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Evaluation Of Serum Vascular Endothelial Growth Factor Receptor 2 Levels In Non Alcoholic Fatty Liver Disease As A Complication Of Hypothyroidism In Iraqi Patients

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Abstract

Vascular endothelial growth factor, also identified as vascular permeability factor, is a powerful vascular permeability factor with strong mitogenic properties for endothelial cells. It acts as the most powerful angiogenesis factor . The purpose of this study was to determine the levels of Vascular endothelial growth factor receptor-2 (VEGF-R2) in hypothyroidism patients with and without fatty liver disease and compare the results to a healthy controls. In addition to examining the association between VEGF-R2 and anthropometric and clinical features such as age, gender, BMI, and duration of hypothyroidism, serum thyroid hormones such as T3, TT4, and TSH, lipid profiles such as serum cholesterol, TG, and HDL, liver enzymes such as GOT , GPT and ALP, kidney function tests such as blood urea and serum creatinine, glycemic parameters such as FBG and HbA1c levels, Serum Hematological Parameters (PCV, MCV and WBC) and Serum Protein Parameters (Total Protein, serum albumin and serum Ferritin) levels.

Ninety people took part in the study and were divided into three groups: G1: Patients who have hypothyroidism and fatty liver disease, G2: Patients who do not have hypothyroidism and fatty liver disease, and G3: Healthy control groups. The Specialized Center for Endocrinology and Diabetes in Baghdad was used to select all study cases. The age ranges of G1 ,G2 and G3 were(3264), (23-67) and (24-40) years respectively. The results showed that the mean serum VEGF-R2 levels in G1 (471.340126.522 pg/ml) and G2 (438.11378.962 pg/ml) were significantly higher (P=0.0001) than the mean serum VEGF-R2 levels in G3 (275.59071.220 pg/ml), while the mean serum VEGF-R2 levels in G1 (471.340126.522 pg/ml) The Receiving Operating Characteristic (ROC) curves analysis for serum VEGF-R2 levels was used as a test to divide subjects into cases and controls, and to determine the "cut-off value" which of optimum sensitivity and specificity to diagnose disease.

Keywords: VEGF-R2 , Non-Alcoholic Fatty liver disease , Hypothyroidism , ROC analysis .

Introduction:

Historically, non-alcoholic fatty liver disease (NAFLD) was viewed as a disease of the industrialized world, primarily linked to metabolic syndrome, diabetes, and obesity [1-3]. NAFLD is classified into two types based on histology: NAFL and NASH [1], NAFL requires more than 5% hepatic steatosis with no evidence of hepatocyte injury, whereas NASH requires more than 5% hepatic steatosis with evidence of inflammation and hepatocyte injury [4]. The non-alcoholic fatty liver disease (NAFLD) can cause severe comorbidities and functional liver damage. NAFLD can progress to steatohepatitis, which can cause progressive liver damage and eventually lead to cirrhosis and hepatocellular carcinoma. NAFLD is considered the hepatic manifestation of the metabolic syndrome because it is associated with metabolic risk factors such as dyslipidemia, insulin resistance, hypertension, and visceral obesity [5]. Obesity is frequently associated with NAFLD. Hypothyroidism, which is also linked to obesity, may play a role in the dysmetabolic state that predisposes to NAFLD [6]. Thyroid hormones play a vital role in body weight regulation, lipid metabolism, and insulin resistance. As a result, thyroid hormones are likely to play a role in the pathogenesis of non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH) [7]. The levels of VEGF and its receptors, vascular endothelial growth factor receptor 1 (VEGFR-1) and VEGFR-2, rise as liver fibrosis progresses [8], and it is well known that vascular growth factors (VEGFs) play a crucial role in angiogenesis regulation via their receptors VEGFRs [9, 10] .

