MEDWIN PUBLISHERS
Committed to Create Value for Researchers

Open Access Journal of Pharmaceutical Research
ISSN: 2574-7797

Exploration of Matrix Metalloproteinase and its Interplay in the Development of Diabetic Nephropathy

Hussain T[1], Faheem A[1], Shahzad Q[2]*, Raza Shah SH[3] and Bukahri SW[4]

[1]Institute of Molecular Biology and Biotechnology, The University of Lahore, Pakistan
[2]Department of Pharmacology, Bahauddin Zakariya University, Pakistan
[3]Department of Chemistry and Biochemistry, University of Agriculture Faisalabad, Pakistan
[4]Institute of Chemical Sciences, Bahauddin Zakariya University, Pakistan

Research Article
Volume 5 Issue 1
Received Date: January 27, 2021
Published Date: March 11, 2021
DOI: 10.23880/oajpr-16000230

***Corresponding author:** Qasim Shahzad, Department of Pharmacology, Bahauddin Zakariya University, Multan, Pakistan, Email: qasim.shahzad71@yahoo.com

Abstract

Introduction: Diabetes mellitus (DM), a metabolic syndrome with abnormality in metabolism of carbohydrates, proteins and lipids, and is characterized by absolute and relative deficiency of insulin secretion. DM leads to its most common and frequent complication –Diabetic kidney disease. Oxidative stress induced by decreased antioxidant defenses and /or increased free radical formation are involved in causative factor and diseases in diabetes, is an evidence based study.

Materials and Methods: The studied group consisted of 50 subjects with diabetes nephropathy recruited from Jinnah Hospital Lahore. Oxidative stress biomarkers (SOD, GSH, Catalase, AOPPs, NO and MDA) were determined by using the method of spectrophotometry. Vitamins (C, E, A and D) and inflammatory markers (TNF-, and IL-6) were analyzed by using commercially available Elisa kits. Results were analyzed through T test by using SPSS version 16.

Results: Hematological profile of diabetic nephropathy patients was observed. Abnormal changes were found in platelets count and lymphocytes predicting coagulation and inflammation inside body. Antioxidants (SOD, CAT, GSH-GPx) and vitamins (A, E, C, D) were decreased. Oxidative markers and inflammatory markers such as MDA, MPO and AOPPs were found to be increased.

Conclusion: It is clear that hyperglycemia activates the various signaling pathways and reactive oxygen species (ROS) formation, which further activates signaling cascades. It causes the structural and functional alterations in kidney that enhance the complications associated with diabetic nephropathy.

Keywords: Diabetic Kidney Disease; Oxidative Stress; Reactive Oxygen Species; Antioxidants; AOPPs

Introduction

Diabetes mellitus is one of the most common cause of chronic kidney failure worldwide [1]. It was estimated by International Diabetic Federation that the incidence of diabetes is 8.8% among the age of 20 -70 years affecting almost 440 million people [2]. It is predicted to cross more than 550 million people by the year 2035 [3]. The most prominent clinical feature of diabetes is its association with chronic tissue complications. It has been seen that short term hyperglycemia condition does not result in serious tissue damage. However, its duration and severity seems to be

major causative factor of organ damage. Early morphological changes of renal injury include nephropathy but extent to this damage is best estimated by detection of proteinuria and glomerular filtration rate (GFR) [2].

Diabetic nephropathy also known as nodular diabetic glomerulosclerosis or inter-capillary glomerulonephritis or Kimmelstiel Wilson syndrome, is a clinical syndrome characterized by macroalbuminuria and microalbuminuria, permanent and irreversible glomerular filtration and arterial hypertension [4]. Diabetic nephropathy is a chronic complication of Type I diabetes mellitus which is due to beta cell destruction or the absolute lack of insulin and Type II diabetes mellitus which is due to insulin resistance or decreased secretion of insulin in the body [5].

Researchers have deduced five different stages of diabetic nephropathy. In stage I GFR is either normal or increased, last five years after the onset of disease. In this stage, 20% increase in the size of Kidney has been noticed along with 10-15% increase in renal blood flow while there is no change in blood pressure and albuminuria. Stage II characterized by the kidney damage with thickening of basement membrane and mesangial expansion but with no clinical signs. However, the stage III is the initial nephropathy characterized by microalbuminuria (Albumin 30-300mg/24 hr.), glomerular damage and increased blood pressure.

Typically, chronic kidney failure is the irreversible stage, termed as stage IV which is characterized by macroalbuminuria (Albumin > 300mg/24 hr.), decreased GFR (below 60 mL/min/1.73m^2) and high blood pressure. Stage V is the terminal kidney failure stage with GFR less than 15mL/min/1.73m^2 that ultimately requires kidney replacement therapies such as hemodialysis, peritoneal dialysis and kidney transplantation [6]. In all these stages, GFR and proteinuria are prescribed as best indicators of degree of damage [7].

