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Research Article

Growth Hormone Receptor Expression in HCV-Related Hepatocellular Carcinoma

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Abstract

Background: Hepatocellular carcinoma (HCC) is unquestionably a major obstacle in the way of health progress in Egypt, considering its high incidence and extreme fatality. Department Meanwhile, one of the major molecular pathways in many solid tumors is that of the GH and its downstream pathways. **Aim of the study:** To assess the state of expression of growth hormone receptor (GHR) in cases of HCC as compared to those with liver cirrhosis and healthy controls. **Subjects & Methods:** sixty patients were enrolled in this study. They were arranged into three equal groups: the first contained 20 HCC patients, while the second consisted of 20 cirrhotic patients, and the third represented 20 healthy controls. We compared the immunohistochemistry results of liver tissue biopsies for GHR expression to patients' demographic data, clinical and laboratory findings, as well as the tumor's clinicopathological characteristics. **Results:** It was found that HCC patients levels of GHR expression were lower than cirrhotic and control groups which were also correlated with features of tumor aggressiveness. **Conclusion:** The down-regulation of GHR was associated with the development and aggressiveness of Hepatitis C-related HCC, regardless of the functional condition of the liver.

Key words: Hepatocellular carcinoma, Growth hormone receptor, Immunohistochemistry.

Introduction

Hepatocellular carcinoma (HCC) with average annual incidence of approximately 850,000 cases worldwide is considered the most prevalent type of primary tumors of the liver, Among tumors it ranks as the sixth in terms of prevalence and the fourth most fatal form[(1)]. HCC has doubled in Egypt over a decade, due to the rise in the frequency and consequences of infection with hepatitis C virus.[(2)]

Unlike many other cancer types, HCC is diagnosed only through radiographic and laboratory criteria, while tissue samples are rarely necessary. This makes it more difficult to identify distinct tumor subtypes and signaling pathways that can be used to build individualized treatment methods, as well as the discovery of novel targeted medicines, which are already widely available and have proven effects[(3)].

One of the main suspected mechanisms in many solid organ malignancies is related to the growth hormone (GH) and its ancillary pathways; namely: signal transducer and activator of transcription 5 (STAT5) and insulin like growth factor 1(IGF-1). [(4–6)]

The anterior pituitary gland is primarily responsible for manufacturing and secreting

GH, which controls postnatal growth via cell secretion, metabolism, survival, and proliferation. It also increases IGF1 gene expression as well as its synthesis in the hepatocytes. GH secretion is tightly controlled by two hypothalamic neuropeptides one called growth hormone releasing hormone (GHRH) and the other called somatostatin (SST). The arcuate nucleus, located at the hypothalamus secretes GHRH, the principal stimulator and regulator of GH production and release. Also in the hypothalamus, the periventricular nucleus produces SST, which mediates the negative feedback of GH upon its own release through G-protein-coupled receptors, SST receptors subtypes 1-5.[7]

When GH interacts to its receptor (GHR), it activates Janus Kinase 2 (JAK2), which is connected with the GHR. JAK2 phosphorylates tyrosines in the GHR. Signaling proteins are attracted to GHR-JAK2 complexes in the cell membrane by those phosphorylated tyrosines. GHR-JAK2 complexes bind and phosphorylate proteins, such as STATs 1, 3, 5a, and 5b, which control the expression of GH-related genes like IGF-1. [8,9]

GHRs are JAK linked receptors that are types of enzyme-coupled receptors. Dehkhoda et al.,. [9] define GHR as a homodimeric receptor with 638 amino acids, and has three domains: a cytoplasmic intracellular domain, a single-pass transmembrane domain, and one cytokine receptor homology domain. Although these receptors lack inherent enzymatic activity, they function through the JAK enzyme family. When such receptors are active, the JAK enzymes are activated, and subsequently activate a specific member of the STAT family. This particular member of the STATs family is then delivered into the nucleus, where it exerts its distinctive impact. [9]

