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Vitronectin (VTN): A Novel Diagnostic and Prognostic Marker for Hepatocellular Carcinoma (HCC) on Top of Chronic Hepatitis C Virus Related Diseases

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ABSTRACT

Background: Hepatocellular carcinoma is the most common liver tumor, which is the fifth most prevalent cancer overall.

Objective: The comparison of alpha-fetoprotein (AFP) and serum vitronectin (VTN) in the diagnosis and prognosis of hepatocellular carcinoma in addition to hepatitis CV-related liver disorders.

Patients and Methods: There were 60 participants in this prospective observational study, and they were split into 4 groups as follows: Group 1 had ten average people. Ten individuals in group 2 with hepatitis C virus infection were included. Twenty individuals with cirrhosis were in group 3. Twenty patients in Group 4 with HCC (in addition to cirrhosis caused by the hepatitis C virus) had tests for vitronectin and AFP before and three months after intervention.

Results: We discovered a statistically significant difference in low platelet count, high serum Cr, high INR, low serum Alb high bilirubin, and greater aspartate aminotransferase between the cirrhotic group and the non-cirrhotic groups. The median level of alfa-fetoprotein in group 4 (Hepatocellular carcinoma patients) was 110 IU/millilitre, which was substantially higher than the median value in the other research groups. The median vitronectin level was unable to differentiate among the different research groups. There was no statistically significant difference in the serum level of vitronectin between the 3 categories in group 3 (cirrhotic patients), according to the Child's classification.

Conclusions: Chronic HCV infection and hepatocellular carcinoma are both health issues in Egypt. As blood AFP levels may be normal in as much as forty percent of patients with hepatocellular carcinoma, particularly in the early stages, it has low specificity for recognising cirrhosis, chronic hepatitis, or cholangiocarcinoma and limited sensitivity for detecting hepatocellular carcinoma.

Keywords: Vitronectin, Hepatocellular carcinoma, HCV, AFP.

INTRODUCTION

In Egypt, HCC is 2.3 percent more common than other malignancies overall. During a ten-year period in Egypt, the prevalence of HCC in those with chronic liver disease nearly doubled, with 48% of cases being associated with liver cirrhosis brought on by the hepatitis C virus (HCV). In fact, after cirrhosis has been diagnosed, it is now widely known that HCC almost usually develops in chronic HCV patients [(1)].

Patients who have chronic HBV or HCV infection should be examined with ultrasound and serum alpha-fetoprotein (AFP) since they are more prone to develop HCC [(2)]. Using imaging or lab methods, HCC can be diagnosed. The three primary radiological diagnostic methods are ultrasound, triphasic CT and dynamic MRI. Ultrasonography sensitivity for the detection of HCC is directly impacted by size of tumor. Other serious problem is how operator-dependent the US is [(3,4)]. HCC can be diagnosed in a laboratory by measuring circulating biomarkers or by invasive fine-needle cytology with intra- or inter-observer variability [(5)].

The American Association for the Study of Liver Diseases recommended that blood levels of AFP >200 ng per millilitre as a substitute for FNC for diagnosis, particularly in people with liver cirrhosis. A third of patients with early-stage HCC and small tumors (less-than 3 centimeters) go undiagnosed despite AFP's modest diagnostic performance (sensitivity: 39-65 percent; specificity: 76-94 percent). But it's vital to consider that certain kinds of tumors, chronic hepatitis, and cirrhosis of the liver all have increased blood AFP levels. Newer markers are needed to address these problems and enable an earlier diagnosis of HCC [(5,6)].

AFP levels can be elevated in a variety of disease presentations, which include metastatic colon tumor and intrahepatic cholangiocarcinoma, for instance [(7)]. Despite the fact that as many as thirty to forty percent of HCC patients have normal AFP levels, AFP has a low level of specificity and sensitivity [(8)].

Vitronectin (VTN or VN), a glycoprotein of the hemopexin family, is widely present in serum, the ECM, and bone [(9)]. VTN is a glycoprotein produced and released by the liver [(10)]. Vitronectin has been shown to effectively attach to and integrate into the extracellular matrix (ECM) of a variety of human tissues, promoting cell adhesion and differentiation while also controlling ECM stability and composition [(11)]. Malignancy may be influenced by vitronectin, according to certain theories [(12)].

The study's aim was to compare the roles of AFP and serum VTN to diagnose and prognose hepatocellular carcinoma as well as liver diseases linked to HCV.

