Assessment of Von Willebrand Factor in Hepatitis C Patients as Biomarker for Liver Fibrosis and Predictor of HCC

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Abstract

Background: Hepatitis C virus (HCV) infection is a progressive disease that may result in chronic active hepatitis, cirrhosis, and hepatocellular carcinoma. Itis estimated that about 160 million individuals are chronically infected with HCV.

Hepatocellular carcinoma (HCC), the fifth most common cancer in men and the ninth in women, represents an urgent clinical problem, being the second leading cause of cancer-related death worldwide.

Aim of the study: To determine whether VWF is a potential biomarker for liver fibrosis in comparison to other markers of fibrosis and predictor for development of Hepatocellular Carcinoma in comparison with Alpha-feto protein and Des Gamma carboxy .prothrombin

Patients and Methods: This study had been carried out on 50 subjects, age range 34-77 year selected from Virology and Hepatology outpatient clinics at Ain shams university hospitals in .Cairo after informed consent were taken from the patients

Subjects were divided as follows:

Group I: Include 20 HCC patients diagnosed by imaging and alpha-fetoprotein.

Group II: Include 20 matched cirrhotic patients without HCC divided according to child – pugh scoring system.

Group III: Include 10 apparently healthy subjects, age and sex matched, having no acute or chronic illness and taking no medications were taken as control group.

Results: In our study, VW factor was statistically significant higher in cirrhotic patients than control group and in patients with HCC than cirrhotic group without HCC, with weak positive correlation with other markers of liver fibrosis (FIB4, APRI), and weak positive correlation with other markers of hepatocellular carcinoma (alpha feto protein, des gamma carboxy prothrombin). VW factor was .statistically significant higher in advanced stages of liver cirrhosis

Conclusion: *In conclusion*; VWfactor is statistically significant higher in patients with Hepatocellular carcinoma than in cirrhotic .patients without HCC

There is weak positive correlation between VW factor as a biomarker .for liver fibrosis and other scores assessing stage of fibrosis

There is a strong correlation between VW factor and child score used to classify stage of liver cirrhosis, so VW factor is valuable predictor for hepatocellular carcinoma and advanced stages of liver cirrhosis.

Keywords: Hepatocellular carcinoma – liver fibrosis- von willebrand factor – alpha feto protein

Introduction:

Hepatitis C virus (HCV) is a major risk factor for chronic liver disease and for the increasing HCC incidence in most Western countries .(ELserag HB.2011)

Hepatocellular carcinoma (HCC), the fifth most common cancer in men and the ninth in women, represents an urgent clinical problem, being the second leading cause of cancer-related death worldwide.

Current methods for HCC diagnosis are classified into the following main categories: imaging [abdominal ultrasonography, contrast-enhanced computed tomography (CT) and magnetic resonance imaging (MRI)] and laboratory biomarker analysis [serum alpha-fetoprotein (aFP) levels]. however, the diagnostic performance of imaging technologies is unsatisfactory, particularly for the diagnosis of small lesions and early-stage HCC *(hung et al., 2015).*

AFP is the most commonly used tumor marker for HCC diagnosis and prognosis prediction, but the false negative rate using AFP level alone is as high as 40% for patients with early-stage HCC. AFP levels remain normal in 15%-30% of all the patients, even patients with advanced HCC *(Zhang et al., 2014).*

Approximately 3% of the world population is infected with HCV, and the severe consequences of virus infection makes HCV one of the most pressing emergencies worldwide. The majority of infected patients are unable to clear the infection and develop a chronic hepatitis C (CHC) infection (Heim MH et al.2014)

CHC results in inflammation-induced lesions in the liver frequently associated with hepatic fat accumulation (steatohepatitis) and progressive fibrosis, which over 20 to 40 years may evolve in cirrhosis (10 to 20% of patients) or HCC (1e5%) (Fig. 1) (westbrook .(RH et al.2014

According to EASL guidelines, treatment should be initiated at least in patients with advanced fibrosis (Metavir score ≥F3) and is strongly considered in patients with moderate fibrosis (F2). Thus, assessment of liver fibrosis is needed prior to anti-viral therapy.