Because malignancy is always thought to be an undesirable excessive development and spread of specific cells, there has been a tendency to accuse GH of producing or encouraging malignancy. This is supported by many observations including: increased incidence of certain cancers such as colorectal carcinoma that was noted in cases of acromegaly[10], and the increased frequency of leukemia in those receiving GH supplemen-tations[11]. On the contrary, patients with GH resistance known as Laron syndrome have lower incidence of malignancies.[12]. Nume-rous researches have studied the expression of GHRs in various solid malignancies. [13], including HCC, but showed conflicting results.[14]

Aims of the study:

To evaluate the level of GHR expression using immune-histochemistry in HCC cases in comparison to cirrhotic patients and healthy controls, and to correlate the results to the patients' demographics and laboratory findings, in addition to the clinicopathological characteristics of the hepatoma.

Subjects & Methods

This retrospective, cross-sectional, comparative study was conducted in the department of Internal Medicine of Minia University Hospital, Egypt, in collaboration with the Pathology Department of both Minia university hospital and Minia Oncology Center, Egypt, in the period between November 2018 and April 2022. In order to have a power of 99%, we obtained a sample size of 20 patients who had HCV-related HCC in this study. It was calculated at a 0.05 level of significance using G Power 3.19.2 Software. Thus, 60 participants were included in the study and split into three equal groups: composed of: HCC patients (group I), patients suffering from liver cirrhosis (group II), and healthy controls (group III).

Eligibility criteria included patients with adequate liver tissue samples and available clinico-pathological data. Exclusion criteria involved patients with advanced renal disease, diabetes mellitus, chronic diseases of the liver excluding hepatitis C, abnormalities in GH-IGF1 axis, hepatic resection, organ transplantation, previous locoregional treatment for HCC, extra hepatic malignancies, and non-HCC liver tumors.

Clinical and laboratory data, as well as pelvi-abdominal ultrasonography and triphasic computed topography data were collected from the patients' medical records. The degree of severity of liver cirrhosis was assessed according to the Child-Pugh and the Model for End-Stage Liver Disease (MELD) scoring

system. The study also evaluated tumor progression using Okuda and Tumor-Node-Metastasis (TNM) staging systems.

Liver biopsy specimens were taken and subjected to routine haematoxylin and eosin staining to assess the hepatic necroinflamatory activity and fibrosis staging[15]. For immune-histochemical studies, tissue sections of 4-µm thickness were taken from ten percent buffered formalin-fixed, paraffin-embedded tissue blocks using the commercially available kits; mouse GHR monoclonal antibody (1/100 dilution, Santa Cruz Biotechnology, Texas, USA), To evaluate proper tissue preparation and staining, positive controls were employed. Every staining run comprised one positive control tissue segment that was prepared just as the patient tissue samples for every antibody. For every example, we prepared one negative control slide by skipping the primary antibody staining step. Absence of cross-reactivity with other non-target cellular elements was indicated by the lack of particular staining in the negative control slides.

The final staining scores for GHR expression were determined by multiplying both the percentage and intensity scores to obtain a score of 0-12. The intensity was assessed as absent: 0, weak: 1, moderate: 2, and strong: 3, whereas the staining percentage of cells was ranked as follows: none: 0, 1: 1-10%, 2:11-33%, 3:34-66%, and 4: 67-100%. Staining scores below 4 denoted low expression, whereas scores above 4 denoted high expression[16] .

Ethical consideration: The Institutional Ethics Committee of Minia University Faculty of Medicine and the Minia Oncology Center Institutional Review Board approved the protocol of this study. The Helsinki Declaration of 1975 and the International Conference on Harmonization of Guidelines for Good Clinical Practice served as the study's guidelines and regulations. Written informed permission was acquired from every participant after providing comprehensive information about the aim and the nature of the study. The issues of privacy and confidentiality of all study participants were considered

Statistical analysis: We analyzed data using international business machines corporation (IBM) - Statistical Package for Social Sciences (SPSS) version 20, The normality of quantitative variables was estimated using the Kolmogorov –Smironov test.