PATIENTS AND METHODS

This was a prospective observational study, conducted over a period of six-months in EL-Mahalla Hepatology Teaching Hospital. The study included 60 cases.

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Study population:

HCC diseased patients (additional to cirrhosis caused by the HCV) were included in the study both before and after the intervention, along with patients with chronic hepatitis C infection, patients with non-cirrhotic hepatitis C without cirrhotic liver, and healthy individuals as controls. Patients with significant co-morbidities, chronic HBV infection, non-viral hepatitis (such as alcoholism, Wilson's disease, hemochromatosis, and AI hepatitis), non-compliant patients, non-alcoholic hepatitis, non-alcoholic steatosis, and non-alcoholic hepatitis were excluded from the study.

Study groups:

The study's participants were divided into four groups: The first group comprised ten common folks. Ten people with the HCV infection made up Group 2. There were twenty cirrhotic people in group 3. Group 4: In just three months, vitronectin levels were checked in twenty individuals with HCC who also had cirrhosis brought on by the HCV.

Study groups patients underwent:

1. Full history taking (smoking and chronic diseases).
2. Full clinical examination.
3. Laboratory investigations:

- LFT (Serum Alb, Serum bilirubin, (INR), ALT and AST).
- PCR detect Anti-HCV and HCV.
- Sr Cr.
- AFP sr levels.
- (VTN) serum levels assessed by ELISA.
- **Measurement of serum vitronectin levels [13]:** on the initial visit, all participants' serum samples were obtained while they were fasting. The manufacturer's instructions and an enzyme-linked immunosorbent test (Takara Bio Inc., Shiga, Japan) were used to measure the amounts of VN (total sixty-five + 75-kDa polypeptide) in serum. The calculated VN levels were calculated using a standard curve. The results were given in µg/millilitre for each serum sample that was analyzed. Laboratory staff made all decisions; they were not privy to any clinical information.

4. Radiology:

Abdominal ultrasonography of the liver, spleen, portal vein, and/or ascites, as well as triphasic tomography abdomen (to diagnose HCC in group 4), were performed using multidetector tomography.

Patients' preparation: Patient was instructed to be fasting at least 2 hours before the exam, to remove clothing and put on a gown, also to remove any metal interferes with the scanning equipment, rechecking of the patient serum creatinine, establishment of good intravenous line for contrast injection and reassurance of the patient.

Image acquisition:

Patient supine, head first, with arms above the head. Images were obtained with a 128 detector rows CT scanner at (120 kV, 200-250 mAs, windows width: 250, window level: 35, FOV: 400 mm, Matrix 512 x 512, pitch: 1.3), rapid collimation and slice thickness 10 mm. Digital scout view was obtained and the precise area to be scanned was selected. Scanning was performed from the lung bases (dome of the liver) down to iliac crest in craniocaudal direction. First, a pre-contrast scan view was obtained. The patient was given 1.5 ml/kg IV non-ionic contrast (scan lux 370 or optiray 350) with an overall dosage ranging from 100-120 ml via power injector at two-three ml/s (pace and volume dependent on IV access, patient weight, and kidney functions). Following an IV contrast material injection, the liver was examined in three phases: arterial (scanning delay, 10-15 seconds), portal (scanning delay, 60-90 seconds), and equilibrium (scanning delay, 2-5 minutes). Image processing: A soft copy workstation (PACS station) review was used for image interpretation and processing.

Ethical approval:

EL-Mahalla Hepatology Teaching Hospital gave its approval to this study. All participants gave written consent after receiving all information. The Helsinki Declaration was followed throughout the study's conduct.

Statistical analysis

SPSS version 22 for Windows® was used. To represent qualitative data, percentages and numbers (frequency) were employed. The groups were compared using the Chi-Square test (X^2). The normality of quantitative data was examined using the Kolmogorov-Smirnov technique. Normally distributed data were displayed as mean±SD. For comparing more than two groups, one-way ANOVA was used (given as F). Non-parametric data were displayed as the median (min - max) and were compared by Kruskal-Wallis test (abbreviated as KW). The relationship between the categorical variable (study group) and the quantitative predictor variable (vitronectin) was examined using the ROC curve and logistic regression analysis. Statistics were regarded as significant when $P<0.05$.

RESULTS

Between the study groups, there was a very statistically significant difference in the mean age of the patients. There were 29 males and 31 females in total, with a substantial sex distribution disparity between the research groups. In the HCC group, males made 80% of the cases (Table 1).