Biomarkers are also used to predict and progression of kidney disease. Since albuminuria has certain limitations and this has led to discovery of more patent and reliable serum and renal biomarkers best known for high sensitivity and specificity [8]. Recently, three new biomarkers are introduced that includes neutrophil gelatinase-associated lipocalin NGAL, beta-trace protein (Beta TP) and microRNA-130b in type II diabetes mellitus [9]. They suggested NGAL and beta TP were significantly high in patients with type II diabetes mellitus; hence they could serve as early tubular and glomerular biomarkers respectively. Several studies have shown the elevated tumor growth factor beta (TGF beta), connective tissue growth factor (CTGF) and vascular endothelial growth factor (VEGF) and they are expected to replace albuminuria in future for better diagnosis [10-12].

It is well established fact that combination of metabolic and hemodynamic alterations and inflammatory reactions are involved in diabetic nephropathy patients. However, studies from past decades have provided enough evidence of pathogenic and molecular events of diabetic nephropathy. It is reported that early and critical sign of progression of diabetic nephropathy is the blood pressure changes that take place inside the kidney. Moreover, the impairment of glomerular microcirculation and intrarenal pressure ultimately causes glomerular sclerosis and hypertrophy. Studies on in vitro models revealed that podocytes and mesangial cells as well as tubular cells experiencing mechanical stretch releases several molecules which causes structural and functional changes in glomeruli [13,14]. These molecules include transforming growth factors, capillary pressure regulators including angiotensin II, Angiotensin converting enzyme (ACE), angiotensin II receptor 1 and type 2 receptor, VEGF and cytokines such as interleukin (IL)-6, IL-18 and MCP1. These molecules induce pathogenic effects via elevating oxidative stress by two means either by activating nicotinamide adenine dinucleotide phosphate hydrogen (NADPH) oxidase or either by activating cellular remodeling signaling. This leads to morphological changes as well as increased synthesis of extra-cellular matrix [15]. In prolong hyperglycemic conditions, advanced glycation end products within renal tissue and plasma [16,17]. There are two pathways through which AGEs induced renal complications occurs. One is through its irreversible binding to type IV collagen and laminin, impair their degradation by matrix metalloproteinases that ultimately leads to fibrosis [18]. Second, by its interaction with the receptor RAGE specifically expressed by podocytes, endothelial and mesangial cells in kidney.

AGEs are also known to provoke profibrotic cytokines such as connective tissue growth factor (CTGF) and TGF-beta, angiogenic growth factor (VEGF) [19]. Furthermore, the ligation of AGEs to RAGE increases the expression of NADPH oxidase and mitochondrial – dependent reactive oxygen species (ROS) generation that induces oxidative stress resulting in glomerular cell proliferation, expansion and hypertrophy. All these morphological changes stimulate injured renal cells to secrete chemo-attractant cytokines which attacks inflammatory cells into the interstitium such as lymphocytes and macrophages. This interstitial infiltration worsens the progression of diabetic nephropathy through the release of several tissue remodeling and inflammatory cytokines which promotes oxidative stress via the activation of NADPH oxidase subunits that include, tumor necrosis factor alpha, interferon gamma and interleukin-I [20]. All

these events lead to tubulointerstitial fibrosis which is the main determinant of disease progression [21].

Materials and Methods

Source of Data: The present study was designed to investigate the key processes involved in the development of diabetic nephropathy. All the selected patients were screened/selected at the Kidney Center of Jinnah Hospital Lahore. Informed consent was obtained before being included in this study. Fifty age and sex- matched clinically and apparently healthy individuals were included as controls (25-40 Years). The experiment protocol was approved by the Research Ethical Committee of The Institute of molecular biology and biotechnology, The University of Lahore. 5 mL of venous blood sample was taken from anti-cubital vein of each participant. The sample bottles were centrifuged within 1 hour of collection after which the serum was separated and stored at -70℃ until assayed.

Inclusion Criteria: The diagnosis of diabetic nephropathy was made on the basis of FBS, GFR and microalbuminuria results.

Exclusion criteria: The subjects with the history of taking drugs (including alcohol and cigarette), pre-diagnosis medications (e.g antiparkinsonian/antipsychotic) were excluded from this study. None of the controls were on any medication, history of chronic infections, malnutrition syndrome, metabolic dysfunction (such as diabetes mellitus, liver diseases, renal diseases, cancer, previous history of hypertension).

Chemicals: All chemical reagents of analytical grades were purchased from Sigma Chemicals Co. (St. Louis, Mo, USA).

Blood Sample Preparation: Sera was separated by centrifugation for 10minutes at 3000rpm and stored at -70℃ until biochemical analysis was performed.

Evaluation of Complete Blood Count (CBC): Complete blood count of selected subjects was performed on the automated hematology blood analyzer by Sysmex (Version XP-2100).

Determination of Malondiaaldehyde (MDA): Lipid peroxidation in sample was estimated calorimetrically by using the method of Ohkawa, et al. [22]. 200µL sample was taken in test tube then 200µL of 8.1% SDS, 1.5mL of acetic acid (20%) and 1.5mL of tetra butyl amine (TBA) (0.8%) was added in test tube and heated for 60min. After cooling, 4mL of n-Butanol was added and centrifuged for 10minutes at 3000rpm. The upper organic layer was separated and absorbance was noted at 532nm against the blank.

