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SERUM VASCULAR CELL ADHESION MOLECULE-1 PREDICTS SIGNIFICANT LIVER FIBROSIS IN NON ALCOHOLIC FATTY LIVER DISEASE

By

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ABSTRACT

Background: Non-alcoholic fatty liver disease (NAFLD) is the most common chronic liver disease worldwide and is strongly associated with obesity, dyslipidemia and insulin resistance. NAFLD often presents as simple steatosis (NAFL) but can progress to non-alcoholic steatohepatitis (NASH) and fibrosis. Current non-invasive biomarkers are not tailored to identify significant (≥F2) fibrosis, although recent guidelines recommend a stringent follow-up of this patient population.

Objective: To investigate the applicability of Vascular Cell Adhesion Molecule-1 (VCAM-1) as non-invasive diagnostic tools for identifying nonalcoholic steatohepatitis -associated fibrosis.

Patients and Methods: This study recruited 100 patients attending at Al-Hussein Hospital, Al-Azhar University and Six October University Hospital between June 2019 and June 2020, divided into four equal groups including patients presented with steatosis, patients presented with steatohepatitis, patients with viral cirrhosis and healthy controls. All participants were subjected to full history, clinical examination, laboratory investigations, abdominal ultrasound, fibroscan, and serum VCAM-1.

Results: Our study identified serum vascular cell adhesion molecule-1 (VCAM-1) as an independent predictor for ≥F2 fibrosis (median 15.33 vs. 11 ng ml−1 in patients with and without significant fibrosis) with an area under the curve (AUROC) for prediction of ≥ F2 fibrosis which had a good predictive value, and the best cutoff for VCAM-1 was 13 ng ml− 1, with a sensitivity of 70.59% and specificity of 100%.

Conclusion: VCAM-1 levels are able to accurately predict significant (≥F2) fibrosis in NAFLD patients.

Keywords: Non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH), Fibroscan, Vascular Cell Adhesion Molecule-1 (VCAM-1).

INTRODUCTION

NAFLD is the hepatic manifestation of obesity and a precursor of an independent risk factor for type 2 diabetes (Lonardo et al., 2015). It is an independent risk factor for cardiovascular disease, with studies unequivocally showing an increased cardiovascular mortality *(Ekstedt et al., 2015).*

The global prevalence of NAFLD and NASH is around 25% and 3%, respectively, although this rises to an estimated 90% and 25%, respectively, in severely obese patients *(Younossi et al., 2016).*

Liver biopsy is still the gold standard for the diagnosis of NASH and the assessment of disease activity, although it has important disadvantages such as its invasive nature and the risk of sampling error *(EASL-EASD-EASO Guidelines, 2016)*. This has inspired the search for non-invasive disease markers, but till now there are no non-invasive markers that can adequately distinguish NAFL from NASH *(Machado MV and Coretz-Pinto, 2013).*

Many markers have shown an acceptable accuracy for the exclusion of advanced fibrosis/cirrhosis (F3-F4) *(McPherson et al., 2013)*. The identification of advanced disease is less accurate, and the distinction between significant (≥ F2) or any (≥ F1) fibrosis versus no fibrosis remains difficult *(EASL-EASD-EASO Guidelines, 2016)*. The latter represents an unmet need, as recent guidelines recommend a closer follow-up of patients with significant fibrosis.

Endothelial dysfunction and pathological angiogenesis in turn predispose the liver to further injury as they increased intra-hepatic vascular resistance, distorted the sinusoidal microvascular architecture, modulated leukocyte infiltration and caused local tissue hypoxia *(Francque et al., 2012, Coulon et al., 2013*, and *Lefere et al., 2016)*. Both processes seem to be early events that precede the development of inflammation and fibrosis *(Pasarin et al., 2011)*, and further substantiate the links between NAFLD and cardiovascular disease *(Francque et al., 2016)*.

Different studies showed that VCAM-1 is a promising marker for ≥ F2 fibrosis *(Lefere et al., 2017)*.

The present study aimed to assess the level of serum vascular cell adhesion molecule-1 (VCAM-1) as non-invasive diagnostic tools for diagnosis NAFLD degree of fibrosis.

PATIENTS AND METHODS

A prospective study has been conducted at Al-Hussein Hospital, Al-Azhar University and Six October University Hospital in Cairo, Egypt between June 2019 and June 2020. This study has been conducted on 100 patients divided into four equal groups:

- Patients have steatosis confirmed by normal liver enzymes and transient elastography.
- Patients have steatohepatitis confirmed by elevated liver enzymes and transient elastography.
- Patients have viral cirrhosis.
- Non obese controls, who were healthy volunteers, have an overall good health, with normal results on liver function tests (SGOT, SGPT), and normal liver on ultrasonography, with a negative history of alcohol abuse.

Inclusion criteria: Patients with normal or elevated (SGOT, SGPT) and hepatomegally with increased echogenicity in abdominal ultrasonography with negative history of alcohol consumption.

Exclusion criteria:

1. Patients diagnosed with liver disease of other etiologies, including alcohol-induced, drug induced liver disease, viral hepatitis, auto-immune hepatitis, metabolic and cholestatic liver diseases, using specific clinical,

biochemical, and/or radiographic criteria.

