Serum Melatonin Levels in Patients with Hepatocellular Carcinoma

Reda Albadawy[a], Yousry Esam-Eldin Abo-Amer [b,] Asmaa Hassan [b], Radwa E. Sharaby[c] , Tamer E. El-Eraky[a]

[a]Hepatology, Gastroenterology & Infectious Diseases Department, Faculty of Medicine- Benha University.
[b]Hepatology, Gastroenterology & Infectious Diseases Department, Mahalla Hepatology Teaching Hospital, Egypt.
[c] Clinical Pathology Department, Faculty of Medicine, Tanta University.

Correspondence to: Asmaa Hassan, Infectious Diseases Department, Mahalla Hepatology Teaching Hospital, Egypt

Email:

docasmaa91@gmail.com

Received: 4 February 2022

Accepted: 5 March 2022

Abstract

Background: HCC accounts for 90% of all primary liver cancers around the world. HCC is the fifth most frequent cancer in the world and the second largest cause of cancer-related death. HCC will be the first indication for liver transplantation by 2030. The need for a marker to detect HCC cases early is critical. Melatonin is a hormone, and when its hemostasis is disrupted by cirrhosis, it may be linked to the development of hepatocellular carcinoma. **The aim** is to assess blood melatonin levels in patients with liver cirrhosis (compensated and decompensated) and HCC. **Subject and Methods:** This was a comparative cross-sectional study with 22 patients with compensated liver cirrhosis, 22 patients with decompensated liver cirrhosis, 22 patients with HCC, and 22 healthy subjects as a control group. Laboratory tests, as well as abdominal ultrasound and spiral CT, were performed as needed. Melatonin levels were measured using an ELISA kit in accordance with the manufacturer's instructions. **Results**: In compensated liver cirrhosis, there was a statistically significant positive correlation between serum melatonin and age, as well as a significant positive correlation between melatonin, (Hb), (WBCs), and (platelets) in decompensated liver cirrhosis. Serum melatonin has low statistical sensitivity, specificity, and predictive value. **Conclusion**: The melatonin levels in the compensated, decompensated and HCC groups were lower than in the control groups, the difference was not statistically significant.

Abbreviations: HCC / Hepatocellular carcinoma. CT/ Computed tomography. ELISA /Enzyme-linked immunoassay. Hb / Hemoglobin. WBCs/ White blood cells

Keywords: Liver Cirrhosis, Hepatocellular Carcinoma (HCC), Apoptosis, Melatonin.

Introduction

Hepatocellular carcinoma (HCC) is the most frequent liver cancer and ranks 5th in terms of global cancer incidence ***(1)***. HCC is a severe public health issue in Egypt, where liver cancer accounts for 11.75 percent of digestive organ malignancies and 1.68 percent of total malignancies. HCC accounts for 70.48 percent of all liver tumors in Egyptians and is regarded as the most serious consequence of cirrhosis ***(2)***. Apoptosis is one of the most important cell death mechanisms, and its inactivation contributes to tumor development and treatment resistance ***(3)***. Cells with a rapid growth rate and high expression of pro-apoptosis genes including Bax, PUMA, and p53 are more susceptible to apoptosis during stress ***(4)***. The intrinsic mechanisms include the release of cytochrome c from the mitochondria and, as a result, the activation of procaspases ***(5)*.** Melatonin is the primary hormone secreted by the human pineal gland. Melatonin and its metabolites, in addition to regulating the sleep-wake cycle, are powerful free-radical scavengers that reduce cellular damage caused by peroxides created during physiological metabolic processes ***(6)*.**

Furthermore, it can stimulate immunity and decrease angiogenesis in a variety of tumors ***(7)***. Melatonin decreased apoptosis in fibroblasts without affecting p53, although it significantly reduced oxidative damage ***(8)***. It suppresses cell growth in a variety of cancer cell lines, including human B-lymphoma, human myeloid leukemia, and human neuroblastoma ***(9)***. In experimental study melatonin reduces AFP expression and promotes apoptosis in HCC through increasing casp 8 expression ***(10)***.