The normally distributed variables were presented as mean ± SD. The Student's t-test was used to compare each two groups, while one-way ANOVA followed by Bonferroni post-hoc tests were used to compare the three groups. The non-normally distributed parameters were expressed as median and interquartile range (IQR). Each two groups were compared using Mann Whitney test while the Kruskal Wallis test was used for the three groups. Categorical data was represented as counts and percentages and techniques such as Chi-square and Fisher exact test were used to compare them. Relationship between continuous variables was evaluated using the Pearson's correlation coefficient, whereas Spearman's correlation coefficient was utilized in case of non-parametric variables. A P-value of less than 0.05 was accepted to be statistically significant.

Results

The participants of the current study were arranged into 3 groups as follows:

Group I: It included 20 cirrhotic patients with hepatocellular carcinoma on top of HCV-related liver cirrhosis, comprised 15 men and 5women. Their ages ranged from 50 to 83 years with mean ± SD of 66. 9±7.8 years.

Group II: It included 20 HCV related cirrhotic patients without HCC comprised 15 men and 5 women. Their ages ranged from 38 to 83 years with mean ± SD of 61.9 ±11.9 years.

Group III: It included 20 healthy controls. They comprised 15 men and 5 women. Their ages ranged from 50 to 75 years with mean ± SD of 63.7±11.1 years

The study groups' demographic data were found comparable as regards age, sex and smoking exposure. As regards body mass index (BMI), there was no significant statistical difference between both HCC and cirrhotic groups, however both groups displayed statistically significant lower values when compared with healthy controls (25.8±3.7 kg/m^2 vs 29.2±2.6 kg/m^2, p<0.001 and 24.3±2.5 kg/m^2 vs 29.2±2.6 kg/m^2, p<0.001, respectively) (**Table 1)**

Table (1): Baseline demographics of the study groups

		Group I HCC	Group II Cirrhotic patients	Group III Control	p value			
		N=20	N=20	N=20	Among 3 groups	I vs II	I vs III	II vs III
Age (years)[1]	***Range*** ***Mean ± SD***	(50-83) 66.9±7.8	(38-83) 61.9±11.9	(50-75) 63.7±11.1	0.389	0.357	0.715	0.821
Sex male/female [n (%)][2]	***Male*** ***Female***	15(75%) 5(25%)	15(75%) 5(25%)	15(75%) 5(25%)	1	1	1	1
BMI (kg/m2)[1]	***Range*** ***Mean ± SD***	(20-34.6) 25.8±3.7	(20-28) 24.3±2.5	(26.8-32.5) 29.2±2.6	***<0.001****	0.104	***<0.001****	***<0.001****
Smoking [n (%)][2]	***No*** ***Yes***	12(60%) 8(40%)	14(70%) 6(30%)	15(75%) 5(25%)	0.583	0.507	0.311	0.723

HCC:hepatocellular carcinoma; **N:** number; **BMI**: Body mass index; **kg/m^2**: Kilogram/meter^2; SD : standard deviation; **ANOVA:** analysis of variance

1= Results are expressed as mean ± SD and compared by One way ANOVA test then by Bonferroni post hoc test.

2= Results are demonstrated as frequency (percentage) and compared by Chi square test or Fisher's exact test

***: Significant level at p value ≤ 0.05**

Table 2 demonstrates that no statistically significant difference was found in any of the studied parameters between cirrhotic patients with HCC and those without. However , the serum levels of total bilirubin, serum glutamate pyruvate transaminase (SGPT), serum glutamic-oxaloacetic transaminase (SGOT), prothrombin time (PT), international normalized ratio (INR) and the tumor marker alpha feto protein (AFP) were significantly higher in patients with HCC compared to the healthy control group [1.2(0.9–1.5)mg/dl vs.0.8(0.7–0.9) mg/dl, p < 0.001; 42.5(34.3–75.8)IU/L vs. 26(18–38)IU/L, p<0.001; 59(37.3–86.5) IU/L vs. 31(25–32)IU/L, p<0.001; 14.1±2.9sec. vs. 12.3±1.1sec., p = 0.042; 1.4±0.5 vs. 1.1±0.1, p = 0.041, and 56.5(22.5–390.3)ng/ml vs.5(3–8) ng/ml, p<0.001, respectively]. However, compared to the healthy control group, HCC patients displayed statistically significant lower blood albumin and platelet count values. [185(130.5-238.8) (1×10^3/µl) vs. 235(190.8-248) (1×10^3/µl), p = 0.013, and 3.3±0.8 gm/dl vs. 4.2±0.5 gm/dl, p = 0.029, respectively]. When HCC patients were classified according to TNM and Okuda staging systems, we found that 5(25%) of them were TNM I+II and 15(75%) were TNM III+IV, while 11(55%) were classified as Okuda stage 1 and 9(45%) were Okuda stage 2 and 3.