2. Any patient on treatment with corticosteroids.
3. Patients diagnosed with hepatocellular carcinoma.
4. Patients diagnosed with inflammatory bowel disease.
5. Patients diagnosed with cancer colon and any type of malignancy.
6. Patients diagnosed with lupus or rheumatoid arthritis.
7. Heavy alcohol consumption (>40g pure alcohol per day).

All participants have been subjected to:

1. **Full medical history:** including age, sex, smoking, alcohol intake, and family history.
2. **Full clinical examination:**

- General: Arterial blood pressure, pulse, respiratory rate, and temperature.
- Local: abdominal contour, abdominal palpation, percussion, and auscultation.
- Cardiac, chest, and neurological examination.

3. **Anthropometric measurements:**

- Body weight was measured to an accuracy of 0.1 kg in light indoor clothing without shoes, and height was measured using a wall-mounted stadiometer.
- Body mass index (BMI) was calculated as body weight/height2 (kg/m2).
- Waist circumference was measured at the umbilicus.

4. **Laboratory investigations including:**

a. Liver function tests (aspartate aminotransferase (AST), alanine Transaminase (ALT), ɣ-glutamyltransferas (GGT), total and direct bilirubin, alkaline phosphatase).

b. Lipid profile: Triglycerides, high density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol and total serum cholesterol.

c. Serological markers (to exclude Wilson's disease, α1-antitrypsin deficiency and bilharziasis).

d. Antinuclear antibody (ANA) test to exclude autoimmune hepatitis.

e. CBC.

f. Kidney functions (Serum creatinine, Blood urea, Na and K).

g. Iron profile to exclude haemochromatosis.

h. Viral markers for hepatitis pattern (HbsAg, HCV IgG, HBc IgG) to exclude occult HBV.

i. Others: Fasting, 2HPP blood glucose, HbA1C, ESR, and C-reactive protein.

5. **Abdominal ultrasonography.**
6. **Transientelastography (Fibroscan).**
7. **Fibrosis score:** The FIB-4 ((age (years) × AST (U l /10))/ (thrombocytes (1000,000,000 per l) × ALT1/2 (U l /10))), and NAFLD Fibrosis Score.
8. **Measurement of serum Vascular Cell Adhesion Molecule-1(VCAM-1).**

An approval from ethical committee at the Faculty of Medicine, Al-Azhar University was obtained.

The procedures and the aim of the study were clearly explained to the patient and family. A written consent was obtained from every patient before enrollment into the study.

Statistical analysis: The data were collected, revised, coded and entered to a personal computer using Statistical Package for the Social Sciences (SPSS) version 23, tabulated and statistically processed.

Data were expressed as Mean ± SD for quantitative parametric measures and both number and percentage for categorized data. Analytical statistics was done using one way ANOVA test followed by Post-hoc tests (Tukey's test and Scheffe's Method). When data found non-parametric, median and inter-quartile range (IQR) were used. Also, qualitative variables were presented as number and percentages. The Comparison between groups with qualitative data was done by using Chi-square test. The comparison between two groups with quantitative data and parametric distribution were done by using Independent t-test. Data with non-parametric distribution were done by using Mann Whitney test. The comparison between more than two groups with quantitative data and parametric distribution were done by using One Way ANOVA test with post hoc analysis by LSD, while data with non-parametric distribution were done by using Kruskall Wallis test.

Receiver operating characteristic curve (ROC) was used in the quantitative form to determine sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and Area under curve (AUC) of VCAM1 between <F2 and ≥F2 fibrosis.

The confidence interval was set to 95% and the margin of error accepted was set to 5%. So, the p-value was considered significant when P value was ≤ 0.05.

RESULTS

In patients presented with steatosis, there were 48% males, 52% females, mean age 42.44 years old, and the mean BMI was 31.64 kg/m2. 32% of them were hypertensive and 44% were diabetics. The ALT serum median level was 22 U/L, AST median level was 20 U/L, total bilirubin median level was 0.8 mg/dl, INR mean was 1.01, and platelet count mean was 273 thousand/ul. The mean level of HDL was 47 mg/dl, LDL was 107 mg/dl, triglycerides was 137 mg/dl, total cholesterol was 162.68 mg/dl, fasting blood glucose was 127.32 mg/dl, 2 hours post prandial blood glucose was 174.76 mg/dl, and hemoglobin A1C was 7.1%. The mean of fibrosis in fibroscan was 6 kilo paskal. The CAP steatosis mean was 262.5db/L, median FIB-4 was 0.55, and median NFS was -3.16. The median of serum VCAM-1 was 2.27ngm/ml and its range was from 1 to 7.35ngm/ml. So, patients with steatosis were older than the control subjects (P = 0.079) and, as expected they had a higher BMI (P> 0.001), waist circumference (P> 0.001), fasting glucose (P = 0.066), 2hpp blood glucose (P= 0.005), HbA1C (P=0.044), triglycerides (P =0.003), lower HDL (P =0.162), and had more type 2 diabetes (P=0.001) and hypertension (P=0.010). Also, patients with steatosis had more steatosis grade (P>0.001) and fibrosis grade (P> 0.001) in fibroscan than the control subjects, but there was no signifcant difference in FIB-4 and VCAM1.