Estimation of Superoxide dismutase (SOD): Superoxide dismutase (SOD) was determined by using the method of Kakkar [23]. 100µL of sample was taken into falcon tube then sodium phosphate buffer (1.2mL; pH 8.3; 0.052M), phenazine methosulphate (0.1mL; 1.86µL), nitro blue tetrazolium (0.3mL; 300µM) and NADH (0.2mL; 750 µM) were also added and reaction was started. After incubation for 90 seconds at 30℃, glacial acetic acid (0.1mL) was added to stop the reaction. 4.0mL of the n-butanol was added in the falcon tubes and centrifuged for 10 minutes at 4000rpm. The upper butanol layer was separated and absorbance was measured at 560nm.

Determination of Catalase (CAT): Catalase was estimated by using the method of Aebi [24]. With the help of spectrophotometer, the estimation of catalase absorbance was taken at 230nm. In the cuvette 0.01 phosphate buffer, 2mM hydrogen peroxide ($H_2O_{2)}$ and sample were added, and reaction was started. The specific activity of catalase was represented by unit/gram of sample. The absorbance values were calculated with the help of standard curve formed by known catalase.

Evaluation of Glutathione (GSH): GSH was estimated by using the method of Moron et al., [25]. A chromophore TNB (absorbance at 412nm) and oxidized glutathione are produced (GSSG) when GSH reacts with Ellman's reagent (DTNB). By this method the calculated GSH is the sum of oxidized (GSSG) and reduced (GSH) from the glutathione. The amount or concentration of unknown sample could be measured by using linear equation produced from several standards of glutathione [26].

Estimation of Glutathione reductase (GRx): Glutathione reductase was estimated by using method of David and Richard [27].

Glutathione peroxidase (GPx) determination: Glutathione peroxidase was determined by the method of spectrophotometer with the help of buffer/ enzyme reagent [28].

Estimation of Advanced oxidative proteins products (AOPPs): Advance oxidation proteins products (AOPPS) were estimated according to the method of Witko-Sarsat, et al. [29].

Evaluation of Vitamin A: Vitamin A was determined by the method of spectrophotometer as an ingredient of pharmacopeial preparation as adopted in 1965 IUPAC US Pharmacopeial Forum [30].

Estimation of Vitamin E: Vitamin E was evaluated in samples by Emmerie-Engel reaction as reported by Rosenberg [31].

Determination of Vitamin C: Vitamin C was estimated by using the method of Chinoy, et al. [32].

Estimation of Nitric Oxide (NO): NO was estimated by using grease's reagent.

Determination of Tumor Necrosis factor-Alpha (TNF-α): TNF-α was determined by the ELISA kit assay by Affimatrix.
Determination of interleukin-6 (IL-6): IL-6 was estimated by the ELISA kit method (R&D systems, MN USA).

Statistical Analysis: For statistical analysis T-test was performed on SPSS version 16.

Results

The fifty diabetic nephropathic patients and fifty healthy subjects taken as control control group, serum samples were included I the study. Circulating stress biomarkers (AOPPs, AGEs), inflammatory biomarkers (IL-6, CRP, TNF-α, MPO etc) and antioxidants (enzymatic and non-enzymatic) were assessed.

Demographic and hematological profile interpretation of diabetic nephropathic patients versus control

The data gathered in table 1 discloses the demographic and hematological profile in diabetic nephropathic patients as compared to normal healthy people. The mean age of DN patients was 45.61±17.89 years and mean age of healthy group was 31.85±5.15 years. The mean weight and body mass index (BMI) of nephropathic patients group was 71.61±11.50 kg and 32.84±5.75 kg/m^2 as compared to normal subjects 41.22 ±2.25 kg and 18.65±2.75 kg/m^2 respectively. The mean systolic and diastolic blood pressure in patients suffering from diabetic nephropathy is recorded to be 127.33±6.75 mmHg and 78.75±2.27 mmHg while the normal control group has 121.91±2.24 mmHg and 78.56 ±1.19mmHg respectively.

The low levels of hemoglobin (8.76± 0.98g/dL) were observed in patients as compared to healthy subjects (11.55 ±0.88 g/dL). The mean RBCs, WBCs, PLT and HCT were 2.28±0.15, 6.44±0.47, 4.26±3.71 and 35.27±2.61 in the control group while the mean values in DN patients were 2.78±0.19, 8.22±1.65, 8.22±5.56 and 32.74±2.45 respectively. The mean prothrombin time assessed in DN patients is increased (11.31±1.67 seconds) as compared to control group (8.65 ±0.95 seconds). The mean serum level of fasting blood sugar (FBS) in diabetic nephropathy disease patients and normal individuals was recorded as 6.36±0.54 mg/dL and 3.01±0.65 mg/dL respectively.

