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Original Research Article

Relationship between Inflammatory Biomarkers (CRP, TNF-alpha, Interleukin 6, and cystatin C) and Renal Function Tests among Type 2 Diabetes Mellitus-Khartoum

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Abstract

Introduction: Always chronic kidney disease has been bound to diabetes mellitus, especially type2. Inflammatory biomarkers, such as CRP, IL6, cystatin C and TNF-alpha) are usually play role in the development and increase risk of type 2 diabetes T2DM, as well as chronic kidney disease, so this study aimed to evaluate renal function tests and inflammatory biomarkers among T2DM and compare evaluated parameters with data of healthy individuals. ***Methods:*** Two hundred (200) subjects were enrolled in this study, 100 of them were patients with T2DM set as case group, , age ranged 33 and 55 years, and the other 100 were healthy individuals were set as control group. Blood samples were collected from both groups in order to assess renal function tests urea, creatinine by means of spectrophotometer based method, while Sodium and Potassium by easylyte device instrument and inflammatory biomarkers CRP, IL6, cystatin C and TNF-alpha by ELISA technique. ***Results:*** Inflammatory biomarkers (IL-6, CRP, Cystatin C and TNF-alpha) levels in diabetic subjects were significantly higher than control group. We also found that serum creatinine and urea were higher in type 2 diabetes patients than normal subjects (p=0.001). Pearson's correlation of serum creatinine and urea with inflammatory biomarkers brought different positive correlation with all parameters. ***Conclusion:*** Elevated levels of inflammatory biomarkers (IL-6, CRP, Cystatin C and TNF-alpha) are positively associated with renal function tests serum creatinine and urea and increased risk of type 2 diabetes.

Keywords: Inflammatory Biomarkers, Chronic Kidney Disease, Spectrophotometer.

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INTRODUCTION

Diabetes mellitus, mainly type 2, is a major health problem with estimates exceeding >380 million peoples worldwide in 2015 [1]. The prevalence of type 2 diabetes has been rapidly rising worldwide. In Korea, the prevalence of diabetes (95% of which were cases of type 2 diabetes) has increased from 1% of the general population in the 1970s to 13.5% of men and 10.7% of women aged 30 years or more in 2000 [2]. Chronic kidney disease (CKD) is one of the major complications of type 2 diabetes, and is the leading cause of end-stage renal disease (ESRD), accounting for approximately 40% of new cases of ESRD every year [3]. Early detection and treatment of CKD in patients with type 2 diabetes can prevent or retard the progression to ESRD [4, 5]. Chronic kidney disease is currently estimated to affect ~10% of the global population and is becoming increasingly common [6]. Patients with CKD display greatly increased morbidity and mortality rates compared with the general population [7]. The presence of low-grade, chronic inflammation has been hypothesized to cause a number of comorbidities observed in CKD, conferring an increase in mortality [8]. However, not all individuals are equally likely to become chronically inflamed, nor are all inflamed patients susceptible to developing various comorbidities. This has given rise to several theories regarding possible roles of various genetic factors as determinants of complications and morbidity and mortality in CKD [9, 10].

Chronic or recurring inflammation contributes to an aberrant continuation of acute phase response and may also lead to further diabetes complications, such as micro and macroangiopathy and impaired healing [11, 12], it is suggested that periodontal disease with increased inflammatory response at local and systemic levels [13] may collaborate to insulin resistance present on T2DM pathogenesis. Although there is scientific evidence on the effects of chronic periodontitis on

diabetes mellitus remains inadequate and inconclusive [14]. Furthermore, whether periodontal therapy may help to control serum levels of inflammatory cytokines still remains controversy [15]. Epidemiological studies have demonstrated an increase in plasma levels of inflammatory markers such as CRP, IL-6 and TNF-α in patients with metabolic syndrome and also in those with clinically overt T2D [16, 17], while other epidemiological study also reported inconsistent findings on the associations of CRP, TNF-α, IL-6, and CKD [18]. Several mechanisms for the effects of CRP and TNF-α on the risk of CKD have been proposed, although the mechanisms leading to the development and progression of renal injury in type 2 diabetes are not fully understood. CRP, produced by the liver tissue, and TNF-α, synthesized primarily by monocytes and macrophages, are cytokines and sensitive indicators of inflammation. It is now evident that activated innate immunity and inflammation are related to the pathogenesis of diabetes. 21 Furthermore, elevated levels of these inflammatory markers in patients with type 2 diabetes, who have chronic low-grade inflammation, play a critical role in the development of microvascular diabetic complications, including CKD, by potentiating endothelial dysfunction [19, 20].

