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ORIGINAL RESEARCH ARTICLE

Correlation between Hyperglycemia, Inflammation and Oxidative Stress in Type-2 Diabetic Nephropathy Subjects

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ABSTRACT

Diabetic nephropathy is one of the major complications of type-2 diabetes and it is currently the leading cause of end-stage renal disease. Hyperglycemia is the driving force for the development of diabetic nephropathy. It is well known that hyperglycemia increases the production of free radicals resulting in oxidative stress. The increases in oxidative stress have been shown to contribute to the development and progression of microvascular complication; the mechanisms by which this occurs are still being investigated. The stimulus for the increase in inflammation in diabetes is still under investigation; however, reactive oxygen species might be a primary source. Therefore the present study has been undertaken to find out the relationship between oxidative stress and inflammatory cytokines in the progression of diabetic nephropathy which will facilitate for the development of new treatment options as well as to improve current therapeutic strategies of diabetic nephropathy in type 2 diabetic subjects. .

Key words: Oxidative stress, inflammation, diabetic nephropathy**.**

Abbreviations: ROS: Reactive oxygen species, IL-6: Interleukin-6, Tnf-α: Tumor necrosis factor- alpha, nF-κB: nuclear factor-κB, PKC: Protein kinase C, RAGE: Receptor advanced glycation end products

INTRODUCTION

Diabetic nephropathy is the most common cause of microvascular chronic complication of type 2 diabetes mellitus which is associated with considerable morbidity and mortality, finally leading to end-stage renal disease [1]. Diabetic nephropathy is a progressive disease that takes several years to develop. It involves various functional clinical abnormalities of the kidney such as elevated creatinine, urea, albuminuria, decline glomerular filtration rate, elevated arterial blood pressure, and fluid retention [2,3]. The pathogenesis of diabetic nephropathy is likely to be multifactorial: it strongly dependent on the duration of diabetes; other risk factors include oxidative stress induced poor glycemic control, hypertension, hypertriglyceridemia, with production of cytokines IL-6 and Tnf-α causing inflammation responsible for endothelial dysfunction [4].

Oxidative stress, defined as an imbalance between oxidants and antioxidants, plays a critical role in the pathogenesis of diabetic vascular complications i.e. diabetic nephropathy. The increased oxidative stress may be caused by hyperglycemia, hyperlipidemia, and elevated free fatty acids, which are commonly seen in patients with diabetes and insulin resistance [5,6]. In vasculatures, increased oxidative stress may cause adverse vessel reactivity, vascular smooth muscle cell proliferation, macrophage adhesion, platelet activation, and lipid peroxidation, which ultimately leads to vascular complications. Many clinical studies have revealed an increased inflammation in diabetic subjects, manifested by the elevated levels of biomarkers for inflammation such as TNF-α, interleukin-6 (IL-6), C-reactive protein, and PAI-1[7].

Possible mechanisms for the induction of inflammation in vascular tissues may include activation of PKC pathway and oxidative stress, upregulation of RAGE, and activation of innate immunity, For instance, carboxymethyllysine-protein adducts due to malondialdehyde, AGEs, can increase the expression of a variety of

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proinflammatory molecules and NF-κB through the interaction with RAGE in renal cells causing diabetic nephropathy in type2 diabetes [8].

The aim of the present study was to investigate the possible associations in diagnosed patients as type-2 diabetic nephropathy subjects via standard screening procedures including (microalbuminuria and ultrasound of kidney) from Department of Nephrology J.A group of Hospitals, G.R Medical College, Gwalior in relation to duration of diabetes, antioxidant enzyme (Gpx), MDA and cytokines (IL-6 and Tnf-α) respectively.

MATERIAL AND METHODS

The study was carried out in Department of Biochemistry in collaboration with Department of Nephrology J.A group of Hospitals, G.R Medical College, and Gwalior. The ethical committee of GRMC has approved this research work. The study was conducted in 300 human subjects with diabetic nephropathy. Serum creatinine and radiological findings were used to differentiate diabetic nephropathy groups. The diabetic nephropathy patients diagnosed by Department of Nephrology in GRMC, J.A group of hospitals were included in this research work by their consent. A structured questionnaire regarding the demographic data such as age, sex, duration of diabetes, height and body weight were measured while wearing light weight clothing, but not shoes. Blood pressure, smoking habit, family history of diabetes, renal disease and hypertension was recorded for each patient. Diabetic patients suffering from any other medical problems were excluded from the study.

10 ml of blood sample was withdrawn from the anticubital vein following overnight fasting. The blood sample was collected in plain, fluoride and EDTA vacutainers. The blood sample was centrifuged for 10 min. at 3000 rpm at room temp. The serum was stored at 4 ºC for biochemical and immunological investigations.

Fasting blood sugar level was estimated by GOD-POD method by Biosystems S.A. Barcelona (spain) Catalogue No: M1503i-09 [9]. Urea and Creatinine was estimated in auto analyzer by kit methods of Biosystems S.A. Barcelona (spain) Catalogue No: M12516i-08 and M12502i-12 respectively [10,11]. Glutathione peroxides (Gpx) was estimated by the method of Bergmeyer **[12]**. Plasma Malondialdehyde (MDA) was estimated by Jean CD [13]**.** Inflammatory markers Tnf-α and Il-6 were estimated by kits available from immunotech company via sandwitch ELISA method [14].