Table (1): Demographic data comparing within the study groups.

Variable	Group 1 No= 10	Group 2 No= 10	Group 3 No=20	Group 4 No=20	Test of sig.
Age (Years)					
Mean ± SD	50.40± 11.6	47.70± 6.8	56.15± 7.9	61.40± 5.2	F= 8.668 P < 0.001*
Gender					
Males	4 (40%)	1 (10%)	8 (40%)	16 (80%)	χ^2=14.750 P = 0.002*
Females	6 (60%)	9 (90%)	12 (60%)	4 (20%)	

*: Significant

The analysis of the different laboratory investigation within the cases of the study groups is shown in table (2). Except for the HGB and WBCs, between the various research groups, all of the analysed metrics showed a considerable level of significance.

Table (2): Lab investigations comparing within the study groups.

Variable	Group 1 No= 10	Group 2 No= 10	Group 3 No=20	Group 4 No=20	Test of sig.
HB (g/dL)					
Mean ± SD	12.94± 1.81	13.46± 1.7	11.52± 2.4	12.37± 2.4	F= 2.030 P = 0.119
PLTs (10^3) (mcL)					
Median (min-max)	258.5 (176-324)	233.5 (177-279)	74.5 (22-136)	134 (80-312)	KW= 39.219 P < 0.001*
WBCs (10^3) (mcL)					
Median (min-max)	6.05 (4.6-10.3)	7.10 (4.7-11.3)	6.26 (2.6-18.6)	6.2 (2.6-10.3)	KW= 3.424 P = 0.331
Creatinine (mg/dl)					
Median (min-max)	0.86 (0.51-1.1)	0.69 (0.61-1.23)	1.38 (0.35-2.7)	0.85 (0.45-1.4)	KW= 18.729 P < 0.001*
INR					
Mean ± SD	1.06± 0.11	1.02± 0.10	1.78± 0.36	1.23±0.20	F= 32.99 P < 0.001*
Alb (g/dL)					
Mean ± SD	4.46± 0.27	4.28± 0.46	2.79± 0.63	3.51±0.69	F=23.847 P < 0.001*
Total bilirubin (µmol/L)					
Median (min-max)	0.73 (0.50-1.1)	0.74 (0.24-1.06)	4.39 (0.30-20.6)	1.09 (0.39-2.11)	KW= 24.364 P < 0.001*
Direct bilirubin (µmol/L)					
Median (min-max)	0.21 (0.19-0.38)	0.29 (0.16-0.7)	1.8 (0.12-12)	0.56 (0.15-.9)	KW= 26.015 P < 0.001*
ALT (U/L)					
Median (min-max)	33 (19-40)	26 (17-66)	43.5 (10-70)	46.5 (16-210)	KW= 11.389 P = 0.010*
AST (U/L)					
Median (min-max)	34 (22-40)	23 (16-81)	57.5 (10-97)	57 (28-320)	KW= 16.359 P = 0.001*

Median, min-max: non-parametric test, **Mean ± SD: parametric test**

*: Significant

The median AFP level in group 4 was greater than the median value in the other research groups, with a high degree of significance. The median level of vitronectin levels in the four research groups did not disclose a statistical significance discrepancy (Table 3).

Table (3): Alfa-fetoprotein and vitronectin levels comparing before treatment in the four study groups

Variable	Group 1 No= 10	Group 2 No= 10	Group 3 No=20	Group 4 No=20	Test of sig.
AFP level before					
Median (min-max)	3.5 (1.2-27.2)	4.45 (1-6.8)	26.5 (1.1-63)	110 (2.6-1800)	KW= 31.803 P < 0.001*
Vitronectin level before					
Median (min-max)	8 (5-66)	7.6 (0-20)	15 (6-60)	11 (0-62)	KW= 3.582 P = 0.310

Median, min-max: non-parametric test.
*: Significant

The serum level of vitronectin didn't show any significant difference between the 3 subgroups of cirrhotic patients, according to the Child's classification.

Table (4): Vitronectin level comparing in the cirrhosis group according to Child- Pugh classification

Variable	CHILD A No= 1	CHILD B No= 6	CHILD C No=13	Test of sig.
Vitronectin level				
Median (min-max)	19	14 (6.5-18)	20 (6-60)	KW= 2.837 P = 0.303

HBsAg was negative for all groups cases. All the cases within the control group were negative for HCV abs analysis while all the cases in the other three groups were positive for HCV abs. A significant degree of difference was found between the various study groups.