In patients presented with steatohepatitis, there were 40% males and 60% females, with mean age 47 years old, with mean BMI 29.78 kg/m2. There were 60% hypertensive patients, and 56% diabetic patients. ALT serum median level was 62 U/L, AST median level was 57 U/L, total bilirubin median level was 0.8 mg/dl, INR mean was 1.04, and platelet count mean was 213 thousand/ul. The mean level of HDL was 41.64 mg/dl, LDL was 142.28 mg/dl, triglycerides was 193.64 mg/dl, total cholesterol was 196.44 mg/dl, fasting blood glucose was 136 mg/dl, 2 hours post prandial blood glucose was 201.16 mg/dl, and hemoglobin A1C was 7.53%. The mean of fibrosis in fibroscan was 8.4 kilo paskal. The CAP steatosis mean was 280.36db/L, median FIB-4 was 1.69, median NFS was -0.58, and the median of serum VCAM-1 was 13ngm/ml and its range was from 11 to 50.96ngm/ml.

Patients with steatohepatitis were older than the control subjects (P > 0.001), and had a higher BMI (P> 0.001), waist circumference (P> 0.001), fasting glucose (P = 0.005), 2hpp blood glucose (P> 0.001), HbA1C (P>0.001), triglycerides (P >0.001), LDL (P >0.001), cholesterol (P =0.038), ALT (P > 0.001), AST (P >0.001), lower HDL(P >0.001), and had more type 2 diabetes (P>0.001) and hypertension (P>0.001). Also, patients with steatohepatitis had more steatosis grade (P>0.001) and fibrosis grade (P> 0.001) in fibroscan than the control subjects with significant FIB-4 (P >0.001), NFS (P >0.001), and VCAM1 (P >0.001).

Patients with steatohepatitis had more often hypertension than those with steatosis (P =0.047), higher ALT, AST, LDL, triglycerides, cholesterol (P> 0.001), and had lower HDL (P= 0.006). Also, patients with steatohepatitis had more steatosis grade (P =0.016) and fibrosis grade (P > 0.001) in fibroscan than those with steatosis with significant FIB-4 (P >0.001), NAFLD fibrosis score (P =0.005), and VCAM-1(P >0.001). Patients with steatohepatitis and steatosis did not differ significantly in Age, BMI, waist circumference, type 2 diabetes prevalence, alkaline phosphatase, bilirubin, INR, and renal functions. To assess the predictive value of serum VCAM-1 levels between steatosis and steatohepatitis, a ROC curve for prediction of steatohepatitis was generated for VCAM-1 which had a good predictive value, with AUROCs 1.000, and the best cutoff for VCAM-1 was 7.35 ng ml− 1, with a sensitivity of 100% and specificity of 100%.

In cirrhotic patients, there were 12% hypertensive patients and 44% diabetic patients. ALT serum median level was 33 U/L, AST median level was 31 U/L, total bilirubin median level was 1.8 mg/dl, INR mean was 1.38, and platelet count mean was 142 thousand/ul. The mean level of HDL was 47.76 mg/dl, LDL was 108.64 mg/dl, triglycerides was 105 mg/dl, total cholesterol was 176.2 mg/dl, fasting blood glucose was 121.56 mg/dl, 2 hours post prandial blood glucose was 176.24 mg/dl, and hemoglobin A1C was 6.63%. The mean of fibrosis in fibroscan was 13.44 kilo paskal, the CAP steatosis mean was 244.44db/L, median FIB-4 was 2.61, and the median of serum VCAM-1 was 1 ngm/ml and its range was from 1 to 4.33ngm/ml.

Patients with cirrhosis were older than patients with steatohepatitis (P=0.012), and had lower BMI (P=0.171), waist circumference (P=0.010), lower HTN prevelance (P>0.001), more DM

(P=0.466). Cirrhotics had lower levels of ALT (P>0.001), AST (P>0.001), albumin (P=0.003), platelet (P>0.001), LDL (P>0.001), triglycerides (P>0.001), cholestrol (P=0.021), higher total bilirubin (P>0.001), and INR (P>0.001) than patients with steatohepatitis. Also, cirrhotic patients had more fibrosis grade (P >0.001), less steatosis grade (P>0.001) in fibroscan, higher FIB-4 (P=0.011) and lower VCAM-1 (P>0.000) than patients with steatohepatitis **(Table 1** and **Table 2)**.

Table (1): Demographic data in studied groups (n= 100)

