CLINICAL SIGNIFICANCE OF DIFFERENTIAL EXPRESSION OF DIAGNOSTIC BIOMARKERS FOR THE DEVELOPMENT OF DIABETIC NEPHROPATHY

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Abstract
Diabetes mellitus (DM) is a metabolic syndrome marked by an absolute and relative lack of insulin secretion and abnormalities in the metabolism of fats, proteins, and carbohydrates. Diabetic kidney damage is the most frequent and common consequence of diabetes mellitus (DM). According to an evidence-based study, oxidative stress caused by weakened antioxidant defences and/or increased production of free radicals plays a role in diabetic disease causation. Fifty patients with diabetic nephropathy were enrolled in this trial from Jinnah Hospital in Lahore. The following oxidative stress biomarkers were identified using spectrophotometric methods: SOD, GSH, Catalase, AOPPs, NO, and MDA. Using commercially available Elisa kits, vitamins (C, E, A, and D) and inflammatory markers (TNF-α and IL-6) were examined. Using SPSS version 16, the T-test was used to analyze the results. Patients with diabetic nephropathy had their hematological profiles examined. Unusual alterations were discovered in the platelet count and lymphocytes, which indicate internal inflammation and coagulation. Vitamins A, E, C, and D, as well as antioxidants (SOD, CAT, and GSH-GPx), were reduced. Increases were observed in oxidative and inflammatory markers, including MDA, MPO, and AOPPs. Based on the information available, it is evident that hyperglycemia...
triggers several signaling pathways and the production of reactive oxygen species (ROS), which in turn triggers additional signaling cascades that lead to structural and functional changes in the kidney that worsen the complications of diabetic nephropathy.

**Keywords:** Diabetic kidney disease, Signaling cascades, Reactive oxygen species, Glutathione, Tumor necrosis factor Alpha, Superoxide Dismutase

**INTRODUCTION**

Diabetes mellitus (DM) is the most common cause of chronic kidney failure in both developed and developing countries. >200 mcg/min or >300 mg/day of albuminuria verified at least twice. The two main characteristics of diabetic nephropathy, sometimes referred to as nodular diabetic glomerulosclerosis, Kimmelstiel-Wilson syndrome, or intercapillary glomerulonephritis, are an irreversible and persistent decrease in glomerular filtration rate (GFR) and arterial hypertension, occurring three to six months apart [1]. Diabetic nephropathy is a chronic consequence that can arise from type 1 diabetes (beta cell death and total lack of insulin) or type 2 diabetes (insulin resistance and/or decreased insulin output). There are five phases in the development of diabetic nephropathy.

It was discovered in the early 1980s that a trace amount of albumin in urine can indicate whether renal impairment will occur in patients with type 1 or type 2 diabetes [2]. The initial stage of kidney injury is called the microalbuminuria stage, or early nephropathy stage. Twenty to thirty percent of individuals have microalbuminuria after the disease has progressed for fifteen years, and fewer than fifty percent develop genuine nephropathy [3].

The aetiology of diabetic nephropathy is complicated and depends on several factors, such as the length of diabetes mellitus, inadequate glucose regulation, oxidative stress, elevated blood pressure, and hypertriglyceridemia [4]. Oxidative stress is a disruption that causes tissue damage and is surrounded by pro and antioxidant factors. It was discovered in the early 1980s that a trace amount of albumin in urine can indicate whether renal impairment will occur in patients with type 1 or type 2 diabetes [2]. The initial stage of kidney injury is called the microalbuminuria stage, or early nephropathy stage. Twenty to thirty percent of individuals have microalbuminuria after the disease has progressed for fifteen years, and fewer than fifty percent develop genuine nephropathy [3]. The aetiology of diabetic nephropathy is complicated and depends on several factors, such as the length of diabetes mellitus, inadequate glucose regulation, oxidative stress, elevated blood pressure, and hypertriglyceridemia [4]. Oxidative stress is a disruption that causes tissue damage and is surrounded by pro and antioxidant factors. Numerous diabetes-related side effects, such as cardiovascular disease, renal problems, and different malignancies, are caused by the positive feedback cycle that involves persistent inflammation, oxidative stress, and the development of insulin resistance. [7]

