JOURNAL OF NUTRITION FASTING AND HEALTH

Plasma levels of vascular endothelial growth factor and its soluble receptor in non-alcoholic fatty liver disease

Mahmoud Belghaisi-Naseri[1], Zahra Dehnavi[1], Farkhonde Razmpour[2], Mahsa Tousi[1], Ali Taghipour[3], Abdolreza Norouzy[1], Mohsen Nematy[1,4], Ali Bahari[5,6], Mohsen Azimi-Nezhad[7,8*]

1. Department of Nutrition, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
2. Department of Clinical Nutrition, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
3. Department of Epidemiology & Biostatistics, Social Determinants of Health Research Center, Cancer Research Center, School of Health, Mashhad University of Medical Sciences, Mashhad, Iran
4. Department of Biochemistry and Nutrition, Endoscopic and Minimally Invasive Surgery and cancer Research Center, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
5. Gastroenterology and Hepatology Department, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
6. Gastroenterology and Hepatology Research Center, Mashhad University of Medical Sciences, Mashhad, Iran
7. Department of Basic Medicine Sciences, MD PhD Assistant Professor of Medical Genetics, Neyshabur University of Medical Sciences (NUMS), Neyshabur, Iran
8. Department of Medical Genetics, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran

ARTICLEINFO

Article type:
Research Paper

Article History:
Received: 21 Jul 2018
Accepted: 08 Dec 2018
Published: 16 Jan 2019

Keywords:
Nonalcoholic Fatty Liver Disease
VEGF
sVEGFR1
Children
Adolescents

ABSTRACT

Introduction: Nonalcoholic fatty liver disease (NAFLD) is a clinical pathologic condition, which leads to hepatocyte inflammation. The present study aimed to compare the plasma levels of vascular endothelial growth factor (VEGF) and soluble VEGF receptor-1 (sVEGFR-1) as inflammation markers in the overweight and obese children and adolescents with and without NAFLD.

Methods: This cross-sectional study was conducted on 70 overweight and obese children and adolescents (37 males and 33 females), who were selected from the patients admitted to a nutrition clinic in Mashhad, located in the northeast of Iran. The diagnosis of NAFLD was confirmed by Fibro Scan, ultrasound, and elevation of liver enzyme. In addition, plasma VEGF and sVEGFR1 were measured in each patient.

Results: Log-transformed VEGF levels had a significant, stepwise increase from grade zero to the first, second, and third grades (P<0.001). However, log-transformed sVEGFR1 showed a regular trend in various grades of NAFLD (P=0.3).The odds ratio (95% confidence interval [CI]) of VEGF across the NAFLD categories was estimated at 1.00, 0.99 (95% CI: 0.97-1.01), 1.02 (95% CI: 0.99-1.04), and 1.04 (95% CI: 1.02-1.06). On the other hand, the odds ratios remained relatively unchanged after the adjustment of age, gender, and body mass index (BMI).

Conclusion: According to the results, there were significant, positive associations between plasma VEGF levels and grades of steatosis in overweight and obese children and adolescents even after the adjustment of age, gender, and BMI.

► *Please cite this paper as*:
Belghaisi-Naseri M, Dehnavi Z, Razmpour F, Tousi M, Taghipour A, Norouzy A, Nematy M, Bahari A, Azimi-Nezhad M. Plasma levels of vascular endothelial growth factor and its soluble receptor in non-alcoholic fatty liver disease. J Nutrition Fasting Health. 2018; 6(2): 107-114. DOI: 10.22038/JNFH.2018. 33580.1124

Introduction

Nonalcoholic fatty liver disease (NAFLD) is a clinical pathologic condition, which is characterized by the accumulation of triglycerides in the hepatocytes of the patients with no or minimal alcohol intake (1). Furthermore, NAFLD is associated with a broad

* *Corresponding author:* Mohsen Azimi-Nezhad, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran. Tel: 00985138002288; Email: Aziminm@mums.ac.ir

spectrum of liver lesions, ranging from simple steatosis to nonalcoholic steatohepatitis (NASH) (2). The prevalence of NAFLD has been reported to be on the rise in many countries across the world, including Iran, with the upward trend estimated at 25.24% and 33.9%, respectively (3, 4).

