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Neopterin as an Inflammatory Biomarker in Patients with Non-alcoholic Fatty Liver Disease

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Abstract

Background: Early diagnosis of non-alcoholic fatty liver diseases (NAFLD) through detection of a new biomarker is crucial to prevent disease deterioration. **Aim:** To evaluate the serum level of neopterin in NAFLD patients and to demonstrate its role as an early biomarker of NAFLD. **Methodology:** This is an observational, cross-sectional study that conducted on 71 newly diagnosed NAFLD patients and 60 healthy individuals as a control group. Liver enzymes (alanine aminotransferase [ALT] and aspartate aminotransferase [AST]) and uric acid were determined. The serum level of neopterin was measured by ELISA and WBC differential count by a Coulter counter analyzer. Erythrocyte sedimentation rate (ESR) was determined by Wintrobe's method. **Results:** NAFLD patients had a significantly higher BMI, waist circumference, and body weight compared to control, also their blood pressure was significantly higher. The serum level of ALT, AST, and uric acid were significantly higher in NAFLD patients than the control. There is non-significant difference between NAFLD patients and controls regarding ESR, lymphocytes and granulocytes, while monocytes and neopterin values were significantly higher among NAFLD patients. **Conclusions:** Serum neopterin level can be a predictor inflammatory marker in NAFLD patients. Clinical Pharmacists as part of healthcare professionals can support NAFLD patients by educating them on lifestyle modification if the disease is diagnosed early before reaching a critical stage.

Introduction

Nonalcoholic fatty liver disease (NAFLD) is a chronic hepatic disease that is considered a common and critical health problem all over the world [1]. It ranges from simple fat deposition in liver cells to liver tissue inflammation/fibrosis and finally cirrhosis and even liver cancer [2,3]. Diagnosis of NAFLD is substantial since it progresses to more critical stages and its diagnosis at early stages can prevent disease deterioration. Even the diagnosis is important when the disease is more progressed, such as in the case of non-alcoholic steatohepatitis (NASH), due to its possibility for progressing to cirrhosis [4]. All these complications of the invasive method stimulate finding more accurate and adorable methods for diagnosis of the different stages of NAFLD [5]. Fat accumulation in the liver stimulates the expression of regulatory protein; hepatokine, which enhances adipose tissue to secrete proinflammatory cytokines such as TNF-α and IL-6 [6, 7].

TNF-α activates harmful pathogenic directions in the progression of NAFLD through reducing HDL-cholesterol and enhancing the expression of cholesterogenic genes [8]. It stimulates the production of hepatic fatty acids (FA) and rises serum levels of triglyceride (TG) [9]. Also, it induces apoptosis and proliferation of hepatic cells

that promote hepatic fibrosis [10]. Neutralization of TNF-α activity has been proven to improve fatty liver disease [11].

Additionally, IL-6 has a crucial role in the pathogenesis of NAFLD [12]. TNF-α and interleukins are responsible for the activation of guanosine triphosphate by cyclohydrolase-1, which catalyzes the conversion of guanosine triphosphate to 7,8-dihydroneopterin [13] which results in improved synthesis of neopterin [14].

Neopterin is a pyrimidine compound that is synthesized and released by monocytes and macrophages in response to interferon (IFN-γ) that is produced by activated T cells. Therefore, neopterin levels are increased in several conditions when T-cell or macrophages are activated [15], including autoimmune diseases [16], neurological diseases [17], peripheral neuropathies [18], gestational diabetes mellitus [19] and in diabetes progression and complications [20]. Also, in alcoholism patients and in those with acute and chronic hepatitis resulting from hepatitis B and C viruses [21].

The serum neopterin level gradually increases in NASH based on the severity of hepatic destruction since neopterin acts as oxidative stress and free radicals participate in the NASH injury mechanism, while the role of serum neopterin as a marker in NAFLD patients need to be find [22]. However, neopterin levels were not studied in patients with NAFLD, therefore, the rationale of this study was to consider the serum neopterin levels in the evaluation of NAFLD.

Methodology

A. Ethical approval

The ethical approval was obtained from the Ethical and Scientific Committees at the College of Pharmacy, University of Sulaimani, Iraq. Patients who enrolled in this work were signed an informed consent form and they acknowledged that they were free and have the right to refuse participation.

B. Study setting

The study was performed in the Department of Clinical Pharmacy, College of Pharmacy, University of Sulaimani in collaboration with Shar Teaching Hospital in Sulaimaniyah city, Iraq between August 2020 to January 2021.