Materials and Methods:

Study Design and Subjects:

Ninety people took part in this study and were divided into three groups: G1: Patients with hypothyroidism and fatty liver disease, G2: Patients without hypothyroidism and G3: healthy control groups . All research cases were selected from the Specialized Center for
Endocrinology and Diabetes in Baghdad between December 2019 and March 2020. The age ranges of G1 ,G2 and G3 were (32-64), (23-67) and (24-40) years respectively. Ten milliliters of venous blood were pulled from the case studies and control samples and placed in a plain tube for (15 minutes) at room temperature before being centrifuged at 4000rpm for 10 minutes to obtain serum, which was stored at (-20oC) unless used immediately. HbA1c levels were calculated using whole blood.The Age, Gender, BMI and Duration of Hypothyroidism,T3, T4 and TSH, Serum Cholesterol, Triglyceride TG and HDL-c, GOT , GPT and ALP ,blood Urea, serum Creatinine, F.B.G, HbA1c , PCV, MCV and WBC, Total Protein, serum albumin and serum Ferritin levels were recorded. The participants' BMI has been calculated as weight (kg)/height squared (m^2). An experienced radiologist diagnosed NAFLD based on increased echogenicity via ultrasound, which is consistent with fatty infiltration of the liver.

Measurement of Serum Levels of VEGF-R2

The enzyme-linked immunosorbent assay (ELISA) VEGF-R2 kit was used to measure VEGF-R2 levels.

Results & Discussion:

Serum Vascular Endothelial Growth Factor Receptor 2 (VEGF-R2) Levels

Data in table(1) demonstrated the mean value of serum VEGF-R2 in G1 (471.340±126.522 pg/ml) which was highly significant increased (P=0.0001) compared with the mean serum VEGF-R2 in G3 (275.590±71.220 pg/ml), while the mean value of serum VEGF-R2 in G1 (471.340±126.522 pg/ml) was slightly increased compared with the mean serum of VEGF-R2 in G2 (438.113±78.962 pg/ml) as shown in figures (1 and 2).

Table (1): Serum levels of VEGF-R2 in G1, G2 and G3.

	G1	G2	G3	P value
Vascular Endothelial Growth Factor Receptor	471.340±126.522 (286.998-676.37)	438.113±78.962 (333.12-588.49)	275.590±71.220 (154.38-410.15)	0.0001#

2 (VEGF-R2) (pg/ml)				
#Significant difference among three independent means using ANOVA test at 0.05 level.				

G1: Hypothyroidism with fatty liver disease patients
G2: Hypothyroidism without fatty liver disease patients
G3: healthy control groups

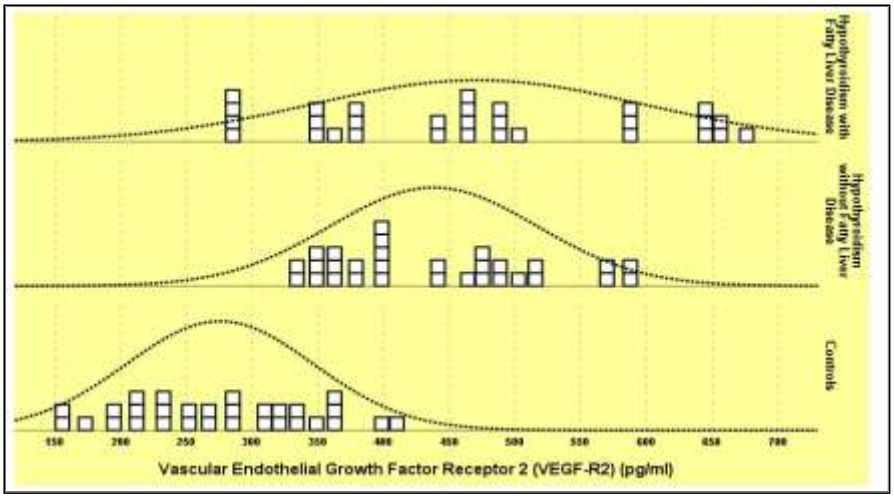

Figure (1): Serum VEGF-R2 levels in **G1**: Hypothyroidism with fatty liver disease patients, **G2** Hypothyroidism without fatty liver disease patients and **G3**: controls.