Excess weight increases the risk of NAFLD, and the mean prevalence of NAFLD in children is reported to be 7.6%, while it reaches 34.2% in obese children (5). The accumulation of lipid droplets in the liver of the patients with NAFLD leads to pathological events in hepatocytes, including sinusoidal blebs, compression of sinusoids with fat-laden hepatocytes, and loss of fenestrae. Consequently, these events may contribute to important alterations in the hepatic microvasculature. Although the pathophysiological mechanisms of NAFLD remain unclear, these alterations could be partly explained based on angiogenic factors (6-8). Moreover, the centrizonal arteries and micro vessels that are indicative of active angiogenesis represent a common finding in NAFLD, even in the early stages of the disease (9).

Vascular endothelial growth factor (VEGF) is a potent angiogenic factor, as well as a highly specific mitogen, for the vascular endothelial cells involved in physiological and pathological angiogenesis (10, 11). VEGF secretion is a hypoxia-inducible factor, 46 kDadimeric glycoprotein with a minimum of five isoforms (VEGF121, VEGF145, VEGF165, VEGF189, and VEGF206), which arise from alternative splicing from a single VEGF gene (12, 13). Among VEGF isoforms, VEGF of 121 amino acids (VEGF121) and VEGF165 are the most abundant and optimally characterized forms with potent angiogenic activity (14). The biological activity of VEGF is modulated based on two receptor tyrosine kinases (VEGFR-1 and VEGFR-2), which play a key role in angiogenesis (15). According to the literature, the soluble, truncated form of VEGFR-1 (sVEGFR-1), which is secreted through the alternative splicing of VEGFR-1 mRNA, could act as a natural, endogenous VEGF inhibitor (15).

Previous findings have denoted the involvement of pro-angiogenic VEGF isoforms and their receptors in the Pathophysiology of NAFLD (16-19).For instance, Jaroszewicz et al. investigated 78 patients with liver cirrhosis and reported significantly higher serum levels of VEGF and sVEGFR-1 in cirrhotic patients compared to healthy controls (16). In another research, Coulonet al. observed that the patients with simple steatosis and NASH had significantly elevated VEGF and sVEGFR-1 levels compared to healthy subjects (17). In contrast, Yilmaz et al. reported no significant difference in the serum levels of VEGF in the patients with NAFLD, while these patients had significantly lower sVEGFR-1 levels compared to healthy subjects (18). Another study in this regard demonstrated lower serum VEGF levels in matched NAFLD patients compared to controls. Moreover, the mentioned study showed significantly lower VEGF levels in the patients with NASH compared to healthy subjects (19). However, serum VEGF may not be a good indicator of circulating VEGF levels in patients, due to the platelet-mediated secretion of VEGF in the clotting process. As such, the plasma levels of VEGF and sVEGFR-1 are better indicators of the circulating levels of VEGF (20).

Considering the discrepancies in previous findings, the present study aimed to compare the plasma levels of VEGF and sVEGFR-1 in the overweight and obese children and adolescents with and without NAFLD.

Material and methods

Sample Population

This cross-sectional study was conducted on70 overweight and obese children and adolescents (37 males and 33 females) aged 11-18 years. The subjects were selected from the patients referring to a nutrition clinic in Mashhad, located in the northeast of Iran. The exclusion criteria were the patients with chronic conditions (e.g., viral hepatitis, autoimmune/congenital liver disease, congenital metabolic diseases, and cancer), surgery within the past six months, and history of heart and kidney artery diseases.

The participants were categorized into four groups based on the severity of liver steatosis. The subjects in the first group (grade zero) were considered as the reference group.