The aim of this study is to assess blood melatonin levels in patients with liver cirrhosis (compensated and decompensated) and HCC.

Subjects and methods

A comparative cross-sectional study was done on 88 subjects at El Mahalla Hepatology Teaching Hospital. The study was conducted from the October 2020 to December 2021, and was approved by the local ethical committee from El-Mahalla Teaching Hospital signed by the Dean.

Subjects were separated into four groups:

Group **A** included 22 patients with compensated liver cirrhosis.

Group **B** included 22 patients with decompensated liver cirrhosis.

Group **C** included 22 patients with HCC.

Group **D** included 22 healthy subjects as a control group.

*** Inclusion criteria:**

a) -Patients > 18 years old.

b) -Cirrhotic patients (compensated and decompensated) whatever the causes.

c) -Patients with HCC.

*** Exclusion criteria:**

a) -Patients< 18 years old.

b) -Patients > 65 years old.

c) -Patients with neurodegenerative disorders.

All the patients were subjected to the following after taking patient consent:

1. *Clinical assessment including history* (Name, Age, Sex, Marital status) and clinical examination (signs of LC eg. Palmer erythema, spider nevi, jaundice, ascites, umbilical hernia, gynecomastia).

2. *Laboratory investigation*: according to the protocol and laboratory advices patients were fasting from 6-8 hrs.

- Complete blood count: including, WBC (total and differential), Hemoglobin and Platelets.
- Liver profile: including, AST, ALT, Bilirubin (total and direct), Serum albumin, Prothrombin time and Alpha fetoprotein for HCC cases.
- Kidney function tests: including, Serum Creatinine and blood urea
- Melatonin: "ELISA" "Morning sample" (10 a.m.): Allow the serum to coagulate at room temperature for 10-20 minutes. Centrifuge for 20 minutes (at 2000-3000 RPM). Collect the supernatants with care. When sediments done during storage, centrifugation should be repeated. Melatonin levels were determined using Competitive-ELISA kits.

3. Radiological investigation:

a) Ultrasonography for evaluation of liver, PV, spleen, kidney and ascites. nodular liver surface, round edge, and hypoechoic nodules in liver parenchyma for cirrhosis, small focal HCC (appears hypoechoic compared with normal liver) and larger lesions are (heterogeneous due to fibrosis, fatty change, necrosis and calcification) .

a) Triphasic spiral CT for HCC cases (focal nodule with early enhancement on the arterial phase with rapid washout of contrast on the portal venous phase of a three-phase contrast scan) or Dynamic MRI for HCC cases (high signal intensity on T2 imaging).

Abbreviations: HCC / Hepatocellular carcinoma. CT/ Computed tomography. ELISA /Enzyme-linked immunoassay. WBCs/ White blood cells. AST/ Aspartate aminotransferase. ALT / Alanine aminotransferase. PRM/ Revolutions per minute. MRI/ Magnetic resonance imaging. PV/ Portal vein. LC/ Liver cirrhosis.

Statistical analysis

The data are provided as the mean standard deviation of three separate experiments. SPSS (statistical package for social science software) version 20.0 was used to tabulate and analyse the data. For quantitative data, the ANOVA test and its post hoc test were used, while for qualitative data, the X2 test and Monte Carlo test, as well as Pearson and Spearman correlation, were used. When the P value was less than 0.05, the difference was considered statistically significant. The Roc curve was also employed.

Results

A comparative cross-sectional was conducted on 88 individuals divided into 4 groups; group A: compensated cirrhosis, group B: decompensated cirrhosis, group C: HCC and group D: as a control group.