Correlation analysis was done by using SPSS version 16.

OBSERVATION

Table 1: Showing mean± SD and correlation of FBS with duration, Gpx, MDA, IL-6 and Tnf-α in type 2 diabetic nephropathy subjects (n=300)

		FBS	Dur	GPx	MDA	IL-6	Tnf-α
	Mean± SD	238± 39	13±2.3	4.74±0.6	6.82±1.1	35.68±11.58	36.52±10.60
FBS	Pearson Correlation	1	0.43**	-.69**	.47**	.94**	.91**
	Sig. (2-tailed)		.00	.00	.00	.00	.00
	N	100	100	100	100	100	100

*Highly Significant (P<0.001)

Table 2: Showing mean± SD and correlation of creatinine with IL-6 and Tnf-α in type 2 diabetic nephropathy subjects (n=300)

		Creatinine	IL-6	Tnf-α
	Mean± SD	3.05±0.99	35.68±11.58	36.52±10.60
Creatinine	Pearson Correlation	1	0.758**	.733**
	Sig. (2-tailed)		0.000	.000
	N	100	100	100

*Highly Significant (P<0.001)

Table 3: Showing mean± SD and correlation of Gpx with IL-6 and Tnf-α in type 2 diabetic nephropathy subjects (n=300)

		Gpx	IL-6	Tnf-α
	Mean± SD	4.74±0.6	35.68±11.58	36.52±10.60
Gpx	Pearson Correlation	1	-.711**	0.667**
	Sig. (2-tailed)		.000	0.000
	N	100	100	100

*Highly Significant (P<0.001)

Abbreviation used: FBS: Fasting Blood Sugar, Dur: Duration, Gpx: Glutathione Peroxidase, MDA: Malondialdehyde, IL-6: Interleukin-6, Tnf-α: Tumor Necrosis Factor.

RESULTS

From our study we found that fasting blood sugar was positively correlated with duration, MDA, serum IL-6 and Tnf-α concentrations in diabetic nephropathy subjects showing significant at (P<0.001). Similarly FBS levels were negatively correlated with antioxidant enzyme GPx and was statistically significant at (P<0.001) (**Table 1**). Serum creatinine levels were positively correlated with serum IL-6 and Tnf-α (**Table 2**) and was statistically significant at (P<0.001). Further more, levels of GPx were negatively correlated with serum IL-6 and Tnf-α (**Table 3**) and was statistically significant at (P<0.001).

DISCUSSION

Oxidative stress has been implicated in the development of diabetic complications, including

diabetic nephropathy, retinopathy, peripheral neuropathy, and cardiovascular disease. Specific cell types, including endothelial cells, in tissues susceptible to diabetic complications are unable to regulate intracellular glucose levels (Orasanu G *et al*, 2009), and hyperglycemia-induced overproduction of superoxide by the mitochondria in the microvasculature is an underlying feature of various complications in type 2 diabetes mellitus (Brownlee *et al*, 2001). Accumulating research suggests that oxidative stress is a significant contributor to the pathogenesis of diabetic nephropathy. The normal kidney generates a substantial amount of oxidative stress because of its high metabolic activity that is unbalanced by an extensive antioxidant system. However, in pathologic states such as hyperglycemia, antioxidant balance shifts toward a pro-oxidant state that accelerates tissue, vascular injury, vascular smooth muscle cell proliferation, macrophage adhesion, platelet activation, and lipid peroxidation, which ultimately leads to vascular complications (Maytin, *et al*, 1999). This oxidative damage progresses concomitant with worsening glucose metabolism finally causing vascular dysfunction, and kidney disease (Nina Vasavad *et al*, 2005).

The role of inflammation in progressive tubulointerstitial renal injury leading DN correlates with a progressive decline in kidney function (Schmid *et al*, 2006; Navarro *et al*, 2006). Recruitment of macrophages into the glomerulus appears to generate reactive oxygen species with production of superoxides by the mitochondria which are considered as a common link between metabolic pathways (Seaquist *et al*, 1989; Pettitt *et al*, 1990), inflammatory cytokines and reactive oxygen species that induces the glomerular damage. In our study there was negative correlation of IL-6 and TNF-α activity with glutathione peroxidase in diabetic nephropathy subjects .Our study highlights the possible role of IL-6 and TNF-α and Gpx in the development and progression of renal injury in diabetic patients, and demonstrates that serum levels of cytokines are increased in diabetic subjects as renal damage progresses. Our results were consistent with the studies of Chen H *et al*; 2007 and Shikano M *et al*.2000**.**

Finally, the relationships between hyperglycemia, oxidative stress and inflammatory state in diabetic nephropathy are complex. A better understanding of the role of inflammatory molecules and oxidative stress in the context of diabetic nephropathy will facilitate the development of new and improved therapeutic targets and strategies that can be translated successfully into clinical applications.

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