Von Willebrand Factor as Non-invasive Marker for Assessing Severity of Chronic Liver Diseases

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Background: Chronic liver disease (CLD) is defined by damage to the liver parenchyma and its regeneration resulting in fibrosis and cirrhosis with progressive deterioration of liver functions. Von Willebrand factor (VWF) has recently been linked to the development and progression of liver illnesses including cirrhosis.**Objective:** to evaluate the level of (VWF) in patients with (CLD).**Patients and Methods:** A case - control study was carried out on 90 children. Have an age ranged between 8 months to 15 years old. Data was collected from children in addition to complete systematic physical examination including stigmata of (CLD) and complications of liver cirrhosis.**Results:** There was significant differences in VWF-Ag level between patients and control groups (*P*= 0.000) and also the level of VWF significance increase in patients with INR>1.1 (*P*= 0.005) and albumin<3.5 g/dl (*P*= 0.002) and There was positive correlation between VWF-Ag level and Pediatric End-Stage Liver Disease sore (*P*= 0.013) and child -Pugh score (*P*= 0.002).**Conclusion:** Level of VWF Ag can be used as a marker for increase severity of chronic liver disease taking in consideration child Pugh and PELD scores.

Keywords: Von Willebrand factor, chronic liver disease.

Introduction

Chronic liver diseases (CLD) can be described as a progressive deterioration of liver functions (for >6 months), CLD is a continuous process of inflammation, destruction and liver regeneration parenchyma, which leads to fibrosis and cirrhosis. It is characterized by a reduction in hepatic function accompanied by chronic inflammation. This may lead to a condition where there is an irreversible destruction of liver cells called cirrhosis[1] .Endothelial dysfunction is a primary contributor in the elevated intrahepatic vascular tone seen in cirrhosis, and Von Willebrand factor antigen markers have been discovered to represent this dysfunction[2,3,4]. VWF has been linked to the progression of liver illnesses such as cirrhosis and liver transplantation-related complications[5,6]. VWF levels are higher in cirrhotic individuals and are related to the degree of fibrosis and the severity of liver disease [7].

Endothelial dysfunction is thought to be a key event in the development of distinct human vascular diseases, including liver cirrhosis and hypertension [8]. The

rise of VWF-Ag indicates development of a prothrombotic state that is related to most complications of end-stage liver disease and it seems that it adds prognostic information in a more straightforward and essential way [9].

In addition to VWF-Ag, the VITRO Score (the Von Willebrand Factor-Ag/thrombocyte ratio) improves the noninvasive diagnostic accuracy of cirrhosis [10], and was established as a marker for cirrhosis and portal hypertension[11].

Among the most common scores used in practice to predict the severity of liver cirrhosis are Child-Pugh score (CPS) [12], Pediatric End-Stage Liver Disease (PELD) score [13], and Model for End-Stage Liver Disease (MELD) score [14], the study aims was to evaluate the level of VWF-Ag in patients with chronic liver diseases, to define the relationship between VWF-Ag and severity of liver disease and to compare between scores such as CPS, PELD and MELD.

Patients& methods:

A case - control study was performed on 90 children from 1st of December 2020 to the 30th of October 2021. Cases group were 45 children who consulted the Gastroenterology outpatient clinic or admitted to gastroenterology ward in Children Welfare Teaching Hospital, Medical city in Baghdad, Iraq whom age ranged between 8 months to 15 years old with signs and symptoms of chronic liver disease. Chronicity was determined by the duration of liver disease (typically >3-6 months) and by evidence of chronic hepatic decompensation (hypoalbuminemia, thrombocytopenia) or physical stigmata of chronic liver disease (clubbing, gynecomastia, skin excoriation, spider telangiectasia and hepatosplenomegaly).

Exclusion criteria were Children below 8 months of age due to normally have high VWF level, Children with severe infection like sepsis, Children with family history of bleeding tendency (factors VIII, IX and VWF deficiency) and Children who had received blood products such as fresh frozen plasma in the last 2 months, due to already increase VWF level in those patients. Control group included 45 child of comparable age to cases and were taken from general outpatient clinic in the same hospital, who consulted the clinic for Non GIT related complaints. Data were collected from both groups of children after taking consent from their parents, including name, age, sex in addition to complete physical examination including signs of chronic liver diseases its complications.

All patients and control groups underwent a complete blood count, blood group and biochemical analysis of blood sample for VWF-Ag, basic coagulation tests (Prothrombin time (PT), Partial Thromboplastin Time (PTT), International

Normalized Ratio (INR), Serum Sodium (S.Na), Creatinine, liver function test including Alanine Transaminase (ALT), Aspartate Aminotransferase (AST), Alkaline Phosphatase (ALP),Total serum billirubin (TSB),Total serum protein (TSP) and serum Albumin (S.ALB), in addition to abdominal ultrasound . The severity of chronic liver disease (CLD) was assessed by using of scoring systems including: PELD, MELD and CTP score.