Table (1) shows regarding age it was significantly higher in HCC group than compensated and control groups (***P2=0.039)***. Also, age was significantly lower in control group than compensated and decompensated groups. It also shows that HB was significantly lower in decompensated group than compensated group and HCC groups (***P1=0.004 .P3=0.006*****).**

S. Albumin was significantly lower in decompensated group than compensated and HCC groups (***P1=0.000 .P3=0.001)*** Also, it shows that INR was significantly higher in decompensated group than compensated group (***P1=0.006***). It shows that AST was significantly higher in decompensated group than compensated group (***P1=0.014***).

Also, it shows that T. bilirubin ***(P1=0.001 P3=0.002*****)** and D. bilirubin (***P1=0.001. P3=0.002*****)** were significantly higher in decompensated group than compensated and HCC groups. As regard PVT it was higher in HCC group than compensated and decompensated groups (***P1= 0.021 .P2 =0.009)***. According to Liver size, shrunken liver was found to be more prevalent in decompensated than compensated and HCC groups ***(P1 = 0.008. P2 = 0.082. P3 = 0.001*****).**

Table (2) shows that Child Pugh score A is lower in decompensated group than compensated and HCC groups, respectively (***P1 0.000***). Also, child score B is higher in HCC groups than compensated and decompensated groups, respectively (***P2 0.001***). It shows that child **C** is higher in decompensated group than compensated and HCC groups respectively (***P30.005***). As regard MELD score, it is higher in decompensated group than compensated and HCC groups respectively (***P1 0.000. P3 0.002***).

Table (3) shows that melatonin level was lower in the compensated, decompensated and HCC groups than control group but, it does not reach the statistical significance. Table (4) shows that there is correlation between age and s. melatonin in compensated group but no correlation in other groups considering age and sex. Table (5) shows that there is positive correlation between melatonin and HB ***(p 0.013)***, WBCs ***(p 0.035),*** Platelets ***(p 0.048)*** and Bilirubin ***(p 0.048)*** in decompensated group. Table (6) shows that there is no significant difference between s. melatonin, Child-Pugh Score and MELD Score in all groups.

Figure (1) shows that cut off point, sensitivity and specificity ***(3.950, 40.9%, 31.8),*** respectively between HCC and control group. Figure (2) shows that cut off point, sensitivity, specificity, PPV, NPV and accuracy ***(4.0, 40.9%, 31.8%, 34.6%, 27.7%, 31.8%)***, respectively between HCC and compensated groups. Figure (3) shows that cut off point, sensitivity, specificity, PPV, NPV and accuracy ***(3.95, 40.9%, 31.8%, 34.6%, 27.7%, 31.8%),*** respectively between HCC and decompensated groups.

Table (1): Comparison between compensated, decompensated and HCC groups considering age, laboratory and radiological findings.

