

Neopterin as an inflammatory prognostic biomarker in male With systemic disease

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Abstract

Background : The serum level of neopterin, a marker associated with cell-mediated immunity, is increased in coronary artery disease and chronic kidney disease (CKD) especially in end stage renal disease (ESRD) and may be a risk marker for adverse outcomes resulting from these diseases.

Objective: to compare the levels of neopterin in two systemic inflammatory diseases cardiovascular disease and end stage disease (CVD & ESRD) and study its correlation with other serum parameters.

Subjects & Methods:

The study involved 38 male patients with ESRD and 40 male patients with CVD (age range of 30- 60 years). They were attending Kidney disease and Transplantation Unit and [Coronary Care Unit](#) (CCU) at Baghdad Teaching Hospital (Medical City) during the period from December 2012 to March 2013 with 58 healthy subjects (control group) of matching age and weight. Fasting venous blood was obtained for glucose, lipid profile, urea and creatinine measurement which were done immediately after separation of the serum by standard routine methods. The determination of neopterin was done using Enzyme-Linked Immuno Sorbent Assay. (ELISA, Sandwich assay).

Results: the results revealed a significant difference in neopterin level between each group of patients (CVD & ESRD) with its control group (45.5 ± 11.9 vs 16.48 ± 3.09) and (95.89 ± 11.3 vs 16.66 ± 2.83) respectively. In addition to inverse correlation between neopterin and estimated glomerular filtration rate (eGFR) in ESRD patients ($p < 0.05$), and significant inverse correlation with triglyceride (TG) in CVD patients ($p < 0.05$).

Conclusion:

The most important results of this study suggest that the neopterin level, which is considered a useful marker of systemic inflammation, in ESRD patients is higher than in CVD patients.

Key words: neopterin, CVD, ESRD,

INTRODUCTION

Cardiovascular disease (CVD) can be defined as a disease that mainly affects the cardiovascular system include cardiac disease and also vascular diseases of the brain and kidney, in addition to the peripheral arterial disease.⁽¹⁾ One of the most important factors that play a key role in various diseases like infections, autoimmune and malignant tumor diseases or in cases of allograft transplantation and considered as one of the causes is the activation of the immune

system. The causes of other diseases like neurological and cardiovascular diseases may be related to immunological processes⁽²⁾. Neopterin which is a new biomarker that could play an important role in pathogenesis of cardiovascular disease and other inflammatory disease.⁽³⁾ Neopterin, which is synthesized by macrophages, is a catabolic product of guanosine triphosphate (GTP), a purine nucleotide, which belongs to the chemical group known as pteridines.⁽⁴⁾ Its synthesis is accompanied with stimulation of the cytokine interferon-gamma and is

indicative of a pro-inflammatory immune status. One of the possible explanation of the enhancement inflammatory processes within vulnerable plaques is due to the effect of neopterin which is serve as marker of cellular immune system activation ,together with pro-inflammatory cytokine , tumor necrosis factor alpha(TNF-a),which stimulates gene transcription for inducible nitric oxide synthase (I NOS), which results in the production of cytotoxic NO free radical.⁽⁵⁾

Chronic kidney disease is a progressive loss in renal function over a period of months or years. There are no specific symptoms of deterioration of kidney function. A reduced appetite and feeling unwell might be the main symptoms of kidney dysfunction. Diabetes and /or high blood pressure are the main risk factors for development of kidney problems.

Chronic kidney disease was classified in five stages begin with stage 1, with mildest and causing few symptoms which progressed to severe illness with poor life expectancy in stage 5 if untreated. This is according to the professional guidelines classification.⁽⁶⁾

Neopterin concentrations are increased in renal disease because of either impairment of its excretion and/or increased its production which is due to systemic inflammation .Although, neopterin is well known as a predictor for cardiovascular mortality, however it is not clear if it is associated with early stage reduced kidney function.⁽⁷⁾

The aim of this study was to compare the levels of neopterin in two systemic diseases, namely the cardiovascular, and end stage renal disease

PATIENTS AND METHODS

Subjects: This study included 78 male patients with ESRD (n= 38) and myocardial infarction (n=40). The range of age was between 30-60 years .They were from Kidney disease and Transplantation Unit and [Coronary Care Unit](#) (CCU) at Baghdad Teaching Hospital during the period from December 2012 to March 2013. The study also included 58 normal male volunteers of matching age and weight.