Group IV (HCC) serum levels of AFP and vitronectin were examined before and after the initial intervention. The median level of AFP showed significantly lower than it had been before to medication whereas there was statistical insignificant decline in vitronectin level (Table 5).

Table (5): Alfa-fetoprotein and vitronectin level in group IV before and after first intervention

	Group IV		Test of significance
	Before first intervention (N=20)	After first intervention (N=20)	
AFP			
Median (min-max)	**110** (2.6-1800)	**70** (1-742)	z=- 3.454 **p= 0.001***
Vitronectin			
Median (min-max)	11 (0-62)	10.75 (3-26)	z=- 0.654 p= 0.513

Median, min-max: non-parametric test.
*: Significant

The predictive value of vitronectin to differentiate between control group and HCV +ve cases is shown in table 6. The cutoff point was 7.75 (Table 6 and figure 1).

Table (6): Vitronectin ability prediction to differentiate between normal and HCV +ve cases

AUC (95 %) CI	P value	Cutoff point	Sensitivity	Specificity	PPV	NPV	Accuracy
0.641 (0.469: 0.813)	**0.162**	**< 7.75**	**52 %**	**68 %**	**70.5 %**	**89 %**	**58 %**

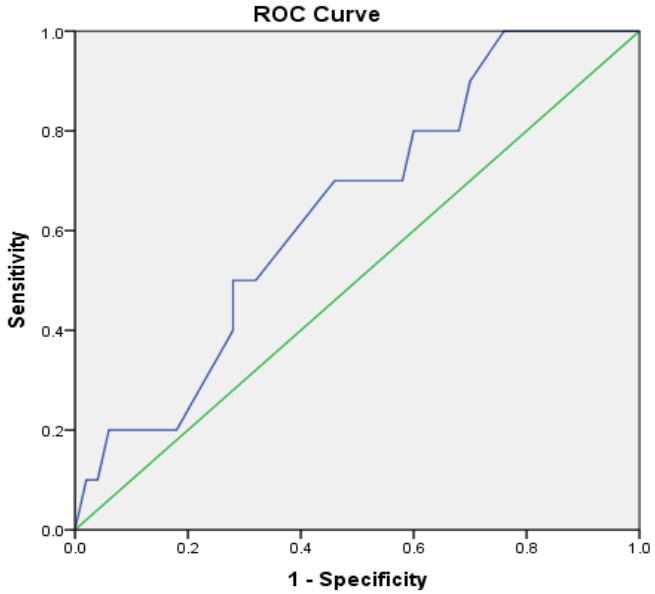

Figure (1): Vitronectin ability prediction to differentiate between normal and HCV +ve cases.

The predictive value of vitronectin to differentiate between HCV +ve and cirrhotic cases is shown in table 7 and figure 2. The cutoff point was 11.1.

Table (7): Prediction of ability of vitronectin for differentiation between HCV +ve cases and cirrhosis

AUC (95 %) CI	P value	Cutoff point	Sensitivity	Specificity	PPV	NPV	Accuracy
0.629 (0.485: 0.773)	**0.106**	***??*** **11.1**	**65 %**	**54 %**	**76.5 %**	**61.2 %**	**60 %**

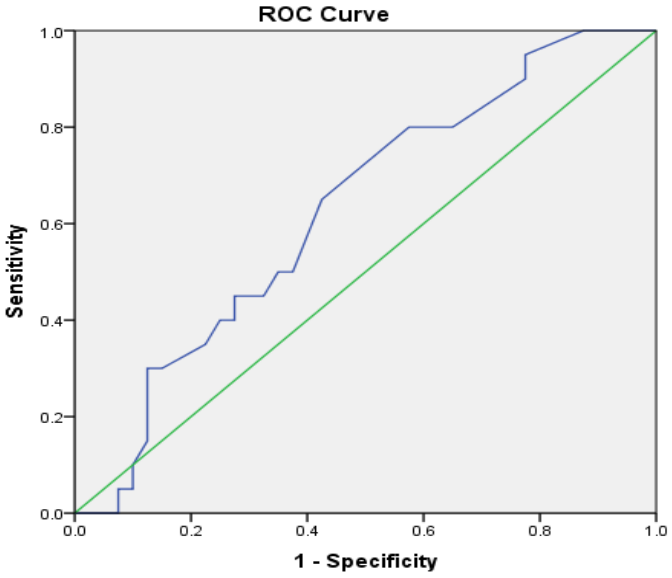

Figure (2): Prediction of ability of vitronectin for differentiation between HCV +ve cases and cirrhosis.

