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ASSESSMENT OF PYRAZINO-PYRIMIDINE COMPOUND AND SOME INFLAMMATORY BIOMARKERS IN PATIENTS WITH TYPE 2 DIABETES

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ABSTRACT: Neopterin, pyrazino-pyrimidine compound, is a metabolite of guanosine triphosphate and is produced by the activated monocytes, macrophages and dendritic cells upon stimulation by interferon gamma produced by T-lymphocytes. **Aim:** This study purposed to analyze the serum level of neopterin and to evaluate its correlation with other markers of inflammation in patients with type 2 diabetes at various stages of diabetic nephropathy. **Methods:** The study done on 80 patients, aged (60 years ±5) with type 2 diabetes, and 60 healthy subjects, aged (50 years±8) recruited from outpatient clinic of nephrology department in Al yarmouk hospital. The serum levels of neopterin, high sensitive C-reactive protein (hs-CRP), interluekin-six (IL-6), tumor necrosis factor-alpha (TNF-α) and interferon-gamma (IFN-γ) were assayed by using enzyme-linked immunosorbent assays (ELISA) kits, and studied. **Results:** The level of serum neopterin was higher in diabetic patients than in control subjects. There were gradual increases of serum neopterin levels from stage two to stage three and four in diabetic patients. Serum neopterin level correlated positively with serum levels of hs-CRP, IL-6, and IFN-γ and correlated negatively with estimated glomerular filtration rate value. **Conclusion:** Serum neopterin level is elevated and correlated with the severity of diabetic nephropathy. It may use as a good biomarker for an accurate identification and prognosis of the diseases associated with the activation of cell-mediated immunity.

INTRODUCTION: Neopterin belonged to pteridine class and formed by fusing pyrimidine and pyrizine rings therefore neopterin sometime called pyrazino-pyrimidine compound. It produced from guanosine triphosphate by activated monocytes, macrophages, dendritic cells, and endothelial cells upon stimulation by interferon gamma (INF-γ) and its release enhanced by tumor necrosis factor [1, 2]. After releases, it enhances macrophage cytotoxicity through its interactions with reactive oxygen, nitrogen, and chloride species [2].

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Increased neopterin concentration in the urine or blood due to activation of cellular immunity and an endogenous release of INF-γ may affect cellular redox state because of interactions between neopterin or its derivative with reactive oxygen or nitrogen intermediates [3].

Neopterin levels are elevated in several conditions including autoimmune diseases such as systemic lupus erythematosus and rheumatoid arthritis [4]; infections such as hepatitis, human immunodeficiency virus, and cytomegalo virus [5]. Elevated neopterin level observed in patients with various cancers and in all cancer types investigated, high neopterin levels were significantly associated with a poor prognosis [6].

The impact of diabetic nephropathy on the increasing population with chronic kidney disease (CKD) and end-stage renal disease (ESRD) is

enormous. Furthermore, prediction and progression of diabetic nephropathy, cardiovascular and renal outcome done by simultaneous evaluation of albuminuria and glomerular filtration rate [7, 8].

The objective of this study is to investigate whether the concentration of neopterin was elevated in serum samples of patients with diabetic nephropathy as compared to control subjects and to evaluate the utility of neopterin as a biomarker of diabetic nephropathy.

MATERIALS AND METHODS: We conducted case-control study among the participants of outpatient clinic of nephrology department in Al yarmouk hospital. This study enrolled 80 patients with diabetic nephropathy and 60 control healthy subjects. Excluded criteria involved patients with known coronary artery disease, heart failure, malignancies, active infections, and patients taking drugs that may effect on inflammatory response.

Estimated glomerular filtration rate (eGFR) was calculated using 2009 CKD-EPI creatinine equation and was classified basing on glomerular filtration rate (GFR) category [9], at which patients on stage (G2) have eGFR =(60–89ml/min/ 1.73m^2), patients on stage (G3) have eGFR = (30–59 ml/min/1.73m^2), and patients on stage (G4) have eGFR = (15–29 ml/min/1.73m^2).