Parameters \ Groups		Control group No.= 25	Steatosis group No.= 25	Steatohepatitis No.= 25	Cirrhosis No.= 25	P-value
Age (years)	Mean±SD	35.32 ± 6.79	42.44 ± 11.74	47.80 ± 11.17	56.12 ± 8.11	<0.001 ǂ
	Range	19 – 48	29 – 68	25 – 65	44 – 73	
BMI (kg/m2)	Mean±SD	25.46 ± 3.33	31.46 ± 4.97	29.78 ± 4.59	28.02 ± 4.91	<0.001 •
	Range	18.41 – 32.05	22.21 – 42.06	22.86 – 38.63	21.44 – 39	
Waist (cm)	Mean±SD	81.48 ± 4.58	93.0 ± 10.07	96.04 ± 11.01	89.24 ± 9.68	<0.01 •
	Range	72 – 91	77 – 110	77 – 123	78 – 110	
ALT SGPT (U/L)	Median (IQR)	25 (18 – 29)	22 (20 – 28)	62 (50.7 – 80)	33 (27 – 34)	<0.001
	Range	11 – 35	14 – 31	30 – 186	8 – 54	
AST SGOT (U/L)	Median (IQR)	22 (18 – 26)	20 (17 – 22)	57 (41 – 72)	31 (29 – 39)	<0.001
	Range	11 – 34	13 – 28	30 – 256	22 – 67	
Platlet (cmm)	Mean±SD	312.96 ± 55.25	273.00 ± 62.85	213.84 ± 65.94	142.28 ± 59.40	<0.001 ǂ
	Range	190 – 420	100 – 401	130 – 471	50 – 275	
HDL (mg/dl)	Mean±S D	53.92 ± 14.71	46.96 ± 7.12	41.64 ± 6.32	47.76 ± 9.23	0.002ǂ
	Range	33 – 90	29 – 58	31 – 56	27 – 60	
LDL (mg/dl)	Mean±SD	103.44 ± 15.15	106.92 ± 21.18	142.28 ± 20.05	108.64 ± 24.77	<0.001•
	Range	79 – 129	63 – 141	110 – 179	64 – 147	
TGs (mg/dl)	Mean±SD	104.92 ± 24.56	137.08 ± 38.90	193.64 ± 39.45	105.00 ± 35.23	<0.001ǂ
	Range	66 – 145	69 – 190	109 – 287	54 – 172	
Total Cholesterol (mg/dl)	Mean±SD	178.36 ± 17.74	162.68 ± 40.07	196.44 ± 33.29	176.20 ± 25.85	<0.001•
	Range	150 – 220	67 – 231	156 – 300	129 – 230	
Fasting blood glucose (mg/dl)	Mean±SD	84.16 ± 8.69	127.32 ± 65.88	136.08 ± 57.02	121.56 ±54.21	0.046ǂ
	Range	69 – 105	66 – 274	72 – 270	70 – 246	
2hpp blood glucose (mg/dl)	Mean±SD	105.48 ± 12.01	174.76 ± 94.59	201.16 ± 87.42	176.24 ± 84.00	<0.001
	Range	87 – 128	87 – 400	95 – 400	86 – 328	
A1C (%)	Mean±SD	5.06 ± 0.35	7.10 ± 2.87	7.53 ± 2.28	6.63 ± 1.94	<0.001ǂ
	Range	4.4 – 5.8	4.6 – 15.6	4.9 – 13.3	4.5 – 11	
Fibroscan fibrosis (kilo.pask)	Mean±SD	5.09 ± 0.42	6.05 ± 0.63	8.40 ± 2.61	13.44 ± 3.40	<0.001 ǂ
	Range	4.4 – 5.8	4.3 – 6.9	4.4 – 17.4	5.8 – 17.6	
Equivalent to grade	F0	25 (100.0%)	10 (40.0%)	1 (4.0%)	0 (0.0%)	<0.001*
	F1	0 (0.0%)	15 (60.0%)	7 (28.0%)	1 (4.0%)	
	F2	0 (0.0%)	0 (0.0%)	8 (32.0%)	5 (20.0%)	
	F3	0 (0.0%)	0 (0.0%)	5 (20.0%)	2 (8.0%)	
	F4	0 (0.0%)	0 (0.0%)	4 (16.0%)	17 (68.0%)	
CAP steatosis (db/L)	Mean±SD	204.20 ± 13.47	262.52 ± 27.65	280.36 ± 28.61	244.44 ± 30.07	<0.001 •
	Range	184 – 220	231 – 342	228 – 330	205 – 319	
Steatosis grade	S0	25 (100.0%)	0 (0.0%)	0 (0.0%)	8 (32.0%)	<0.001 *
	S1	0 (0.0%)	14 (56.0%)	5 (20.0%)	10 (40.0%)	
	S2	0 (0.0%)	9 (36.0%)	14 (56.0%)	5 (20.0%)	
	S3	0 (0.0%)	2 (8.0%)	6 (24.0%)	2 (8.0%)	
FIB-4	Median (IQR)	0.65 (0.5 – 0.75)	0.55 (0.5 – 0.72)	1.69(0.98– 2.47)	2.61(1.52– 4.01)	<0.001 ǂ
	Range	0.33 – 1.12	0.33 – 2.7	0.43 – 5.86	0.72 – 11.2	
NAFLD fibrosis Score	Median (IQR)	-3.40 (-3.90 – -3.23)	-3.16 (-3.40 – -0.65)	-0.58 (-1.70 – 0.42)	--	0.005ǂ
	Range	-4.44 – -2.26	-4.44 – 1.35	-4.37 – 2.03	--	
VCAM1 (ngm/ml)	Median (IQR)	2.56 (1 – 2.71)	2.27 (1 – 3.26)	13 (12 – 18.26)	1 (1 – 2.12)	<0.001ǂ
	Range	1 – 4.26	1 – 7.35	11 – 50.96	1 – 4.33	