Diabetic nephropathy disease patients versus control group inflammatory markers and oxidative stress markers

Data concerning inflammatory biomarkers profile in table 3 illustrated to be statistically significant. The mean serum level of IL-6 in diabetic nephropathy disease patients and normal individuals was recorded as 6.87±0.81 pg/mL and 4.78±0.59 pg/mL respectively, displaying the raised levels of IL-6 in DN patients as compared to normal and statistically significant (p=0.032). The data analysis of TNF-α and MPO showed statistically significant enhanced levels in DN group 27.34±1.45 pg/mL and 25.47±2.87 in comparison with normal subjects 18.39±4.44 pg/mL and 17.51±2.89 respectively. The mean CRP serum level in DN patients was 3.45±1.26 nmol/mL while in control group was 0.945±0.54 nmol/mL and also found to be statistically significant (p≥0.05). The level of GSH in DN patients was raised as compared to control group. The nitrosative stress biomarker, NO in diseased patients (21.47 µmol/L) is remarkably increased as compared to normal subjects (19.49 µmol/L). The mean serum level of AGEs in DN disease patients and normal peoples were documented as 11.46±0.98 U/mL and 2.78±0.97 U/mL respectively presenting the increased levels in DN patients in comparison to control subjects. The data interpretation of AOPPs has shown significant raised levels in DN group (10.54±2.74 mmol/L) as compared with normal subjects (4.56±1.71 mmol/L). The mean level of GGT in fifty DN patients was found to be increased (54.18±4.94IU/L).

The results represented in table 3 reflecting the activity investigated anti-oxidants SOD (superoxide dismutase), CAT (catalase), and vitamin E, vitamin A, glutathione peroxidase (GPx) and glutathione reductase (Gr) in normal subjects and their altered behavior of anti-oxidants in response to diabetic nephropathy disease. The data shows statistically highly significant between and within groups. The significant falling trend of SOD was recorded in DN patients contrary to normal entities. Serum CAT levels drops in DN individuals 2.16±1.56 nmol/mL and was highly significant (p=0.033). The GSH, GPx and GRx levels of DN patients are displaying the reducing trend as compared to normal individuals and also witnessed that it is satatistically significant. Vitamin E measured in DN patients was 10.78±3.49 µg/mL and in healthy persons was 21.54±5.47 µg/mL. Vitamin A and C levels in patients suffering from diabetic nephropathy decreases as compared to control group. Vitamin D levels in DN patients has been evident to be decreased (9.27±1.29 ng/mL) as compared to healthy people group (13.65±0.99 ng/mL). So, according to data interpretation the anti-oxidant activity in diabetic nephropathy has been reported to be decreased.

Variable	Control (n=50)	Subjects (n=50)
Weight (Kg)	41.22±2.25	71.61±11.50
Age (Years)	31.85±5.15	45.61±17.89
BMI (m^2)	18.65±2.75	32.84±5.75
SBP (mmHg)	121.91±2.24	127.33±6.75
DBP (mmHg)	78.56±1.19	78.75±2.27
Hb (g/dL)	11.55±0.88	8.76±0.98
RBCs	3.28±0.15	2.78±0.19
WBCs	6.44±0.47	8.22±1.65
FBS	3.01±0.65	6.36±0.54
PLTs	4.26±3.71	8.22±5.56
Hct (%)	35.27±2.45	32.74±2.61
Prothrombin Time (s)	8.65±0.95	11.31±1.67

Table 1: Demographic and Hematological Profile.

Variable	Control (n=50)	Subjects (n=50)	P Value
MDA (nmol/mL)	0.945±0.54	3.45±1.26	0.021
SOD (µg/mL)	2.78±0.24	0.490±0.12	0.023
GSH (µg/mL)	7.79±1.27	4.24±1.68	0.020
CAT nmol/mol	3.89±0.84	2.16±1.56	0.033
GGT (IU/L)	41.15±5.56	54.18±4.84	0.015
CRP (ng/mL)	1.87±0.98	11.56±0.48	0.023
IL-6 (pg/mL)	4.78±0.59	6.87±0.81	0.032`
TNF-α (pg/mL)	18.39±4.44	27.34±1.45	0.014
AOPPs (mmol/L)	4.56±1.71	10.54±2.74	0.023
AGEs (U/mL)	2.78±0.97	11.46±0.98	0.000
MPO	17.51±2.89	25.47±2.87	0.015
Vit-A (µg/mL)	27.59±3.49	14.56±4.47	0.023
Vit-C (µg/mL)	19.58±0.88	3.54±0.99	0.014
Vit-E (µg/mL)	21.54±5.47	10.78±3.49	0.023
Vit-D (ng/mL)	13.65±0.99	9.27±1.29	0.032
NO (µmol/L)	19.49±0.47	21.47±0.89	0.014
GPx (U/L)	7.27±0.67	4.86±0.87	0.025
GRx (U/L)	4.52±0.98	1.98±0.94	0.000

Table 2: Oxidative Stress Markers and Inflammatory Markers.

Parameters	Correlation Coefficient
MDA Vs. TNF-α	0.154*
MDA Vs. Vit D	-0.529*
MDA Vs. NO	0.647*
MPO Vs. MDA	0.356*
MPO Vs. SOD	-0.480*
MPO Vs. GSH	-0.450*
MPO Vs. CAT	-0.249*
IL-6 Vs. CAT	-0.454*
TNF-α Vs. Vit D	-0.199*
TNF-α Vs. IL-6	0.389*

Table 3: Pearson's Correlation among the parameters of subjects with DN.

