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RESEARCH ARTICLE

Comparative Study of the Antioxidant Status and Inflammatory Markers in Type 2 Diabetic Male and Female Nephropathy Subjects

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ABSTRACT

Background: Diabetic nephropathy is one of the microvascular complications of Type2 diabetes mellitus (T2DM). Free radicals are formed disproportionately in diabetes by glucose oxidation, nonenzymatic glycation of proteins, and the subsequent oxidative degradation of glycated proteins. An abnormally high level of hyperglycemia generates reactive oxygen species (ROS) with simultaneous decline of antioxidant levels causing increased lipid peroxidation in T2DM leading microvascular complications. **Aims and Objective:** The study aimed to predict the development of diabetic nephropathy due to oxidative stress in type-2 male and female diabetic nephropathy subjects. **Material and Methods:** Serum levels of inflammatory markers (Interleukin-6 [IL-6] and Tumor necrosis factor alpha [Tnf-α]), antioxidants, [Glutathione reductase (GR) and Glutathione peroxidase (GPx)], plasma malondialdehyde (MDA), fasting blood sugar, urea and creatinine levels were estimated in controls (n=50, males and n=50, females) and T2DM with diabetic nephropathy ((n=50, males and n=50, females) and comparison was done. A Student's t-test was used to estimate differences between the groups. All parameters were given as mean ± standard deviation. The criterion for significance was $P < 0.05$. **Conclusion:** From our study, it was concluded that inflammatory markers, IL-6 and TNF-α, were found to be increased in type 2 female diabetic nephropathy subjects than male nephropathy subjects. Antioxidant Gpx enzyme levels are found to be decreased in the erythrocyte of both female diabetic nephropathy and male nephropathy subjects, however, decrease in Gpx level in female subjects was more than in male nephropathy subjects of type 2 diabetes.

Keywords: Diabetic nephropathy, inflammation, oxidative stress

INTRODUCTION

Diabetes is one of the most common chronic diseases with metabolic disorders characterized by hyperglycemia causing late microvascular complications, among which diabetic nephropathy is the most deleterious. Diabetic nephropathy has become the single most frequent condition causing end-stage renal disease, and it is fully justified to call it "a medical catastrophe of worldwide dimension."[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] 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, production of cytokines interleukin-6 (IL-6), and tumor necrosis factor alpha (TNF-α) causing renal endothelial dysfunction.[3] Oxidative stress, defined as an imbalance between oxidants and antioxidants, plays a critical role in the pathogenesis of diabetic vascular complications, that is, diabetic nephropathy. Hyperglycemia, a

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prominent clinical feature of diabetes, is a major cause responsible for the increased production of reactive oxygen species (ROS) and intensified oxidative stress.[4,5] There is a strong belief that renal glomeruli are particularly sensitive to oxidative stress,[6] suggesting the involvement and participation of ROS in the pathogenesis of diabetic nephropathy. Possible mechanisms for the induction of inflammation in vascular tissues may include activation of protein kinase C pathway and oxidative stress,[7] upregulation of recent advanced glycation end products (RAGE),[8] and activation of innate immunity.[9] For instance, carboxymethyllysine-protein adducts due to malondialdehyde (MDA), AGEs, can increase the expression of a variety of pro-inflammatory molecules and nuclear factor-kappa B through the interaction with RAGE in renal cells causing diabetic nephropathy in T2DM.[10,11]

MATERIALS AND METHODS

The study was carried out in collaboration with the Department of Biochemistry, Chitwan Medical College and Department of Nephrology in G.R Medical College, Gwalior. The ethical committee of GRMC has approved this research work. The diabetic nephropathy patients attending the department of nephrology were included in this research work by their consent.

Details of study are as follows:

Experimental designs of study are as follows: (Age matched).

Total number of subjects (experimental): 200.

- 100 – T2DM subjects
- 100 – T2DM with diabetic nephropathy
  - 50 – Male diabetic nephropathy subjects
  - 50 – Female diabetic nephropathy subjects.
- 100 – T2DM subjects without microvascular complications.
  - 50 – Male T2DM subjects
  - 50 – Female T2DM subjects.

Ten milliliters of blood sample will be drawn from the antecubital vein following overnight fasting. The blood sample will be collected in plain, fluoride, and ethylenediaminetetraacetic acid Vacutainer. The blood sample was centrifuged for 10 min at 3000 rpm at room temperature. The whole blood and serum were stored at 4°C for biochemical and immunological investigations.

Fasting blood sugar level was estimated by glucose oxidase-peroxidase method. Urea and creatinine were estimated by autoanalyzer through kit methods (diacetyl monoxime and Jaffe's method). Glutathione peroxides (Gpx) were estimated by the method of Hafeman *et al*. (1974). Glutathione reductase (GR) was estimated by the method of Horn HD (1963). Plasma MDA was estimated by Jean *et al*. (1983)**.** Hemoglobin was measured by cyanmethemoglobin method. Inflammatory markers TNF-α and IL-6 were estimated by kits available from ImmunoTech company by sandwich enzyme-linked immunosorbent assay method.

Statistical analysis was done using Student's *t*-test. A Student's *t*-test was used to estimate differences between the groups. All parameters were given as mean ± standard deviation. The criterion for significance was $P < 0.05$.

RESULTS

It was found from the study that both male and female diabetic nephropathy subjects have significant increase of fasting blood sugar, serum urea, creatinine, MDA, and inflammatory markers (IL-6 and TNF-α) as compared to both male and female diabetic subjects without microvascular complication. All the parameters are showing highly significant at $P < 0.05$.

Antioxidant enzyme Gpx being decreased in both male and female diabetic nephropathy subjects as compared to both male and female diabetic subjects without microvascular complication and it was highly significant at $P < 0.05$, whereas GR was found to be normal range (3–13 U/g of Hb) in both diabetic nephropathy and diabetic subjects without microvascular complication and was found non-significant at $P > 0.05$.

