = 0.235$ ) (Table 1).

AST, ALT, direct bilirubin and total proteins levels were significantly higher in group II than group I ( $p = 0.001^*$ ,  $0.001^*$ ,  $0.026^*$  and  $0.021^*$ , respectively), while serum albumin level showed no significant difference between the two groups. Also GGT and ALP were significantly higher in group II than group I ( $p = 0.043^*$  and  $0.041^*$ , respectively) (Table 2).

No significant difference between both groups as regards HB level, WBC count, prothrombin activity and INR was found (Table 3).

Platelets were significantly lower in group II than group I ( $p = 0.036^*$ ).

APRI score was significantly higher in group II than group I with a  $p$  value  $< 0.001^*$  (Table 3).

AFP serum concentration was significantly higher in group II showing a median value of [462.0(22.7–10271)] ng/ml than in group I which showed median value of [10.30 (1.58–63.90)] ng/ml with  $p3 < 0.001^*$  (Table 4).

Also the serum concentration of AFP in both groups was significantly higher than the control group which showed a median value of [5.10 (2–10)] ng/ml with [ $p1 < 0.001^*$  and  $p2 < 0.001^*$ ] (Table 4).

The serum concentration of serotonin in group II was [478.35 (266.60–1577.40)] ng/ml which was significantly higher than in group I which showed a median value of [119.40 (44.90–337.40)] ng/ml with  $p3 < 0.001^*$ , also serotonin serum concentration in both groups was significantly higher than the control group which showed a median value of [51.50 (42.50–75)] ng/ml, [ $p1 < 0.001^*$  and  $p2 < 0.001^*$ ] (Table 4).

Serotonin serum concentration was significantly higher in child class C than child class B and A ( $p = 0.023^*$ ) (Table 5).

There was a significant positive correlation between serum serotonin concentration and AFP in group I and group II ( $r = 0.298$ ,  $p = 0.047^*$ ,  $r = 0.468$ ,  $0.009^*$ , respectively) (Table 6).

Serotonin showed negative significant correlation with APRI score in group II ( $r = -0.363$ ,  $p = 0.049^*$ ), while in group I the negative correlation between serotonin and APRI score was not significant ( $r = -0.064$ ,  $p = 0.675$ ) (Table 7).

**Table 4** AFP and serotonin concentration in control and patient groups.

|                         | Control (n = 10) | Group I (n = 45) | Group II (n = 30) | p value        |
|-------------------------|------------------|------------------|-------------------|----------------|
| <i>AFP (ng/ml)</i>      |                  |                  |                   |                |
| Min–Max                 | 2.0–10.0         | 1.58–63.90       | 22.70–10271.0     | $p1 = 0.022^*$ |
| Mean ± SD               | 5.73 ± 3.03      | 15.89 ± 17.30    | 1406.96 ± 2213.02 | $p2 < 0.001^*$ |
| Median                  | 5.10             | 10.30            | 462.0             | $p3 < 0.001^*$ |
| <i>Serotonin(ng/ml)</i> |                  |                  |                   |                |
| Min–Max                 | 42.50–75.0       | 44.90–337.40     | 266.60–1577.40    | $p1 < 0.001^*$ |
| Mean ± SD               | 55.60 ± 12.36    | 148.22 ± 75.77   | 652.86 ± 343.56   | $p2 < 0.001^*$ |
| Median                  | 51.50            | 119.40           | 478.35            | $p < 0.001^*$  |

Group I = patients with liver cirrhosis.  
 Group II = patients with liver cirrhosis and hepatocellular carcinoma.  
 AFP =  $\alpha$  fetoprotein.