Table 2: Baseline characteristics of the study groups

		Group I HCC	Group II Cirrhotic patients	Group III Control	p value			
		N=20	N=20	N=20	Among 3 groups	I vs II	I vs III	II vs III
Haemoglobin gm/dl) [1]	*Range Mean ± SD*	(7.8-15.9) 12.5±1.8	(7.8-15.2) 13±1.6	(11.7-15.5) 13.1±1.5	*0.496*	*0.624*	*0.512*	*0.982*
WBCs (1×103/µl)²	*Median IQR*	7.5 (5.3-9.8)	7.5 (4.9-9.2)	4.9 (4.6-12)	*0.417*	*0.797*	*0.192*	*0.356*
Platelets (1×103/µl)²	*Median IQR*	130.5 (185 -238.8)	183 (158.3-214)	235 (190.8-248)	***0.005****	*1*	***0.013****	***0.001****
Total Bilirubin (mg/dl) [2]	*Median IQR*	1.2 (0.9-1.5)	1 (0.8-1.2)	0.8 (0.7-0.9)	***<0.001****	*0.338*	***0.001****	***0.001****
SGPT (IU/L) [2]	*Median IQR*	42.5 (34.3-75.8)	42.9 (36-65.9)	26 (18-38)	***0.001****	*0.892*	***0.001****	***0.001****
SGOT IU/ L) [2]	*Median IQR*	59 (37.3-86.5)	57.9 (44.6-81.6)	31 (25-32)	***<0.001****	*0.924*	***<0.001****	***<0.001****
Albumin (gm/dl) [1]	*Range Mean±SD*	(2.3-4.6) 3.3±0.8	(2.3-4.9) 3.9±0.8	(3.6-4.3) 4.2±0.5	***0.035****	*0.207*	***0.029****	*0.634*
PT (seconds)[1]	*Range Mean ±SD*	(11.3-24) 14.1±2.9	(11.3-24) 13.7±3.1	(11-14) 12.3±1.1	***0.046****	*0.737*	***0.042****	*0.200*
INR[1]	*Range Mean±SD*	(1-2.4) 1.4±0.5	(1-2.4) 1.3±0.4	(1-1.3) 1.1±0.1	***0.046****	*0.794*	***0.041****	*0.224*
Urea (mg/dl)[2]	*Median IQR*	37 (27-44.5)	30.5 (27-37.8)	29 (26-49.3)	*0.529*	*0.244*	*0.438*	*0.724*
Creatinine (mg/dl)[2]	*Median IQR*	0.9 (0.6-1.1)	0.9 (0.7-1)	0.7 (0.6-0.8)	*0.076*	*0.935*	*0.084*	0.1480
AFP (ng/dl) [2]	*Median IQR*	56.5 (22.5-390.3)	20 (5.1-400)	5 (3-8)	*<0.001**	*0.078*	*<0.001**	<0.001*
TNM stage [n. (%)] I+II III+IV	*5 (25%) 15(75)*	–	–	–	–	–	–	
Okuda stage [n. (%)] 1 2+3	*11(55) 9(45%)*	–	–	–	–	–	–	

HCC: hepatocellular carcinoma; N: number; SD: standard deviation;* ANOVA: *analysis of variance; IQR: interquartile range; WBCs: white blood cells; SGPT: serum glutamate pyruvate transaminase; SGOT: serum glutamic-oxaloacetic transaminase; PT: prothrombin time; INR: international normalized ratio; AFP: alpha fetoprotein. TNM: tumor, nodes and metastasis.

1= Results are expressed as mean ± SD and compared by One way ANOVA test then by Bonferroni post hoc test.