The predictive value of vitronectin to differentiate between cirrhotic and HCC cases is shown in table 8 and figure 3. The cut-off point was 17.2.

Table (8): Prediction vitronectin ability for differentiation between cirrhosis and HCC

AUC (95 %) CI	P value	Cut-off point	Sensitivity	Specificity	PPV	NPV	Accuracy
0.543 (0.389: 0.696)	**0.594**	**> 17.2**	**73 %**	**48 %**	**77 %**	**80 %**	**75 %**

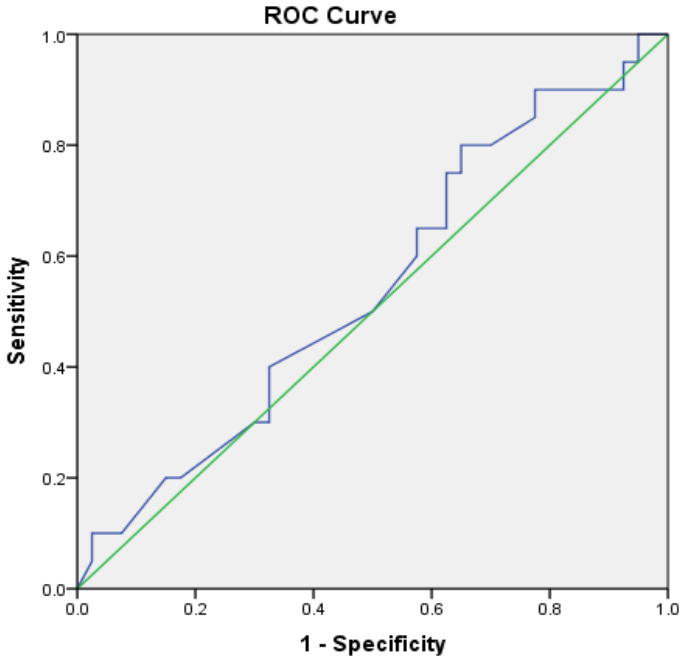

Figure (3): Prediction vitronectin ability for differentiation between cirrhosis and HCC.

Regarding the type of intervention performed in cases with HCC; 10 cases (50%) underwent trans-arterial chemo-embolization (TACE), 6 cases (30%) underwent microwave ablation (MW), 2 cases (10%) underwent radio frequency ablation (RFA) and 2 cases (10%) underwent surgical resection.

DISCUSSION

It is now believed that hepatocellular carcinoma is one of the most common malignancies and a major reason of death in Egypt due to chronic HCV linked cirrhosis high incidence [(14)]. When a tumor is discovered, it has frequently reached a point where there are no effective treatments left. Therefore, early diagnosis would benefit the patient and stop the increase in healthcare costs [(15)].

Hepatocytes are the main producers of the acute-phase glycoprotein called vitronectin, which is secreted. Through interactions with integrins, it is found in serum and the ECM and promotes cell attachment and dissemination [(16)]. It is present in small amounts in healthy ECM, but its concentrations increase in chronic liver inflammation, where its deposition in the sinusoids is an indication of progressing fibrosis [(17)]. Unfavourable prognostic variables are linked to increased VTN in hepatocellular carcinoma [(18)].

The study sixty participants were divided into four groups: a healthy control group, an HCV-positive group, a cirrhotic group, and hepatocellular carcinoma group. **Yang *et al.*** [(13)] inquiry for diagnostic and prognostic value of serum vitronectin evaluation among HCC patients showed a similar study design. However, they added a group of HBV positive individuals rather than HCV cases, and they included more people in each group for a total of hundred and five subjects in the study.

The mean age of the patients varied significantly between the study groups (P<0.001) in terms of statistics. In total, there were twenty-nine men and thirty-one women, with a significant gender difference amongst the research groups. Eighty percent of the cases in the HCC group were made by men. In contrast, **Yang *et al.*** [(13)] claimed that there were no appreciable differences in age or gender amongst the four groups.

According to radiographic findings from another study, 492 individuals were split into 3 groups (fibrotic, cirrhotic, and hepatocellular carcinoma). The study discovered that the median age was 58 years old and that men made up 76.4 percent of individuals who received an HCC diagnosis [(14)]. This is in accordance with comprehensive studies, including one by **Carrilho *et al.*** [(19)], who reported that seventy-eight percent of their 1405 HCC patients in Brazil were men after undertaking a multicenter survey over a 6-year period. According to **Alves *et al.*** [(20)] examination of 210 patients suffering from hepatocellular carcinoma, 83.3 percent (76.6 and 83.3 percent) of them were males.