	Compensated N=22 Mean ± SD	**Decompensated N=22 Mean ± SD**	**HCC N=22 Mean ± SD**	**Test used**	
				P value	
Age (yrs.) (mean±SD)	52.18±7.51	59.77±8.85	60.09±5.42	P1=0.052 **P2=0.039*** P3=0.998	
				ANOVA	**Post hoc test**
				P-value	**P-value**
HB(g/dL)	11.50±1.97	9.48±1.60	11.42±1.82	0.000*	**P1=0.004*** P2=0.999 **P3=0.006***
WBC(thousands/cmm)	6.19±4.63	6.47±5.2555	5.33±2.82	0.668	P1=0.977 P2=0.810 P3=0.690
Platelets(thousands/cmm)	104.27±43.33	91.68±54.04	128.23±83.01	0.151	P1=0.800 P2=0.449 P3=0.160
S. Albumin (g/dL)	3.55±0.61	2.69±0.57	3.39±0.58	**0.000***	**P1=0.000*** P2=0.675 **P3=0.001***
INR	1.20±0.30	1.61±0.54	1.31±0.31	**0.004***	**P1=0.006*** P2=0.648 P3=0.064
ALT(U/L)	30.68±11.07	43.86±44.88	39.46±18.02	0.306	P1=0.319 P2=0.599 P3=0.878
AST(U/L)	30.14±13.78	58.09±43.47	48.41±27.52	**0.013***	**P1=0.014*** P2=0.152 P3=0.582
T. Bilirubin(mg/dL)	1.91±1.04	5.77±5.23	1.82±1.35	**0.005***	**P1=0.001*** P2=0.996 **P3=0.002***
D. Bilirubin(mg/dL)	0.84±0.62	3.65±3.98	0.93±1.15	**0.003***	**P1=0.001*** P2=0.993 **P3=0.002***
AFP(ng/ml)	6.66±5.35	11.35±10.53	2054.39±6065.92	0.090	P1=1.000 P2=0.161 P3=0.162
S. Creatinine(mg/dL)	1.32±0.49	1.55±0.91	1.19±0.51	0.202	P1=0.520 P2=0.817 P3=0.211
				X2 test P-value	
Splenomegaly	10 (45.5) 12(54.5)	7 (31.8) 15 (68.2)	5 (22.7) 17 (77.3)	P1=0.353 P2=0.112 P3=0.498	
Liver size					
Normal	0(0)	0 (0)	0 (0)	**P1 = 0.008***	
Enlarged	19 (86.4)	2 (9.1)	14 (63.6)	**P2 = 0.082***	
Shrunken	3(13.6)	20 (90.9)	8 (36.4)	**P3 = 0.001***	
Ascites					
No	22 (100)	0 (0)	9 (40.9)	**P1 =0.000***	
Mild	0	6 (27.3)	4(18.2)	**P2= 0.004***	
Moderate	0	4 (18.2)	5 (22.7)	**P3=0.0020***	
Marked	0	12 (54.5)	4 (18.2)		
PVT				**P1 = 0.021***	
Yes	0(0)	6 (27.3)	7 (31.8)	**P2 =0.009***	
No	22(100)	16 (72.7)	15 (68.2)	P3= 0.977	
PV diameter				**P1= 0.000***	
Average	22 (100)	8 (36.3)	12 (54.5)	**P2=0.004***	
Dilated	0(0)	14(63.6)	10 (45.5)	P3=1.000	

* The significance level is ≤ 0.05, P1 Compensated& Decompensated, P2 Compensated & HCC, P3 Decompensated &HCC

Table (2): Child and MELD score in the compensated, decompensated, HCC groups

		Compensated (n=22) No. (%)	Decompensated (n=22) No. (%)	HCC (n=22) No. (%)	Monte Carlo test /ANOVA P-value
Child-Pugh Score	**A**	17 (77.3%)	1 (4.5%)	3 (13.6%)	**P1=0.000***
	B	5 (22.7%)	4 (18.2%)	16 (72.7%)	**P2=0.001***
	C	0 (0%)	17(77.2%)	3 (13.5%)	**P3=0.005***
MELD score (mean±SD)		12.91±4.22	19.50±6.22	12.73±4.71	**P1=0.000*** P2=0.999 **P3=0.002***

* The significance level is ≤ 0.05, P1 Compensated& Decompensated, P2 Compensated & HCC, P3 Decompensated &HCC

Table (3): Serum melatonin in all studied groups

	Compensated N=22 Mean±SD	Decompensated N=22 Mean±SD	HCC N=22 Mean±SD	Control N=22 Mean±SD	ANOVA P-value	Post hoc test P-value
S. Melatonin(pg/ml)	7.55±10.90	4.38±0.86	7.89±14.19	9.96±12.40	0.403	P1=0.599 P2=0.994 P3=0.533 P4=0.732 P5=0.567 P6=0.432

* The significance level is ≤ 0.05, P1 Compensated & Decompensated, P2 Compensated & HCC, P3 Decompensated & HCC, P4 Control & Compensated, P5 Control & Decompensated, P6 Control & HCC

Table (4): Correlation between S. Melatonin, age and sex in the Compensated, decompensated groups and HCC groups.