Discussion

The present study confirmed the increased levels of proinflammatory cytokines such as IL-6 and TNF-α in patients of diabetic nephropathy as compared with healthy individuals. Sekizuka, et al., study reported very high levels of IL-6 in patients with type 2 diabetic nephropathy, compared to diabetic patients without nephropathy [33]. He further analyzed the biopsies of these patients by high resolution in situ hybridization and confirmed mRNA encoding glomerular cells that ultimately affects the structural dynamics of the kidney. Furthermore studies in type 2 diabetic patients confirmed the significant correlation of IL-6 with glomerular basement thickening, a prominent feature of diabetic nephropathy and act as a renal disease progression predictor [34,35].

Current study shows a significant increase in TNF-α in patient as compared with control subjects. It is pleiotropic cytokine not only produced by macrophages, monocytes and T-cells but also expressed by intrinsic renal cells including mesangial, glomerular, endothelial and renal tubular cells [36]. Recent studies demonstrate that TNF-α can be stored within cells and its converting enzyme rapidly increases its secretion when stimulated [37]. Several experimental studies of diabetic rats have reported its increased concentration and mRNA encoding in glomerular in glomerular and tubular cells. This support its role in the development of renal hyper functioning as it stimulate sodium-dependent solute uptake in cultured mouse proximal tubular cells who exhibited sodium retention and renal hypertrophy [38,39]. Clinical studies of patients with diabetic nephropathy have shown high values of cytokine in serum and urine samples as the disease progresses which shows its direct involvement in the development and progression of disease [36].

Through the activation of multiple patways of cellular metabolism, oxidative stress is achieved by the changes in normal redox reactions due to unlike distribution of oxidant to antioxidant ratio [40]. Several enzymatic and non-enzymatic antioxidants are produced in response to increased oxidative stress created by elevated ROS levels such as catalase (CAT), superoxide dismutase (SOD), glutathione S-transferase (GST), glutathione peroxidase (GPx) and some non-enzymatic antioxidants like vitamins A, C and E, reduced glutathione (GSH) and uric acid. Superoxide anion (0_2^-) dismutase are used by GPX and CAT to form water. Hydrophilic forms of reactive electrophilic species are converted by the enzymatic antioxidant GST and its elimination from the body is mediated through conjugation with GSH. Non enzymatic antioxidants like vitamin C and E are evident in the termination of lipid peroxidation [41,42].

This present study compared the oxidative stress status of diabetic nephropathic patients with the healthy group. There is a significant decrease in the activity of antioxidants such as CAT, GSH-Px and SOD as compared with non-diseased group. This decrease breaks the balance between antioxidant and oxidant which leads to the redical mediated cellular injury. It is also demonstrated in another study conducted by Pan, et al., 2009, they investigated the oxidative stress level of patients with diabetic nephropathy and diabetic patients without nephropathy. It reported significant decrease in serum SOD, CAT and GSH-Px activity of patients with diabetic nephropathy [43].

Vitamin E also known as tocopherol is a lipid soluble vitamin that generally detoxifies free radicals and helps in the recycling processes to form vitamin A and C. It is potent immune stimulator, prevents lipid peroxidation and also stops the implication of genetic changes by inhibiting DNA damage caused by elevated level of ROS. Several studies have mentioned its lower level in diabetic patients with complications as compared with controls [44]. This study confirms the significant reduction in the levels of vitamin E, A and C of patients as compared to controls.

Different studies have s shown inverse relationship of vitamin D with the diabetic complications. The current study shows decrease in vitamin D as compared with control. It also indicates a significant inverse correlation of vitamin D with pro-inflammatory cytokines (IL-6 and TNF-α) which promotes renal injury and also increases oxidative stress. Some previous studies have demonstrated that supplementation of vitamin D improved the symptoms of diabetic kidney and can reverse its progression through inhibiting RAS activation by improving glucose metabolism and reducing fibrosis [45]. Another inverse correlation of serum vitamin D with albuminuria has been reported so far.

Albuminuria is main indicator of micro and macro vascular complications caused by hyperglycemia. A study reported that kidney tissue has enormous number of vitamin D receptors, hence it is considered to be the vital target of vitamin D activity. All these studies suggest that vitamin D can delay the progression of diabetic nephropathy [46].

This study reports AGEs, another parameter with significantly higher concentration in patients as compared with the controls. Several other studies have confirmed its significantly increased concentrations in diabetic of both types [47]. Since low molecular weight AGEs accumulates in the kidney and then filtered by proximal tubular cells lead to reduced glomerular filtration rate and tubulointerstitial cell damage in patients with diabetic nephropathy [48,49]. It is a promising statement that AGE-RAGE interaction is involved in the pathogenesis of diabetic nephropathy. RAGE is a signal transducer receptor for AGEs to produce inflammatory reactions [50]. Podocytes and mesangial cells are highly expressed by the RAGE expression [51]. It has a crucial role in the development and progression of diabetic nephropathy. A study reported the diabetic homozygous RAGE null mice failed to develop glomerular basement membrane thickening and mesangial expansion. Same authors also claimed that its reaction enhances the glomerular infiltration of inflammatory cells which worsens the disease [52]. AGE-RAGE interaction further enhances the activation of NF-$_k$B that further plays part in the enhancement of ROS levels [53].