Written informed consent was obtained from the parents or legal guardians of the subjects, and the study protocol was approved by the Ethics Committee of Mashhad University of Medical Sciences (MUMS).

Data Collection and Measurements

Data were collected on the height (cm), weight (kg), and body mass index (BMI; kg/m^2) of the subjects based on standard protocols. Height was measured to the nearest millimeter with a tape measure, and body weight was measured to the nearest 0.1 kilogram using electronic scales. BMI was calculated as weight (kg) divided by the square of the height (m^2). In addition, we measured the systolic blood pressure (SBP) and diastolic blood pressure (DBP) twice using a sphygmomanometer. To do so, the subjects remained seated at rest for 15 minutes before measuring the blood pressure on their left arm. If the first two readings differed by more than 25 mmHg (SBP) and more than15 mmHg (DBP), the measurements would be repeated in triplicate.

Biochemical Measurements

Fasting blood samples were collected after overnight fasting (10-12 hours) in order to determine the levels of fasting blood glucose (FBG),fasting insulin (FI), hemoglobin A1c, platelet count, and full-fasting lipid profile, including the levels of triglyceride (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C).

To estimate insulin resistance (IR), the homeostasis model assessment (HOMA) was calculated using the following formula:

FI (IU/ml) ×FBG (mmol/l)/22.5

The level of serum high-sensitivity C-reactive protein (hs-CRP), the immunoturbidimetry was applied with the detection limit of 0.06 mg/l (Pars Azmoun, Karaj, Iran) (21). Moreover, the concentrations of liver function enzymes were measured in each patient, including aspartate aminotransferase (AST), alanine aminotransferase (ALT), and gamma-glutamyl transpeptidase (GGT).

Histological Analysis

The status of liver steatosis and fibrosis was identified using transient elastography (Fibro Scan) and controlled attenuation parameter (CAP) (Echosens, Paris, France).CAP is a device used to examine liver stiffness and hepatic steatosis, which could be considered a singular choice instead of liver biopsy in NAFLD patients. CAP identifies the patients with steatosis grades S1 (11-33%), S2 (34-66%), and S3 (more than 66% of the involved hepatocytes), with the area under curve (AUC) values of 0.85-0.88. In this study, the subjects with the CAP results of ≤10% were classified as the control group, and the others were categorized as NAFLD patients.

Measurement of Plasma Angiogenic Proteins

Approximately 10 milliliters of blood was collected in an EDTA vacuum tube for each patient, and the blood samples were centrifuged at 1000×g for 15 minutes. The plasma samples were stored at the temperature of -80°C until immediately before analysis. Plasma levels of VEGF (BMS277/2, eBioscience, Vienna, Austria) and sVEGFR1 (DVR100B, R&D Systems, Minneapolis, U.S.A) were determined using the commercial ELISA kit. The sensitivity of the VEGF assay was 7.9 pg/ml, while the intra-assay variation was 6.2%, and the inter-assay variation was 4.3%. The obtained values for sensitivity, intra-assay coefficient, and inter-assay coefficient were estimated at 3.5 pg/ml, 2.6%, and 5.5% for the sVEGFR1 assay, respectively.

Statistical Analysis

Data analysis was performed in SPSS version 18 (SPSS Inc., IL, USA) using the Kolmogorov-Smirnov test to evaluate the normal distribution of the data. In addition, descriptive statistics (mean, frequency, and standard deviation [SD]) were used for all the variables, which were expressed as median and interquartile range for the data with non-normal distribution and mean and SD for the normally distributed data. To compare the qualitative variables, we used Chi-square, and the analysis of variance (ANOVA) was performed for the variables with normal distribution.

Multivariate analysis was employed to estimate the risk as approximated by the odds ratio (OR). The ORs were obtained using multivariate logistic regression at 95% confidence intervals (CI) in order to determine the effects of potential confounders (e.g., age, gender, and BMI), and β-coefficients were obtained from the univariate linear regression.