**Table 5** Relation between child class and serotonin in total patients.

|                  | Child class          |                       |                       | <i>p</i> |
|------------------|----------------------|-----------------------|-----------------------|----------|
|                  | A ( <i>n</i> = 9)    | B ( <i>n</i> = 40)    | C ( <i>n</i> = 26)    |          |
| <i>Serotonin</i> |                      |                       |                       |          |
| Median (Min–Max) | 172.10(81.10–311.10) | 196.05(44.90–1317.70) | 365.25(49.10–1577.40) | 0.023*   |
| Mean ± SD        | 167.53 ± 71.99       | 297.09 ± 283.39       | 463.63 ± 393.42       |          |

*p*: *p* value for Kruskal Wallis test.

\* Statistically significant at *p* ≤ 0.05.

**Table 6** Correlation between AFP and serotonin.

|     |                      | Serotonin |         |          |
|-----|----------------------|-----------|---------|----------|
|     |                      | Control   | Group I | Group II |
| AFP | <i>r<sub>s</sub></i> | 0.442     | 0.298*  | 0.468*   |
|     | <i>p</i>             | 0.200     | 0.047   | 0.009    |

Group I = patients with liver cirrhosis.

Group II = patients with liver cirrhosis and hepatocellular carcinoma.

AFP = α fetoprotein.

*r<sub>s</sub>*: Spearman coefficient.

\* Statistically significant at *p* ≤ 0.05.

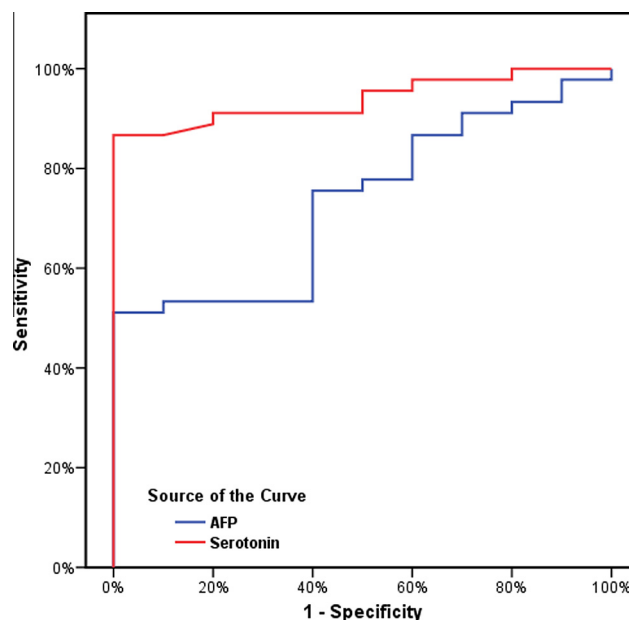
**Table 7** Correlation between serotonin and APRI.

|      |                      | Serotonin |          |
|------|----------------------|-----------|----------|
|      |                      | Group I   | Group II |
| APRI | <i>r<sub>s</sub></i> | 0.064     | −0.363*  |
|      | <i>p</i>             | 0.675     | 0.049    |

Group I = patients with liver cirrhosis.

Group II = patients with liver cirrhosis and hepatocellular carcinoma.

APRI = aspartate aminotransferase-to-platelet ratio index.



**Figure 1** ROC curve for AFP and serotonin in liver Cirrhosis group.

4.1. ROC curve

4.1.1. A; Group I: (Fig. 1)

\*AFP area under ROC curve (AUROC) at cut off (10 ng/ml) was 0.733

*p* = 0.074 showing 51.11% sensitivity 100% ppV, 100% specificity, 31.25% NpV and 60% accuracy.

\*Serotonin AUROC with a cut off value of 75 ng/ml (0.939 *p* = 0.031) showed 86.67% sensitivity and 100% specificity with 62.50% NPV and 89.09% accuracy.