Numerous studies have demonstrated the crucial role that inflammatory molecules, including chemokines, cytokines, and adhesion molecules, play in the pathogenesis of diabetic nephropathy [8]. Although the etiology of diabetic nephropathy is becoming better known, the precise pathway that leads from a chronic hyperglycemic state to the formation of diabetic nephropathy remains unclear. Given that hyperglycemia is known to activate oxidative stress and inflammatory pathways, it is hypothesized that the interaction of these two pathways results in kidney injury brought on by hyperglycemia [9]. These findings have spurred investigation into a biomarker of inflammation and oxidative stress that could be useful in the treatment of diabetic nephropathy [10]. The primary aim of the present investigation is to ascertain the concentrations of several stress biomarkers and inflammatory cytokines, as well as their correlation with the progression of diabetic nephropathy.

**MATERIALS AND METHODS**

**SELECTED SAMPLES**

The goal of the current study was to examine the major mechanisms behind the onset of diabetic nephropathy. At the Jinnah Hospital in Lahore, each of the 100 patients who had been chosen was screened. Prior to being incorporated into this research, informed consent was acquired. In this study,
100 patients with diabetic nephropathy and 100 healthy persons served as controls. The research ethics committee at the Institute of Molecular Biology and Biotechnology, The University of Lahore, Pakistan approved the experimental protocol. Every participant had a five-milliliter venous blood sample drawn from their antecubital vein. Within an hour of sample collection, the bottles were centrifuged, and the serum was separated and kept at -70°C until analysis. Participants having a history of drug use, including alcohol and cigarettes, as well as those using pre-diagnosis drugs like antiparkinsonian or antipsychotics, were not allowed to participate in this study. A history of chronic infections, malnutrition syndrome, metabolic dysfunction (such as diabetes mellitus, liver disorders, renal illness, cancer, prior history of PE and HTN, presence of vascular disease and CVA), and no control group was taking any medication.

CHEMICALS
The source of all analytical-grade chemical reagents was Sigma/Invitrogen Chemical Co. (St. Louis, Mo., USA).

BIOCHEMICAL INVESTIGATIONS
Using an automated Sysmex hematology blood analyzer (version XP-2100), a complete blood count was conducted on the chosen subjects. Superoxide dismutase (SOD) levels were measured using the Kakkar et al. (1984) technique [11]. Using a spectrophotometer, the Aebi (1984) method was used to test catalase. The GSH concentrations were determined using the Moron et al. (1979) technique [12]. The Mills (1957) method was utilized to measure the quantity of glutathione peroxidase (GPx). The Racker (1955) method [13] was used to measure the glutathione reductase levels. Using the Griess test technique, the concentration of NO was ascertained [14]. The methodology of Witko Sarsat et al. (1996) [15] was used to determine the amounts of AOPPs. Spectrophotometric analysis was performed to ascertain the MDA levels using the Ohkawa et al. (1979) [16] technique. Using the Odetti et al. (1992) procedure, advanced glycation end-product pentosidine is thought to be a biomarker of non-enzymatic protein glycation [17]. The method of Rosenberg and Culik (1959) [18] was used to measure the level of vitamin C. Using the Rutkowski et al. (2006) [19] procedure, the amount of Vitamin A in the serum sample was calculated. The Emmerie-Engel reaction, first described by Evans and Bishop (1922) [20] was used to measure vitamin E. The enzymatic colorimetric test was used to measure the amount of gamma-glutamyl transferase. The human diagnostic ELISA kit (Invitrogen Elisa Kit) was utilized to determine the various amounts of Vitamin D, MPO, IL-6, HsCRP, and TNF-α.

STATISTICAL CONSIDERATIONS
SPSS version 10 was used for the data processing and statistical analysis. The comparison of patients with diabetic nephropathy and a healthy control group was done using an independent sample t-test. In this investigation, the Pearson correlation coefficient (r) was also employed. Every result was given as Mean±SD, with p<0.05 designating statistically significant results.