In recent study, it is stated that myeloperoxidase (MPO) is derived from leucocytes and plays an important role in inflammation and generating ROS from inflammatory sites [54]. Only few studies have been able to find out the relationship between MPO and diabetic patients but their results are controversial. In one study the serum levels of MPO, rate of ROS and levels of glucose found higher in diabetic patients [55]. Findings of this study are in accordance with the reported study as it also shows the higher levels of MPO in patients with diabetic nephropathy. There is also a significant correlation of MPO and MDA and inverse correlation of MPO with SOD, CAT and GSH.

For the detection of progression of diabetic nephropathy in patients is done by the use of several biomarkers such as MDA, AOPPs, GGT and CRP. The findings of current study indicated a significant increase all biomarkers performed on serum samples of diabetic nephropathy as compared with healthy subjects. These increased values indicate the severity of disease and pointed towards the oxidative stress status in patients. Free radicals attack lipid membranes and initiates lipid peroxidation, producing large amounts of reactive products. MDA is a marker widely used. It is decomposition product of per oxidized polyunsaturated fatty acids. A study conducted in diabetic patients and diabetic nephropathic patients indicated significantly higher values of MDA in patients with diabetic nephropathy as compared with diabetic without renal complications. Same study also indicated the higher values of AOPPs and showed that these two parameters are more pronounced in patients with renal complication [43]. Furthermore, urinary biomarkers can detect tubular damage, thus this study also performed GGT test to diagnose diabetic nephropathy. This test also appears with very high values than control subjects. A study was conducted to detect the accuracy of urinary GGT and ALP in diabetic nephropathy. It indicated that urinary GGT and ALP were threefold higher in type 2 diabetic patients with nephropathy. It also proposed that urinary GGT and ALP have potential value in the diagnosis of nephropathy in type 2 diabetic patients, but GGT has slightly higher ability to discriminate nephropathy than ALP [56].

A non-specific systemic inflammatory marker C reactive protein (CRP) was performed. As indicated in the results of this study, it is also found to be very high in subjects as compared with control group. Comparative study of CRP and metabolic variables of type 2 diabetes with and without nephropathy was conducted and that indicated the significantly higher values of CRP in patients with diabetic nephropathy. It also showed as increasing trend serum CRP with the degree of microalbumin excretion and the severity of nephropathy in type 2 diabetic patients [57].

Conclusion

The local and systemic oxidative stress that underlines the pathological characteristics of DN is the result of the imbalance within the production of oxidants/antioxidants. Various aspects of diabetic nephropathy associated with oxidative stress coupled with the damage induced by ROS are anticipation to yield an impetus of designing new generation of specific antioxidants that are potentially more effective to reduce reno-vascular complications of diabetes. Several molecular pathways are participated in the generation of reactive oxygen species (ROS), the important ones includes the formation of AGEs and AOPPs, since the ROS appears to be the common denominator for the causation of renal injury they may be the most suitable targets for developing novel therapeutic agents to ameliorate reno-vascular complications of diabetes. Moreover, there are complex interplay that need to be carefully examined in order to piece together a detailed specific understanding of the pathologic steps responsible for oxidative stress and diabetic nephropathy.

References

1. Reutens AT, Prentice L, Atkins R (2008) The epidemiology of diabetic kidney disease. The epidemiology of diabetes mellitus 2: 499-518.

2. Zhang J, Liu J, Qin X (2018) Advances in early biomarkers of diabetic nephropathy. Revista da Associação Médica Brasileira (1992) 64(1): 85-92.

3. Andersen AR, Christiansen JS, Andersen JK, Kreiner S, Deckert T (1983) Diabetic nephropathy in type 1 (insulin-dependent) diabetes: an epidemiological study. Diabetologia 25(6): 496-501.

4. Adler AI, Stevens RJ, Manley SE, Bilous RW, Cull CA, et al. (2003) Development and progression of nephropathy in type 2 diabetes: The United Kingdom Prospective Diabetes Study (UKPDS 64). Kidney international 63(1): 225-232.

5. Vrhovac B, Jakšić B, Reiner Ž, Vucelić B (2008) Interna medicina. Medicus 17(1_Nutricionizam): 157-157.

6. Mogensen CE (2000) Microalbuminuria, blood pressure and diabetic renal disease: origin and development of ideas. In: The Kidney and Hypertension in Diabetes Mellitus, pp: 655-706.

7. Buchan IE (1997) Arcus QuickStat Biomedical version. Cambridge: Addison Wesley Longman Ltd.

8. MacIsaac RJ, Ekinci EI, Jerums G (2014) Markers of and risk factors for the development and progression of diabetic kidney disease. American journal of kidney diseases 63(2): S39-S62.

9. Motawi TK, Shehata NI, ElNokeety MM, El-Emady YF (2018) Potential serum biomarkers for early detection of diabetic nephropathy. Diabetes Res Clin Pract 136: 150-158.

10. Nguyen TQ, Tarnow L, Jorsal A, Oliver N, Roestenberg P, et al. (2008) Plasma connective tissue growth factor is an independent predictor of end-stage renal disease and mortality in type 1 diabetic nephropathy. Diabetes care 31(6): 1177-1182.