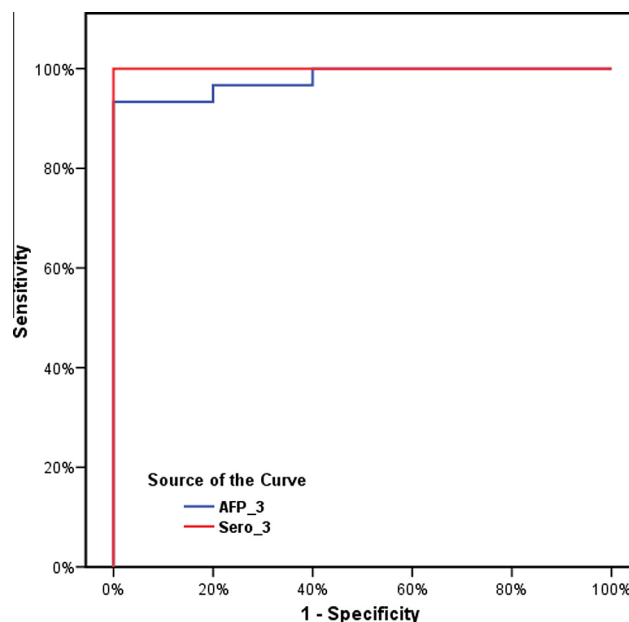
4.1.2. B; Group II: (Fig. 2)

AFP at cut off of 10 ng/ml in the area under ROC curve was 0.980 *p* = 0.018 showing 93.33% sensitivity with 100% PPV and 100% specificity with 83.3% NPV and 95% accuracy .

Serotonin with cut off of 75 ng/ml was 1.000 *p* < 0.001 showing 100% diagnostic performance.

5. Discussion

Hepatocellular carcinoma is the fifth leading cause of cancer and the third leading cause of cancer death.<sup>49</sup> This cancer varies widely in incidence throughout the world, with rising



**Figure 2** ROC curve for AFP and serotonin in liver Cirrhosis and HCC group.

incidence in Egypt.<sup>5,8</sup> In general, almost all HCC cases are preceded by chronic hepatitis or liver cirrhosis which is mainly caused by hepatitis B and hepatitis C.<sup>50</sup> Despite the surveillance programs for high-risk patients, it is still a medical issue that many patients have an unresectable HCC at the time of diagnosis.<sup>51</sup>

Ultrasound (US) screening is superior to AFP assay for the detection of HCC but combined use of AFP monitoring and US is recommended in patients with chronic HCV.<sup>52,53</sup>

Serotonin (5HT), a well-known neurotransmitter within the central nervous system, also regulates a wide range of physiological actions in the gastrointestinal tract.<sup>54</sup> 5HT is a potent mitogen for many different cell types, including hepatocytes.<sup>55</sup> Within the liver, 5HT has the ability to regulate hepatic blood flow at both the portal and sinusoidal levels and it may play a role in hepatic regeneration.<sup>19</sup> Serotonin has been shown to mediate the pathology of many liver diseases, such as steatohepatitis, chronic cholestasis, viral hepatitis and liver cirrhosis.<sup>30,35</sup> All these conditions are involved in the tumourigenesis of HCC.<sup>56</sup>

After the application of serotonin inhibitors, portal pressure is decreased in patients with liver cirrhosis, confirming the importance of serotonin in the pathogenesis of portal hypertension.<sup>57</sup> On the other hand, higher serotonin levels are associated with improved antiviral treatment outcomes in patients with HCV.<sup>58</sup> These findings make serotonin both a friend and foe of the liver.<sup>59</sup>

AFP seems to be of prognostic value at the time of tumour diagnosis. A high concentration in HCC is associated with greater tumour size, bilobar involvement, portal vein invasion, and a lower median survival rate.<sup>60</sup>

Farinati et al.<sup>61</sup> concluded that AFP was not a sensitive marker to detect the presence of HCC. Also, the prognostic value of AFP is limited, but it is correlated with the overall survival in untreated patients.