Type	Pearson/spearman correlation		S.Melatonine
Compensated	Age	R	-0.537
		P-value	**0.010***
	Sex	R	0.137
		P-value	0.544
Decompensated	Age	R	-0.291
		P-value	0.190
	Sex	R	0.230
		P-value	0.303
HCC	Age	R	0.070
		P-value	0.757
	Sex	R	0.040
		P-value	0.859

* The significance level is ≤ 0.05

Table (5): Correlation between serum melatonin and other laboratory investigations in compensated, decompensated, control and HCC groups.

Pearson correlation		Compensated	Decompensated	HCC	Control
		Serum Melatonin			
HB	**R**	0.059	0.521	-0.046	-0.106
	P- value	0.794	**0.013***	0.840	0.638
WBC	**R**	0.034	0.452	0.121	-0.220
	P- value	0.882	**0.035***	0.590	0.325
Platelets	**R**	-0.009	0.426	-0.165	0.064
	P- value	0.969	**0.048***	0.462	0.778
S. Albumin	**R**	0.192	-0.390	-0.230	0.071
	P- value	0.391	0.073	0.304	0.754
INR	**R**	0.210	0.244	-0.039	0.056
	P- value	0.349	0.275	0.864	0.806
ALT	**R**	-0.217	-0.203	-0.109	0.248
	P- value	0.331	0.364	0.630	0.266
AST	**R**	-0.038	0.037	0.196	-0.291
	P- value	0.866	0.869	0.383	0.189
T. Bilirubin	**R**	-0.242	0.425	0.238	-0.027
	P- value	0.277	**0.048***	0.287	0.906
D. Bilirubin	**R**	-0.236	0.464	0.033	0.005
	P- value	0.290	**0.048***	0.884	0.984
S. Creatinine	**R**	-0.039	-0.176	-0.164	0.098
	P- value	0.863	0.434	0.466	0.666
AFP	**R**	-0.200	-0.105	-0.114	0.205
	P- value	0.373 0.373	0.642 0.642	0.614 0.614	0.361 0.361

* The significance level is ≤ 0.05

Table (6): Correlation between S. Melatonin, Child-Pugh Score and MELD score in the compensated, decompensated and HCC groups.

		Spearman's correlation	S. Melatonin
Child pugh score	Compensated	R	0.060
		P-value	0.792
	Decompensated	R	0.146
		P-value	0.517
	HCC	R	-0.284
		P-value	0.201
MELD	Compensated	R	-0.063
		P-value	0.782
	Decompensated	R	0.147
		P-value	0.514
	HCC	R	0.013
		P-value	0.953

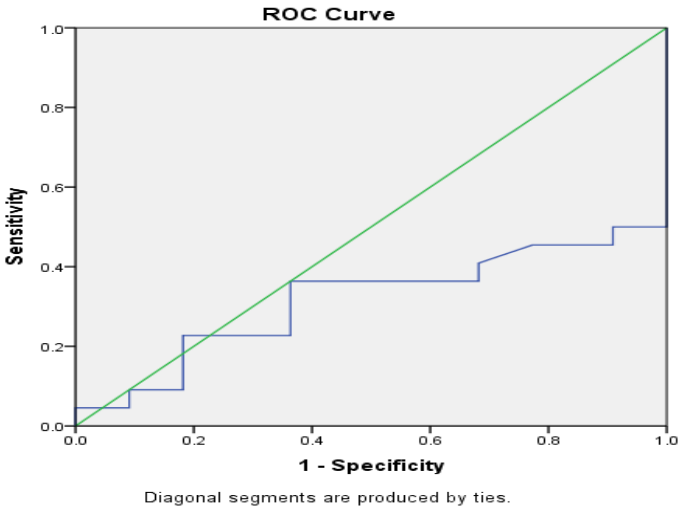

Figure (1): Roc curve for Diagnostic performance of serum melatonin in HCC and control groups.