11. Nguyen TQ, Tarnow L, Andersen S, Hovind P, Parving HH, et al. (2006) Urinary connective tissue growth factor excretion correlates with clinical markers of renal disease in a large population of type 1 diabetic patients with diabetic nephropathy. Diabetes care 29(1): 83-88.

12. Pfeiffer A, Middelberg-Bisping K, Drewes C, Schatz H (1996) Elevated plasma levels of transforming growth factor-β1 in NIDDM. Diabetes care 19(10): 1113-1117.

13. Forbes JM, Fukami K, Cooper ME (2007) Diabetic nephropathy: where hemodynamics meets metabolism. Experimental and clinical endocrinology & diabetes 115(02): 69-84.

14. Siragy HM, Carey RM (2010) Role of the intrarenal renin-angiotensin-aldosterone system in chronic kidney disease. American journal of nephrology 31(6): 541-550.

15. Cao Y, Hao Y, Li H, Liu Q, Gao F, et al. (2014) Role of endoplasmic reticulum stress in apoptosis of differentiated mouse podocytes induced by high glucose. International journal of molecular medicine 33(4): 809-816.

16. Bucala R, Vlassara H (1995) Advanced glycosylation end products in diabetic renal and vascular disease. American journal of kidney diseases 26(6): 875-888.

17. Daroux M, Prévost G, Maillard-Lefebvre H, Gaxatte C, D'agati VD, et al. (2010) Advanced glycation end-products: implications for diabetic and non-diabetic nephropathies. Diabetes & metabolism 36(1): 1-10.

18. Campion CG, Sanchez-Ferras O, Batchu SN (2017) Potential role of serum and urinary biomarkers in diagnosis and prognosis of diabetic nephropathy. Canadian journal of kidney health and disease 4: 2054358117705371.

19. D'agati, V, Schmidt AM (2010) RAGE and the pathogenesis of chronic kidney disease. Nature Reviews Nephrology 6(6): 352-60.

20. Navarro-Gonzalez JF, Mora-Fernandez C (2008) The role of inflammatory cytokines in diabetic nephropathy. Journal of the American Society of Nephrology 19(3): 433-442.

21. Zeisberg M, Neilson EG (2010) Mechanisms of tubulointerstitial fibrosis. Journal of the American Society of Nephrology 21(11): 1819-1834.

22. Ohkawa H, Ohishi N, Yagi K (1979) Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Analytical biochemistry 95(2): 351-358.

23. Kakkar P, Das B, Viswanathan PN (1984) A modified spectrophotometric assay of superoxide dismutase. Indian J Biochem Biophys 21(2): 130-132.

24. Aebi H (1974) Catalase. In: Methods of enzymatic analysis. pp: 673-684.

25. Moron MS, Depierre JW, Mannervik B (1979) Levels of glutathione, glutathione reductase and glutathione S-transferase activities in rat lung and liver. Biochim Biophys Acta 582(1): 67-78.

26. Tietze F (1969) Enzymic method for quantitative determination of nanogram amounts of total and oxidized glutathione: applications to mammalian blood

and other tissues. Anal biochem 27(3): 502-522.

27. David M, Richard JS (1983) Glutathione reductase. Methods of Enzymatic Analysis. Bermeyer, Hans Ulrich Jr (Eds.), pp: 258-265.

28. Albrecht W (1980) Glutathione peroxidase. Enzymatic basis of detoxication 1: 333-353.

29. Witko-Sarsat V, Friedlander M, Capeillère-Blandin C, Nguyen-Khoa T, Nguyen AT, et al. (1996) Advanced oxidation protein products as a novel marker of oxidative stress in uremia. Kidney international 49(5): 1304-1313.

30. Evans LC, Gariepy RF (2015) Measure theory and fine properties of functions.

31. Rosenberg HR (1992) Chemistry and physiology of the vitamins. New York: Inter science, pp: 452-453.

32. Chinoy NJ, Rao MV, Seethalakshmi L (1979) Effects of gonadectomy & sex hormone replacement on tissue distribution of ascorbate in male & female albino rats. Indian journal of experimental biology 17(11): 1171-1175.

33. Sekizuka K, Tomino Y, Sei C, Kurusu A, Tashiro K, et al. (1994) Detection of serum IL-6 in patients with diabetic nephropathy. Nephron 68(2): 284-285.

34. Nosadini R, Velussi M, Brocco E, Bruseghin M, Abaterusso C, et al. (2000) Course of renal function in type 2 diabetic patients with abnormalities of albumin excretion rate. Diabetes 49(3): 476-484.

35. Dalla Vestra M, Mussap M, Gallina P, Bruseghin M, Cernigoi AM, et al. (2005) Acute-phase markers of inflammation and glomerular structure in patients with type 2 diabetes. Journal of the American Society of Nephrology 16(3): S78-S82.

36. San Juan R, Aguado JM, Lumbreras C, Fortun J, MuñozP, et al. (2008) Impact of current transplantation management on the development of cytomegalovirus disease after renal transplantation. Clinical infectious diseases 47(7): 875-882.