Liver biopsy is considered the ‘gold standard’ for determination of fibrosis stage, but has drawbacks like sample size, sampling error, high cost, inter- and intra-observer variance. Furthermore, it is associated with patient discomfort, although the risk of major

complications is low, but also includes mortality (1/4000–1/10000).10, 11

Therefore, many non-invasive fibrosis tests have been developed. These indirect biomarkers of fibrosis are composed of easy available variables with one or more fibrosis predicting panels like AST to platelet ratio index (APRI); fibrosis index (FI), fibrosis cirrhosis index (FCI), FIB 4 score and Forns Index.

Transient Elastography (TE) has also the ability to assess fibrosis. TE detects cirrhosis (AUROC 0.87–0.98) more adequately than significant fibrosis (AUROC 0.75– 0.93).

Von Willebrand factor (vWF) is a large important adhesive protein for both platelet adhesion and aggregation. Estimation of vWF-Ag is a well-established method with small inter-laboratory variability.

vWF is mediated by two platelet membrane receptors, glycoprotein (Gp) 1b and Gp IIb/IIIa, in a co-ordinated and synergistic manner.17, 18 For adhesion of vWF to Gp 1b, large vWF-multimers are needed.

vWF-Ag is elevated in liver disease it might be a key player in establishing liver fibrosis.18, 23 vWF-Ag was established as a valuable marker for prediction of varices, portal hypertension and mortality in patients with liver cirrhosis.(La Mura V,et al.2011)

vWF-Ag increases with every Child–Pugh stage.(Lisman T,et

(al.2006

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Patients and Methods: This study had been carried out on 50 subjects, age range 34-77 year selected from Virology and Hepatology outpatient clinics at Ain shams university hospitals in Cairo after informed consent were taken from the patients

Subjects were divided as follow:

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❖ **Groups were age and sex matched**

Inclusion criteria:

1- Adult ≥18years old patients form both sexes.

2- Patients with positive HCV antibody &positive PCR HCV(RNA).

Exclusion criteria:

1- Pregnancy and lactation

2- Malignancy other than HCC

3- HIV co infected patients

4- HBV co infected patients

Methods:

1- Pre enrollment assessment and work up:

All patients were subjected to the following

Full history taking including history of chronic liver disease, symptoms of hepatic decompensation such as lower limb edema, ascites, hepatic encephalopathy, hematemesis or melena as well as history of extra hepatic manifestations and other system affection

2- Full clinical examination: general and local, for the stigmata of chronic liver disease

3- Intial laboratory assessment including:

Liver profile: Alanine transaminase (ALT),Aspartate transaminase (AST),serum albumin level, serum total bilirubin level, international normalized ratio (INR), serum alpha feto protein (AFP)

Complete blood count with differential (CBC)

HCV quantitative RNA assay via polymerase chain reaction (PCR)

HBsAg

HIV Ab

Pregnancy test for females in child bearing period

Von willebrand Ag

Des gamma carboxy prothrombin

Abdominal Ultrasound: performed after overnight fasting (7 hours) with the patient lying in a supine position with emphasis on liver size, liver echogenicity (bright or coarse echo pattern), splenic bi-polar diameter, portal vein diameter.