Collection and Panels Specimen: 1ml of blood was drawn from each patient by using tube with Sodium Citrate 3.2%, blue top Centrifugation: 2000-2500 g for 10 min. Storage plasma sample should be frozen, the specimen is stable for 12 months at -70°C, VWF-Ag level was measured by ELISA method using BioSource VWF Ag Test Kit. Reference ranges are as follows [15,16]:

Newborn = < 6 month = 60-230%.
Children = 1-10 years = 50-160%.
Adults = 60-160% (blood type O); 70-200% (non-O blood type).

Statistical analysis: Data were analyzed using computer software programs of Statistical Package of Social Science (SPSS) version 26. Descriptive statistics reported as frequency distribution tables, number and percentage for qualitative data: mean, standard deviation and range for quantitative data. Unpaired t-test, One Way ANOVA test, and Chi-square test were used to identify the significant difference between study groups of cases and control. Pearson's correlation and univariate linear regression model were used to identify the correlation among different quantitative parameters. A P-value of < 0.05 was used as the criterion for determining statistical significance.

Results:

About 45 children were enrolled diagnosed with chronic liver diseases and 45 normal children as control group. The mean level of VWF-Ag was significantly higher among cases group than that of controls group (5.4533 ± 3.91672 vs. 0.9911 ± 0.39475) with mean differences of -4.46222 (t= 7.604, df:88, 95%CI -3.29602, P= 0.000).

Regarding mean levels differences of Von Willebrand Factor among different clinical parameters of cases group, only cases who had encephalopathy and portal hypertension found to show significant differences, as mean level of Von Willebrand Factor was significantly higher among children who presented with

encephalopathy and portal hypertension than those who did not (7.6000 ± 4.38529 vs. 4.4516 ± 3.30291) (*t*= -2.669, df:43, *P*= 0.011) and (6.0625 ± 4.12872 vs. 3.8000 ± 2.66083) (*t*= -2.180, df:43, *P*= 0.036) respectively. Other clinical feature like ascites shows no significant differences regarding the mean level of clotting factor (P> 0.05).

With respect to correlation between Von Willebrand factor and other lab parameters of cases group, Albumin was found to have weak inverse relation with VWF (r2 = 0.170, P= 0.005), while INR was found to have strong positive relation with INR (r2 = 0.201, P= 0.002) (Table 1) (Figure 1,2).

Table 1 Correlation between Von Willebrand Factor and Lab parameters among cases group (n= 45).

Parameter	Von Willebrand Factor			
	Pearson Correlation	R square	95% CI	*P*-value
S. ALB	**-0.413**	**0.170**	**-0.610 – 0.178**	**0.005**
S. creatinine	-0.035	0.001	-0.261 – 0.226	0.818
INR	**0.449**	**0.201**	**0.112 – 0.641**	**0.002**

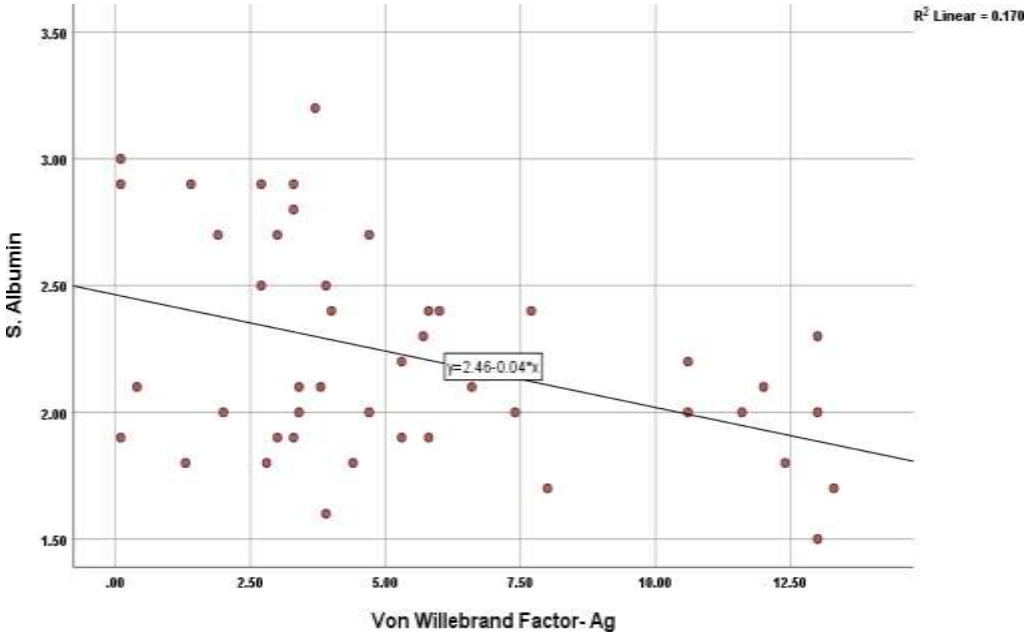

Figure 1: Scatter plot correlation between VWF-Ag and serum albumin among cases group (n= 45).