Methods: Ten milliliters (10ml) of fasting venous blood were withdrawn from both patients and controls. The blood sample was collected in a plain tube and centrifuged for 15 minutes at 3000 rpm after being allowed to clot at room temperature for 30 minutes. The separated serum was divided into aliquots and stored frozen at (-20 c°) to be used later for neopterin determination by Enzyme-Linked Immune Sorbent Assay (ELISA) . While blood glucose, lipid profile,

urea and creatinine analysis were done immediately after separation of the serum.

The quantitative determination of Glomerular filtration rate is calculated by CKD EPI Calculator - four variables MDRD CKD EPI equation With SI Units using standardized serum creatinine, age, race, gender, white or other race male.⁽⁸⁾

Body mass index was calculated as body weight (in Kg/Sq height (meter))⁽⁹⁾

Statistical study:

All values were expressed as mean $\pm$ standard deviation (mean $\pm$ SD). All Statistical analysis was performed using Statistical Package for the Social Sciences (SPSS version 15.0). Independent student t-test was performed to assess differences between two means. Pearson correlation coefficient was used to determine the correlation between quantitative data. P value < 0.05 was considered significant.

RESULT

There are no significant differences in age (years) and BMI (kg/m²) between ESRD patients and control group (46.66 $\pm$ 10 vs. 44.6 $\pm$ 8.17) and (26.34 $\pm$ 3.91 vs. 27.77 $\pm$ 2.91) respectively (p > 0.05) , while there are significant differences in other biochemical parameters as shown in table 1, except for serum glucose .

Table (1) : Comparison between serum variables in patients with end stage

renal disease (ESRD) and their controls as mean $\pm$ SD.

Parameters	ESRD (mean±SD) (n=38)	Control(mean±SD) (n=35)	P value
Neopterin	95.89 ± 11.3	16.66± 2.83	<0.0001**
glucose	5.74±1.14	5.36±1.12	0.1563
triglycerides	2.55±0.64	1.6±0.54	<0.0001**
Cholesterol	5.78±0.68	4.27±0.70	<0.0001**
HDL -cholesterol	0.85±0.13	1.18±0.22	<0.0001**
LDL -cholesterol	3.4 ±0.7	2.35±0.78	<0.0001**
AI	4.17±1.26	2.6±0.76	<0.0001**
Urea	28.58±10.94	5.85±0.74	<0.0001**
Creatinine	551.0±326.15	74.4±7.79	<0.0001**

P* < 0.05 , p** < 0.0005 , p*** < 0.0001

All values are expressed in mmol / L , except for neopterin (n mol /L) and creatinine (μ mol /L), AI = Atherogenic Index .

There are no significant differences in age (years) and BMI (kg/m²) between CVD patients and control group (46.43 ±9.8 vs .44.17±8.1) and 30.65 ± 5.53 vs

30.09±5.27) respectively. (p> 0.05) , while there are significant differences in other biochemical parameters except glucose as shown in table 2.