*:Chi-square test; •: One Way ANOVA test; ǂ: Kruskall Wallis test

Table (2): Comparison between P value of different parameters of all groups

	Post Hoc analysis					
	P1	**P2**	**P3**	**P4**	**P5**	**P6**
Age (years)	**0.079**	**<0.001**	**<0.001**	**0.120**	**<0.001**	**0.012**
BMI (kg/m2)	**<0.001**	**<0.001**	**0.047**	**0.190**	**0.008**	**0.171**
Waist (cm)	**<0.001**	**<0.001**	**0.004**	**0.245**	**0.151**	**0.010**
ALT SGPT (U/L)	**-0.370**	**<0.001**	**0.003**	**<0.001**	**<0.001**	**<0.001**
AST SGOT (U/L)	**-1.080**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Platlet (cmm)	**0.037**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
HDL (mg/dl)	**0.162**	**<0.001**	**0.210**	**0.006**	**0.793**	**0.015**
LDL (mg/dl)	**0.551**	**<0.001**	**0.374**	**<0.001**	**0.768**	**<0.001**
TGs (mg/dl)	**0.003**	**<0.001**	**0.648**	**<0.001**	**0.004**	**<0.001**
Total Cholesterol (mg/dl)	**0.071**	**0.038**	**0.802**	**<0.001**	**0.119**	**0.021**
Fasting blood glucose (mg/dl)	**0.066**	**0.005**	**0.082**	**0.567**	**0.749**	**0.299**
2hpp blood glucose (mg/dl)	**0.005**	**<0.001**	**0.004**	**0.184**	**0.869**	**0.268**
A1C (%)	**0.044**	**<0.001**	**0.003**	**0.140**	**0.946**	**0.111**
Fibroscan fibrosis (kilo.pask)	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
FIB-4	**0.861**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**0.011**
CAP steatosis (db/L)	**<0.001**	**<0.001**	**<0.001**	**0.016**	**0.015**	**<0.001**
NAFLD fibrosis Score	**0.018**	**<0.001**	**--**	**0.005**	**--**	**--**
VCAM1	**0.831**	**0.000**	**0.159**	**0.000**	**0.170**	**0.000**

P1: Control group Vs Steatosis group, P2: Control group Vs Steatohepatitis, P3: Control group Vs Cirrhosis, P4: Steatosis group Vs Steatohepatitis, P5: Steatosis group Vs Cirrhosis, P6: Steatohepatitis VS Cirrhosis

Steatohepatitis patients with ≥ F2 fibrosis were older than steatohepatitis patients with <F2 fibrosis (mean 50.82 and 41.38, P = 0.054), had a higher waist circumference (P=0.002), more type 2 diabetes with insignificant P value, lower platelet count (P=0.003), significant FIB-4 score (P=0.013) and had significant NAFLD fibrosis score (P<0.001). Serum VCAM-1 levels were higher in patients with > F2 fibrosis compared to the patients with <F2 fibrosis (median 15.33 ngm ml− 1, ranging from 11.13 to 50.96 ngm ml− 1 with significant P value=0.003) **(Table 3)**.

Table (3): Comparison between Steatohepatitis patients without significant fibrosis (<F2) versus those with significant fibrosis (≥F2):

Parameters / Groups		<F2 No.= 8	≥F2 No.= 17	P- value
Age (years)	Mean±SD	41.38 ± 12.83	50.82 ± 9.20	**0.054**
	Range	25 – 57	35 – 65	
BMI (kg/m2)	Mean±SD	29.27 ± 5.78	30.02 ± 4.09	0.712
	Range	23.77 – 38.63	22.86 – 38.51	
Waist circumference (cm)	Mean±SD	86.75 ± 7.38	100.41 ± 9.73	**0.002**
	Range	77 – 97	85 – 123	
ALT SGPT (U/L)	Median (IQR)	64 (54.35 – 131.5)	60 (50 – 68)	0.281
	Range	50 – 186	30 – 180	
AST SGOT (U/L)	Median (IQR)	53.15 (33 – 91.5)	59 (42 – 69)	0.884
	Range	30 – 256	36 – 100	
Platelets (cmm)	Mean±SD	266.75 ± 85.24	188.94 ± 35.83	**0.003**
	Range	198 – 471	130 – 250	
HDL (mg/dl)	Mean±SD	41.63 ± 4.53	41.65 ± 7.14	0.725
	Range	35 – 46	31 – 56	
LDL (mg/dl)	Mean±SD	138.13 ± 10.12	144.24 ± 23.35	0.489
	Range	126 – 156	110 – 179	
TGs (mg/dl)	Mean±SD	188.13 ± 38.81	196.24 ± 40.66	0.838
	Range	109 – 230	130 – 287	
Cholesterol (mg/dl)	Mean±SD	196.0 ± 21.71	196.65 ± 38.16	0.965
	Range	159 – 220	156 – 300	
Fasting blood glucose (mg/dl)	Mean±SD	119.0 ± 66.28	144.12 ± 52.34	0.180
	Range	73 – 255	72 – 270	
2hpp blood glucose (mg/dl)	Mean±SD	164.88 ± 84.48	218.24 ± 85.86	0.137
	Range	100 – 320	95 – 400	
A1C (%)	Mean±SD	6.95 ± 2.28	7.81 ± 2.30	0.541
	Range	5.2 – 10.9	4.9 – 13.3	
Fibroscan fibrosis (kilo.paskal)	Mean±SD	6.40 ± 0.82	9.34 ± 2.64	**<0.001**
	Range	4.4 – 6.8	7 – 17.4	
CAP steatosis (db/L)	Mean±SD	275.00 ± 33.15	282.88 ± 26.95	0.532
	Range	235 – 330	228 – 330	
FIB-4	Mean±SD	1.22± 0.85	2.23 ± 1.23	**0.013**
	Range	0.43 – 3.21	0.77 – 5.86	
NAFLD fibrosis Score	Median (IQR)	-3.27 (-3.68 - -2.26)	-0.15 (-0.75 – 0.74)	**<0.001**
	Range	-4.37 – 0.51	-3.08 – 2.03	
VCAM1(ngm/ml)	Median (IQR)	11 (11 – 13)	15.33 (12.2 – 20.89)	**0.003**
	Range	11 – 13	11.13 – 50.96	