Calculation of child-pugh score according to the original formula

Scoring:

Measure	1 point	2 points	3 points
Total bilirubin (mg/dl)	(<2)	(2-3)	(>3)
Serum Albumin	>3.5	2.8-3.5	<2.8
PT, INR	<1.7	1.71-2.3	>2.3
Ascites	None	Mild	Moderate to severe
Hepatic encephalopathy	None	Grade 1-2 (or suppressed with medication)	Grade 3-4(or refractory)

interpretation:

Points	class	One year survival	Two year survival
5-6	A	100%	85%
7-9	B	81%	57%
10-15	C	45%	35%

Calculating APRI score

It is an AST to Platelet Ratio Index

(AST Level IU/AST upper normal level IU)/platelate count x100

- Score greater than 0.7 had a sensitivity of 77% and a specificity of 72 %for predicting significant hepatic fibrosis
- Score greater than 1 had a sensitivity of 76% and specificity of 72%
- Using an APRI cutoff of 2.0 was more specific (91%) but less sensitive (46%).
- The lower the APRI score (less than 0.5), the greater the negative predictive value (and ability to rule out cirrhosis) and the higher the value (greater than 1.5) the greater the positive predictive value (and ability to rule in cirrhosis);mid range values are less helpful.

Calculating FIB -4 SCORE

- The Fibrosis 4 score helps to estimate the amount of scarring in the liver
- FIB-4=Age (years)X AST Level (u/l) /Platelet count (109/L)x (ALT [U/l])$^{1/2}$)
- The FIB-4 index is a new noninvasive test for the assessment of liver fibrosis. A score of <1.45 and >3.25 enables the correct identification of patients who have moderate or significant fibrosis, respectively, and could avoid LB examination. The FIB-4 index proved to be

concordant with FibroTest results. Because the FIB-4 index is readily available, inexpensive, and easily reproducible, it could rapidly replace expensive and/or invasive methods to assess liver fibrosis, especially in emerging countries, to detect patients who need antiviral treatment and to monitor liver fibrosis progression (or regression). Other studies are now required to validate this new score in combination with other noninvasive tests to enhance its diagnostic performance, especially for intermediate values.

- Using a lower cutoff value of 1.45, a FIB-4 score <1.45 had a negative predictive value of 90% for advanced fibrosis. In contrast, a FIB-4 > 3.25 would have a 97% specificity and a positive predictive value of 65% for advanced fibrosis

Principle of th Assay:

This kit is a sandwich Enzyme-Linked Immunosorbent Assay (ELISA). vWFAg is added to the wells pre-coated with vWFAg monoclonal antibody. After cubation a biotin-conjugated anti-human vWFAg antibody is added and binds to human vWFAg. After incubation unbound biotin-conjugated anti-human vWFAg antibody is washed away during a washing step. Streptavidin-HRP is added and binds to the biotin-conjugated anti-human vWFAg antibody. After incubation unbound Streptavidin-HRP is washed away during a washing step. Substrate solution is then added and color develops in proportion to the amount of human vWFAg. The reaction is terminated by addition of acidic stop solution and absorbance is measured at 450 nm.

Specimen collection:

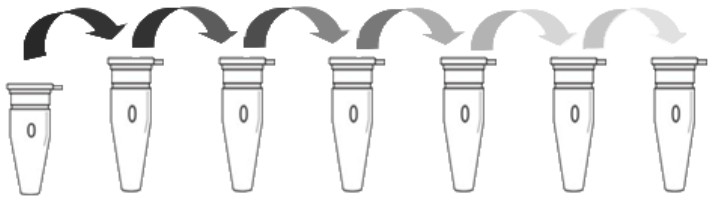
Serum Allow serum to clot for 10-20 minutes at room temperature. Centrifuge at 2000-3000 RPM for 20 minutes.

Plasma Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 minutes at 2000-3000 RPM at 2 - 8°C within 30 minutes of collection.

