Evaluation of Some Inflammatory Cytokines and Glycated Hemoglobin in Uncontrolled Type 2 Diabetes Mellitus with Nephropathy

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Abstract

Background: Chronic hyperglycemia causes diabetic nephropathy(DN), which is a typical microvascular complication of type 2 diabetes mellitus. The pathogenesis of DN is not fully understanding. The inflammation may possess a significant role in the progression of DN in diabetic patients.

Method: The study accomplished at teaching laboratories of medical city, Baghdad, Iraq.It was included 50uncontrolled diabetic type 2 patients with nephropathy, age range (40 –78) years and 42 controlled diabetics type 2 without nephropathy, age range (35 – 52) years as a control group.The participants divided in to two groups according to HbA1c measurement which is described as follows: < 7.5% of HbA1c describes controlled diabetes, and > 9% of HbA1c describes uncontrolled diabetes. The calculation of the Glomerular filtration rate (GFR) was used to determine the renal function for each participant. A venous blood sample was obtained to estimate HbA1c,fasting serum glucose (FSG), urea, creatinine, interleukin-6(IL-6),interleukin-1 beta(IL-1β)and tumor necrosis factor – alpha(TNF- α.).

Results : Significantly higher serum urea and creatinine levels in uncontrolled diabetic patients with nephropathy than controlled diabetic without nephropathy. The mean of HbA1c of DN group was higher as compare to diabetic control (DC) group, Results of IL-1β, IL-6 and TNF-α exhibits a significant raised level for diabetics with nephropathy, ($p< 0.05$) for IL-1β, ($p< 0.05$) for IL-6 and ($p< 0.01$) for TNF-α .

Conclusion : Raised serum levels of TNF- α, IL-1βand IL-6 in uncontrolled diabetic patients with nephropathy concurrently with raising the average HbA1c in them indicate that the inflammation may have a role in the advancement of DN in diabetic patients with poor glycemic control.

Keywords: *inflammation, interleukins, tupe2 diabetes mellitus, HbA1c, hyperglycemia*

Introduction

Diabetic nephropathy (DN) isanadvanced renal disease designated by persistent albuminuria with reduction of glomerular filtration rate (GFR) due to uncontrolled hyperglycemia [1]. Metabolic changes occurred in diabetes causes deterioration of glomerular functions leading to DN that recognized by an accumulation of nitrogenous compoundslike creatinine and urea with a consecutive deterioration in the GFR followed by arterial hypertension give raise torenal damage[2].Immunological and inflammatory employssignificantroles in DN and its progression, particularlyIL-8, IL-6,IL-1b,and TNF-α, by modifying disease via various mechanisms. Regulation of inflammatory and immune responses by these cytokines through secondary pathways which are contributes to the pathophysiology of many diseases including diabetes mellitus (DM) [3].Pathological alterations in DN are renal hypertrophy and hyperfiltrationthat are associated with inflammation processes which included release of proinflammatory and anti-inflammatory cytokines from many immune cells [4]. IL-1b isproinflammatory cytokines produced by macrophages and renal in inflammatory based diseases. It was involved in the declining of glomerular functions, leading to renal

damage in DN [5].

Interleukine-6 (IL-6) has both a pro-inflammatory cytokine and an anti-inflammatoryeffectin human,causesglomerular basement membrane thickening. ,enhance endothelial permeability and mesangial cell proliferation [6]. Tumor necrosis factor alpha (TNF-α), an inflammatory cytokine modifies glomerular functions and increased vascular endothelium permeability infiltration by inflammatory cells, grow extracellular matrix, generation of reactive oxygen species and blood flow interrupt are additional notable effects in renal structures [6]. The production of these proinflamatory cytokines, which triggered by the hyperglycemic status that happens in diabetic patients, followed in renal deterioration, either glomerulus or different structures [7].