2=Results are displayed as median (IQR) and compared by Kruskal Wallis test followed by Mann Whitney test between each two groups.

****: Significant level at p value ≤ 0.05***

Table 3 shows that there was no statistically significant difference between cirrhotic patients having HCC and those without as regard the different hepatic functional scoring systems applied in this study.

Table 3: Comparison of scores of hepatic functional status in cirrhotic patients with hepatocellular carcinoma against those without.

		Group I HCC	Group II Cirrhotic patients	P value
		N=20	N=20	
Child-Pugh score[1]	*Range* *Mean ± SD*	(5-8) 6.3±1.3	(5-8) 5.6±0.9	*0.053*
Child-Pugh class [2]	*Class A* *Class B*	14(70%) 6(30%)	16(80%) 4(20%)	*0.465*
MELD score[3]	*Median* *IQR*	10 (7.3-13)	7 (6 -11)	*0.154*

HCC: hepatocellular carcinoma; N: number; IQR: interquartile range; MELD: model for end-stage liver disease,

1.Data are expressed as mean ± SD and compared using Student's t-test

2. Data are displayed as frequency(percentage) and compared using Chi-square and Fisher's exact test when appropriate.

3.Data are displayed as median (IQR) and compared using Mann Whitney test

Table 4 shows that HCC cases showed significantly lower levels of GHR expression in comparison to cirrhotic group without HCC (0.5(0–3.8) vs. 4(0.8-6), p=0.024) and control group (0.5(0–3.8) vs. 6(4-6), p<0.001)

Table 4: Comparison of hepatic growth hormone receptor expression in the study groups.

epatic expression		Group I HCC	Group II Cirrhotic patients	Group III Control	P value			
		N=20	N=20	N=20	Among 3 groups	I vs II	I vs III	II vs III
GHR score	*Median* *IQR*	0.5 (0-3.8)	4 (0.8-6)	6 (4-6)	***<0.001****	***0.024****	***<0.001****	***0.013****

HCC: hepatocellular carcinoma; N=number; IQR: interquartile range ; GHR:growth hormone receptor, Data are displayed as median(IQR) and compared using Kruskal Wallis test then by Mann Whitney test .

****: Significant level at p value ≤ 0.05***

Table 5 shows that the hepatic GHR expression was positively correlated with BMI (r = 0.31; p = 0.016). On the contrary it was negatively correlated as regard age (r= -0.490; p<0.001), AFP (r= -0.39, p=0.019).

Table 5: Correlations of hepatic expression of growth hormone receptor with different clinicobiochemical parameters.

	N	*GHR*	
		r	***P value***
Age (years)(p)	60	***-0.490***	***<0.001****
BMI (kg/m^2)(p)	60	***0.312***	***0.016****
Smoking (S)	60	*0.095*	*0.469*
Hemoglobin (gm/dl)(P)	60	*-0.017*	*0.900*
WBCs (x10^3/ml)(P)	60	*0.062*	*0.638*
Platelets (x10^3/ml)(P)	60	*0.007*	*0.956*
Total bilirubin (mg/dl) (P)	60	*-0.067*	*0.612*
SGPT (IU/l)(P)	60	*-0.006*	*0.721*
SGOT (IU/l) (P)	60	*0.241*	*0.432*
Albumin (gm/dl) (P)	60	*0.166*	*0.204*
PT (seconds)(P)	60	*-0.216*	*0.098*
INR (P)	60	*-0.116*	*0.377*
Urea (mg/dl)(P)	60	*0.181*	*0.167*
Creatinine (mg/dl) (P)	60	*-0.064*	*0.628*
AFP (ng/ml)(P)	60	***-0.39***	***0.019****
CHILD-Pugh Class (S)	40	*0.013*	*0.935*
Child-Pugh score (P)	40	*-0.063*	*0.700*
MELD score (P)	40	*0.051*	*0.754*

N: number of patients; GHR: growth hormone receptor, BMI: body mass index; WBCs: white blood cells; SGPT: serum glutamate pyruvate transaminase; SGOT: serum glutamic-oxaloacetic transaminase; AFP: alpha fetoprotein; PT: prothrombin time; INR: international normalized ratio; MELD: model for end-stage liver disease. (P) Pearson's correlation; (S) Spearman's correlation.