Reagent preparation:

- All reagents should be brought to room temperature before use.
- Standard It is strongly recommended that all standards and samples be run in duplicate. If the standard hasn't been run out, keep the remain at -20°C. Diluted standard can’t be reused. Dilution of standard solutions suggested are as follows:

120ng/	Standard	120µl Original Standard + 120µl
60ng/m	Standard	120µl Standard No.5 + 120µl Standard
30ng/m	Standard	120µl Standard No.4 + 120µl Standard
15ng/m	Standard	120µl Standard No.3 + 120µl Standard
7.5ng/m	Standard	120µl Standard No.2 + 120µl Standard



Standard	S5	S4	S3	S2	S1
240ng/m	120ng/m	60ng/ml	30ng/ml	15ng/ml	7.5ng/m

- Wash Buffer Dilute 20ml of Wash Buffer Concentrate 30x into deionized or distilled water to yield 500 ml of 1x Wash Buffer. If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved.

:Results

Table 1:comparison between the three groups regarding the mean value of VW factor (n=50)

VW factor (ng/ml)	Groups			ANOVA		TUKEY'S Test		
	HCC	Cirrhotic	Control	F	P-value	H&CI	H&CO	CI&CO
Range	208.98 - 678.4	177 - 334	65 - 90	51.472	<0.001*	<0.001*	<0.001*	<0.001*
Mean ±SD	416.971 ± 131.260	236.728 ± 50.813	76.620 ± 8.256					

.Von willebrand factor among the study groups

Table 1: As regard VW factor, it ranged from 208.98 to 678.4 ng/ml in HCC patients with mean ±SD =416.971± 131.260; from 177 to 334 ng/ml in cirrhotic patients without HCC with mean±SD =236.728± 50.813; from 65 ng/ml to 90 ng/ml in control group with mean±SD = 76.620±8.256, being statistically significant higher in HCC group than in cirrhotic patients without HCC with (p – value= <0.001), statistically significant higher in HCC patients than in control group (p-value =<0.001), in cirrhotic patients without HCC than in control group with (p – value <0.001).

Table 2: Comparison between the three groups regarding the mean value (of alpha feto protein (n=50

AFP (ng/ml)	Groups			ANOVA	
	HCC	Cirrhotic	Control	F	P-value
Range	1.44 - 52061	0.5 - 48871	1.5 - 4.5	0.479	0.623
Mean ±SD	3914.957 ± 12041.067	2447.147 ± 10927.042	3.013 ± 1.026		

.Alpha feto protein among the study groups

Table 2: Alpha _feto protein as a marker for hepatocellular carcinoma was ranging from 1.44 – 52061 ng/ml with mean ±SD = 3914.957± 12041.067, from 0.5 to 48871 ng/ml in cirrhotic patients without HCC with mean ± SD = 2447.147 ± 10927.042, from 1.5 to 4.5 ng/ml in control group with mean ±SD = 3.013 ±1.026 with no statistically .significant difference between the study groups

Table 3: comparison between the three groups regarding the mean value (of des gamma carboxy prothrombin. (n=50

DCP (mAU/ml)	Groups			ANOVA		TUKEY'S Test		
	HCC	Cirrhotic	Control	F	P-value	H&CI	H&CO	CI&CO
Range	19 - 50.6	8.3 - 30	1.5 - 5.4	53.122	<0.001*	<0.001*	<0.001*	<0.001*
Mean ±SD	29.965 ± 8.388	17.718 ± 6.425	3.450 ± 1.243					

Table 3: des gamma carboxy prothrombin as a marker for HCC was ranging from 19 to 50.6 mAU/ml in patients with HCC with mean± SD = 29.965 ± 8.388 and from 8.3 to 30 mAU/ml with mean ± SD = 17.718 ± 6.425 and ranged from 1.5 to 5.4 with mean± SD = 3.450 ±1.243, being statistically significant higher in HCC patients than in cirrhotic patients (p-value = <0.001), in HCC patients than in control group with (p-value= <0.001), in cirrhotic patients than in control group with (p- value .(=<0.001

Table 4: VW factor mean value regarding patients' gender, child score .and portal vein invasion in HCC group