Table (2) Comparison between serum variables in patients with cardiovascular

disease (CVD) and their controls as mean±SD;

Parameter	CVD(mean±SD) (n=40)	Control(mean±SD) (n=46)	P value
neopterin	45 .5± 11.9	16.48±3.09	<0.0001**
glucose	6.07±1.64	5.39±1.26	0.0349*
triglycerides	2.4±0.71	1.61±0.54	<0.0001**
cholesterol	5.79±0.72	4.77±0.71	<0.0001**
HDL-cholesterol	0.91±0.22	1.18±0.21	<0.0001**
LDL -cholesterol	3.64 ±0.67	2.95 ±0.59	<0.0001**
AI	4.25 ± 1.40	2.15±0.87	<0.0001**
urea	7.42±1.95	5.94±0.71	<0.0001**
creatinine	88.55±25.67	74.18±7.47	0.0014*

P* < 0.05 , p** < 0.0005, p*** < 0.0001

All values are expressed in mmol / L , except for neopterin (nmol /L) and creatinine (μ mol /L). AI = AtherogenicIndex.

Figure 1 shows the presence of significant inverse correlation between neopterin and serum TG in the CVD patients, while Fig.2 and 3 show significant inverse correlation of neopterin with estimated glomerular filtration rate (eGFR) and direct correlation with serum creatinine.

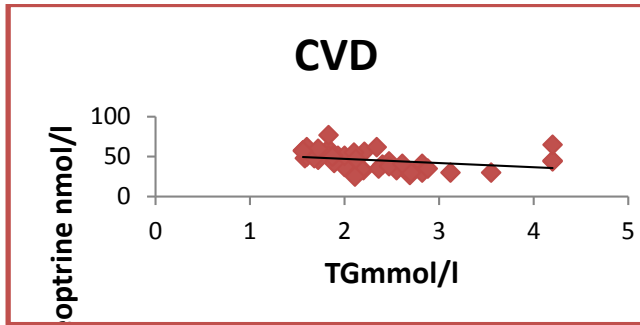


Figure (1): Correlation between serum neopterin and triglycerides

($r = -0.314$, $p = 0.048$) in cardiovascular disease.

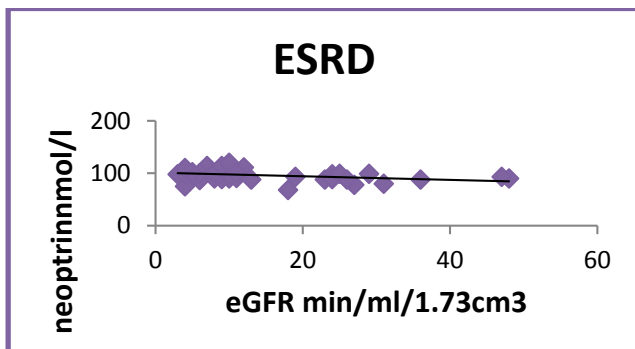


Figure (2): correlation between serum neopterin, and estimated glomerular filtration rate ($r = -0.35$, $p = 0.03$) in end stage renal disease.

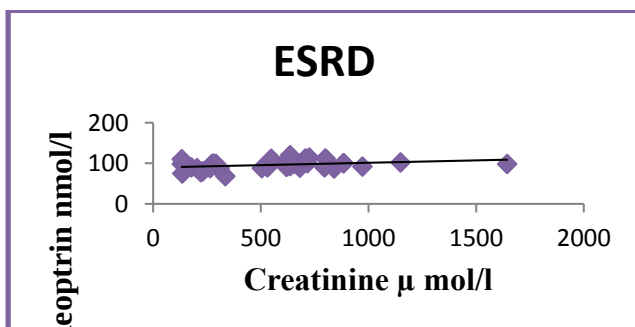


Figure (3): correlation between serum neopterin, and creatinine

($r = -0.38$, $p = 0.03$) in end stage renal disease.