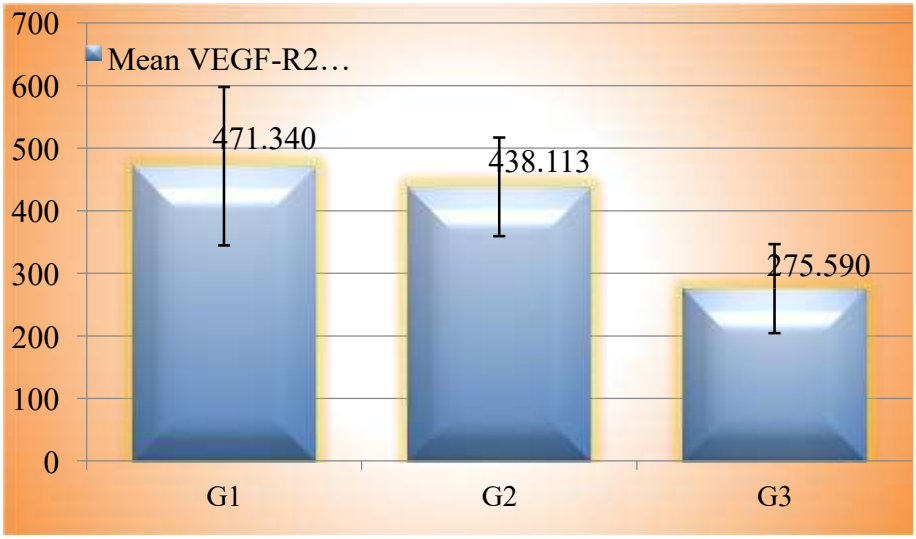

Figure (2): Mean Values of Serum VEGF-R2 levels in **G1**: Hypothyroidism with fatty liver disease patients, **G2**: Hypothyroidism without fatty liver disease patients and **G3**: controls.

The link between hypothyroidism and NAFLD may be supported by the association between thyroid dysfunction and metabolic syndrome. A recent study discovered a significant relation between thyroid dysfunction and metabolic syndrome parameters, which supports the link between thyroid dysfunction and NAFLD [11]**.** One more study suggests that hypothyroidism and NAFLD are linked due to the active influence of thyroid hormones on lipid metabolism, carbohydrate, protein, and energy exchange [7]. The majority of previous studies revealed the role of the VEGF-VEGFR2 complex in the vascular changes and angiogenesis process caused by cirrhosis and hepatocellular carcinoma. The most potent angiogenic factor is vascular endothelial growth factor, also known as vascular permeability factor. VEGF expression was noticeably higher in cirrhotic livers than in non-cirrhotic livers. The expression of the potent angiogenic factor VEGF and its receptors, VEGFR-1 (vascular endothelial growth factor receptor 1) and VEGFR-2, has been shown to increase during the progression of liver fibrosis [8]. Vascular growth factors (VEGFs) are well known to play a crucial role in the regulation of angiogenesis via the receptors VEGFRs [9]. The accumulation of red blood cells

revealed a faulty vasculature resulting in impaired blood flow through the liver. In addition to the decreased hepatic lipid stores, it is possible that these accumulated red blood cells contribute to the mutant liver's color phenotype. The authors demonstrated that VEGF signaling in the liver is required for the development of functional sinusoidal vasculature, which is needed for efficient plasma lipoprotein uptake [12].

Serum VEGF-R2 Level in Relation to Clinical Characteristics:

Data in Table (2) showed the statistical analysis of the relationship between serum Vascular Endothelial Growth Factor Receptor-2 (VEGF-R2) levels and grading of the related clinical characteristics in all studied groups. Results in Table (2) showed a significant change in VEGF-R2 levels with age categories in G1 (P=0.009) and with BMI categories in G1 (P=0.008).

Table (2): Relationship Between Serum VEGF-R2 Levels and Grading of The Related Clinical Characteristics in G1, G2 and G3.