HCC		VW factor (ng/ml)				T-Test or ANOVA	
		N	Mean	±	SD	T or F	P-value
Sex	Male	14	408.747	±	133.266	-0.419	0.680
	Female	6	436.160	±	136.615		
Child	Child A	12	362.890	±	105.666	3.403	0.057
	Child B	4	471.043	±	152.388		
	Child C	4	525.143	±	117.292		
Fibroscan	F1	2	451.800	±	179.747	0.303	0.743
	F3	3	364.333	±	108.187		
	F4	15	422.855	±	136.376		
PV invasion	No	16	397.766	±	128.391	-1.335	0.198
	Yes	4	493.790	±	129.850		

Table 4: VW factor in patients with HCC as regard sex, child score, fibroscan, PV invasion

Among HCC group, there was no statistically significant difference between VW factor results between males and females or between the 3 child groups or between different fibroscan grades, or regarding portal .vein invasion

Cirrhotic		VW factor (ng/ml)				T-Test or ANOVA	
		N	Mean	±	SD	T or F	P-value
Sex	Male	16	228.763	±	46.962	-1.441	0.167
	Female	4	268.590	±	60.280		
Child	Child A	7	196.580	±	11.804	4.837	0.022*
	Child B	7	253.000	±	57.868		
	Child C	6	264.583	±	45.470		
Fibroscan	F3	3	184.333	±	6.429	-2.105	0.050*
	F4	17	245.974	±	49.552		

Table 5: VW factor in cirrhotic group without HCC as regard sex, child .score, Fibroscan

VW factor was statistically significant higher in patients with Child C than Child B than Child C with mean±SD = 196.580±11.804 in Child A, mean±SD = 253.000±57.868 in child B, mean ±SD = 264.583±45.470 in .child C WITH (P-VALUE = 0.022)

VW factor was statistically significant higher in F4 patients than F3 patients With mean±SD = 184.333±6.42 in F3, mean±SD = .245.974±49.552 in F4, (p-value = 0.050)

Table 6: VW factor among the study population as regard lab, imaging .data, child score, other markers of HCC and Liver fibrosis

Correlations				
	VW factor (ng/ml)			
	HCC		Cirrhotic	
	R	P-value	r	P-value
Age	-0.318	0.171	-0.203	0.391
TLC	-0.215	0.362	-0.341	0.142
HB	0.058	0.807	-0.151	0.526
PLT	-0.154	0.516	-0.578	0.008*
ALT	0.030	0.900	-0.106	0.657
AST	0.286	0.221	0.121	0.610
Albumin	-0.273	0.244	-0.577	0.008*
Bilirubin	0.630	0.003*	0.037	0.878
INR	0.156	0.510	0.563	0.010*
AFP (ng/ml)	0.399	0.081	0.242	0.304
DCP (mAU/ml)	0.093	0.697	0.595	0.006*
PV diameter (mm)	0.033	0.890	0.162	0.494
Liver size (cm)	0.386	0.092	-0.529	0.016*
S. Create	0.269	0.251	0.325	0.162
Spleen size (cm)	-0.121	0.612	0.454	0.044*
Child Score	0.482	0.032*	0.452	0.045*
HCV PCR	0.138	0.687	0.031	0.933

APRI	0.321	0.168	0.351	0.129
FIB4 score	0.166	0.485	0.241	0.306
HCC size (cm)	0.327	0.159	-	-

As regard age and liver enzymes, there was no statistical significance between cirrhotic patients and HCC group.

Regarding different parameters of complete blood picture (CBC), there was no statistically significant difference between hemoglobin and total leucocytic count while platelets were statically significant lower among .cirrhotic patients without HCC (P-VALUE =0.008)

As regard synthetic functions of the liver, Albumin was statistically significant lower among cirrhotic patients without HCC, while bilirubin was statistically significant higher among HCC group. International normalized ratio (INR) was statistically significant higher in cirrhotic .patients without HCC

Markers of hepatocellular carcinoma; there was no statistically significant difference as regard alpha feto protein, while desgamma carboxyprothrombin was statistically significant higher in patients with HCC (p- value = 0.006).