After recruitment, participants asked to be in a fasting state. Blood samples were collected; serum was separated and stored at -80 °C until analysis. Commercially available enzyme-linked immunosorbent assay (ELISA) kits were used to measure serum levels of hs-CRP, IL-6,TNF-α, IFN-γ and neopterin and all the samples were analyzed in duplicate. Moreover, spectrophotometric assay kits were used to measure serum levels of albumin, creatinine, and urea.

All eligible participants provided written informed consent to partake in this study. The study protocol conforms to the ethical guidelines and approved by the institution's ethics committee.

Results are shown as mean ± SD with 95% confidence interval (CI), and *P* values of (0 <0.05) were regarded to be statistically significant. All statistical analyses performed using series SPSS version 18.

RESULTS: Details and clinical data of the studied groups showed in **Table 1**. There were no differences in age and gender distribution observed between control and patients. Of the 80 patients with diabetic nephropathy, 28 were in stage two (G2), 30 in stage three (G3), and 22 in stage four (G4).

TABLE 1: THE DEMOGRAPHIC AND CLINICAL DATA OF THE STUDIED GROUPS

Variables	Control	Diabetic patients
Number	60	80
Gender (Male/Female)	(25/35)	(33/47)
Age (years)	50 ± 8	60 ± 5
Serum Albumin (g/L)	40.2 ± 3.6	32.4 ± 5.7 ***
Serum Creatinine (mg/dl)	0.67 ± 0.35	1.58 ± 0.46 ***
Serum Urea (mg/dl)	30.7 ± 8.5	68.3 ± 12.4 ***
eGFR (ml/min)	97.6 ± 7.5	50.7 ± 12.4 ***
Serum Neopterin (nmol/L)	7.5 ± 1.2	35.7 ± 3.36 ***
Serum hs CRP (mg/L)	2.25 ± 1.07	43.7 ± 4.8 ***
Serum IL-6 (pg/ml)	2.05 ± 1.2	19.2 ± 2.3 ***
Serum TNF-α (pg/ml)	3.8 ± 0.98	8.2 ± 2.42 ***
Serum INF-γ (pg/ml)	2.13 ± 0.64	8.87 ± 1.5 ***

Data are presented as mean ±SD (standard deviation) for continuous variables; *** P<0.001 high significant difference versus control.

Abbreviations: g/L, gram per liter; mg/dl, milligram per deciliter; nmol/L, nanomole per liter; pg/ml, picogram per milliliter; Number, sample size of the participants; hs- CRP, high sensitive C-reactive protein; eGFR, estimated glomerular filtration rate; IL-6, Interluekin-six; TNF-α, tumor necrosis factor-alpha; IFN-γ , Interferon-gamma

In contrast to healthy subjects, serum neopterin level was notably higher in patients with diabetic nephropathy (**Table 1**) and it is seemed to be 5 folded more than its level in control subjects (**Fig. 1**).

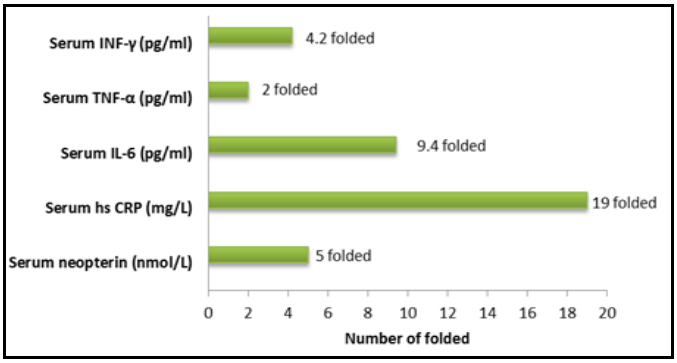

FIG. 1: BAR GRAPH ELUCIDATED THE NUMBER OF FOLDED INCREASES IN SERUM LEVELS OF INFLAMMATORY BIOMARKERS FOR DIABETIC PATIENTS VERSUS CONTROL LEVELS

Abbreviations: hs- CRP, high sensitive C-reactive protein; IL-6, Interluekin-six; IFN-γ, Interferon-gamma; TNF-α, tumor necrosis factor-alpha

As well, there were graded increases in serum neopterin levels from stage two to four as indicated in **Fig. 2**.