•: Independent t-test; ‡: Mann Whitney test

To assess the predictive value of serum VCAM-1 levels between <F2 and ≥F2 fibrosis in steatohepatitis patients, a ROC curve for prediction of ≥ F2 fibrosis was generated for VCAM-1 which had a good predictive value, with AUROCs 0.868, and the best cut off for VCAM-1 was 13 ng ml− 1, with a sensitivity of 70.59% and specificity of 100% **(Figure 1** and **Table 4)**.

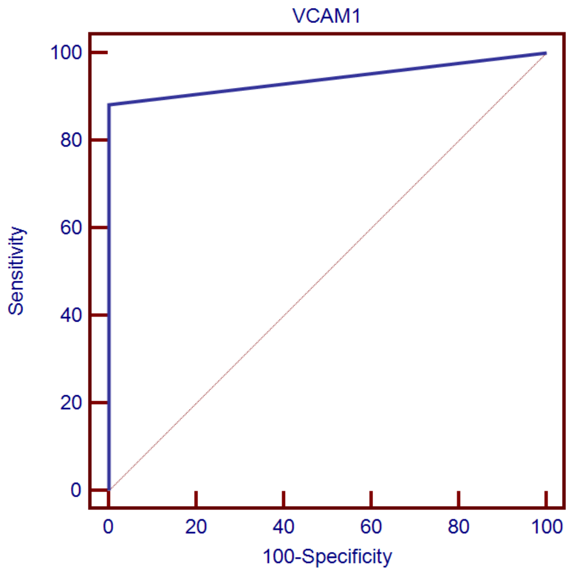

Figure (1): ROC curve of VCAM 1 as a predictor between <F2 and ≥F2 in Steatohepatitis patients.

Table (4): ROC curve of VCAM 1 as a predictor between <F2 and ≥F2:

Parameter	AUC	Cut of Point	Sensitivity	Specificity	PPV	NPV
VCAM1	0.868	>13	70.59	100.0	100.0	61.5

DISCUSSION

The main purpose of this study was the evaluation of seum vascular cell adhesion molecule-1 in distinguishing between various NAFLD disease stages. Our results pointed to vascular cell adhesion molecule-1 (VCAM-1) as a promising marker for ≥ F2 fibrosis.

In our study, patients presented with steatosis were older than the control subjects and had a higher BMI, waist circumference, fasting glucose, 2hpp blood glucose, A1C, triglycerides, lower HDL, more type 2 diabetes and hypertension. Also, patients with steatosis had more steatosis grade and fibrosis grade in fibroscan than the control subjects but there was no signifcant difference in FIB-4 and VCAM1.

Similar to our results, *Lefere et al. (2017)* stated that NAFLD patients were older than the control subjects, had a higher BMI, waist circumference, fasting glucose, ALT, AST, more type 2 diabetes and hypertension, but with insignificant difference in VCAM-1 between NAFLD and control group.

In agreement with our study, *Bilgir et al. (2015)* showed that the levels of adhesion molecules in patients with NAFLD were higher than those in the control subjects but only a significant difference in seum E-selectin levels between the NAFLD and control groups was observed. However, there were no statistically significant differences in sICAM-1 and sVCAM-1 levels between NAFLD group and control group.

Our results, as regards patients presented with steatohepatitis, they were older than the control subjects, had a higher BMI, waist circumference, fasting glucose, 2hpp blood glucose, HbA1C, triglycerides, LDL, cholesterol, ALT, AST, more type 2 diabetes and hypertension. Also, patients with steatohepatitis had more steatosis grade and fibrosis grade in fibroscan than the control subjects with significant FIB-4, NFS, and VCAM1.

In our study, we also detected a statistically significant difference between patients with steatohepatitis and patients with steatosis, as patients with steatohepatitis had more often hypertension than those with steatosis, had higher ALT, AST, LDL, triglycerides, and cholesterol. Also, patients with steatohepatitis had more steatosis grade and fibrosis grade in fibroscan than those with steatosis with significant FIB-4, NAFLD fibrosis score, and VCAM-1. Patients with steatohepatitis and steatosis did not differ significantly in age, BMI, waist circumference, type 2 diabetes prevalence, serum HDL cholesterol, alkaline phosphatase, bilirubin, and INR.