There was no statistically significant difference as regard portal vein .diameter and serum creatinine

Liver size was statistically significant lower in patients with HCC (p-value = 0.016), while spleen size was statistically significant higher in .(HCC group (p-value = 0.044

Child score was statistically significant higher in both cirrhotic patients without HCC and HCC group.(p- value = 0.032), (p- value = 0.045) .respectively

There was no statistically significant difference as regard FIB4, APRI, .HCV PCR

:Discussion

Chronic hepatitis C virus (HCV) infection is one of the leading causes of liver cirrhosis and hepatocellular carcinoma (HCC) worldwide***.(Hajarizadeh B, et al.2013)***

The extent of hepatic fibrosis, a predictor of disease progression, determines the need for antiviral treatment and may help to select the

optimal duration of therapy as well as the most appropriate regimen.***(Ferenci P, et al.2015)***

Hepatocellular carcinoma is the fifth most common tumor worldwide and the second most common cause of cancer-related death. It is a major health problem and its incidence is increasing. The presence of liver cirrhosis is the major risk factor and worldwide this is largely due to chronic hepatitis C virus (HCV) and hepatitis B virus (HBV) infection ***(Heimbach et al., 2018)***

Early detection of patients with HCC is attractive because it gives better prognosis as HCC tends to grow slowly and stay confined to the liver. Early detection is possible with ultrasound scanning and AFP monitoring, although the use of AFP as a screening test is complicated by frequent false positive and false negative results, so early diagnosis of HCC would not be difficult if tumor markers and medical imaging were combined ***(Smith, 2015).***

The best way to effectively diagnose HCC in a timely fashion is to enter patients who are at high risk for development of this tumor in a regular surveillance program using ultrasound imaging every six months. In patients who are not in a routine surveillance program, the diagnosis of HCC may be first entertained in a patient with underlying liver disease (eg, cirrhosis, chronic viral hepatitis) who develops a rising serum alpha-fetoprotein (AFP) level ***(Smith, 2015).***

The AASLD recommends surveillance of adults with cirrhosis because it improves overall survival, and it suggests surveillance using ultrasound (US), with or without alpha-fetoprotein (AFP), every 6 months ***(Singal et al., 2014)***

Liver biopsy is the gold standard for determination of fibrosis stage, but has drawbacks, it is associated with patient discomfort, although the risk of major complications is low, but also includes mortality (1/4000–1/10000).10, 11Up to40% of patients do not agree to liver biopsy. ***(Beinhardt S, et al.2012)***

SO, many non-invasive fibrosis tests have been developed. These indirect biomarkers of fibrosis are composed of easy available variables with one or more fibrosis-predicting panels like AST to platelet ratio index (APRI); fibrose index (FI),fibrosis cirrhosis index (FCI), FIB 4score and Forns Index. ***(Castera L.2012).***

Von Willebrand factor (vWF) is a large important adhesive protein for both platelet adhesion and aggregation. AsvWF-Ag is elevated in liver disease it might be a key marker in establishing liver fibrosis**.(Iannacone M,et al.2005)**

vWF-Ag was established as a valuable marker for prediction of varices, portal hypertension and mortality in patients with liver cirrhosis. **((Ferlitsch M,et al.2012**

The aim of this study was to determine whether VWF is a potential biomarker for liver fibrosis compared to APRI and FIB 4 score and predictor for HCC development in comparison with other available .markers like alpha feto protein and des gamma carboxy prothrombin

This study was carried out on 50 patients classified into 3 groups: Group I included 10 randomly selected apparently healthy individuals as a control group, Group 2 included 20 cirrhotic patients with hepatitis C virus-related hepatocellular carcinoma diagnosed by imaging and alpha feto protein . Among the HCC patients there were 4 patients with portal vein invasion. Group 3 included 20 patients with liver fibrosis and cirrhosis only without HCC classified according to child score into child A, child .B, child C