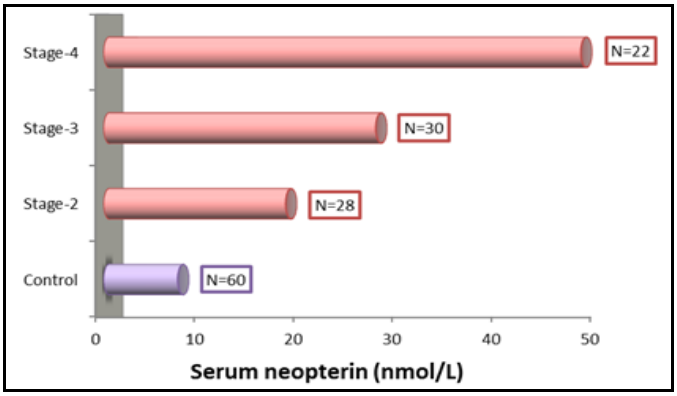

FIG. 2: BAR GRAPH SHOWED THE MEAN SERUM NEOPTERIN LEVELS (nmol/L) AND THE NUMBER (N) OF THE PARTICIPANTS IN HEALTHY CONTROL AND IN DIFFERENT STAGES OF DIABETIC NEPHROPATHY

The serum neopterin level exhibited a high significant inverse correlation with eGFR, and had a positive association with hsCRP, IL-6, and IFN-γ in patients with diabetic nephropathy. Whereas a non-significant correlation noted between serum neopterin level and TNF-α in those patients, **Table 2**.

TABLE 2: CORRELATION COEFFICIENT (r) OF SERUM NEOPTERIN LEVEL WITH THE ESTIMATED GLOMERULAR FILTRATION RATE VALUE AND WITH THE SERUM LEVELS OF OTHER INFLAMMATORY MARKERS IN DIABETIC PATIENTS

	R	P-value
eGFR	-0.42	P <0.001
Serum hs-CRP	0.33	P <0.01
Serum IL-6	0.28	P <0.05
Serum INF-γ	0.36	P <0.001
Serum TNF-α	0.21	P > 0.05

P > 0.05 non-significant correlation; P <0.05 significant correlation; P <0.01, P <0.001 high significant correlation.

Abbreviations: eGFR, estimated glomerular filtration rate; hs-CRP, high sensitive C-reactive protein; IL-6, Interluekin-six; IFN-γ, Interferon-gamma; TNF-α, tumor necrosis factor-alpha

There were high significant increases in mean serum levels of other inflammatory biomarkers found in patients with diabetic nephropathy as compared to control subjects, at which hsCRP increased 19 folded , IL-6 increased 9.4 folded, TNF-α increased 2 folded and IFN-γ increased 4 folded , **Fig. 1.**

DISCUSSION: The current study showed that the patients with diabetic nephropathy exhibited high significant increase in serum level of neopterin compared to healthy subjects, and this increase correlated positively with the increased circulating levels of several inflammatory biomarkers (hsCRP, IL-6, and IFN-γ).

Serum level of neopterin reveal continuous increases with the decreasing GFR, these finding is consistent with previous studies [10, 11] that proposed the decreased renal elimination and/or increased inflammatory reaction on renal function are behind the progressive increased in neoptrin levels.

Other study showed that the elevated activity of the pteridine pathway leads to the generation of singlet oxygen, hydroxyl radical and nitric oxide [12], which may affect renal function and decreased renal elimination.