Recent data suggest that the use of AFP as a diagnostic test is less specific than was once thought. Since it can be elevated in liver cirrhosis and other malignancies, it is recommended that it is no longer be used for the diagnosis of HCC.<sup>62</sup> In our work, serum AFP was significantly higher in patients than healthy subjects ( $p1 = 0.022^*$  and  $p2 < 0.001^*$ ) also it was significantly higher in group II than group I ( $p3 < 0.001^*$ ), although it was higher than normal in some patients with liver cirrhosis without HCC (Table 4).

Baig et al.<sup>63</sup> concluded that AFP was a significant marker for HCC and also an indicator of HCC risks mostly in patients with cirrhosis and HCV/HBV infections. In a study conducted in Chinese patients with chronic hepatitis B, 44 patients were found to have an elevated AFP. Of these, only six had HCC.<sup>64</sup>

In our work, plasma serotonin level was significantly higher in patients than healthy subjects ( $p1 < 0.001^*$  and  $p2 < 0.001^*$ ) also it was significantly higher in group II than group I ( $p3 < 0.000^*$ ) (Table 4).

Serum serotonin concentration was significantly higher in child class C than child class A and B ( $p = 0.023^*$ ) (Table 5).

Culafic et al.<sup>38</sup> found a statistically significant difference between serotonin plasma values in patients with liver cirrhosis and healthy subjects, moreover they found that its level was significantly higher in Child-pugh grade A/B than in grade C patients but platelets serotonin content was not significantly different between Child-pugh grade C and grade A/B and concluded that plasma serotonin is a better marker of liver insufficiency than platelet serotonin content.

In this work, platelets serotonin was not measured and platelets count was low in both groups but was significantly lower in group II than group I ( $p = 0.036^*$ ) (Table 3).

Marasini et al.<sup>65</sup> described a significant reduction of serotonin in platelets of patients with liver cirrhosis, although the level of plasma serotonin was within the normal range. In the study of Beaudry et al.,<sup>66</sup> the whole blood serotonin level was significantly lower in patients with cirrhosis than in age matched controls, and no correlation was found between these levels and the severity of cirrhosis but the unconjugated plasma serotonin level, an indication of the active form of serotonin, was significantly higher in patients with cirrhosis than in the controls.

Platelets are able to attract serotonin from the gut and lung. Serotonin is present in high concentration in platelets, where it accumulates from the plasma via the active transport system SERT. Thus, serotonin participates in the aggregation of platelets and the coagulation of blood.<sup>67</sup> Operating as buffers, platelets maintain low levels of free circulating serotonin. As the carrier and reservoir, platelets store serotonin in dense electron granules.<sup>68</sup>

Lesurtel et al.<sup>69</sup> identified platelets as the major source of serotonin that drives liver regeneration in partial hepatectomy of (PHx) mice. They found that liver regeneration in thrombocytopenic mice following PHx was restored by supplementing the mice with platelet-rich plasma containing near weight levels of serotonin.

Laffi et al.<sup>32</sup> gave evidence for significant reduction of substances that are deposited in thick and in alpha granules in patients with liver cirrhosis, a condition defined as "platelet exhaustion".

Culafic et al.<sup>38</sup> concluded that plasma serotonin was significantly higher in patients with cirrhosis than in the controls and represents the degree of liver insufficiency (Table 8). It was noticed that liver regeneration and repair were significantly impaired in platelet-depleted animals. Mice lacking peripheral serotonin showed a failure of hepatocyte proliferation after ischaemia, but otherwise displays normal tissue remodelling. The results suggest that platelets may not cause postschaemic liver injury, but mediate tissue repair through modulation of inflammation and the release of serotonin.<sup>31</sup>

**Table 8** Agreement (sensitivity, specificity and accuracy) for AFP and serotonin in liver Cirrhosis group.

|           |      | Control | Liver Cirrhosis | Sensitivity | Specificity | PPV   | NpV   | Accuracy |
|-----------|------|---------|-----------------|-------------|-------------|-------|-------|----------|
| AFP       | > 10 | 10      | 22              | 51.11       | 100.0       | 100.0 | 31.25 | 60.0     |
|           | ≤ 10 | 0       | 23              |             |             |       |       |          |
| Serotonin | > 75 | 10      | 6               | 86.67       | 100.0       | 100.0 | 62.50 | 89.09    |
|           | ≤ 75 | 0       | 39              |             |             |       |       |          |