MATERIAL AND METHOD

This cross-sectional case control study was conducted among diabetic patients mainly diagnosed with type 2, 100 were set as case group, enrolled in this study. Also, 100 healthy men selected as control group. under hygienic condition blood samples were collected, fluoride oxalate added blood containers were for measurement of fasting blood glucose (spectrophotometry based method), ethylene diamin tetra acetic acid (EDTA) added blood containers for assessment of glycated hemoglobin (HbA1c) by means of immunodetection method using ichroma device and kits, plane containers were used to serum formation after complete clotting of whole blood at room temperature for ,measurement electrolytes (Na and K) by easylyte instrument and reagent, urea, creatinine (spectrophotometry based method) and inflammatory biomarkers, CRP. TNF- α, IL6 and cystatin C by enzyme linked immunoassay (ELISA).

Ethical Consideration

This study was approved with the ethical committee of Alneelain university- faculty of graduate and medical laboratory science. Every diabetic center and personnel involved did give consent to be recruited for this study.

Statistical Analysis

Data obtained was analyzed using statistical package of social science (SPSS) version 22. Fasting blood glucose, HbA1c and Inflammatory biomarkers were used for statistical analysis. All the data were expressed as mean ± SD (standard deviation), the statistical significance of differences between the values was assessed by ANOVA or Mann–Whitney U test (as appropriate). Logistic and Multiple regressions were performed among on the parameters. A two-tailed p value of <0.05 was considered as statistically significant.

RESULTS

In this case control study diabetic patients, type 2 were enrolled to assess inflammation biomarkers, beside renal function tests (urea, creatinine, Na and K) and glucose with glysated hemoglobin. 100 T2DM patients were sorted according to age to more than 50 years 18 (36%) and less 50 years were 35 (64%) as in Figure-1.

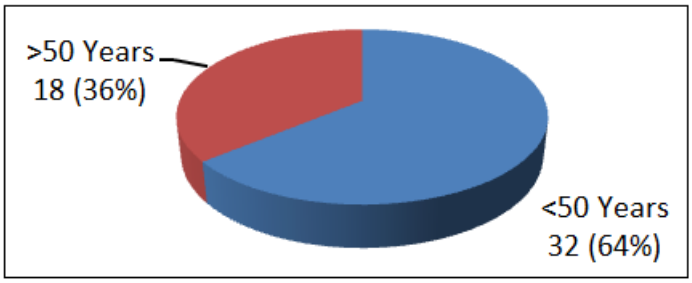

Fig-1: Distribution of Patients According To Age Group

Comparing measured parameters (FBG, Hb A1c, urea, creatinine, Na, K, cysatin C, IL6, serum CRP and TNF-α) for case group (T2 DM) and control group, revealed that most of them were increased among case group except Na and they bought significant difference as p value for each was less than 0.05 as in Table-1.

Table-1: Mean comparison of study parameters in case versus control

Parameters	Case (Mean±SD)	Control (Mean±SD)	*P-value*
FBG	180.30±35.91	96.36±9.49	<.001
HBA1c	7.74±1.12	5.28±0.37	<.001
Urea	40.44±10.66	33.20±5.61	<.001

Creatinine	1.21±0.47	0.96±0.16	0.001
Na	135.16±5.00	138.16±3.47	0.001
K	4.24±0.52	4.03±0.39	0.027
Cystatin C	1.88±1.23	0.74±0.14	<.001
s-CRP	6.05±2.47	3.18±1.67	<.001
IL-6	13.96±3.72	11.72±2.56	0.001
TNF-α	13.50±3.11	9.02±1.92	<.001

Significant difference p value <0.05

Pearson's correlation of urea and creatinine with Na and K with each of the inflammatory biomarkers brought different positive correlation with all parameters except Na as in Table-2

Table-2: Correlation between study parameters with urea and creatinine

Parameters	urea		creatinine	
	R value	P value	R value	P value
Creatinine	0.893	0.000		
Na	-0793	0.000	-0.840	<.001
K	0.596	0.596	0.645	<.001
Cystatin C	0.553	0.553	0.393	0.005
CRP	0.311	0.311	0.223	0.120
IL-6	0.347	0.347	0.155	0.284
TNF-α	0.447	0.447	0.243	0.088

While correlations Na and K with inflammatory biomarkers gave entirely negative and positive respectively as in Table-3.