Grading of correlation coefficient:0-0.29=mild;0.30-0.49=moderate; 0.50-1=marked

****: Significant level at p value ≤ 0.05***

Table 6 demonstrates that HCC patients with lower hepatic expression of GHR had a significantly higher frequency of cases whose AFP > 100 ng/ml. (76.4% vs. 23.6%; p = 0.040); tumor diameter >5cm (78.5% vs. 21.5%; p =0.01); vascular invasion (85.7 %vs. 14.3%; p = 0.001), and advanced TNM stage (80 % vs. 20%; p = 0.010)

Table 6: Relationship between hepatic expression of growth hormone receptor, and clinicopathological characteristics of the tumor in hepatocellular carcinoma patients

variable	Hepatic expression of GHR			
	N	**No/low N.(%)**	**High N.(%)**	**p-value**
Age (year)				
<60	4	4(100)	0(0)	0.197
>60	16	11(68.7)	5(31.3)	
Gender				
Male	14	10(71.4)	4(28.6)	0.573
Female	6	5(83.3)	1(16.7)	
Child-Pugh class				
A	14	10(71.4)	4(28.6)	0.573
B+C	6	5(83.3)	1(16.7)	
MELD score				
<=12	15	11(73.3)	4(27.7)	0.766
>12	5	4(80)	1(20)	
AFP				
≤100 ng/ml	3	1(33.3)	2(66.7)	**0.040**
>100 ng/ml	17	13(76.4)	4(23.6)	
Tumor number				
Single	12	10(83.3)	2(16.7)	0.292
Multiple	8	5(62.5)	3(37.5)	
Tumor diameter(cm)				
=<5	6	2(33.3)	4(66.7)	**0.010**
>5	14	11(78.5)	2(21.5)	
Vascular invasion				
No	6	2(33.3)	4(66.7)	**0.001**
Yes	14	12(85.7)	2(14.3)	
Lymphatic permeation				
No	18	13(72.2)	5(27.8)	0.389
Yes	2	2(100)	0(0)	
TNM [n.()]				
I	5	2(40)	3(60)	**0.010**
II+III+IV	15	12(80)	3(20)	
Okuda [n.()]				
1	11	7(63.7)	4(36.3)	0.194
2+3	9	8(88.9)	1(11.1)	

N: number of patients; MELD: model for end-stage liver disease; AFP: alpha fetoprotein; GHR: growth hormone receptor, TNM: tumor, nodes and metastasis.
Data are expressed as proportions and percentages, and compared using Chi-square statistic or Fisher's exact test.
****: Significant level at p value ≤ 0.05***

Figure 1 (a-c), shows the hepatic expression of GHR in the studied groups.

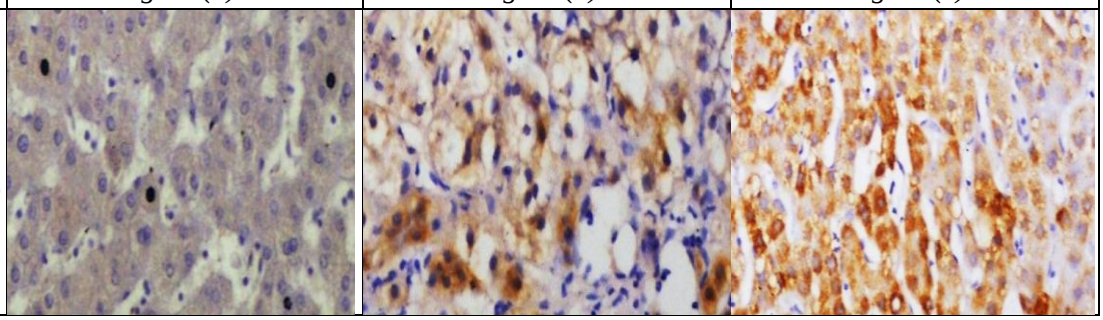
	Control	Cirrhosis	HCC
	Figure (a)	*Figure (b)*	*Figure (c)*
GHR			

Figure 1:(a-c): Hepatic expression of growth hormone receptor in the study groups
Magnification 200 X scale bare 100um.
GHR expression: a) in healthy control: b) in cirrhotic patients; c) in HCC patients; (mainly cytoplasmic)
GHR: growth hormone receptor; HCC: hepatocellular carcinoma.

