ISSN 0976 – 3333

Available Online at www.ijpba.info

International Journal of Pharmaceutical & Biological Archives 2012; 3(5):1203-1206

ORIGINAL RESEARCH ARTICLE

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

Deepak Kafle[1], Neelima Singh*[1], S.K.Singh[1], Nivedita Singh[1] and Nazrul Islam[2]

[1]*Department of Biochemistry, GR Medical College, Gwalior (M.P) , India*
[2]*Department of Physiology, Chitwan Medical College, Bharatpur, Nepal*

Received 27 May 2012; Revised 29 Sep 2012; Accepted 08 Oct 2012

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: Recent advanced glycation end products.

INTRODUCTION

Diabetic nephropathy is the most common cause of microvascular chronic complication of type-2 diabetes. It 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, which increases production of cytokines IL-6 and Tnf-α from endothelial cells casing inflammation in type-2 diabetes [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. 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 [6]. Many clinical studies have revealed that with long duration of disease inflammation in diabetic subjects may cause nephropathy.

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 BMI, duration of diabetes, antioxidant enzymes (Gpx and GR), MDA and cytokines (IL-6 and Tnf-α) respectively. Serum creatinine was measured as a marker of renal damage in nephropathy subjects.

MATERIALS 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

***Corresponding Author:** Dr. Neelima Singh**, Email:** neelima48@rediffmail.com

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.

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.

Details of study are as follows:

Total number of subjects (experimental): 150

50 – Type-2 diabetic subjects with diabetic nephropathy

50 – Type-2 diabetic subjects without nephropathy

50 – Control subjects (age matched)

Fasting blood sugar level was estimated by GOD-POD method by Biosystems S.A. Barcelona (spain) Catalogue No: M1503i-09 [7]**.** 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 [8]. Glutathione peroxides (Gpx) was estimated by the method of Bergmeyer [9]. Plasma Malondialdehyde (MDA) was estimated by Jean CD [10]**.** Inflammatory markers Tnf-α and Il-6 were estimated by kits available from immunotech company via sandwitch ELISA method [11].

Correlation analysis was done by using SPSS version 16.

Table 1: showing mean ± Standard deviation between healthy control, type 2 diabetes without any microvascular complication (group 1) and type 2 diabetes with diabetic nephropathy subjects (group2).

S. No	Parameters	Healthy control (n=50)	(group 1, n=50,)	(group2, n=50)
1	Age (years)	50-60 yrs	50-60 yrs	55-65 yrs
2	Duration of diabetes	----------	5.8	13.16
3	BMI (Kg/m^2)	24.17±2.09	27.98 ± 1.70 #	29.29±4.41*+
4	FBS (mg/dl)	77.36±11.74	146.16 ±33.10 #	182.14±51.95*+
5	Urea (mg/dl)	23.52±4.03	30.32 ± 7.62 NS	122.83±47.31**++
6	Creatinine(mg/dl)	0.87±0.26	1.01 ± 0.35 NS	2.73±1.29**++
7	MDA (µmol/liter of plasma)	2.28±0.43	5.31±1.10 #	7.32±1.62*+
8	Gpx(U/mg of Hb)	8.06±0.51	6.46±0.84 #	4.81±0.74*+
9	GR (U/g of Hb)	6.12±0.55	5.55±1.02NS	5.09±0.82NS NS
10	IL-6 (pg/ml)	9.18±1.3	13.59±1.96*	33.95±10.79**++
11	Tnf-α (pg/ml)	9.49±1.04	13.36±1.81*	40.87±12.32**+
12	Hemoglobin(g%)	12.92±1.03	12.36±0.95NS	7.57±1.14*+

***** *-- Significant at (P<0.05) between group I and group II.*

****** *-- Highly Significant at (P<0.001) between group I and group II.*

 # -- Significant at (P<0.05) between control and group I

+ -- Significant at (P<0.05) between control and group II.

NS --- Non significant (P>0.05)

RESULTS

It was found from the study that group 2 subjects have significant increase of fasting blood sugar, serum urea , creatinine, malondialdehyde and inflammatory markers (IL-6 and Tnf-α) levels with decreasing in antioxidant GPx as compared to both group 1 and healthy controls. All the parameters are showing highly significant at (P<0.05, Table 1).

Fasting blood sugar was positively correlated with MDA, serum IL-6 and Tnf-α concentrations (for group 1: r = 0.43, P<0.05; r =0.867, P<0.001; r =0.867, P<0.001 and for group 2: r = 0.47, P<0.05; r = 0.94, P<0.001; r=0.91, P<0.001; respectively). Persistent hyperglycemia levels was negatively correlated with antioxidant status i.e. GPx levels (for group 1: r = -0.68, P<0.001 and for group 2: r = -0.74, P<0.001 respectively). Serum creatinine levels were positively correlated with serum IL-6 and Tnf-α only in group 2 subjects (r = 0.75, P<0.001; r = 0.71, P<0.001; respectively). Furthermore the decreasing levels of GPx were positively correlated with serum IL-6 and Tnf-α (for group 1: r = 0.62, P<0.05; r = 0.47, P<0.05 and for group 2: r = 0.71, P<0.001; r = 0.66, P<0.001 respectively)

DISCUSSIONS

Oxidative stress depicts the existence of products called free radicals and reactive oxygen species (ROS) which are formed under normal physiological conditions, become deleterious when not being quenched by the antioxidant systems [12]. The decreased GPx in diabetic nephropathy (group 2) in comparison to group 1 and control subjects may be due to the overproduction of hydrogen peroxide (H_2O_2) via hyperglycemia induced oxidative stress. Glutathione reductase was in normal range in all

the groups which might be due to utilization of NADPH in the polyol pathway in type-2 diabetes. Further more free radicals are generated disproportionately in long standing diabetes by glucose auto-oxidation, polyol pathway and non enzymatic glycation of proteins as well.