This observational, cross-sectional study included patients with NAFLD that have been diagnosed and recruited by consultant gastroenterologists. According to the laboratory tests, the participants were recruited into Group I that were 71 NAFLD patients (29 males and 42 females) with a mean age of 43 ± 8.78 years and Group II that were 60 apparently healthy individuals as a control group (20 males and 40 females) with mean age of 42.1 ± 6.36 years.

C. Inclusion criteria

Both sexes aged > 18 years old with high serum hepatic enzymes (ALT and AST) and with positive findings of hepatic fatty infiltration on ultrasonography were included in this study.

D. Exclusion criteria

Patients with alcoholism, drug addicts, other forms of liver diseases (such as viral hepatitis and autoimmune hepatitis), human immunodeficiency virus infection, pregnant women, and lactating mother were excluded from this study.

E. Assessments

Bodyweight (kg), height (m), and waist circumference (cm) were measured using a sensitive electronic balance, standard stadiometer, and a steel tape of measurement, respectively. Body mass index (BMI) was calculated as BMI (kg/m^2) = body weight (kg)/height $(m)^2$ [23].

In a sitting position, the blood pressure (mm Hg) of both arms of all study participants was measured at rest using an electronic sphygmomanometer. Later, the mean of the readings was taken. Under aseptic conditions and 12 hours of overnight fasting, 5 mL venous blood were drawn from each patient, from which 2 mL of the whole blood was added to the EDTA tube for determination of WBC differential count using a Coulter counter analyzer (Celltac α MEK-6500K, Nihon Kohden Europe) and ESR using Wintrobe's method. Simultaneously, 3 mL of the collected blood was centrifuged at 3000 rpm for 15 minutes at test tubes free of anticoagulants for separation of the serum and determination of liver enzymes (ALT and AST) and uric acid using a Cobas-311 autoanalyzer according to the instruction of manufacturer. The serum level of neopterin was also measured using ELISA (BioTek ELx 800- USA).

2.6. *Statistical analysis*

The results were expressed as mean ± SD. The continuous variables of the two groups were compared using a two-tailed independent sample t-test while categorical variables were analyzed using a Chi-square test. Prediction of the serum neopterin level was calculated using a multivariable regression analysis with post-hoc analysis of variance (ANOVA). A $p \leq 0.05$ was considered statistically significant. For analyzing data, SPSS - 24 program was used.

Results

Table 1 shows the demographic and basic features of the participants. There is insignificant difference in sex distribution between NAFLD patients and the control group. The mean ± SD of the age of NAFLD patients was comparable with that of the control. Concomitant illnesses (diabetes, hypertension, and dyslipidemia) were observed in 61.9% of NAFLD patients and their blood pressure was significantly ($p \leq 0.05$) higher than control. NAFLD patients have a significantly ($p \leq 0.05$) greater BMI, waist circumference, and body weight compared to the control. The serum level of ALT, AST, and uric acid was significantly ($p \leq 0.05$) more in patients than the corresponding level of control.

Table 1. Demographic and basic characteristics of the NAFLD patients and healthy subjects.

Characteristic	Group I (n=71)	Group II (n=60)	P-value
Gender (%)			
Male	29 (40.9%)	20 (33.3%)	
Female	42 (59.1%)	40 (66.7%)	0.479
Age (year)	43 ± 8.78	42.1 ± 6.36	0.67
Concomitant illness			
Diabetes	16 (22.5%)	0 (0%)	
Hypertension	24 (33.8%)	0 (0%)	
Dyslipidemia	4 (5.6%)	0 (0%)	
None	27 (38.1%)	60 (100%)	< 0.001*
Body mass index (kg/m^2)	33.7 ± 5.7	29.4 ± 3	< 0.001*
Waist circumference (cm)	107.4 ± 9.6	96 ± 7.4	< 0.001*
Body weight (kg)	90.7 ± 14.9	76 ± 10	< 0.001*

Height (cm)	164.4 ± 7.8	163.7 ± 8.9	0.342
Blood pressure (mmHg)			
Systolic	137.9 ± 18.2	112.3 ± 7.3	< 0.001*
Diastolic	86.7 ± 11.9	75.2 ± 5	< 0.001*
Serum level of (IU/L)			
Alanine aminotransferase	62.3 ± 21.9	22.3 ± 6.9	< 0.001*
Aspartate aminotransferase	48 ± 13.1	22.3 ± 4	0.001*
Uric acid (mg/dL)	6.3 ± 1.3	4.4 ± 1.1	0.001*

The results are expressed as number, percent and mean± SD. P-value was calculated by using a Chi-square test for categorized data and two-tailed independent two-sample t-test for comparing NAFLD patients (Group I) with control subjects (Group II). * Symbol denotes significant difference between the two groups.