Similar to our results, *Lefere et al. (2017)* showed that patients with NASH were older than those with NAFL and had a higher fasting glucose level, and more often had type 2 diabetes. Patients with NAFLD and NASH did not differ significantly in BMI, serum triglycerides, cholesterol, LDL cholesterol, HDL cholesterol, ALT, AST, thrombocytes, C-reactive protein, or the presence of hypertension.

In our study, we detected a significant difference in serum VCAM-1 level between the statosis and steatohepatitis groups, and to assess the predictive value of serum VCAM-1 levels between steatosis and steatohepatitis, a ROC curve for prediction of steatohepatitis was generated for VCAM-1 which had a good predictive value, with AUROCs 1.000, and the best cutoff for VCAM-1 was 7.35 ng ml− 1, with a sensitivity of 100% and specificity of 100%.

Similar to our results, *Mosa et al. (2011)* concluded that there was a significant increase in circulating levels of ICAM-1, VCAM-1 and E-selectin in NAFLD compared to healthy control subjects and it may be used to comprehensively using the ability of circulating VCAM-1, E-selectin and ICAM-1 to predict fatty liver disease.

Our study found that steatohepatitis patients with ≥ F2 fibrosis were older than steatohepatitis patients with <F2 fibrosis, had a higher waist circumference, more type 2 diabetes with insignificant P value, lower platelet count, significant FIB-4 score and NAFLD Fibrosis Score.

In close to our results, *Lefere et al. (2017)* showed that apart from VCAM-1, only the presence of type 2 diabetes, and serum LDL and total cholesterol were significantly associated with - F2 fibrosis in the NASH patients.

In our study, we found that serum VCAM-1 levels were higher in patients with - F2 fibrosis compared to the patients with <F2 fibrosis (median 15.33 and 11 ngm ml− 1 respectively with significant P value). To assess the predictive value of serum VCAM-1 levels, a ROC curve for prediction of - F2 fibrosis was generated for VCAM-1 which had a good predictive value, with AUROCs 0.868, and the best

cutoff for VCAM-1 was 13 ng ml− 1, with a sensitivity of 70.59% and specificity of 100%.

Similar to our results, Lefere et al. (2017) showed that serum VCAM-1 levels were higher in patients with ⩾ F2 fibrosis compared to the patients with <F2 fibrosis (median 14.0 and 8.7 ng ml− 1, respectively). They assessed the predictive value of serum VCAM-1 levels, a ROC curve for prediction of - F2 fibrosis was generated for VCAM-1, and they found that VCAM-1 had a good predictive value. The best cutoff for VCAM-1 was 13.2 ng ml− 1, with a sensitivity of 80% and specificity of 83%. Given the prevalence of significant fibrosis of 0.33 in their population, and this corresponded to a positive and negative predictive value of 70% and 89%, respectively. Furthermore, they recruited a second cohort of obese patients undergoing bariatric surgery to make external validation of VCAM-1, and they found that serum VCAM-1 levels were higher compared to patients without significant fibrosis. The AUROC for F2 fibrosis was 0.89. A low cutoff (15.6 ng ml− 1) had a sensitivity of 100% and specificity of 68.4%, whereas a higher cutoff of 18.4 ng ml− 1 had a sensitivity and specificity of 66.7% and 84.2%, respectively.

In agreement with our results, *Kar et al. (2019)* found that VCAM-1 levels were elevated by 55% and 40% in the advanced and mild fibrosis groups compared to no fibrosis cohort, respectively. Also, VCAM-1 positively correlated with FIB4. Furthermore, VCAM-1 demonstrated better performance to distinguish between no fibrosis from advanced stages (AUROC = 0.87) and mild fibrosis from advanced fibrosis (AUROC = 0.79). However, sensitivity was considered poor for distinguishing no fibrosis compared to mild fibrosis (AUROC = 0.53). They stated that addition of biomarkers such as IL-6 and VCAM-1 to panels may yield increased sensitivity and specificity for staging of NASH.

VCAM-1 has been recognized as a good biomarker of NASH fibrosis by *Yoshimura et al. (2016)* who performed a robust clinical examination of 261 biomolecules in 132 NASH patients. Diagnostic biomarkers of NASH fibrosis were determined based on data mining in a “factor module” scheme, where multiple mutually correlated results were considered as a single dataset. Within the factor module, VCAM-1 stood out as a biomarker of interest for NASH fibrosis and formed the basis of the FM-Fibro Index.

Okanoue et al. (2018) displayed diagnostic accuracy over 0.90 by AUROC when comparing mild (F0-2) to advanced (F3-4) fibrosis stages. On the other hand, in a large, multicenter study in biopsy-proven NASH patients, *Itoh et al. (2018)* found that FM-Fibro index had lower, although sufficient accuracy for predicting NASH-related fibrosis (AUROC ~0.70), yet excellent positive predictive value.

In our study, regarding the cirrhotic patients, we found that patients with cirrhosis were older than patients with steatohepatitis, had lower BMI, waist circumference, lower HTN prevelance, and more DM prevelance. Cirrhotic patients had lower levels of ALT, AST, albumin, platelet, LDL, triglycerides, cholesterol, higher total bilirubin, and INR than patients with steatohepatitis. Also, cirrhotic patients had more fibrosis grade in fibroscan, higher FIB-4, but lower seum VCAM-1 than patients with steatohepatitis.