The values of plasma VEGF and sVEGFR-1 were logarithmically transformed (log10) before analysis in order for normal distribution.

Additionally, Mann-Whitney U test was applied for serum hs-CRP as it was non-normally distributed even after the logarithmical transformation. In all the statistical analyses, P-value of less than 0.05 was considered significant.

Results

In total, 70 subjects with the mean age of 12.7±2.8 years were enrolled in the study, and 53% were male. Demographic and biochemical characteristics of the sample population are presented in Table 1. The mean age of the subjects in the NAFLD and control groups was 13.07±2.76 and 12.08±2.88 years, respectively. The subjects were stratified into two groups based on the presence of NAFLD.

Table 1. Demographic and Biochemical Characteristics of NAFLD Patients and Controls

	NAFLD (n=48)	CONTROL (N=22)	P-value
Gender (M/F)	26/22	11/11	0.4
Age (year)	13.0±2.7	12.0±2.8	0.2
BMI (kg/m^2)	29.4±5.1	25.0±3.3	<0.001
HOMA-IR	51.6±37.6	32.8±19.8	0.008
SBP (mmHg)	116.2±19.5	110.5±14.5	0.2
DBP (mmHg)	67.7±11.3	63.1±10.7	0.1
AST (U/l)	26.2±6.3	24.6±6.8	0.3
ALT (U/l)	28.9±15.1	24.3±12.5	0.2
GGT(mg/dl)	22.3±9.2	21.5±6.8	0.7
TC (mg/dl)	144.8±22.5	150.5±27.0	0.3
HDL-C (mg/dl)	39.4±7.3	39.7±8.0	0.8
LDL-C (mg/dl)	78.2±18.1	83.3±14.0	0.2
TG (mg/dl)	101.4±34.0	94.8±34.2	0.4
Platelet (10^9)	326.1±70.2	274.0±78.7	0.007
hs-CRP (mg/dl)	2.45 (0.72-5.62)	1.45 (0.77-2.72)	0.4
Steatosis (%)	50±24	7.5±1.5	<0.001
Steatosis Grade	S1=17 S2=8 S3=23	S0=22	

Values expressed as mean±SD for variables with normal distribution, and median and interquartile range for hs-CRP as a non-normally distributed variable; BMI: body mass index; HOMA-IR: homeostasis model of insulin resistance; SBP: systolic blood pressure; DBP: diastolic blood pressure; AST: aspartate aminotransferase; ALT: alanine aminotransferase; GGT:gamma-glutamyltranspeptidase; TC: total cholesterol; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; TG: triglyceride; hs-CRP: high-sensitivity C-reactive protein

According to the information in Table 1, there were 22 healthy individuals (31%) and 48 NAFLD patients (69%) in the study. No significant differences were observed between the groups in terms of age, SBP, DBP, AST, ALT, GGT, TC, HDL-C, LDL-C, TG, and hs-CRP (Table 1).Moreover, the patients with NAFLD had significantly higher BMI (P<0.001), HOMA-IR (P=0.008), and platelet count (P=0.007).

The NAFLD patients were further categorized based on the grade of steatosis (Table 2). The log-transformed VEGF levels had a significant, stepwise increase from grade zero to the first, second, and third grades (P<0.001). However, log transformed sVEGFR1 showed no regular trend between various grades of NAFLD (P=0.3).