ROS produced in hyperglycemia may be one of the reasons to increase peroxidation of cellular membrane lipids as well as oxidation of proteins yielding protein carbonyl derivatives, producing high level of MDA in the diabetic nephropathy subjects which is a suggestive feature of oxidative stress in complications of type-2 diabetes. Our results are also consistent with (Adam *et. al*. 2001) [13].

The long duration of diabetes in uncontrolled hyperglycemia induces advanced glycation end products activating nf-kb factor which directly acts on nucleus and via transcription releases cytokines (TNF-α, IL-6) which are cytotoxic to glomerular, mesangial, and epithelial cells inducing direct renal damage. The released cytokines may have effect on the protein permeability barrier of the glomerulus causing alterations in hemodynamic factors of renal cells like glomerular basement membrane thickening, renal mesangial cell expansion and hyperplasia of extracellular matrix, a crucial lesion of diabetic nephropathy in long standing type-2 diabetic patients. Furthermore our study results are also consistent with (Dalla Vestra M *et al*. 2005) [14], showing increasing levels of cytokines (TNF-α and IL-6) in type2 diabetic nephropathy subjects.

The decrease in hemoglobin level in diabetic nephropathy patients are due to decrease in production of hormone erythropoietin which is produced from the renal cells of kidney which was inconsistent with (Ravanan *et al*. 2007) [15].

So, finally our study concludes that hyperglycemia generates intracellular reactive oxygen species (ROS) in mesangial and tubular epithelial renal cells inducing cytokines, IL-6 and Tnf-α in diabetic kidney. Inflammatory markers are increased and antioxidant enzyme levels are decreased in the plasma of diabetic nephropathy patients due to hyperglycemia induced oxidative stress. The renal risk score for diabetic nephropathy emphasizes the importance of the identification of levels of inflammatory markers and antioxidant levels, as well as increased serum creatinine to predict the development of diabetic nephropathy in type- 2 diabetes.

REFERENCES

1. Raine AEG (1993) Epidemiology, development and treatment of end-stage renal failure in type 2 (non-insulin-dependent) diabetic patients in Europe. *Diabetologia* 36:1099–1104
2. Wolf. G, Ziyadeh. FN (2007) Cellular and molecular mechanisms of proteinuria in diabetic nephropathy. Nephron Physiol 106:26–31
3. Balakumar.P,Chakkarwar.VA ,Kishan .P (2009) Vascular endothelial dysfunction: a tug of war in diabetic nephropathy? Biomed Pharmacother 63:171–179
4. Diabetes Control and Complications Trial Research Group (1993) The effect of intensive treatment of diabetes on the development and progression of long term complications in insulin-dependent diabetes mellitus. *N Engl J Med* 329:977–986
5. Kuroki T, Isshiki K, King GL (2003) Oxidative stress: the lead or supporting actor in the pathogenesis of diabetic complications. *JAm Soc Nephrol*, 14:S216–S22
6. Maytin M, Leopold J, Loscalzo J (1999) Oxidant stress in the vasculature. *Curr Atheroscler Rep*, 1:156–16
7. Trinder P. Ann.Clin.Biochem, 6, 24 (1969)
8. Fabinyl DL, Ertingshausen G. Automated reaction rate method for determination of serum creatinine with CentrifiChem. Clin Chem 1971; 17:696-700
9. Bergmeyer HV. Method in Enzymatic Analysis, New York Academic Press (1963), pp875-9.
10. Jean CD, Maryse T, Marie JF. Plasma Malondialdehyde levels during Myocardial infarction. *Clinica Chimica Acta*. 1983; 129: 319-22.
11. Enzyme immunoassay for the in vitro determination of Tnf-α in serum. Kit for determination of Tnf-α, 9 6 wells (cat. # im1121 and cat. # im1120)
12. Fang Y.Z, S. Yang, G. WU (2002) Free radical, antioxidant and nutrition, *Nutrition*, 18,872–890.
13. Adams S, Green P, Claxton R, Simcox S, Williams MV, Walsh K, Leeuwenburgh C.(2001) Reactive carbonyl formation by oxidative and non-oxidative pathways. Front Biosci; 6:A17–24.

14. Dalla Vestra M, Mussap M, Gallina P, Brueghin M, Cernigoi AM, Saller A, Plebani M, Fioretto P (2005) Acute-phase markers of inflammation and glomerular structure in patients with type 2 diabetes. *J Am Soc Nephrol* 16 [Suppl 1]: S78–S82

15. Ravanan.R & J. R. Spiro & P. W. Mathieson &R. M. Smith (2007) Impact of diabetes on haemoglobin levels in renal disease. DOI 10.1007/s00125-006-0514-y