This was consistent with studies on the prevalence and epidemiological traits of HCC conducted in Egypt, which included 321 hepatocellular carcinoma patients, of which 82.55 percent were men and 17.45 percent were women [(21)]. The findings of **Hussein *et al.*** [(22)] that there were more male patients (75%) than female patients (25 percent), were consistent with this as well. **Goncalves *et al.*** [(23)] discovered a ratio of 3.4:1 man to every woman in a different multicenter survey carried out during a three-year research period. The larger prevalence of risk factors in males, such as smoking, DM, HCV, and exposure to industrial aflatoxins, as well as a putative sex hormone role, can be linked to this sex distribution.

In our study, the mean age of the cases within the HCC groups was 61.40± 5.2 years, which has

higher statistically significant difference as compared to the other groups. In a different study, patients with cirrhosis ranged in age from twenty-three to sixty-two (mean 46.729.03 years), whereas patients with HCC ranged in age from forty-two to 70 (mean 58.705.76 years). Between the two groups, there was a highly significant difference in age [24]. This demonstrated that although the incidence of hepatocellular carcinoma varies by country, it increases progressively with age.

The cirrhotic group had lower platelet count, higher serum creatinine, higher INR, lower serum Alb, higher bilirubin, and higher aspartate aminotransferase, than the non-cirrhotic groups, according to the lab features of the groups under investigation. In contrast, **Baghdady *et al.*** [24] found a significant difference between the groups with and without HCC, with the first group having higher bilirubin, lower albumin, and longer PT. **Okonkwo *et al.*** [25] also discovered that although the mean value of serum Alb was not substantially different between HCC patients and those with liver cirrhosis, it was significantly lower in HCC patients than in those with chronic hepatitis. In hepatocellular carcinoma patients, the average blood bilirubin level was four times the upper limit of normal.

In cirrhotic individuals, the average bilirubin level was 2.8 times the upper limit of normal, and 57.5 percent of them had elevated bilirubin levels. **Elgamal *et al.*** [14] findings that HCC is associated with raised aspartate aminotransferase, thrombocytopenia, hypoalbuminemia, increased bilirubin, and delayed PT, with P less than 0.001 for all measurements, were in agreement with this.

In a different study, there was a statistically significant difference in serum AST levels between the HCC and non-HCC groups ($P<0.05$) [24]. This agrees with findings by **Lopez *et al.*** [8] and **Okonkwo *et al.*** [25] showing the serum AST level in hepatocellular carcinoma was 1.39 times the upper limit of normal and the mean value of AST in HCC was 3.5 times the upper limit of normal, respectively. **Lopez *et al.*** [8] also found that there was a statistical significance discrepancy in ALT serum level between the HCC group and the non-HCC group. These findings make sense given that HCC frequently arises from a background of chronic liver illness.

The median alfa-fetoprotein level in group 4 was 110 IU/millilitre, which was higher than the median value in the other research groups with a high degree of significance ($P<0.001$). The results of **Yang *et al.*** [13] who demonstrated that patients with HCC had blood AFP levels that were significantly higher than those of patients with liver cirrhosis, chronic hepatitis patients, and healthy controls, were consistent with this. Another study found that patients with HCC had median AFP levels that were considerably higher than those with cirrhosis and fibrosis at 69.1 IU/millilitre[14]. This was also in line with a second study [24], which found that HCC patients had noticeably higher AFP serum levels. Furthermore, **Battaglia *et al.*** [26] found that untreated HCC patients had mean plasma concentrations of AFP that were significantly greater than those with chronic liver disease.

According to the median level of vitronectin, there was no statistically significant difference between the various research groups ($p=0.310$). It didn't seem like its value was considerably increasing or decreasing. According to a study by **Peng *et al.*** [27] that looked at changes in serum protein levels, including VTN, in the evolution of the disease, the levels of VTN decreased as hepatitis B advanced from chronic HBV to HBV-induced acute or CLF.

The finding of reduced VTN levels was in agreement with a study by **Tomihira** [28], which showed that plasma VTN levels were lower in chronic liver disease. This lower level of VTN may have been due to a decrease in synthesis, an accumulation of damaged tissues, or a combination of the two. The same authors also discovered that variations in the levels of glycoproteins involved in cell attachment played a significant role in the progression of hepatic fibrosis in patients with CLD. These patients also had lower plasma VTN levels, which were linked to hepatic dysfunction.