RESULTS
DIABETIC NEPHROPATHY PATIENT DEMOGRAPHIC PROFILE VS. HEALTHY CONTROL
Table 1 compares the demographic distribution of patients with diabetic nephropathy to that of healthy individuals. When comparing diabetic nephropathy patients to a healthy control group, the following measurements were made: weight (62.71±11.39 kg vs. 41.52±4.33 kg), age (42.64±17.69 Yrs vs. 36.83±4.06 Yrs), SBP (1.23±6.68 mmHg vs. 1.20±1.33 mmHg), DBP (80.56±2.10 mmHg vs. 78.64±1.13 mmHg), and body mass index (70.64±2.61 Kg/m3 vs. 19.51±1.41 Kg/m3).
TABLE 01: DEMOGRAPHIC PROFILE OF DIABETIC NEPHROPATHY PATIENTS VERSUS HEALTHY CONTROL

<table>
<thead>
<tr>
<th>Variables</th>
<th>Control (n=50)</th>
<th>Subject (n=50)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (kg)</td>
<td>41.52±4.33</td>
<td>62.71±11.39</td>
</tr>
<tr>
<td>Age (Yrs)</td>
<td>36.83±4.06</td>
<td>42.64±17.69</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>1.20±1.33</td>
<td>1.23±6.68</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>78.64±1.13</td>
<td>80.56±2.10</td>
</tr>
<tr>
<td>Body Mass Index (Kg/m²)</td>
<td>19.51±1.41</td>
<td>70.64±2.61</td>
</tr>
</tbody>
</table>

SBP: systolic blood pressure; DBP: diastolic blood pressure; BMI: body mass index

FIGURE: 01 PROPHETIC VARIABLE LEVELS VERSUS HEALTHY CONTROL IN PATIENTS WITH DIABETIC NEPHROPATHY

- Fasting blood glucose (mg/dL)
- RBGs (x10⁹/L)
- WBCs (x10⁹/L)
- Hb (g/dL)
- PLTs (x10⁹/L)
- HCT (%)
- PTI (Sec)
- MDA (nmol/ml)
Clinical Significance Of Differential Expression Of Diagnostic Biomarkers For The Development Of Diabetic Nephropathy

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DIABETIC NEPHROPATHY PATIENTS’ HAEMATOLOGICAL PROFILE VS. HEALTHY CONTROL

Figure 01 illustrates the measurement of the hematological profile in individuals with diabetic nephropathy in comparison to a healthy control group. When comparing the mean fasting blood glucose level of patients with diabetic nephropathy (4.23±0.60 mg/dL) to that of a healthy control group (3.01±0.61 mg/dL), a substantial rise was seen. Comparing the patient group (3.20±0.21 x10^9/L, 6.31±1.03 g/dL, 167±6.55 x10^9/L, and 24.87±2.43%) to the healthy individual group (24.87±2.43 x10^9/L, 12.67±0.78 g/dL, 317±4.27 x10^9/L, and 51.29±2.59%), a decrease in RBCs, Hb, platelets, and HCT were observed in the patient group. Conversely, the average WBC and PTT
values in patients with diabetic nephropathy were found to be considerably higher (9.24±1.69 x10⁹/L and 13.31±1.75 Sec) in comparison to the healthy control group (7.57±0.40 x10⁹/L and 9.64±0.93 Sec), respectively.

**PROPHETIC ANTIOXIDANTS LEVELS VERSUS HEALTHY CONTROL IN PATIENTS WITH DIABETIC NEPHROPATHY**

The information depicted in Figure 01 illustrates the levels of different stress variables and their noteworthy contribution to the development of diabetic nephropathy in patients relative to control subjects. In comparison to control individuals, the mean values of MDA (3.80±1.11 nmol/mL vs. 1.43±0.36 nmol/mL), GGT (58.35±8.64 IU/L vs. 43.12±6.82 IU/L), HsCRP (1.47±0.10 mg/dL vs. 1.04±0.12 mg/dL), IL-6 (6.73±0.82 pg/mL vs. 5.64±0.51 pg/mL), TNF-α (55.78±4.03 pg/mL vs. 29.90±1.14 pg/mL), AOPPs (1.45±1.06 µmol/L vs. 0.84±0.04 µmol/L), AGEs (2.77±0.29 nmol/mL vs. 203±4.81 µg/L), and MPO (236±25.01 µg/L vs. 203±4.81 µg/L) were significantly increased in diabetic nephropathy patients as compared to healthy individuals.