Hyperglycemia or poor glycemic control was considered a good promotor of DN.Tightly controlled of blood glucose level is required to prevent or delay diabetic kidney disease [8] .Glycosylated hemoglobin (HbA1C) is biomarker of glycemic control in DM condition because it describes blood glucose levels in the last 60-90 days. Severalstudies determined that HbA1c were significantly correlated withcytokine concentrations in DN [9,10].The purpose of this research to define the relation between glycated Hemoglobin and some inflammatory cytokines (IL-1β, IL-6 and TNF- α) in DN .

Patients & Method

The study accomplished at teaching laboratories of medical city, Baghdad, Iraq, a total 50 uncontrolled diabetics type 2 with stage 3-4 of nephropathy(UDN), age range (40 –78) years and 42 controlled diabetics type2 without nephropathy(DC), age range (35 – 52) years as a control groupwere enrolled in the study. The ethics committee approved the study of college of pharmacy, university of Baghdad, Baghdad, Iraq (No. UBCP-RECA 26102019). Exclusion criteria included: type 1 DM, gestational DM, multivitamin supplements, bleeding disorder, anemia, infections (e.g. hepatitis, malignant diseases).The glycemic file of the participants did evaluated by estimating HbA1c, FSG levels. The participants divided in to two groups according to HbA1c measurement which is described as follows: < 7.5% of HbA1c describes controlled diabetes, and > 9% of HbA1c describes uncontrolled diabetes[11]. Venous blood sample was collected to measure HbA1c using HPLC assay(Arkray, Germany) and fasting serum glucose (FSG) levels by enzymatic colorimetric method (Siemens USA). Serum urea nitrogen was measured by using urease/glutamate dehydrogenase coupled enzymatic technique (Dimension clinical chemistry System ,DF21 Siemens, USA). Serum creatinine was measured by using modified kinetic Jaffe technique (Dimension clinical chemistry System, DF33B Siemens, USA). Enzyme linked immunosorbent assay(ELISA) technique was used for quantitative measurement of IL-1β,IL6 and TNF-α by using human ELISA kit . Estimated Glomerular Filtration Rate (eGFR) was measured by modified diet renal disease (MDRD) equation for Creatinine and the equation adjusted for age and sex. [12], as shown in table (1). GFR measurement gives the basis for discovery and classification of chronic renal disorder. The eGFR of the UDN involved in the research classified as mild to moderate GFR reduction. The eGFR of the DCgroup classifiedas normal GFR.

Table 1:Stages of kidney disease according to GFR

Stages	GFR(ml/min/1.73m2)	Description
1	≥ 90	Normal or elevated GFR
2	60-89	Mild GFR reduction
3	30-59	Moderate GFR reduction
4	15-29	Sever GFR reduction
5	<15	Renal failure

Statistical Analysis

Statistical analysis was made employing SAS (Statistical Analysis System. Version 21). An Independent t-test sample was used to compare the serum levels of all parameters between UDN and DCgroups .The variations between the groups were reflected to be significant at a p-value of ≤ 0.05.

Results

The laboratory and descriptive analysis for the participantswere installed in table 2, which revealed the mean, standard deviation for these parameters and

p value. Calculations displays the mean of eGFR for patients group is 33.65, this value classified as moderate GFR reduction. The mean of eGFR for control group is 100.58 which classified as normal GFR values. Mean HbA1c of UDN group was 10.45±1.52%and that of DC group was 6.17±1.02 %. The mean of HbA1c of UDN group was higher as compare to DCgroup, so the increase of HbA1c values associated with increasedincidence of nephropathy in type2 DM. Results of IL-1b, IL-6 and TNF-α displays a significant increased levels for UDN group, ($p< 0.05$) for IL-1b, ($p< 0.05$) for IL-6 and ($p< 0.01$) for TNF-α (table2).