Additionally, a different study by **Kobayashi** [17] revealed a relationship between the severity of liver disease and the plasma VTN concentration, which was much lower in CLD than in healthy controls. Notably, patients with chronic liver disease exhibited significantly higher levels of VTN in their liver tissue compared to healthy controls. These results demonstrated that during the healing process, VTN was deposited in injured tissue and functioned as an adhesive protein.

A different study [13], however, found that HCC patients had significantly higher serum VTN levels than the other groups. We also disagreed with **Ferrín *et al.*'s findings** [18], which demonstrated that HCC patients had considerably greater blood levels of VTN whether they had persistent HCV infection or not. In contrast also, **Hwang *et al.*** [29] discovered that the serum VTN level was higher in hepatocellular carcinoma patients compared to healthy controls.

There was no statistically significant difference between the study groups in terms of the median level of HCV RNA as determined by PCR ($p=0.890$).

The cutoff point for the predictive value of vitronectin to distinguish between the control group and HCV positive cases was 7.75, with a fifty-two percent sensitivity, 68 percent specificity, and a fifty-eight percent overall accuracy**.** The cutoff point for vitronectin's predictive value for distinguishment between HCV positive cases and cirrhotic cases was 11.1 with a 65% sensitivity, 54% specificity, and sixty percent overall accuracy. The cutoff point for vitronectin's predictive value to distinguish between cirrhotic and HCC cases was 17.2,

with a 73 percent sensitivity, 48 percent specificity, and a 75 percent overall accuracy.

Our results differed from those of **Yang *et al.* [(13)]**, who showed that VTN levels had significant diagnostic value for distinguishing HCC from control, chronic hepatitis, and cirrhotic liver with accuracy of 88.7%, 84.6%, and 80% respectively.

CONCLUSION

In none of the research groups, vitronectin failed to show a statistically significant difference, and in comparison, to AFP, it had a lower sensitivity and specificity. Therefore, based on the findings of our study, we are unable to use vitronectin alone to diagnose or forecast the fate of HCC in addition to cirrhosis brought on by an HCV +ve infection. More study and investigation are required, according to the evidence that is emerging on VTN, liver cirrhosis, and HCC.