Joanna et al. (2014) conducted a study on patients who underwent HCC resection. Preoperative serum levels of soluble VCAM-1 were measured. Serum VCAM-1 level in HCC patients was inversely correlated with platelet count and serum albumin level, but positively correlated with serum bilirubin level. Serum VCAM-1 level was not associated with tumor characteristics. Serum VCAM-1 level was significantly higher in HCC patients with cirrhosis compared with those without cirrhosis.

CONCLUSION

- While many markers have shown an acceptable accuracy for the exclusion of advanced fibrosis/cirrhosis (F3-F4), the identification of advanced disease is less accurate, and the distinction between significant (≥F2) and any (≥F1) fibrosis vs no fibrosis remains difficult.
- VCAM-1 may be a useful biomarker in distingushing steatosis from steatohepatitis, and for the diagnosis of significant (≥F2) liver fibrosis in steatohepatitis patients.
- The non-invasive diagnosis of moderate stages of fibrosis in NAFLD represented an important unmet clinical need so, this study has identified vascular cell adhesion molecule 1 (VCAM-1) as a promising marker for diagnosing significant (≥F2) fibrosis in patient with steatohepatitis.

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جزيء الالتصاق 1 بالخلايا الوعائية في مصل الدم يتنبأ بمدى تليف الكبد في مرض الكبد الدهني غير الكحولي

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خلفية البحث: مرض الكبد الدهني غير الكحولي هو أكثر أمراض الكبد المزمنة شيوعًا في جميع أنحاء العالم ويرتبط بشدة بالسمنة وخلل دهون الدم وزيادة مقاومة الأنسولين. غالبًا ما يظهر مرض الكبد الدهني غير الكحولي على شكل تجمع دهني بسيط بالكبد ولكن يمكن أن يتطور إلى التهاب الكبد الدهني غير الكحولي والتليف. الدلائل الغير جراحية الحيوية الحالية لا تستطيع ان تحدد درجة تليف الكبد الهامة من الدرجة الثانية فأكثر، على الرغم من أن الإرشادات الحديثة توصي بمتابعة صارمة لهذه المجموعة من المرضى.

هذه الدراسة وغيرها أبلغت عن دور تكوين الأوعية الدموية المرضية في التسبب في مرض الكبد الدهني غير الكحولي، مع إبراز العوامل المؤيدة لتولد الأوعية المرضية كدلائل تشخيصية محتملة.

الهدف من البحث: البحث في قابلية تطبيق جزيء الالتصاق 1 بالخلايا الوعائية كدلالات تشخيصية غير جراحية لتحديد تليف الكبد الناتج عن التهاب الكبد الدهني غير الكحولي.

المرضى وطرق البحث: أجريت هذه الدراسة على 100 مريضا في مستشفى الحسين - جامعة الأزهر ومستشفى السادس من أكتوبر الجامعي في الفترة ما بين يونيو 2019 إلى يونيو 2020، وقد تم تقسيمهم الى أربعة مجموعات متساوية واشتملت على 25 مريضا يعانون من تجمع دهني بالكبد و 25 مريضا مصابين بالتهاب الكبد الدهني و 25 مريض مصابا بتليف الكبد الفيروسي و 25 شخصا من الأصحاء. وخضع جميع المشاركين إلى التاريخ المرضي الكامل، والفحص

السـريري، والفحوصـات المخبريـة، والموجـات فـوق الصـوتية علـى الـبطن، والفيبروسكان، وتحليل مصل جزيء الالتصاق 1 بالخلايا الوعائية.

نتـائج البحـث: حـددت دراسـتنا جـزيء إلتصـاق 1 بالخلايـا الوعائيـة فـي الـدم كمتنبـئ مسـتقل لتليـف الكبـد مـن الدرجـة الثانيـة او أكثـر (الوسـيط 15.33 مقابـل 11 نـانوغرام/ مـل فـي المرضـى الـذين يعـانون مـن تليـف الكبـد الهـام و المرضـى الـذين لايعنـون مـن تليـف الكبـد الهـام علـى الترتيـب ؛ وكانـت قيمـة معامـل الاحتمـال <0.003) مـع وجـود منطقـة تحـت المنحنـى تسـاوي 0.868 للتنبـوء بتليـف الكبـد المسـاوي اوالاكثـر مـن الدرجـة الثانيـة و كـان ذلـك لـه قيمـة تنبؤيـة جيـدة، وكـان أفضـل حـد لـجزيء الالتصـاق 1 بالخلايـا الوعائيـة هـو 13 نـانوغرام/ مـل، مـع نسـبة حساسية 70.59٪ ونسبة دقة 100٪.

الاسـتنتاج: مسـتويات جـزيء الالتصـاق 1 بالخلايـا الوعائيـة فـي الـدم قـادرة علـى التنبـوء بدقـة بتليـف الكبـد الهـام مـن الدرجـة الثانيـة او أكثـر فـي مرضـى الكبـد الـدهني غير الكحولي.

الكلمـات الدالـة: مـرض الكبـد الـدهني غيـر الكحـولي، التهـاب الكبـد الـدهني غيـر الكحولي، الفيبروسكان، جزيء الالتصاق 1 بالخلايا الوعائية.