Table 2. Logarithmically Transformed VEGF and sVEGFR1 Levels Based on Grades of NAFLD

	Steatosis Grade				P-value
	0 (n=22)	1 (n=17)	2 (n=8)	3 (n=23)	
Log VEGF (pg/ml)	1.62±0.30	1.63±0.24	1.85±0.14	1.93±0.28	<0.001
S Log sVEGFR1 (pg/ml)	1.33±0.32	1.42±0.30	1.20±0.29	1.30±0.27	0.3

Values expressed as mean±SD; VEGF: vascular endothelial growth factor; sVEGFR1: soluble vascular endothelial growth factor receptor-1

According to the multivariate analyses, the patients with grade zero NAFLD were the reference group (Table 3). The ORs (95% CI) for VEGF and sVEGFR1 across the patient categories were estimated at 1.00, 0.99 (95% CI: 0.97-1.01), 1.02 (95% CI: 0.99-1.04), and 1.04 (95% CI: 1.02-1.06), and 1.00, 1.01 (95% CI: 0.98-1.05), 0.96 (95% CI: 0.91-1.03), and 0.99 (95% CI: 0.95-1.02), respectively. However, the ORs remained relatively unchanged even after the adjustment of age, gender, and BMI.

Table 3. Crude and Adjusted Odds Ratios of First, Second, and Third Grade of NAFLD Associated with VEGF andsVEGFR1

	Steatosis Grade		
	Reference Group and Group 1	Reference Group and Group 2	Reference Group and Group 3
Crude OR-VEGF (pg/ml)	0.99 (95% CI: 0.97-1.01)	1.02 (95% CI: 0.99-1.04)	1.04 (95% CI: 1.02-1.06)***
Crude OR-sVEGFR1 (pg/ml)	1.01 (95% CI: 0.98-1.05)	0.96 (95% CI: 0.91-1.03)	0.99 (95% CI: 0.95-1.02)
Adjusted OR-VEGF (pg/ml)	0.99 (95% CI: 0.97-1.01)	1.01 (95% CI: 0.98-1.04)	1.03 (95% CI: 1.01-1.05)**
Adjusted OR-sVEGFR1 (pg/ml)	1.01 (95% CI: 0.97-1.04)	0.95 (95% CI: 0.89-1.01)	0.97 (95% CI: 0.94-1.01)

Odds ratios with 95% confidence interval (CI) according to multiple logistic regression both crude and adjusted for potential confounders (i.e., age, gender, and BMI); *P<0.05, **P<0.01, ***P<0.001

The β-coefficients were used to evaluate the associations of VEGF andsVEGFR1 with demographic and biochemical markers (Table 4).In addition, the examination of the β-coefficients indicated that VEGF was correlated with the rate of steatosis (β=0.7; P<0.001), age (β=4.6; P=0.007), and BMI (β=2.9; P=0.002).

Table 4. β-coefficients on Association of VEGF/sVEGFR1with Demographic and Biochemical Parameters

	Steatosis (%)	Age (year)	BMI (kg/m^2)	HOMA-IR	SBP (mmHg)	DBP (mmHg)	AST (U/l)	ALT (U/l)	GGT (mg/dl)	TC (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)	TG (mg/dl)	Platelet (10^9)	hs-CRP (mg/dL)
VEGF	0.7***	4.6**	2.9**	0.15	0.17	0.02	1.8*	0.5	0.8	-0.2	-0.5	-0.3	0.08	0.04	0.6
sVEGFR1	-0.05	0.5	0.3	0.01	0.09	0.2	-0.2	0.2	0.3	-0.1	0.2	-0.1	-0.1	-0.06*	-0.1

Unstandardized (β) coefficients according to univariate linear regression; BMI: body mass index; HOMA-IR: homeostasis model of insulin resistance; SBP: systolic blood pressure; DBP: diastolic blood pressure; AST: aspartate aminotransferase; ALT: alanine aminotransferase; GGT:gamma-glutamyltranspeptidase; TC: total cholesterol; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; TG: triglyceride; hs-CRP: high-sensitivity C-reactive protein; VEGF: vascular endothelial growth factor; sVEGFR1: soluble vascular endothelial growth factor receptor-1; *P<0.05, **P<0.01, ***P<0.001

Discussion

According to the results of the present study, the plasma levels of VEGF were positively associated with the grade of steatosis in the overweight and obese children and adolescents even after the adjustment of age, gender, and BMI. However, plasma sVEGFR1 levels showed no specific patterns across the four grades of steatosis.