On the other hand, non-significant difference between NAFLD patients and control regarding the level of inflammatory markers such as ESR, lymphocytes and granulocytes were seen, while monocytes and neopterin were significantly ($p \leq 0.05$) higher among NAFLD patients compared with control (Table 2).

Table 2. Comparison between the NAFL patients and Control in the measured inflammatory markers.

Variable	Group I (n=71)	Group II (n=60)	P-value
WBC ($\times 10^3$)	7.5 ± 2	7 ± 1.4	0.145
Granulocytes	4.7 ± 1.6	4.4 ± 1.2	0.336
Lymphocytes	2.2 ± 0.6	2.2 ± 0.5	0.807
Monocytes	0.5 ± 0.30	0.4 ± 0.07	0.012*
ESR (mm/h)	22.5 ± 10.8	17.9 ± 5.5	0.709
Neopterin (nmol/L)	10.4 ± 2.6	7.3 ± 2.2	0.013*

The results are expressed as mean± SD. P-value was calculated by using two-tailed independent two-sample t-test for comparing NAFLD patients (Group I) with control subjects (Group II). * Symbol denotes significant difference between the two groups.

Discussion

Generally, most NAFLD patients are overweight/obese and suffered from insulin resistance [24 - 26]. This is in agreement with our results since our NAFLD patients have a significantly higher BMI compared to the healthy individual and 22.5% of them were diabetics.

In obesity, the secretion of proinflammatory cytokines increases due to the expansion of visceral adipocytes that increases the release of FA, infiltration, and activation of macrophage within adipose tissue [27-29]. Therefore, TNFα and IL-6 are increased in obesity and lead to the development of fatty liver [30]. Additionally, these pro-inflammatory cytokines are also might results in cardiovascular disorders [31]. This is also confirmed in this study since the high number of NAFLD patients were hypertensive and their blood pressure was significantly ($p \leq 0.05$) higher than the healthy individuals.

Studies on NAFLD patients demonstrated that increased levels of AST and ALT predict the risk of progression to fibrosis [32, 33], while many studies have established that NAFLD patients have normal serum aminotransferases level such as in the study that conducted in USA in which, 79% of adult with fatty liver had normal aminotransferase level [34]. The same result was observed in 55% of the Italian adults with fatty liver [35]. Another study by Gholam et al. showed that 46% of steatohepatitis patients had normal serum transaminase levels [36]. Additionally, in the current study, the ALT and AST levels were normal among NAFLD patients but they were significantly ($p \leq 0.05$) higher than the control.

In this study, serum uric acid was significantly ($p \leq 0.05$) higher among NAFLD patients compared to the control. Other studies in Korea and USA had shown that elevated uric acid level was unconventionally related to NAFLD [37, 38]. The correlation between serum uric acid and NAFLD was caused by excessive fat deposition in hepatocytes, which is strictly related to insulin resistance and obesity. Hence, insulin resistance indorses lipolysis of peripheral adipose tissue and rises free FA entry into the liver, which finally results in hyperinsulinemia, thus rises the uric acid production and decreases the renal excretion of uric acid [39].

The WBC count is routinely measured as a marker for systemic inflammation, which is inexpensive and can be done simply. In this respect, the result of Park et al. 2004 showed that there were no significant associations between NAFLD and the total WBC count. Thus, it was realized that WBC count is not interpreter of NAFLD while the increased monocyte level related to NAFLD and the occurrence of NAFLD was more in the patients with a high monocyte level. Our current findings showed a relationship between the monocyte level and NAFLD and approved that the high monocyte level might be a valuable marker for NAFLD and may contribute to the pathogenesis of NAFLD [40].

Inflammation is found to be involved in the pathogenesis of NAFLD, and monocytes are one of the markers of inflammation [41]. The exact mechanism beyond raised monocytes in NAFLD patients is not known exactly [41-43]. Our study more confirmed the previous studies since the total WBC in NAFLD patients was non-significantly different from healthy individuals while monocyte count was significantly ($p \leq 0.05$) higher in NAFLD patients.

Many researches have shown that the plasma level of TNF-α is higher in patients with NAFLD and NASH, compared to control groups [44, 45] while some studies established a considerable increase in the serum level of TNF and IL-6 in steatohepatitis [46]. This is maybe the reason for increasing neopterin level significantly ($p \leq 0.05$) among our NAFLD patients since increases in TNF-α and IL-6 are consequently contributed to the elevation of neopterin level.

Conclusion

In conclusion, serum neopterin level can be a predictor inflammatory marker in NAFLD and this follow-up can be easily performed by a clinical pharmacist in cooperation with other health care professionals such as internists, nurses, and laboratory staff for preventing NAFLD progression through lifestyle modification.

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