DISCUSSION

In recent years, a big attention was paid to the association between inflammation and atherosclerosis. Many studies have demonstrated that local and systemic inflammation plays an important role in the formation and development of atherosclerosis which involved different types of inflammatory cells, and cytokines.⁽¹⁰⁾ This study showed significantly high levels of neopterin in CVD patients, in comparison with the normal control group. These results confirm previous findings of other studies dealing with the same subject, with the involvement of monocytes in the pathological processes related to the development of acute coronary syndromes.^(11, 12)

In several studies it was demonstrated that there were significant correlations between neopterin concentration and certain direct immunological activity measures like concentrations of interferon gamma, interleukins. A distinct advantage of neopterin, in contrast to substances like interferon gamma, is its considerable chemical stability making it a sensitive and reliable marker for clinical practical use.^(13,14, 15)

Activated T lymphocytes secrete γ -interferon, which leads to mononuclear macrophages excitation and neopterin production. Therefore, neopterin levels directly reflect the activation of macrophages, T lymphocytes, and cellular immunity.⁽³⁾ In addition to this relation between neopterin and immunity, neopterin can easily penetrate into the blood circulation and because of its high chemical stability and low molecular weight, its concentration in coronary artery blood can be determined by measurement of neopterin level in peripheral blood, which can be used to assess the degree of coronary artery lesions.⁽¹⁶⁾

From table 2 there is a significant difference in neopterin level between patients with ESRD and healthy control groups. The decline in GFR of ESRD and dialysis patients have greater chances for developing CVD and this will increase the mortality percentage within ESRD patients.^(17,18) The negative correlation between neopterin with each of GFR and positive correlation with creatinine in this study (fig 2 & 3) confirms the clinical significance of neopterin as a biomarker for the presence of a high chance for developing inflammatory state or CVD in these patients. Although it is well established that a dysfunction of the immune system is induced by the uremic state, this disturbance has not been systemically studied as a potential contributing cause of

premature deaths resulting from CVD and infections in ESRD.

One brief review describes disorders of the immune systems in ESRD and studying the possibility, that a state of acquired immune dysfunction in uremia could be an important contributing factor for both CVD and inflammatory complications.⁽¹⁹⁾

Two previous studies had shown that the link between chronic inflammation and ESRD, which progress to atherosclerotic disease and CVD had a great interest in understanding the mechanism of this association.^(20, 21)

The possible explanation of this relationship is that the activated T cells formed as a result of inflammation may play an important role in CVD mediation through the elevated serum neopterin.⁽⁷⁾

Of interest in this study is the presence of a significant negative correlation between neopterin and hypertriglyceridemia in CVD patients group as shown in figure (1). There is, also, inverse correlation, but not significant, between neopterin and both LDL, and HDL. The mechanism of this correlation may be due to the activation of the immune system, which is indicated by the activation of the immune system, and indicated by high neopterin level, attended by the release of interleukin 6, up-regulation of LDL receptors, with a consequent lowering of LDL-C and triglycerides.^(22, 23, 24)

In addition to all above, the most important result in this study, which represent the main objective of it, is that the level of neopterin in ESRD patients is significantly higher than its level in CVD patients which could be attributed to either increased its production that mean the degree of inflammation is higher than CVD, or to delayed clearance through the kidneys or combination of both mechanisms. Inflammation and increased oxidative stress are common features in uremic patients.⁽²⁵⁾

One of these features was the presence of oxidative damaged protein products named advanced oxidation protein products (AOPPs) in the plasma of those patients.⁽²⁶⁾

Advanced oxidation protein products were found to be elevated at early stages of chronic renal failure and are closely related to monocyte activation markers including neopterin, TNF- α and its soluble receptors.⁽²⁷⁾

The second possible explanation of increasing its level is due to decrease its clearance since neopterin is not metabolized in the blood⁽²⁸⁾ or combination of both causes.

One recent study concluded that the more impaired the renal function, the higher is the increase in serum neopterin concentration.⁽²⁹⁾ Further studies and measurement of other inflammatory factors are needed to confirm the results of this speculation.

So in conclusion, this study shows marked difference in the neopterin levels between CVD and ESRD this can be explained by three probabilities, either the differences in the degree of the inflammation between these two diseases (which increase with increasing the severity of CKD), or related to the defect in clearance pathway of this inflammatory marker or combination of both.

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