Table2: Laboratory and descriptive analysis for UDN and DC groups

Biochemical parameters	UDN No: 50	DC No: 42	P-value*
Male /female ratio	28:22	26:16	
Age (years	50.79±16.45	32.72±6.37	<0.001
HbA1c (%)	10.45±1.52	6.17±1.02	<0.001
FSG (mg/dl)	179.3±70.25	99.17±9.75	<0.05
Urea(mg/dl)	55.46±27.25	30.32±4.83	<0.001
Creatinine (mg/dl)	2.22±0.59	0.85±0.16	<0.001
eGFR (ml/min/1.73m2)	33.65±11.95	107.13±13.96	<0.001
IL-1b (ng/ml)	4.24±0.231	3.44±0.25	<0.05
IL-6 (pg/ml)	6.56±3.98	3.5±1.93	<0.05
TNF-α (pg/ml)	30.80±4.75	15.84±4.43	<0.01

*p-value < 0.05 wasanalyzed significant and p-value<0.01 wasanalyzed highly significant

Discussion

Chronic hyperglycemia and inflammation are the major causative agent of DN[13].Several metabolic, biochemical and hemodynamic changesact as promotor to inflammation in DN . DN is critical micrcvascular complications of type 2 diabetes mellitus that required early diagnosis and management to prevent irreversible renal failure[14]. Therefore, adequate control of blood glucose levels is needed to delay the progression of DN.Important markers were applied to determinethe renal function which are blood urea and creatinine levels . The measurements of eGFR is depend on the amount of creatinine detected in a blood specimen. Raising serum creatinine levels result in increased GFR. Renal disorders are happenedwhen eGFR is < 60ml / min / 1.73m2. Activation of the immune system and chronic inflammation are both implicated in pathogenesis of DN, researches have demonstrated that chemokines, cytokines, as well as immune cells as monocytes, lymphocytes, and macrophages are involved in pathogenesis and complications of type 2 diabetes mellitus [15]. In the current study, it noticed that IL-6 and IL-1β serum levels are significantly higher in UDN group than the levels mentioned in DC group as correspond with other studies[16,17]. IL-1 is recognized to be implicated in the deterioration of glomerular functions. It related to the secretion of prostaglandin by mesangial cells of kidney leading to changes in renal structure which are important in the development of DN[18]. In addition to this study ,Sari MI et alalsodetermined that serum IL-6 levels raised in patients with diabetic nephropathy [19]. Also a previous study has reported a significant difference in the IL-6 levels between a group of diabetic patients and a group of healthy people [20].IL-6 also encourages growth and proliferation of mesangial cells. It has been recognized that increase proliferation

and action of mesangial cells producing in extracellular matrix growth, glomerular basement membrane thickening, and glomerulosclerosis, eventually lead to DN [7]. Furthermore, IL-6 has non immune mediated mechanism that influence glucose metabolism by action on skeletal muscle cells, pancreatic islet cells and adipocytes [21,22]. Additionally, this research reveals that serum TNF-α levels in UDN group are significantly more than the serum TNF-α levels in DCgroup. Similarly, other studies show that increase risk of diabetic kidney disease associated with the increase levels of TNF-α in patients with type 2 DM [23,24].TNF-α exert different effects to cells including apoptotic and necrotic cell death by direct and autocrine mechanism, also changes of endothelial permeability.TNF-α lead to impairment of glomerular capillary wall barrier function, hence increasing albuminuria [7].

In this study, the averageof HbA1c in UDN group was significantly more than that of DC group, thus HbA1c may be used as an indicator of prognosis in the development of DM complications. Similar results were observed by other studieswhich found that increase HbA1c levels above 7.0% were significantly correlated with increased prevalence of nephropathy[25,26].Past studies pronounced that age was crucial factor linked to renal function derangement .The old age and duration of diabetes were significant risk factors for nephropathy as an deal with this study[27,28] . Poor glycemic control shown by elevated mean HbA1c, and fasting serum glucose levels were significantly connected with increased predominance of nephropathy in this study.

Conclusion

Raised serum levels of IL-1b, IL-6and TNF- α in uncontrolled diabetic patients with nephropathy concurrently with raising theaverage HbA1c in themindicate that the inflammation may have a significant role in the progression of DN .The occurrence and risk of DN increased with advanced age and poor glycemic control , so that the incidence and worsening of DN among type 2 diabetic patients could be limited or restricted by keeping tightly controlled HbA1c. The perception of the inflammatory response in DN is urged to identify novel anti-inflammatory plans for the intensive therapy to patients with DN.

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