		Vascular Endothelial Growth Factor Receptor 2 (VEGF-R2) (pg/ml)					
		G1		G2		G3	
		No	Mean ±SD(Range)	No	Mean ±SD(Range)	No	Mean ±SD(Range)
Age (years)	20---29	-	-	3	436.82±70.14 (358.265-493.159)	25	272.04±65.50 (154.381-367.255)
	30---39	5	386.71±47.18 (351.877-438.801)	3	413.87±88.35 (348.046-514.283)	4	312.28±108.09 (191.547-410.150)
	40---49	8	534.81±112.53 (375.871-676.374)	12	457.53±91.50 (333.121-588.491)	1	217.52±
	50---59	11	410.75±129.03 (286.998-593.193)	7	394.02±51.49 (348.627-500.092)	-	-
	60---69	6	568.32±84.08 (489.890-645.209)	5	468.56±75.54 (364.048-573.718)	-	-
	P value		0.009#		0.436		0.423
Gender	Male	6	390.80±110.40 (289.928-493.522)	9	404.33±61.96 (335.025-514.283)	8	286.64±87.45 (154.381-410.150)
	Female	24	491.47±124.17 (286.998-676.374)	21	452.59±82.29 (333.121-588.491)	22	271.57±66.28 (157.007-367.255)
	P value		0.081		0.127		0.617
BMI (Kg/m2)	Normal (18.5-24.9)	4	613.76±41.74 (592.283-676.374)	5	407.35±81.87 (335.025-514.283)	26	275.23±73.31 (154.381-410.150)
	Overweight (25-29.9)	11	460.99±119.49 (286.998-656.447)	7	465.41±82.98 (365.004-573.718)	4	277.95±65.12 (208.926-345.380)
	Obese I (30-34.9)	4	327.22±74.39 (289.928-438.801)	12	424.18±73.77 (333.121-582.377)	-	-
	Obese II (=>35)	11	482.31±115.28 (351.877-645.209)	6	459.77±86.88 (348.627-588.491)	-	-
	P value		0.008#		0.511		0.945
Duration of hypothyroidism	<1 year	8	527.46±98.22 (437.989-645.209)	5	506.86±78.02 (393.591-588.491)	-	-
	1---4	12	496.28±145.57 (286.998-676.374)	18	426.44±79.74 (333.121-582.377)	-	-
	5---9	5	454.07±91.92 (290.028-505.613)	5	414.95±48.45 (348.627-476.348)	-	-

	=>10 years	5	338.96±44.86 (289.928-375.112)	2	429.18±100.29 (358.265-500.092)	-	-
	P value		0.045#		0.202		-
*Significant difference between two independent means using Student-t-test at 0.05 level.							
#Significant difference among three independent means using ANOVA test at 0.05 level.							

G1: Hypothyroidism with fatty liver disease patients
G2: Hypothyroidism without fatty liver disease patients
G3: Controls.

Receiving Operating Characteristic (ROC) for VEGF-R2:
The ROC-curves analysis for serum VEGF-R2 levels, when used as test to diagnosis subjects into hypothyroidism with fatty liver cases (G1) and control groups(G3), showed the area-under-the curve (AUC) of serum VEGF-R2(pg/ml) was (0.918) with confidence interval (95% CI) and lower bound (0.852) and upper bound (0.983).

Serum VEGF-R2 correlation study:
Table (3) summarizes the relationship between serum VEGF-R2 and the investigated clinical and biochemical indicators of the study population. For G1 patients, serum VEGFR2 levels were highly positively and significantly correlated with (F.B.G and HDL). Additionally, ALP in G1 strongly positively linked with serum VEGF-R2 levels. Similarly, serum VEGF-R2 levels were significantly negatively proportionate to G1 (duration of hypothyroidism, S.creatinine, GOT, and GPT). VEGF-R2 's correlation with (Age, T_3, HbA1C, MCV, WBC, S.Albumin, S.ferritin) for G1, with (Age, BMI, TT4, TSH,
S.Creatinine, MCV, S.Cholesterol, GOT, ALP and S.ferritin) for G2, and with (T_3 , B.urea, S.Creatinine, Hb, PCV, WBC, TG and ALP) for G3 , is positive but not significant change.
VEGF-R2's correlation with (BMI, TT_4, TSH, B.urea, Hb, PCV, s. cholesterol, TG and T.Protein) for G1 , with (Duration of hypothyroidism, T_3, F.B.G, HbA1C, B.urea, Hb,
PCV, WBC, TG, HDL, GPT, T.Protein and S.Albumin) for G2 and with (Age, BMI, TT_4 , TSH, F.B.S, HbA1C, MCV, s. cholesterol, HDL, GOT, GPT, T.Protein, S.Albumin and S.Ferritin) for G3, is negative but not significant change.