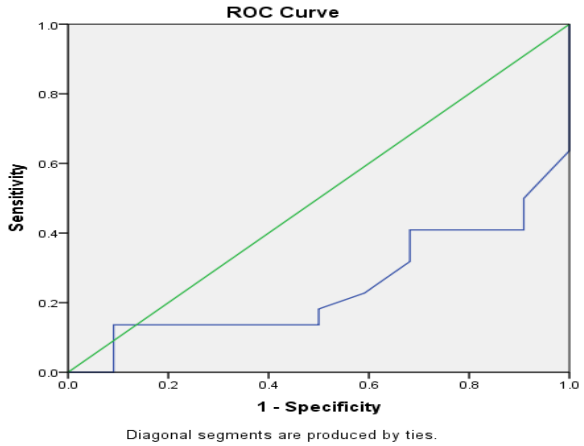

Figure (2): Roc curve for Diagnostic performance of serum melatonin in HCC and compensated groups.

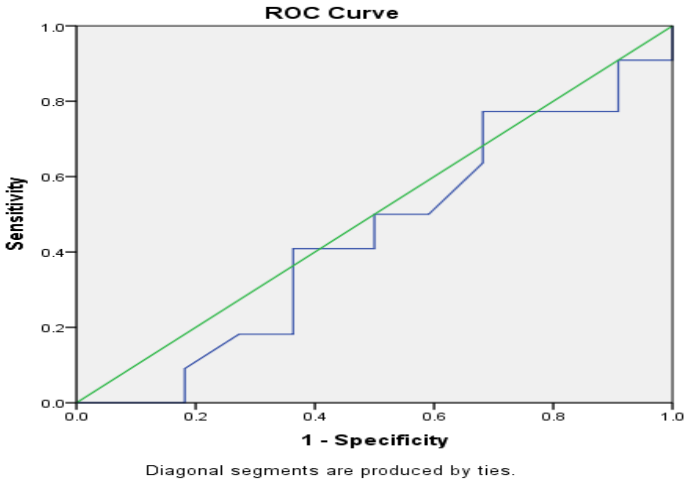

Figure (3): Roc curve for Diagnostic performance of serum melatonin in HCC and decompensated groups.

Abbreviations: HCC / Hepatocellular carcinoma. HB / Hemoglobin. WBCs/ white blood cells. AST/ Aspartate aminotransferase. ALT / Alanine aminotransferase. INR/ international normalized ratio. MELD/ Model for end-stage liver disease. PVT/ Portal vein thrombosis. ANOVA/ Analysis of variance. AFP/ Alpha fetoprotein.

Discussion

Melatonin (MT), also known as N-acetyl-5-methoxytryptamine, which is extracted from the pineal gland and regulates a variety of physiological processes ***(11)***. Melatonin not only has a powerful antioxidant action that protects cells and tissues from free radical damage, but it also suppresses proinflammatory cytokines during the progression of hepatic fibrosis ***(12)***.

This was a comparative cross-sectional trial that looked at blood melatonin levels in 88 individuals with liver cirrhosis (compensated and decompensated) and HCC. In the current study, the HCC group was significantly older than the compensated and control groups. This is comparable to the findings of *Subramaniam* et al. ***(13)***, who found that the average age of their research participants was 54.26 years old, with a high frequency between 51 and 60 years. Furthermore, Kew discovered that the incidence of HCC in South Africa was associated with a mean age of 50.9 and 51.0 years ***(14).*** Furthermore, the age of the control group was significantly lower than that of the compensated and decompensated groups. This was similar to what Lee and colleagues reported in a study on 145 patients with CLD and 101 healthy individuals ***(15).***

Child Pugh score A is lower in the decompensated groups than in the compensated and HCC groups. This is consistent with Ampuero's study, which found that child Pugh score A was considerably lower in cirrhosis progression than in stable cirrhosis (p-value=0.0001) ***(16)***. It demonstrates that child Pugh score C is greater in the decompensated group than in the compensated and HCC groups. This is consistent with the Ripoll et al. research, which found a statistically significant increase in Child Pugh score A in compensated liver cirrhosis versus decompensated liver cirrhosis, with a p-value of 0.0001***(17)***. In the current study, 31.8 percent of patients are BCLC stage A, 27.3 percent are in stage B, 27.3 percent are in stage C, and 13.6 percent are in stage D. In a previous research by Kim et al., the majority of HCC patients (64%) were in stage A, followed by stage C (21.5%), stage B (12.5%), and stage D (2%) ***(18)***.