Figure 2(a-b), shows the hepatic expression of GHR, in liver sinusoidal endothelial cells, in HCC patients versus cirrhotic patients**.**

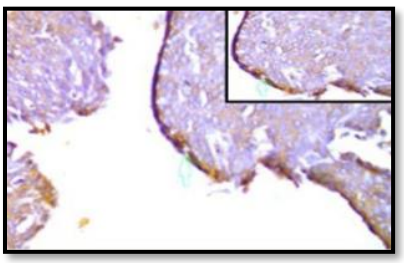
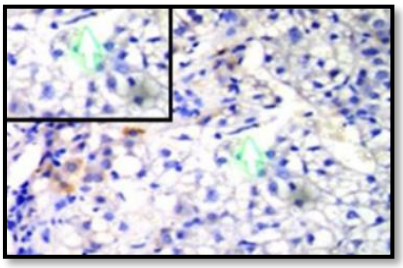
Immunohisto-chemical marker and detection site	Hepatocellular carcinoma	Liver cirrhosis
GHR expression in liver sinusoidal endothelial cells	 **(a): positive cytoplasmic immunoexpression**	 **(b): negative cytoplasmic immunoexpression**

Figure 2: The expression of growth hormone receptor in liver sinusoidal endothelial cells in hepatocellular carcinoma patients versus cirrhotic patients Images are presented at 200X magnification power with 400X zoom in boxes.
HCC= hepatocellular carcinoma, GHR=growth hormone receptor,

Discussion

As far as we know, the role of expression of GHR in those with HCV-associated HCC is not well investigated yet.

Numerous researchers have revealed that hepatic expression of GHR was elevated in human tumors[17–20] due to its nuclear localization that triggers cell cycling and oncogenesis[18]. Others claimed that binding of GH to those abnormally manufactured GHR in tumor cells motivate the JAK2, and settles epithelial-to-mesenchymal transition (EMT) which assists tumor progression[19].

On the contrary, in this study, the hepatic expression of GHR was shown to be significantly suppressed in HCC patients in comparison with cirrhosis and control groups.

This observation can be attributed to hepatocellular dysfunction resulting from chronic liver insult and the burden of the tumor[21]. Our results agreed with the few researches that are available in the literature. [14]. However, different methods were used in these investigations to measure GHR hepatic expression. Lower levels of GHR have been linked to a condition of resistance to GH action, which might be connected to impaired GH binding to GHR, reduced GH clearance, and poor IGF-1 production as a result of hepatocyte injury [22]

GH performs its pro-oncogenic actions by inducing a pro-tumorigenic environment through a variety of mechanisms. This phenomena originates from the fact that excessive growth hormone affects Deoxy Ribonucleic Acid (DNA) repair, allowing damaged DNA to accumulate unrepaired, increasing the chance of oncogenic aberrations[23]. Furthermore, GH suppresses numerous tumor suppressor genes, including p53 which promotes cell division[4]. Additionally, elevated GH might trigger the EMT by repressing the expression of the cell adhesion protein E-cadherin [18]. Moreover, Kong et al.,.[25] had reported the effect of GH on EMT in hepatoma patients. GH promotes cellular migration and invasion in addition to helping tumor cells acquire characteristics similar to cancer stem cells by suppressing Claudin-1, another tight junction protein, through STAT3 activation in HCC [20].

According to Lee et al.,[27]**,** HCV infection might cause a variety of inflammatory and fibrotic mediators in HCV-related HCC patients, including stellate cell activation and the production of proinflammatory cytokines, reactive oxygen species, and cell death signals[22].