**Table 9** Agreement (sensitivity, specificity and accuracy) for AFP and serotonin in liver Cirrhosis and the HCC group.

|           |      | Control | Liver Cirrhosis + HCC | Sensitivity | Specificity | PPV   | NPV   | Accuracy |
|-----------|------|---------|-----------------------|-------------|-------------|-------|-------|----------|
| AFP       | > 10 | 10      | 2                     | 93.33       | 100.0       | 100.0 | 83.33 | 95.0     |
|           | ≤10  | 0       | 28                    |             |             |       |       |          |
| Serotonin | > 75 | 10      | 0                     | 100.0       | 100.0       | 100.0 | 100.0 | 100.0    |

APRI score was significantly higher in group II than group I ( $p < 0.001^*$ ) also a significant negative correlation was found between serotonin level and APRI score in group II patients who had both higher serotonin and APRI score ( $r = -0.363$ ,  $p = 0.049^*$ ). Similar results obtained by Loftis et al<sup>58</sup> who demonstrated an association between higher serotonin level and lower APRI score suggesting that higher serotonin levels might be a surrogate marker of less advanced liver disease.

The involvement of serotonin in the induction of hepatocyte DNA synthesis was first investigated in primary cultures of adult rat hepatocytes by Balasubramanian et al.,<sup>70</sup> who showed that 5-HT could significantly induce hepatocyte proliferation in the presence of insulin and EGF (epidermal growth factor) (Table 9).

Among serotonin receptors the 5-HT<sub>2B</sub> receptor has been strongly associated with increased hepatic stellate cells (HSCs) proliferation and liver fibrosis. HSCs have been reported to secrete numerous factors that influence hepatocyte proliferation. The role of serotonin as a mitogen for HSCs during liver regeneration remains hugely unknown.<sup>30,71</sup>

Serotonin signalling seems to play a pivotal role in determining the balance between regeneration and fibrogenesis in chronic liver disease and it has been reported that the activation of 5-HT<sub>2B</sub> receptor on fibrogenic HSCs suppresses hepatocyte proliferation through augmented production of TGFβ<sub>1</sub>.<sup>72</sup> At the severe end of the spectrum, 5-HT has been involved in the pathogenesis of human HCC through increased 5-HT<sub>2B</sub> expression, which seems to facilitate the survival of carcinoma cells and to inhibit autophagy.<sup>48</sup> It has also been reported to exert a proliferative effect on cholangiocytes and to promote cholangiocarcinoma growth.<sup>73</sup> There are also some interesting reports suggesting that serotonin can potentially contribute to liver tissue hypoperfusion following hepatic ischaemia and reperfusion in canines raising new dilemmas about its effects on hepatic regeneration.<sup>19,74</sup>

Serotonin can be potentially associated with either beneficial or detrimental effects on liver regeneration and these actions are mediated through many different receptor subtypes located either centrally or peripherally.<sup>71</sup>

A significant positive correlation was found between serum serotonin and AFP in group I and group II ( $r = 0.298$ ,  $p = 0.047^*$ ,  $r = 0.468$  and  $0.009^*$ , respectively) (Table 6), so higher AFP is associated with higher serotonin. This signifies the association between AFP and serotonin and so the importance of serum serotonin as a marker of HCC and together with the results of ROC curve in both groups can consider serotonin as a good marker for the diagnosis of HCC).

## 6. Conclusion

plasma serotonin levels are significantly higher in patients with cirrhosis and HCC than in cirrhosis in the control groups and

is involved in the tumourigenesis of hepatocellular carcinoma the third cause of cancer-related death worldwide.

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