Although melatonin inhibits growth in a variety of cancer cell lines, research on its oncostatic effects in hepatocellular carcinoma is limited ***(19)***. In our investigation, no significant variation in s. melatonin levels was found across all study groups with low sensitivity, specificity, and predictive value for compensated, decompensated liver disease, and hepatocellular carcinoma. Previously, Carbajo-Pescador and colleagues had reported that melatonin significantly reduced cAMP levels with significant interplay between melatonin and cytosolic quinone reductase type-2 (NQO2) receptors in liver cancer cells ***(19).***In contrast to another study by Chojnacki et al. study which was performed on 90 alcoholic patients with hepatic encephalopathy and 30 healthy volunteers and found a significant higher Melatonin level in hepatic encephalopathy than control group with p-value <0.01 ***(20)*** . prior study found that melatonin reduces AFP expression and promotes apoptosis in HCC through stimulating casp 8 expression, making it a suitable adjuvant for chemotherapy in HCC ***(10)***.

On the other hand, this contradicts another study by Chojnacki et al., which was conducted on 90 alcoholic patients with hepatic encephalopathy and 30 healthy volunteers and discovered a significantly higher Melatonin level in hepatic encephalopathy than control group with p-value 0.01. This revealed that increased concentration of melatonin in blood of patients with liver cirrhosis may be the result of both hepatic insufficiency and transport of melatonin from gastrointestinal tract to systemic circulation through the portosystemic shunts. Melatonin levels in the blood may alter the clinical characteristics of hepatic encephalopathy ***(20).***

In the decompensated group, there is a positive connection between S. Melatonin and Platelets. This is consistent with the Esmaeili et al. research, which compared Melatonin to placebo as an antipruritic agent in CLD and discovered a statistically significant positive connection between Melatonin and platelets with a p-value of 0.001 ***(21).***

Our research discovered a statistically significant beneficial relationship between S. Melatonin, HB, and WBCs. Tarocco et al. conducted a study to investigate the effect of melatonin on some haematological parameters and discovered that melatonin significantly increased RBCs count, Hb concentration, and total leukocyte count, clarifying the effect of melatonin on

improving health and immune status ***(22)***. This can also be explained by Melatonin's biological functions, which include apoptosis regulation, oxidative phosphorylation regulation, reactive oxygen species regulation, homeostasis, cytoskeletal function, and anti-proliferative actions ***(23)***. Finally, our investigation found that blood melatonin levels did not differ substantially between compensated, decompensated, and hepatocellular cancer patients.

Conclusion

The melatonin levels in the compensated, decompensated and HCC groups were lower than in the control group, the difference was not statistically significant.

Recommendations: Further longitudinal studies with large sample size are needed for further estimation of melatonin level. Also, doing the study on more than one melatonin sample to demonstrate the circadian melatonin rhythm.

Limitations: The primary limitations of this study are that only daily melatonin levels were measured, therefore no information on potential disruptions in the circadian melatonin rhythm was collected. In addition, there is a paucity of longitudinal data that allows for the tracking of individual melatonin variations as liver cirrhosis progresses.

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To cite this article: Reda Albadawy, Yousry Esam-Eldin Abo-Amer , Asmaa Hassan , Radwa E. Sharaby , Tamer E. El-Eraky. Serum Melatonin Levels in Patients with Hepatocellular Carcinoma. BMFJ 2022;39(1):295-308. DOI: 10.21608/bmfj.2022.120033.1541