Table-3: Correlation between study parameters with Na and K

parameters	Na		K	
	R value	P value	R value	P value
Urea	-0.793	0.00	0.565	<.001
Creatinine	-0.893	0.00	0.645	<.001
Cystatin C	-.461	.001	.452	<.001
CRP	-.294	.038	.193	.001
IL-6	-.286	.044	.313	.178
TNF-α	-.320	.024	.477	.027

DISCUSSION

Identifying inflammatory biomarkers predictive of estimated glomerular rate (eGFR) decline could point to pathways that may help identify new therapeutics. With the recognition that type 2 diabetes itself may be a chronic inflammatory state, the role of potentially modifiable lipid and inflammatory biomarkers has been of particular interest [21]. Chronic kidney disease (CKD) has become a public-health problem [22]. GFR is the most important marker of kidney function, but it cannot be easily measured in most clinical or research settings, and therefore estimating equations are based on filtration markers such as serum creatinine and cystatin C [23]. Within the last decade, a hypothesis was proposed to explain the pathogenesis of T2DM that connects the disease to a state of subclinical chronic inflammation 105-106. Inflammation is a short-term adaptive response of the body elicited as a principle component of tissue repair to deal with injuries and microbial infections (as cold, flu). It also can be elevated in chronic conditions such as peripheral neuropathy, chronic kidney disease and fatty liver. Current thinking suggests that abnormal levels of chemokines released by the expanding adipose tissue in obesity activate monocytes and increase the secretion of pro-inflammatory adipokines. These cytokines in turn enhance insulin resistance in adipose and other tissues, thereby increasing the risk for T2DM [24, 25]. In this study targeting T2DM to see how inflammatory biomarkers b (CRP. TNF- ***α***, IL6 and cystatin C) and renal function (urea and creatinine) tests with electrolytes (Na and K) to figure how persistant increased levels of glucose and HbA1c can be associated with increased inflammatory biomarkers and increased RFTs in comparison with data of healthy individuals with no T2DM or renal issues. 100 T2DM patients and 100 healthy subjects were recruited for this study, set as case and control groups respectively, increased levels of fasting blood glucose, HbA1c, urea, creatinine, CRP. TNF- α, IL6 and cystatin C and decreased Na among case group more than control group brining significant difference when compared with control group. Correlation levels of RFTs and electrolytes with biomarkers of inflammation, brought urea and creatinine with Na and K with each of the inflammatory biomarkers brought different positive

correlations, while correlations Na and K with inflammatory biomarkers gave entirely negative and positive. Many studies conducted in order for evaluate T2 DM patients or binding diabetes mellitus with some issues in order to predict complication or something like that. An agreement with our study found with a study conducted as the same manner of this study, but differ in the aim, it was a review article based on growing evidence that inflammatory markers play a role in the development of type 2 diabetes. It to systematically review prospective studies on the associations of elevated levels of interleukin-6 (IL-6) and C-reactive protein (CRP) with increased risk of type 2 diabetes by conducting a meta-analysis. The meta-analysis, including 10 prospective studies, with a total of 19,709 participants and 4,480 cases, detected a significant dose-response association of IL-6 levels with type 2 diabetes risk. For CRP, the meta-analysis involving 22 cohorts, with a total of 40,735 participants and 5,753 cases, showed that elevated CRP levels were significantly associated with increased risk of type 2 diabetes Sensitivity and subgroup analyses further supported the associations [26]. Other study assessed inflammatory biomarker TNF-α among type 2 diabetes mellitus to prove the evidence suggests that chronic subclinical inflammation plays a key role in the pathogenesis and progression of diabetic nephropathy. 80 were enrolled and clinical and laboratory data were recorded. Albumin-creatinine ratio (ACR) was calculated in first-morning urine samples. Serum and urinary tumor necrosis factor-α (TNF-α) levels were determined for 45 patients had normoalbuminuria, 33 microalbuminuria, and 2 macroalbuminuria. Patients with microalbuminuria were older, with higher glycosylated hemoglobin levels (HbA1c) and they more frequently had diabetic retinopathy, neuropathy, and cardiovascular disease. In patients with type 2 DM, urinary, but not serum, TNF-α level are associated with the presence and severity of microalbuminuria [27]. A partial agreement with study conducted among T2DM subjects, aimed to determine the association of inflammatory biomarkers with glycemic control in subjects with T2D and poor glycemic control, performed comparing 122 subjects with T2D with 54 control subjects. Patients assess for inflammatory biomarkers (C-reactive protein, Interleukin-6 and others) and were evaluated according to the degree of glycemic control. 42 subjects with T2D were studied before and after 3 months of improving glycemic control by different strategies. Patients with T2DM had higher C-reactive protein (CRP) compared to controls [28].

CONCLUSION

This study involved T2DM and control group of healthy individuals to compare with laboratory results, it shown that increased of inflammatory biomarkers, renal function tests, potassium and decreased level of sodium, binding these parameters with developing consequences on many levels of body's systems, lead to renal function deterioration . Significant association of elevated levels of IL-6, CRP, cystatin C and TNF-alpha with type 2 diabetes risk. Increased of inflammatory biomarkers significantly and associated with the progression of Diabetic Nephropathy in type 2 diabetic patients.

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