Conflicting results have been proposed regarding the association of liver histology with the serum levels of VEGF as a potent angiogenic factor. According to the literature, serum VEGF levels are higher(16, 17), lower(19) or the same (18) in the patients diagnosed with NAFLD compared to healthy controls.

In a study in this regard, Coulon et al. measured the concentrations of inflammatory and angiogenic cytokines in the serum of an obese population with simple steatosis (n=30) and NASH (n=32), comparing the findings with age-and gender-matched healthy controls (n=30). Consistent with our findings, the results of the mentioned study indicated a significant elevation in the VEGF levels in the patients with simple steatosis, as well as a borderline significant elevation in the patients with NASH compared to the serum levels of the healthy subjects(17). Similarly, Jaroszewicz et al. conducted a research on 78 cirrhotic patients so as to evaluate the plasma concentration of VEGF in liver cirrhosis. The obtained results indicated the significant increase of plasma VEGF in liver cirrhosis compared to controls (16).

In another research, Steenkiste et al. denoted

that the serum circulating level of placental growth factor (PlGF), which belongs to the VEGF family, increased in the patients with cirrhosis, which showed its association with the stage of fibrosis (22). Similarly, Li et al. investigated the role of VEGF and basic fibroblast growth factor (bFGF) in the neovascularization and formation of spider angiomas in the patients with liver cirrhosis. Furthermore, elevated levels of plasma VEGF and bFGF were reported in these patients, especially those with spider angiomas(23). Therefore, it could be inferred that angiogenesis plays a pivotal role in the progression of NAFLD.

In contrast to our findings, Papageorgiouaet al. reported lower VEGF levels in matched NAFLD patients compared to healthy controls. Moreover, the VEGF levels in the patients with NASH were observed to be lower compared to those with simple fatty liver. The findings also demonstrated that patients with NASH had significantly lower VEGF levels compared to healthy controls (19).

In another study, the association of VEGF concentrations with liver histology was assessed in 99 patients with biopsy-confirmed NAFLD and 75 healthy controls. No significant difference was denoted in serum VEGF levels in the patients with NAFLD compared to the controls (18). This discrepancy could be partly explained based on the heterogeneity of the patients and controls in various studies.

There have been conflicting reports in the literature regarding the association of liver histology and plasma sVEGF1 levels (16-18). For instance, Yilmaz et al. reported that sVEGF1levels were significantly lower in the patients with NAFLD even after the adjustment of potential confounders, including age, gender, BMI, diabetes mellitus, HOMA-IR, BPs, metabolic syndrome, liver enzymes, lipid variables, and hs-CRP(18).On the other hand, the findings of another study indicated that the concentration of sVEGFR1 increased significantly in the serum of the patients with simple steatosis and NASH compared to healthy controls. However, no significant difference was observed in the concentration of sVEGFR2 between the patients with simple steatosis and NASH and controls (17). Januszkiewiczet al. have also claimed that plasma sVEGFR1 increase in all cirrhotic patients in terms of the degree of liver insufficiency, while this finding did not apply to the level of sVEGFR2 (16).

While the majority of the patients with NAFLD remain asymptomatic, the disease progresses in approximately 20% and develops to NASH, thereby leading to cirrhosis, portal hypertension, and hepatocellular carcinoma (HCC)(24, 25). The VEGF gene has been reported to be transcribed and expressed, and the VEGF protein has been reported to be secreted by HCC cells (26, 27). In a study by Yamaguchi et al., VEGF expression in the HCC tissues was reported to be correlated with the histological grade (28). Therefore, it could be concluded that VEGF plays a key role in tumor angiogenesis.

The present study was the first to compare the plasma levels of VEGF and sVEGFR-1 in the overweight and obese children and adolescents with and without NAFLD. One of the strengths of the current research was the use of accurate plasma VEGF rather than serum VEGF levels, as well as the measurement of sVEGFR1 as an important factor involved in angiogenesis. Moreover, the effects of the potential confounders (e.g., age, gender, and BMI) were controlled.