37. Wang L, Du F, Wang X (2008) TNF-α induces two distinct caspase-8 activation pathways. Cell 133(4): 693-703.

38. Di Petrillo K, Gesek FA (2004) Pentoxifylline ameliorates renal tumor necrosis factor expression, sodium retention, and renal hypertrophy in diabetic rats. American journal of nephrology 24(3): 352-359.

39. Schreiner GF, Kohan DE (1990) Regulation of renal transport processes and hemodynamics by macrophages and lymphocytes. American Journal of Physiology-Renal Physiology 258(4): F761-F767.

40. Böttinger EP, Bitzer M (2002) TGF-ß signaling in renal disease. Journal of the American Society of Nephrology 13(10): 2600-2610.

41. McClelland AD, Herman-Edelstein M, Komers R, Jha JC, Winbanks CE, et al. (2015) miR-21 promotes renal fibrosis in diabetic nephropathy by targeting PTEN and SMAD7. Clinical Science 129(12): 1237-1249.

42. Oikari S, Makkonen K, Deen AJ, Tyni I, Kärnä R, et al. (2016) Hexosamine biosynthesis in keratinocytes: roles of GFAT and GNPDA enzymes in the maintenance of UDP-GlcNAc content and hyaluronan synthesis. Glycobiology 26(7): 710-722.

43. Pan HZ, Zhang L, Guo MY, Sui H, Li H, et al. (2010) The oxidative stress status in diabetes mellitus and diabetic nephropathy. Acta diabetologica 47(1): 71-76.

44. Thomas MC (2016) Epigenetic mechanisms in diabetic kidney disease. Current Diabetes Reports 16(3): 31.

45. Nasri H, Behradmanesh S, Ahmadi A, Rafieian-Kopaei M (2014) Impact of oral vitamin D (cholecalciferol) replacement therapy on blood pressure in type 2 diabetes patients; a randomized, double-blind, placebo controlled clinical trial. J nephropathology 3(1): 29-33.

46. Sönmez MF, Dündar M (2016) Ameliorative effects of pentoxifylline on NOS induced by diabetes in rat kidney. Renal failure 38(4): 605-613.

47. Makita Z, Radoff S, Rayfield EJ, Yang Z, Skolnik E, et al. (1991) Advanced glycosylation end products in patients with diabetic nephropathy. New England Journal of Medicine 325(12): 836-842.

48. Vincent MM, Bautista O, Kenny D, Sell DR, Fogarty J, et al. (1999) Skin collagen glycation, glycoxidation, and crosslinking are lower in subjects with long-term intensive versus conventional therapy of type 1 diabetes: relevance of glycated collagen products versus HbA1c as markers of diabetic complications. DCCT Skin Collagen Ancillary Study Group. Diabetes Control and Complications Trial. Diabetes 48(4): 870-880.

49. Saul G, Sun W, Cleary P, Sell DR, Dahms W, et al. (2005) Glycation and carboxymethyllysine levels in skin collagen predict the risk of future 10-year progression of diabetic retinopathy and nephropathy in the diabetes control and complications trial and epidemiology of diabetes interventions and complications participants with type 1 diabetes. Diabetes 54(11): 3103-3111.

50. Yamagishi SI, Matsui T (2010) Advanced glycation end products, oxidative stress and diabetic nephropathy. Oxidative medicine and cellular longevity 3(2): 101-108.

51. Tanji N, Markowitz GS, Fu C, Kislinger T, Taguchi A, et al. (2000) Expression of advanced glycation end products and their cellular receptor RAGE in diabetic nephropathy and nondiabetic renal disease. Journal of the American Society of Nephrology 11(9): 1656-1666.

52. Wendt TM, Tanji N, Guo J, Kislinger TR, Qu W, et al. (2003) RAGE drives the development of glomerulosclerosis and implicates podocyte activation in the pathogenesis of diabetic nephropathy. The American journal of pathology 162(4): 1123-1137.

53. Bierhaus A, Schiekofer S, Schwaninger M, Andrassy M, Humpert PM, et al. (2001) Diabetes-associated sustained activation of the transcription factor nuclear factor-κB. Diabetes 50(12): 2792-2808.

54. Zhang C, Yang J, Jennings LK (2004) Leukocyte-derived myeloperoxidase amplifies high-glucose—induced endothelial dysfunction through interaction with high-glucose—stimulated, vascular non—leukocyte-derived reactive oxygen species. Diabetes 53(11): 2950-2959.

55. Kapur P, Peña-Llopis S, Christie A, Zhrebker L, Pavía-Jiménez A, et al. (2013) Effects on survival of BAP1 and PBRM1 mutations in sporadic clear-cell renal-cell carcinoma: a retrospective analysis with independent validation. The lancet oncology 14(2): 159-167.

56. Elks CM, Reed SD, Mariappan N, Shukitt-Hale B, Joseph JA, et al. (2011) A blueberry-enriched diet attenuates nephropathy in a rat model of hypertension via reduction in oxidative stress. PloS one 6(9): e24028.

57. Shaheer AK, Tharayil JK, Krishna PW (2017) A comparative study of high sensitivity C-reactive protein and metabolic variables in type 2 diabetes mellitus with and without nephropathy. Journal of clinical and diagnostic research: JCDR 11(9): BC01-BC04.