In our study, the body mass index (BMI) was found to be increased in both male and female diabetic nephropathy subjects as compared to both male and female diabetic subjects without microvascular complication and it was found significant at 5% ($P < 0.05$) [Table 1].

The hemoglobin levels was found to be decreased in both male and female diabetic nephropathy subjects as compared to both male and female diabetic subjects without microvascular complication and it was found significant at $P < 0.05$ but the hemoglobin levels were found within the normal range in both male and female diabetic subjects without microvascular complication.

DISCUSSION

Our results are consistent with those of other studies on oxidative stress but for IL-6 and TNF-α till date, very few researches have been done with regard to inflammatory markers so that the exact mechanism for increase in the level of these inflammatory markers in type-2 female diabetic nephropathy as compared to type-2 male diabetic nephropathy is still unknown. It might be hyperglycemia-induced ROS, advanced glycation end-products, etc., may play an important role in the generation of cytokines (IL-6 and TNF- α) in renal cells in diabetic nephropathy subjects. More recent studies in type 2 diabetic patients demonstrate a significant association between IL-6 and glomerular basement membrane thickening, a crucial lesion of diabetic nephropathy, and a strong predictor of renal progression.[12]

The increase in protein oxidation and lipid peroxidation as reflected by increase in plasma levels of MDA in T2DM is due to hyperglycemia inducing overproduction of oxygen free radicals, suggesting a feature of oxidative stress in type 2 diabetes. The increased levels of thiobarbituric acid reactive substances in T2DM diabetic nephropathy patients may be due to the following reasons: (1) Oxidative stress in diabetic patients. (2) Compositional changes in low-density lipoproteins may lead to conformational changes, possibly resulting in a different exposure of fatty acids to oxygen free radicals that enhance a faster rate of lipid peroxidation.[13]

Antioxidant enzyme Gpx was found decreased more in T2DM female nephropathy subjects as compared to T2DM male nephropathy subjects, suggesting female T2DM nephropathy subjects in more stressed condition. This might be due to hyperglycemia induced oxidative stress, due to low hemoglobin concentration in female subjects of diabetic nephropathy and due to excess utilization of NADPH in renal mesangial cells through polyol pathway in type 2 diabetic nephropathy subjects. Our study results were also consistent with Srivastava *et al.,* 2004.[14] The decrease in hemoglobin level in diabetic nephropathy subjects

Table 1: Mean±standard deviation between age-matched T2DM male and female without microvascular complications and diabetic nephropathy subjects

S. No.	Parameters	T2DM without microvascular complications (males, *n*=50)	T2DM with diabetic nephropathy (males=50)	T2DM without microvascular complications (females, *n*=50)	T2DM with diabetic nephropathy (females=50)
1	Age	45–60 years	45–60 years	45–60 years	45–60 years
2	Duration	5.5 years	14 years	6.0 years	13 years
3	BMI	28.98±1.70	29.98±4.00*	28.78±1.80	29.29±4.41*^{NS}
4	FBS	146±33.10	193.13±22.62*	156±35.10	210±25.59*^{NS}
5	Urea	30±7.62	109±33.32*	28±6.62	115±34.10*^{NS}
6	Creatinine	1.01±0.35	3.36±1.75*	1.13±0.55	3.42±1.60*^{NS}
7	MDA	5.31±1.10	6.92±1.33*	5.00±1.00	7.03±1.62*^{NS}
8	Gpx	6.46±0.84	4.897±0.895*	5.40±1.02	4.41±0.36*^{NS}
9	GR	6.55±1.02	6.90±0.895^{NS}	6.00±1.32	6.50±0.738^{NS}
10	IL-6	13.59±1.96	30.40±15. 63*	12.50±1.70	40.28±22.10*$^{+}$
11	TNF-α	13.36±1.81	36.88±14.70*	14.00±1.95	56.07±19.72*$^{+}$
12	Hb	12.36±0.95	7.60±0.95*	12.00±0.90	6.37±1.14*$^{+}$

*Significant at $P<0.05$ within type 2 male diabetic nephropathy and T2DM without microvascular complications male subjects. *Significant at $P<0.05$ within type 2 female diabetic nephropathy and T2DM without microvascular complications female subjects. $^{+}$Significant at $P<0.05$ within type 2 diabetic nephropathy male and female subjects. Abbreviations with their units: BMI: Body mass index (Kg/m^2), FBS: Fasting blood glucose (mg/dl), Urea (mg/dl), Creatinine (mg/dl), MDA: Malondialdehyde (μmol/liter of plasma), Gpx: Glutathione peroxidase (U/mg of Hb), GR: Glutathione reductase (U/g of Hb), IL-6: Interleukin-6 (pg/ml), TNF-α: Tumor necrosis factor-α (pg/ml), Hb: Hemoglobin (gm/dl)

as compared to regulated diabetes might be due to decrease in production of hormone erythropoietin which is produced from the renal cells of kidney, which was also reported by Ravanan *et al*., 2007.[15]

CONCLUSION

Hyperglycemia induces intracellular ROS in glomerular mesangial and tubular epithelial cells which induces cytokines, IL-6, and TNF-α production in diabetic kidney. From our study, it was concluded that inflammatory markers, IL-6 and TNF-α, were found to be increased in type 2 female diabetic nephropathy subjects than male nephropathy subjects. Antioxidant Gpx enzyme levels are found to be decreased in the erythrocyte of both female diabetic nephropathy and male nephropathy subjects, however, decrease in Gpx level in female subjects was more than in male nephropathy subjects of type 2 diabetes. The level of GR was found within the normal range in all the subjects.

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