Some of the main limitations of the present study included the use of Fibro Scan for the diagnosis of NAFLD rather than biopsy (gold standard method) and not measuring the plasma levels of sVEGFR-2, VEGF-B, and PlGF as the important factors involved in angiogenesis.

Conclusion

According to the results, there was a significant, positive association between the plasma levels of VEGF and grades of steatosis in overweight and obese children and adolescents even after the adjustment of age, gender, and BMI.

Acknowledgments

Hereby, we extend our gratitude to Mashhad University of Medical Sciences (MUMS) for assisting us in this research project.

Conflict of interest

None declared.

References

1. Angulo P. Nonalcoholic fatty liver disease. N Engl J Med. 2002; 346(16): 1221-31.
2. Harrison SA, Neuschwander-Tetri BA. Nonalcoholic fatty liver disease and nonalcoholic steatohepatitis. Clin Liver Dis. 2004; 8(4): 861-79.
3. Moghaddasifar I, Lankarani K, Moosazadeh M, Afshari M, Ghaemi A, Aliramezany M, et al. Prevalence of Non-alcoholic Fatty Liver Disease and Its Related Factors in Iran. Int J Organ Transplant Med. 2016; 7(3): 149.
4. Younossi ZM, Koenig AB, Abdelatif D, Fazel Y, Henry L, Wymer M. Global Epidemiology of Non-Alcoholic Fatty Liver Disease–Meta-Analytic Assessment of Prevalence, Incidence and Outcomes. Hepatology. 2016; 64(1): 73-84.
5. Anderson EL, Howe LD, Jones HE, Higgins JP, Lawlor DA, Fraser A. The prevalence of non-alcoholic fatty liver disease in children and adolescents: a systematic review and meta-analysis. PloS one. 2015; 10(10): e0140908.
6. Francque S, Laleman W, Verbeke L, Van Steenkiste C, Casteleyn C, Kwanten W, et al. Increased intrahepatic resistance in severe steatosis: endothelial dysfunction, vasoconstrictor overproduction and altered microvascular architecture. Laboratory investigation. 2012; 92(10): 1428-39.
7. Farrell GC, Teoh N, McCuskey R. Hepatic microcirculation in fatty liver disease. Anat Rec. 2008; 291(6): 684-92.
8. Coulon S, Legry V, Heindryckx F, Van Steenkiste C, Casteleyn C, Olievier K, et al. Role of vascular endothelial growth factor in the pathophysiology of nonalcoholic steatohepatitis in two rodent models. Hepatology. 2013; 57(5): 1793-805.
9. Gill RM, Belt P, Wilson L, Bass NM, Ferrell LD. Centrizonal Arteries and Microvessels in Non-Alcoholic Steatohepatitis. Am J Surg Pathol. 2011; 35(9): 1400.
10. Sung H-K, Michael IP, Nagy A. Multifaceted role of vascular endothelial growth factor signaling in adult tissue physiology: an emerging concept with clinical implications. Curr Opin Hematol. 2010; 17(3): 206-12.
11. Ferrara N. Role of vascular endothelial growth factor in physiologic and pathologic angiogenesis: therapeutic implications. Semin Oncol. 2002; 29(6 Suppl 16): 10-4.
12. Houck KA, Ferrara N, Winer J, Cachianes G, Li B, Leung DW. The vascular endothelial growth factor family: identification of a fourth molecular species and characterization of alternative splicing of RNA. Mol Endocrinol. 1991; 5(12): 1806-14.
13. Tischer E, Mitchell R, Hartman T, Silva M, Gospodarowicz D, Fiddes JC, et al. The human gene for vascular endothelial growth factor. Multiple protein forms are encoded through alternative exon splicing. J Biol Chem. 1991; 266(18): 11947-54.