Grossmann *et al*. in 2015, noted that neopterin level was positively associated with the prevalence of the diabetes disease [13]. As well, neopterin considered as a marker of diabetic progression and complication [14].

The diabetic patients in the current study showed inflammatory activation and increases in the inflammatory biomarkers (CRP, IL-6, IFN-γ, and TNF-α) levels which are consistent with other study done on patients with chronic kidney disease [11].

Conversely, other study reveal weak association of CRP level with diabetes prevalence after adjusting other cardiovascular risk factors and comorbidities and this occur may be due to the early activation of the immune system [13].

CRP is one of the inflammatory biomarkers for type 2 diabetes–associated cardiovascular diseases [15, 16]. The regulatory functions of CRP include enhancement of leukocyte reactivity, complement fixation, and modulation of platelet activation [17].

Furthermore, cytokines such as IL-6, or TNF-α that may contribute to islet cell autoimmunity in type 2 diabetes through activation of T cells, B cells, and macrophages may increase expression of β-cell antigens, and subsequent β-cell apoptosis [18].

In 2004, Bodlaj *et al*. documented that the presence of type 2 diabetes mellitus in addition to end stage renal disease (ESRD) was not associated with the further increases in serum levels of the inflammatory parameters, *i.e*. the deteriorated prognosis of diabetic ESRD patients is probably not associated with the activation of inflammatory processes [19].

Many studies have proven a strong association of neopterin with cardiovascular disease [20], increased cardiac events rates [21] and atheromatous plaque activity and progression in patients with coronary artery disease [22]. In vascular smooth muscle cells, elevated neopterin level cause subsequent increase in nitric oxide (NO) produsction [23] and induce programmed cell death by activated the transcriptional nuclear factor (NF)-κB [24].

Glucocorticoids drugs may cause suppression of cell-mediated immunity and consequently result in decreased neopterin levels. As well, statin drug, which has anti-inflammatory effects by inhibiting HMG-CoA (hydroxyl methyl glutaryl – CoA) can decrease serum level of neopterin [25]. Therefore, patients taking these drugs excluded in this study.

CONCLUSION: As the neopterin level serves as an indirect indicator for oxidative stress thereby impaired renal elimination and increased inflammatory reaction that occur during diabetic nephropathy might be associated with the stepwise increase in serum neopterin level. More studies needed to confirm the association between the neopterin elevation and the clinical events accompanied diabetic nephropathy.

CONFLICT OF INTERESTS: There is no conflict of interests to declare.

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REFERENCES:

1. Berdowska A and Zwirska-Korczala K: Neopterin measurement in clinical diagnosis. J Clin Pharm ther 2001; 26(5): 319–329.
2. Hofmann G, Wirleitner B, and Fuchs D: Potential role of immune system activation- associated production of neopterin derivatives in humans. Inflam Res 2003; 52(8): 313–321.
3. Feldman DM. Neopterin a novel marker of immune function in hepatitis C. 2008. Oct 09, Available from: http://wwwhcvadvocateorg/hcsp/articles/feldman-1.html
4. Aulitzky W E, Tilg H, Nicderwieser D, *et al*.: Comparison of serum neopterin levels and urinary neopterin excretion in renal allograft recipients, Clin Nephro 1988; 29(5): 248–252.
5. Halota W, Jaruga B, and Pawłkowska M: Serum neopterin and β_2-microglobulin concentration as prognostic markers of AIDS natural history. Pols ki Merkuriusz Lekarski 2002; 13(74):126–128.
6. Sucher R, Schroecksnadel K, Weiss G, Margreiter R, Fuchs D, Brandacher G: Neopterin, a prognostic marker in human malignancies. Cancer Lett 2010; 287:13–22.
7. Levey AS, deJong PE, Coresh J, El Nahas M, Astor BC, *et al*.: The definition, classification, and prognosis of chronic kidney disease: a KDIGO Controversies Conference report. Kidney Int 2011; 80:17–28.
8. Ninomiya T, Perkovic V, deGalan BE, Zoungas S, Pillai A, *et al*.: Albumin uria and kidney function in dependently predict cardiovascular and renal out comes in diabetes. JAmSoc Nephro 2009; l20:1813–1821.
9. Kidney Disease: Improving Global Outcomes (KDIGO) CKD Work Group. KDIGO 2012 Clinical Practice Guideline for the Evaluation and Management of Chronic Kidney Disease. Kidney inter., Suppl. 2013; 3:1–150.
10. Pecoits-Filho R, eimb¨ urger O, P.B´ ar´ any, *et al*.: Associations between circulating inflammatory markers and residual renal function in CRF patients. Ame J Kid Dis 2003; 41(6): 1212–1218.
11. Yadav Ashok K, Vinod S, and Vivekanand J. Association between Serum Neopterin and Inflammatory Activation in Chronic Kidney Disease. Media Inflam 2012; 10:1- 6.
12. Razumovitch JA, Semenkova GN, Fuchs D, Cherenkevich SN. Influence of neopterin on the generation of reactive oxygen species in human neutrophils. FEBS Lett. 2003; 549:83–6.
13. Grossmann V, Volker HS, Tanja Z, Marina PN, *et al*.: Profile of the Immune and Inflammatory Response in Individuals with Prediabetes and Type 2 Diabetes. Diabetes Care 2015; 38:1356–1364.
14. Weiss MF, Rodby RA, Justice AC, *et al*.: Free pentosidine and neopterin as markers of progression rate in diabetic nephropathy. Kid Int1998; 54(1):193-202.
15. Donath MY, Shoelson SE. Type 2 diabetes as an inflammatory disease. Nat Rev Immunol 2011; 11:98–107.
16. Herder C, Brunner EJ, Rathmann W, *et al*.: Elevated levels of the anti-inflammatory interleukin-1 receptor antagonist precede the onset of type 2 diabetes: the Whitehall II study. Diabetes Care 2009; 32:421–423.
17. Pradhan AD, Manson JE, Rifai N, Buring JE, Ridker PM. C-reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus. JAMA 2001; 286:327–334.
18. Itariu BK, Stulnig TM. Autoimmune aspects of type 2 diabetes mellitus - a mini-review. Gerontology 2014; 60: 189–196.
19. Bodlaj G, Jörg B, Robert P, Thomas S, and Georg B. Type 2 diabetes in patients with end- stage renal disease is not associated with further increased serum inflammatory parameters. J nephrol 2004; 17: 112-117.
20. Auer J, Berent R, Lassnig E, Weber T, and Eber B: Prognostic significance of immune activation after acute

coronary syndromes. JAme Col Card 2002; 9 (11):1878.

21. Sasaki T, Takeishi Y, Suzuki S, *et al.*: High serum level of
22. Avanzas P, Arroyo-Espliguero R, Cosı´n-Sales J, Aldama G, Pizzi C, Quiles J, Kaski JC. Markers of inflammation and multiple complex stenosis (pancoronary plaque vulnerability) in patients with non-ST segment elevation acute coronary syndromes. Heart 2004; 90:847–852.
23. Gieseg SP, Crone E M, Flavall E A, Amit Z.: Potential to inhibit growth of atherosclerotic plaque development through modulation of macrophage neopterin/7,8-dihydroneopterin synthesis. Brit J Pharm 2008; 153 (4): 627–635.

neopterin is a risk factor of patients with heart failure. Inter J Card 2010; 145(2):318.

24. Schobersberger W, Hoffmann G, Grote J, Wachter H and Fuchs D: Induction of inducible nitric oxide synthase expression by neopterin in vascular smooth muscle cells. FEBS Letters, 1995; 377 (3): 461–464.
25. Mulder D J, van Haelst P L, Wobbes M H, Gans R O, *et al.*: The Effect of Aggressive Versus Conventional Lipid-lowering Therapy on Markers of Inflammatory and Oxidative stress. Cardiovasc Drugs Ther 2007; 21:91–97.

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