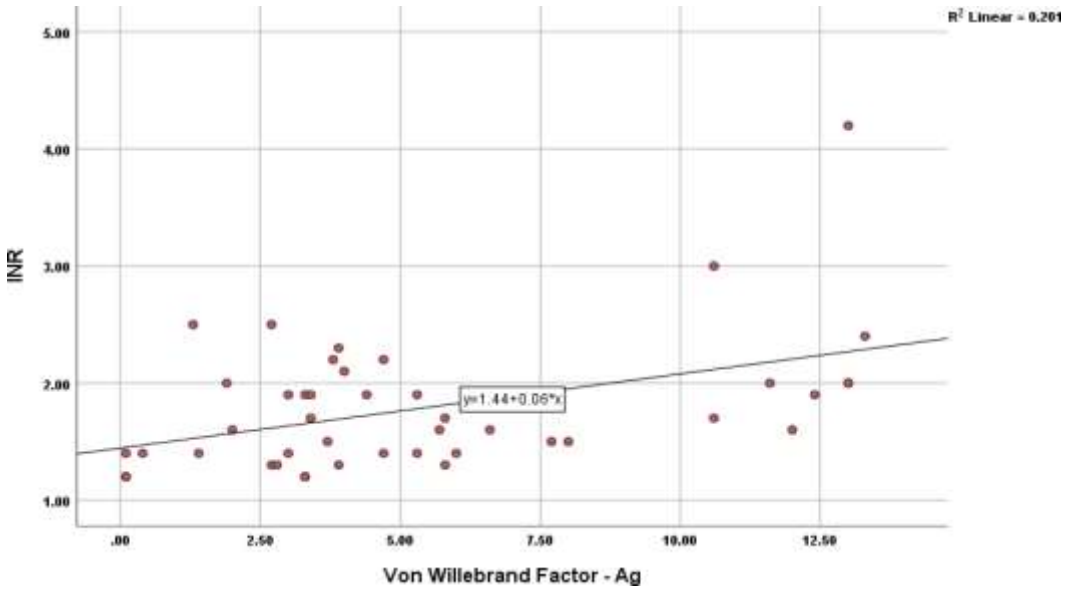

Figure 2: Scatter plot correlation between VWF-Ag and INR among cases group (n= 45).

Apart from children who aged 12+ years old, there is a significant correlation between pediatric end-stage liver disease score (PELD) and Von Willebrand Factor using a correlation and linear regression model, (r2 = 0.163, P= 0.013) (Table 2) that was clearly illustrated by scatter plots correlation between them, (Figure 3).

Table 2: Correlation between Von Willebrand Factor and PELD score among cases group (n= 37).

PELD Score				
Variables	**Pearson correlation**	**R square**	**95% CI**	***P*-value**
Von Willebrand Factor	0.404	0.163	0.002 – 0.683	**0.013**