14. Neufeld G, Cohen T, Gengrinovitch S, Poltorak Z. Vascular endothelial growth factor (VEGF) and its receptors. FASEB J. 1999; 13(1): 9-22.
15. Ferrara N, Gerber H-P, LeCouter J. The biology of VEGF and its receptors. Nat Med. 2003; 9(6): 669-76.
16. Jaroszewicz J, Januszkiewicz M, Flisiak R, Rogalska M, Kalinowska A, Wierzbicka I. Circulating vascular endothelial growth factor and its soluble receptors in patients with liver cirrhosis: possible association with hepatic function impairment. Cytokine. 2008; 44(1): 14-7.
17. Coulon S, Francque S, Colle I, Verrijken A, Blomme B, Heindryckx F, et al. Evaluation of inflammatory and angiogenic factors in patients with non-alcoholic fatty liver disease. Cytokine. 2012; 59(2): 442-9.
18. Yilmaz Y, Yonal O, Kurt R, Alahdab YO, Ozdogan O, Celikel CA, et al. Circulating levels of vascular endothelial growth factor A and its soluble receptor in patients with biopsy-proven nonalcoholic fatty liver disease. Arch Med Res. 2011; 42(1): 38-43.
19. Papageorgiou M-V, Hadziyannis E, Tiniakos D, Georgiou A, Margariti A, Kostas A, et al. Serum levels of vascular endothelial growth factor in non-alcoholic fatty liver disease. Ann Gastroenterol. 2017; 30(2): 209.
20. Webb NJ, Bottomley MJ, Watson CJ, Brenchley PE. Vascular endothelial growth factor (VEGF) is released from platelets during blood clotting: implications for measurement of circulating VEGF levels in clinical disease. Clin Sci (Lond). 1998; 94(4): 395-404.
21. Tayefi M, Shafiee M, Kazemi-Bajestani SMR, Esmaeili H, Darroudi S, Khakpouri S, et al. Depression and anxiety both associate with serum level of hs-CRP: A gender-stratified analysis in a population-based study. Psychoneuroendocrinology. 2017; 81: 63-9.
22. Van Steenkiste C, Ribera J, Geerts A, Pauta M, Tugues S, Casteleyn C, et al. Inhibition of placental growth factor activity reduces the severity of fibrosis, inflammation, and portal hypertension in cirrhotic mice. Hepatology. 2011; 53(5): 1629-40.
23. Li C-P, Lee F-Y, Hwang S-J, Lu R-H, Lee W-P, Chao Y, et al. Spider angiomas in patients with liver cirrhosis: role of vascular endothelial growth factor and basic fibroblast growth factor. World J Gastroenterol. 2003; 9(12): 2832.
24. Shimada M, Hashimoto E, Taniai M, Hasegawa K, Okuda H, Hayashi N, et al. Hepatocellular carcinoma in patients with non-alcoholic steatohepatitis. JHepatol. 2002; 37(1): 154-60.
25. Caldwell SH, Oelsner DH, Iezzoni JC, Hespenheide EE, Battle EH, Driscoll CJ. Cryptogenic cirrhosis: clinical characterization and risk factors for underlying disease. Hepatology. 1999; 29(3): 664-9.
26. Miura H, Miyazaki T, Kuroda M, Oka T, Machinami R, Kodama T, et al. Increased expression of vascular endothelial growth factor in human hepatocellular carcinoma. J Hepatol. 1997; 27(5): 854-61.

27. Suzuki K, Hayashi N, Miyamoto Y, Yamamoto M, Ohkawa K, Ito Y, et al. Expression of vascular permeability factor/vascular endothelial growth factor in human hepatocellular carcinoma. Cancer Res. 1996; 56(13): 3004-9.

28. Yamaguchi R, Yano H, Iemura A, Ogasawara S, Haramaki M, Kojiro M. Expression of vascular endothelial growth factor in human hepatocellular carcinoma. Hepatology. 1998; 28(1): 68-77.