Table (3): Correlation of VEGF-R2 to clinical and biochemical parameters in G1, G2 and G3.

		Vascular Endothelial Growth Factor Receptor 2 (VEGF-R2) (pg/ml)		
		G1	G2	G3
Age (years)	r	0.212	0.019	-0.036
	P	0.260	0.922	0.849
BMI (Kg/m2)	r	-0.153	0.146	-0.180
	P	0.420	0.442	0.341
Duration of hypothyroidism (years)	r	-0.462*	-0.131	-
	P	0.010	0.492	-
T3 (0.92-2.33 nmol/L)	r	0.098	-0.101	0.008
	P	0.607	0.594	0.968
TT4 (60-120 nmol/L)	r	-0.347	0.084	-0.071
	P	0.060	0.660	0.711
TSH (0.25-5 mulu/ml)	r	-0.309	0.045	-0.172
	P	0.097	0.815	0.364
Fasting blood glucose (F.B.G) (8-12 hrs:3.6-5.5 for 6--hrs; 11.1 mmol/l)	r	0.478**	-0.208	-0.032
	P	0.008	0.271	0.868
HbA1C (4.1-5.6%)	r	0.335	-0.098	-0.057
	P	0.071	0.606	0.764

Blood urea (3.5-7.5 mmol/L)	r	-0.114	-0.017	0.095
	P	0.550	0.930	0.616
Serum creatinine (Male; 53-115 for Female; 44-80 umol/L)	r	-0.437*	0.015	0.054
	P	0.016	0.936	0.779
Haemoglobin (Male'; 13-17 for Female;11.5-15 g/dl)	r	-0.122	-0.201	0.108
	P	0.520	0.288	0.571
PCV.(Male: 40-50% for Female; 36-45%)	r	-0.122	-0.201	0.108
	P	0.520	0.288	0.571
MCV (Male; 83-101 for Female; 80-100 um3)	r	0.207	0.039	-0.085
	P	0.272	0.838	0.654
WBC (4.5-10 x 103)	r	0.247	-0.106	0.081
	P	0.188	0.579	0.670
Serum cholesterol (>5 mmol/L)	r	-0.250	0.002	-0.041
	P	0.183	0.991	0.828
Serum triglycerides (>2 mmol/L)	r	-0.337	-0.044	0.218
	P	0.069	0.817	0.248
HDL (1-1.5 mmol/L)	r	0.466**	-0.058	-0.346
	P	0.010	0.760	0.061
SGOT (Male >50 for Female >35 U/L)	r	-0.412*	0.030	-0.043
	P	0.024	0.875	0.822
SGPT (Male; 13-40 for Female; 10-28 U/L)	r	-0.460*	-0.022	-0.097
	P	0.010	0.907	0.610
Serum ALP (21-92 U/L)	r	0.443*	0.079	0.084
	P	0.014	0.680	0.659
Total protein (66-91 gm/L)	r	-0.019	-0.133	-0.027
	P	0.922	0.485	0.887
Serum albumin (35-50 mg/L)	r	0.328	-0.134	-0.329
	P	0.076	0.481	0.076
Serum ferritin (Male: 70-435, Female cyclic: 10-160; Menopausal: 25-280 ng/ml)	r	0.050	0.071	-0.062
	P	0.793	0.709	0.746
*Significant correlation at 0.05 level. **Highly significant correlation at 0.01 level.				

G1: Hypothyroidism with fatty liver disease patients ,
G2: Hypothyroidism without fatty liver disease patients , **G3:** controls

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