Collectively, these occurrences create a cirrhotic environment that is permissive to the formation of cancer, a process described as "field cancerization" that starts and accelerates hepatocarcinogenesis and likely dysregulates GHR expression[23]. One of the cell cycle inhibitors that can result in an aggressive form of HCC is CDKN1A, which is down regulated by the viral core protein[24]. Carroti et al.,[31] reported a suppressive effect on GH-IGF1 axis induced by HCV infection. In a study done by Lin et al.,.[14], it was found that the suppressed GHR expression in HCV patients was reversible upon treatment with direct acting antiviral drugs.

This study demonstrated a strong negative correlation between hepatic expression of GHR and age of the patients in the HCC group. In early life, the GH-IGF-1 axis is more active due to the much production of sex hormones [32]. With aging, the activity of this axis declines to protect DNA from the negative effects of GH on its repair.[33]. This may provide a reasonable explanation for our findings. We found a positive correlation between hepatic expression of GHR and patients' BMI. Chronic liver illnesses can cause malnutrition due to insufficient food intake, impaired metabolism, and malabsorption[34,35]. Malnutrition can alter the GH-IGF-1 pathway by causing GH resistance, suppressing hepatic production of GHR and IGF-1 mRNA, and lowering IGF-1 bioactivity [36] However, the exact mechanism(s) remains unknown [37]

Furthermore, a relation was found between some factors associated with a worse prognosis in HCC patients and lower expression of GHR. Those factors included elevated serum AFP levels, larger tumor size, vascular invasion, and advanced histopathological stage. Lin et al.,. [14], reported nearly equivalent relationships which indicates that lower expression of GHR and the resulting state of GH resistance might be associated with more carcinogenic state and more loss of the proapoptotic function of GH [14,38].

Another important finding in this study is the observation of GHR expression in the liver sinusoidal endothelial cells (LSECs), which constitutes with a range of cells the HCC microenvironment. Patten et al.,[39], mentioned that the LSECs permit the acquisition of angiogenesis, the aggravation of fibrosis, the persistence of viral infections, and EMT. All of which increase the risk of chronic liver injury and, consequently, cancer.

Certainly, this study has some limitations; first, the quite small size of the studied sample. Second, the retrospective design of the study.

Lastly, we couldn't rule out the impact of HCV on GHR expression in the liver.

In conclusion, by utilizing the immunohisto-chemistry (IHC) approach, we discovered that down-regulation of GHR was associated with the development of HCV-related HCC, as well as tumor aggressiveness regardless of liver function. Nevertheless, further validation through extensive prospective, multi-center studies was required to validate the practical significance of these findings.

Authors contribution:
All authors contributed to the Material preparation, study conception and design were performed by **[**Prof. Dr. Mona Abdel Rahman Abu El-Makarem**]** data collection and analysis were performed by **[**Prof. Dr. Mona Abdel Rahman Abu El-Makarem**]** and **[**Alaa Al-Deen Mostafa Mohamed**].** The first draft of the manuscript was written by **[**Alaa Al-Deen Mostafa Mohamed**]** Pathologic examination was done by **[**Prof. Dr: Mariana Fathy Kamel] and [Dr. Mahmoud Gamal Amin] and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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ABBREVIATIONS

AFP	Alpha Feto Protein
ANOVA	Analysis Of Variance
BMI	Body Mass Index
DNA	Deoxy Ribonucleic Acid
EMT	Epithelial-To-Mesenchymal Transition
GH	Growth Hormone
GHR	Growth Hormone Receptor
GHRH	Growth Hormone Releasing Hormone
HCC	Hepatocellular Carcinoma
HCV	Hepatitis C Virus
IBM	International Business Machines Corporation
IGF-1	Insulin Like Growth Factor One
INR	International Normalized Ratio
IQR	Inter-Quartile Range
JAK2	Janus Kinase 2
LSECS	Liver Sinusoidal Endothelial Cells
MELD	Model For End-Stage Liver Disease
PT	Prothrombin Time
SD	Standard Deviation
SGOT	Serum Glutamic-Oxaloacetic Transaminase
SGPT	Serum Glutamate Pyruvate Transaminase
SPSS	Statistical Package For Social Sciences
SST	Somatostatin
STAT	Signal Transducer And Activator Of Transcription
TNM	Tumor, Node and Metastasis
WBC	White Blood Cells

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