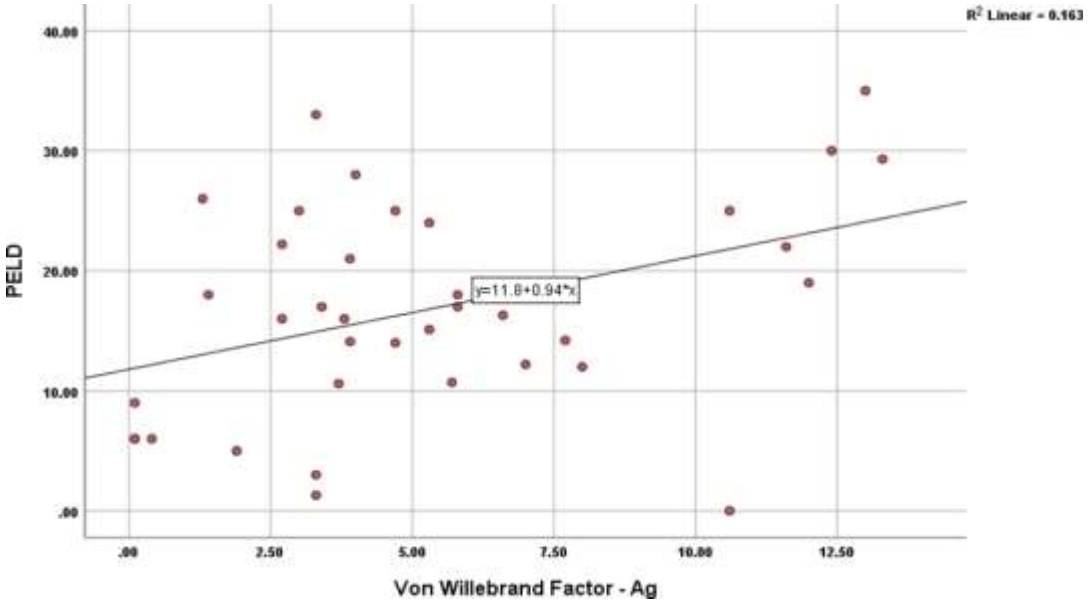

Figure 3 : Scatter plot correlation between Von Willebrand Factor and PELD score among cases group (n= 37).

Among children who aged 12+ years old, there is no significant correlation was found between child's model for end-stage liver disease score (MELD) and Von Willebrand Factor using a correlation and linear regression model, (r^2 = 0.017, *P*= 0.761) (Table 3) .

Table 3 Means correlation between Von Willebrand Factor and MELD score among cases group (n= 8)

Variables	MELD			
	Pearson correlation	R square	95% CI	*P*-value
Von Willebrand Factor	0.129	0.017	-0.638 – 0.839	0.761

Regarding Child PUGH score, significant difference was found between means of VWF-Ag among child Pugh classes, as the mean of VWF-Ag of child PUGH class C was significantly higher than that of Class B which subsequently higher than that of class A (6.7194 ± 3.91386 vs. 3.0545 ± 2.01264 vs. 1.1667 ± 1.84752) (F= 7.266, *P*= 0.002) (Table 4).

Table 4 Comparison of VWF-Ag among Child PUGH classes among cases group (n=45).

Child Pugh Classes [a]	No.	Von Willebrand Factor- Ag		
		(Mean ± SD)	St. error	Mean square [b]
Class A	3	1.1667 ± 1.84752	1.06667	
Class B	11	3.0545 ± 2.01264	0.55207	86.488
Class C	31	6.7194 ± 3.91386	0.70295	

F= 7.266, P= 0.002, a: One-way ANOVA test, b: Mean square between groups (classes).

Discussion:

Cirrhosis of the liver is characterized by loss of liver function and increased resistance to portal flow, as well as a rise in portal system pressure. VWF levels are higher in cirrhotic individuals and are related to the degree of fibrosis and the severity of the illness [17].

In agreement with this study regarding VWF significance between cases and control [6, 10 , 18 ,19] reached the same conclusion. This increase in the level of VWF-Ag can be the result of both increases the release from megakaryocytes and activated endothelial cells and a decrease in its clearance due to reduced liver mass [20].

The same results which this study concluded where VWF-Ag level among cases group has shown significant differences with portal hypertension was supported by many studies [21,22,23]. VWF-Ag level among cases group has shown significant differences with encephalopathy as clinical complication of liver diseases this result could be attributed to High VWF-Ag was closely related to plasma endotoxin, proinflammatory cytokines and poor prognosis mainly in acute on chronic hepatic failure [19] , similar result to studies carried out in Turkey [23] and Italy [24].

Ascites has shown no significant differences regarding the level of clotting factor In disagreement to other studies [22,25] . International normalized ratio of blood clotting has found positive correlations with Von Willebrand factor similar to that seen in a study from Macedonia [26] ,and Turkey [23] As hepatocellular failure

develops and progresses , the synthesis of clotting factors decrease and eventually prothrombin time increases.

In this study, we observed serum albumin level inversely related with VWF-Ag level in liver diseases ,This was also detected a study from Austria [22] ,Albumin is a protein synthesized in liver and decreases with hepatic failure. There was significant correlation between PELD score and VWF-Ag similar to that seen in a study from Turkey[23].

There was no significant correlation between MELD score and their VWF-Ag In disagreement to a study carried out in Vienna in 2020 [27], and other systematic review article 19. this result can be explained due to small sample size of patients over the age of 12 who participated in the study.Regarding Child PUGH, a significant difference was identified between VWF-Ag among child PUGH classes, similar to that seen a study from Turkey in 2020 [23], and Italy in 1996 [24] and Austria in 2019 [19].

Conclusion: Serum VWF–Ag can be used as a predictor for the severity in chronic liver disease patients (especially portal hypertensive and Encephalopathtic).

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