REFERENCES

1. **El-Zayadi A, Badran H, Barakat E *et al.* (2005):** Hepatocellular carcinoma in Egypt: a single centre study over a decade. World J Gastroenterol., 11 (33): 5193-5198.
2. **Bruix J, Sherman M (2005):** Practice Guidelines Committee, American Association for the Study of Liver Diseases Management of hepatocellular carcinoma. Hepatology, 42: 1208-1236.
3. **Dodd I, Miller W, Baron R *et al.* (1992):** Detection of malignant tumors in end-stage cirrhotic livers: efficacy of sonography as a screening technique. Am J Roentgenol., 159: 727-733.
4. **Befeler A, Di Bisceglie A (2002):** Hepatocellular carcinoma: diagnosis and treatment. Gastroenterology, 122: 1609-1619.
5. **Debruyne E, Vanderschaeghe D, Vlierberghe H *et al.* (2010):** Diagnostic value of the hemopexin N-glycan profile in hepatocellular carcinoma patients. Clin Chem., 56: 823-831.
6. **Liu A, Yao T, Wang W *et al.* (2012):** Circulating miR-15b and miR-130b in serum as potential markers for detecting hepatocellular carcinoma: a retrospective cohort study. BMJ Open, 2: e000825. doi: 10.1136/bmjopen-2012-000825.
7. **Wang N, Wang C, Li W *et al.* (2014):** Prognostic value of serum AFP, AFP-L3, and GP73 in monitoring short-term treatment response and recurrence of hepatocellular carcinoma after radiofrequency ablation. Asian Pac J Cancer Prev., 1539–1544.
8. **Lopez J, Balasegaram M, Thambyrajah V *et al.* (1996):** The value of liver function tests in hepatocellular carcinoma. Malaysian Journal of Pathology, 18: 95-100.
9. **Boron W, Boulpaep E (2012):** Medical Physiology. Saunders, Pp. 1097. https://www.academia.edu/44402343/Medical_Physiology_Boron_and_Boulpaep_2UE_2012_
10. **Yasumitsu H, Seno N, Misugi E *et al.* (1993):** Vitronectin secretion by hepatic and non-hepatic human cancer cells. In Vitro Cell Dev Biol., 29: 403-407.
11. **Preissner K (1991):** Structure and biological role of vitronectin. Annu Rev Cell Biol., 7: 275-310.
12. **Hurt E, Chan K, Serrat M *et al.* (2009):** Identification of vitronectin as an extrinsic inducer of cancer stem cell differentiation and tumor formation. Stem Cells, 28: 390-98.
13. **Yang X, Zhou L, Yang Q *et al.* (2016):** Diagnostic and prognostic roles of serum vitronectin in hepatitis B-related hepatocellular carcinoma. Cancer Biomarkers, 17(3): 271-279.
14. **Elgamal S, Ghafar A, Ghoneem E *et al.* (2018):** Characterization of patients with hepatocellular carcinoma on the way for early detection: one center experience. The Egyptian Journal of Internal Medicine, 30(4): 231-35.
15. **Plentz R, Malek N (2015):** Early detection of hepatocellular carcinoma: how to screen and follow up patients with liver cirrhosis according to the GERMAN S3 guideline? Diagnostics, 5(4): 497-503.
16. **Xu J, Mosher D (2011):** Fibronectin and other adhesive glycoproteins the extracellular matrix: an overview, Springer. pp. 41-75. DOI:10.1007/978-3-642-16555-9_2
17. **Kobayashi J, Yamada S, Kawasaki H (1994):** Distribution of vitronectin in plasma and liver tissue: relationship to chronic liver disease. Hepatology, 20(6): 1412-1417.
18. **Ferrín G, Ranchal I, Llamoza C *et al.* (2014):** Identification of candidate biomarkers for hepatocellular carcinoma in plasma of HCV-infected cirrhotic patients by 2-D DIGE. Liver International, 34(3): 438-446.
19. **Carrilho F, Kikuchi L, Branco F *et al.* (2010):** Clinical and epidemiological aspects of hepatocellular carcinoma in Brazil. Clinics, 65(12): 1285-1290.
20. **Alves R, Harriz M, Vanini H *et al.* (2011):** Advanced hepatocellular carcinoma. Review of targeted molecular drugs. Annals of Hepatology, 10(1): 21-27.
21. **El-Zayadi A, Abaza H, Shawky S *et al.* (2001):** Prevalence and epidemiological features of hepatocellular carcinoma in Egypt—a single center experience. Hepatology Research, 19(2): 170-179.
22. **Hussein M, Ibrahim A, Khattab N *et al.* (2010):** Serum transforming growth factor beta1 in hepatitis c virus related chronic liver disease and hepatocellular carcinoma patients. The Medical Journal of Cairo University, 78(2): 702-709.
23. **Goncalves C, Pereira F, Gayotto L (1997):** Hepatocellular carcinoma in Brazil: report of a national survey (Florianópolis, SC, 1995). Revista do Instituto de Medicina Tropical de São Paulo, 39(3): 165-170.
24. **Baghdady I, Fouad F, Sayed M *et al.* (2014):** Serum markers for the early detection of hepatocellular carcinoma in patients with chronic viral hepatitis C infection. Menoufia Med J., 27: 744-750.
25. **Okonkwo U, Nwosu M, Nnadozie O *et al.* (2011):** Is liver function test of any diagnostic relevance in patients presenting with hepatocellular carcinoma? Orient Journal of Medicine, 23(1-4): 17-23.
26. **Battaglia S, Benzoubir N, Nobilet S *et al.* (2009):** Liver cancer-derived hepatitis C virus core proteins shift TGF-beta responses from tumor suppression to epithelial-mesenchymal transition. PloS One, 4(2): e4355. doi: 10.1371/journal.pone.0004355.
27. **Peng L, Liu J, Li Y *et al.* (2013):** Serum proteomics analysis and comparisons using iTRAQ in the progression of hepatitis B. Experimental and Therapeutic Medicine, 6(5): 1169-1176.
28. **Tomihira M (1991):** Changes in plasma vitronectin, fibronectin, and serum laminin P1 levels and immunohistochemical study of vitronectin in the liver of patients with chronic liver diseases. Hukuoka Acta Medica., 82(1): 21-30.
29. **Hwang H, Lee J, Lee H *et al.* (2014**): In-depth analysis of site-specific N-glycosylation in vitronectin from human plasma by tandem mass spectrometry with immunoprecipitation. Analytical and Bioanalytical Chemistry, 406(30): 7999-8011.