In this study HCC was found to be more prevalent in men 14 (70.0%) than in women 6 (30.0%). This was in accordance to ***El-Zayadi et al. (2005)*** who showed higher incidence of HCC among male patients. The results were contrary to what was found by ***Tokushige et al. (2016)*** in their study. This may be explained in part by the differences in sample size, exposure to risk factors and sex hormones. It has been speculated that estrogens, androgens, degree of iron deposition and difference in ethnicity could modulate hepatocarcionogenesis and explain the higher incidence of HCC in men ***(Nishida et al., 2012)***.

In the current study, the ages of patients with HCC ranged between 45-77 years with a mean 57.850 ± 8.4 years and this is probably attributed to the duration of the underlying liver disease. Those results were consistent with *Konstantin et al. (2015)* who found that the mean age of patients with HCC was 63.79 ± 9.99 years. Also those results were close to those of *Oliver et al. (2013)* who stated that the mean age of patients with HCC was 59.7 ± 10.4 years. This approves the prevalence of HCC in the fifth .and sixth decades of life

In this study, we evaluate the diagnostic value of vWF-Ag as a novel non-invasive biomarker in the assessment of liver fibrosis and predictor for .hepatocellular carcinoma

In this study, VWF: Ag was related to liver fibrosis progression of chronic hepatitis C and inversely correlated with albumin and platelet count that goes in agreement with ***(Takaya, et al.2018)*** that stated that that plasma levels of VWF: Ag increase according to hepatic spare ability decline in patients with LC. VWF: Ag was related to liver fibrosis progression of chronic hepatitis and inversely correlated with albumin, prothrombin time and platelet count. VWF is observed more brightly in hepatic ECs in histological findings according to liver fibrosis progression.

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In the current study we concluded that VW Ag level was higher in patints with hepatocellular carcinoma than cirrhotic patients without HCC than the control group (p-value=<0.001), there was positive correlation between VW Ag and liver cirrhosis stage assessed by child score among patients with HCC,and cirrhotic patients without HCC (p-value =0.032), (p-value=0.045) respectively that goes with ***(Takaya,et al.2018)*** that stated that VWF: Ag levels of patients who developed HCC with absent to moderate liver fibrosis and severe fibrosis were higher than those of patients without HCC. Thus, patients who develop HCC have higher VWF: Ag levels than patients without HCC who have the same liver .fibrosis stage (p-value <0.005)

In our study, Des gamma carboxy prothrombin was statistically significant higher in HCC group than cirrhotic patients without HCC than control group (p-value=<0.001), while there was positive correlation as regard alpha feto protein between the study groups (p-value=0.623). it was higher in HCC group with mean±SD =3914.9±12041 than in cirrhotic patients without HCC than control group.

There was positive correlation between VW Ag and other markers of hepatocellular carcinoma alpha feto protein and des gamma carboxy prothrombin in both HCC (p-value =0.081, 0.697) respectively, cirrhotic ;patients without HCC (p-value =0.3, 0.006) respectively

With des gamma carboxy prothrombin being statistically significant with von willebrand factor in cirrhotic patients; these results didn't match with ***(Takaya,et al.2018)*** who reported that The VWF: Ag was not shown to

be correlated with AFP and DCP. To detect predictive biomarkers for HCC development, they performed univariate analysis. Univariate analysis showed that VWF: Ag, age, albumin, aspartate aminotransferase, platelet count and liver fibrosis stage were useful predictive biomarkers for HCC development, whereas AFP and DCP were not. They also performed multivariate analysis of predictive biomarkers for HCC development which showed that only VWF: Ag was a useful predictive biomarker for HCC development.

There was a weak positive correlation between VW Ag in different fibrosis stages and FIB 4 and APRI score assessing the stage of liver fibrosis in HCC group and cirrhotic patients without HCC that also goes with ***(Takaya,et al.2018)*** that stated that The plasma levels of VWF: Ag increased according to the progression of liver fibrosis and were directly correlated with liver fibrosis stage assessed by liver biobsy ; VWF: Ag level was higher in patients with severe fibrosis (F3 and F4) than in patients with absent to moderate liver fibrosis (F0 to F2). The area under the curve (AUC) of VWF: Ag for the diagnosis of severe liver fibrosis stage was 0.721, which has moderate accuracy in the receiver operating characteristic curve.

It also goes with ***(A. Maieron,et al.2013)*** that stated that vWF-Ag levels were increasing with stage of fibrosis: in patients with fibrosis stage 0,vWF-Ag was median 136.5% (IQR 96.0–181.5); in fibrosis stage I, 140.6% (IQR 97.0–189.0); in fibrosis stage II,157.5% (IQR 127.0–196.0); in fibrosis stage III, 171.0%(IQR 139.5–218.0) and in fibrosis stage IV, 252.0% (IQR201.0–325.0);$P<0.001$.

(A. Maieron,et al.2013) stated that the diagnostic performance of vWF-Ag in predicting liver fibrosis in comparison to other fibrosis scores was analysed by AUROC: with 0.703, vWF-Ag is one of the best markers to differentiate patients with fibrosis(F1-F4) from patients without fibrosis (F0).

(A. Maieron,et al.2013) also reported that none of the existing fibrosis scores is good in distinguishing mild or no fibrosis (F0, F1) from significant fibrosis (≥F2); vWF-Ag shows an AUROC of 0.7 (IQR 0.592–0.781). In their sample, APRI score performed best with an AUROC of 0.752 (IQR 0.679–0.826) to distinguish between≤F1and≥F2. The diagnostic performance of vWF-Ag in comparison to other fibrosis scores to differentiate from significant fibrosis (≥F2) is comparable to all the other scores. APRI score is performing best in the group, with an

AUROC of 0.75 (IQR: 0.681–0.813); however, not significantly better than vWF-Ag with an AUROC of 0.7 (IQR 0.616–0.778) (P=0.2) or VITRO score with an AUROC of 0.72 (IQR: 0.647–0.79) (P=0.3), which are performing as third and second best.

In our study, VW Ag isn't strongly correlated with other scores assessing liver fibrosis, but strongly correlated with child score assessing liver cirrhosis, thus we concluded that VW Ag is more valuable assessing late stages of liver cirrhosis that went in agreement with ***(A. Maieron,et al.2013)*** that stated that throughout their literature, most fibrosis scores show poor performance in detecting mild fibrosis stages.

vWF-Ag and VITRO score show comparable results,

but no significant improvement in detection of mild

fibrosis stages.

As transient elastography shows a lack of accuracy in distinguishing among F1, F2 and F3, evaluating the precise stage of fibrosis still remains the domain of liver biopsy. While vWF-Ag and VITRO score gain diagnostic accuracy in detecting significant fibrosis and cirrhosis.

(A. Maieron,et al.2013) clearly demonstrates the diagnostic value of vWF-Ag as a novel non-invasive biomarker in the assessment of liver fibrosis. We were able to show vWF-Ag as predictor of advanced fibrosis (F3) and cirrhosis (F4) in patients with CHC with a NPV of 91.5%.

The AUROC of vWF-Ag is 0.79 for advanced fibrosis

and 0.84 for detecting cirrhosis.

In conclusion; VWfactor is statistically significant higher in patients with .Hepatocellular carcinoma than in cirrhotic patients withot HCC

There is weak positive correlation between VW factor as a biomarker for .liver fibrosis and other scores assessing stage of fibrosis

There is a strong correlation between VW factor and child score used to classify stage of liver cirrhosis, so VW factor is valuable predictor for .hepatocellular carcinoma and advanced stages of liver cirrhosis

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