Contrary to that, the levels of SOD, GSH, CAT, Vit-A, Vit-C, Vit-E, GPx and GRx were significantly decreased in diabetic nephropathy patients (0.09±0.03 IU/mg Protein, 4.23±1.64 µmol/L, 2.20±0.26 IU/L, 428±95.78 mcg/dL, 0.36±0.22 mg/dL, 0.24±0.09 µg/mL, 9.44±1.21 mg/mL, 6.61±0.37 nmol/min/mL and 1.47±0.22 nmol/min/mL) as compared to healthy individuals (0.49±0.04 IU/mg Protein, 9.79±1.22 µmol/L, 3.95±0.21 IU/L, 615±44.52 mcg/dL, 0.56±0.08 mg/dL, 0.29±0.05 µg/mL, 13.22±0.81 ng/mL, 8.22±0.69 nmol/min/mL, 4.36±1.59 nmol/min/mL) respectively. Patients with diabetic nephropathy showed higher trends of NO (57.67±8.87 µmol/L vs. 19.42±1.42 µmol/L) when compared to healthy people. Diabetic nephropathy patients had significantly higher levels of AGEs (2.77±0.29 nmol/mL vs. 2.56±0.10 nmol/mL), MPO (236±25.01 µg/L vs. 203±4.81 µg/L), AOPPs (1.45±1.06 µmol/L vs. 0.84±0.04 µmol/L), and 29.90±1.14 pg/mL, respectively, as compared to control subjects.

**DISCUSSION**

Diabetic nephropathy is the main consequence of diabetes. The only remaining primary cause of end-stage renal disease is glomerulonephritis. Because of its complex metabolic problems, end-stage renal disease is sometimes harder to cure than other kidney disorders. Prompt prophylaxis and treatment are therefore essential to prevent diabetic nephropathy [21]. This study looked into the role of oxidative stress-induced mitochondrial damage in diabetic nephropathy. The degree of oxidation that results from the body being exposed to various harmful stimuli, leading to an imbalance in the body's antioxidant system and tissue damage, is referred to as "oxidative stress". Recent research has connected the production of reactive oxygen species (ROS) to several chronic metabolic diseases, such as diabetes and atherosclerosis [22]. Previous studies have indicated that oxidative stress plays a major role in the development of diabetic nephropathy. Malondialdehyde (MDA) is a primary consequence of membrane lipid peroxidation, and its production may exacerbate membrane damage. The current study's finding of ROS and MDA elevation in the kidneys supported the presence of hyperoxidative stress in diabetes individuals [21]. Table 02 indicates that a positive connection (r=.653*) was found in the current investigation between MDA and NO. By converting it into glutathione (GSH), the thiol-containing antioxidant N-acetyl cysteine (NAC) protects tissue in vivo. According to early research, NAC lessened lung damage by stopping vascular endothelial cells and neutrophils from producing adhesion molecules. Furthermore, it penetrated leukocytes and transformed into physiological antioxidants to increase intracellular reduction levels, which resulted in the inactivation of ROS in cells and medium [23]. Glutathione, a thiol-containing tripeptide, is present in significant amounts in living cells in its reduced form (GSH). It joins forces with ROS to generate glutathione radical, which glutathione reductase activity subsequently reduces to its original form. The patient group showed a significant decrease in GSH concentration during this experiment, which may be connected to the overproduction of reactive oxygen species, which causes the conversion of GSH from its reduced to
its oxidized form. Sodium oxide dismutase (SOD) catalyzes superoxide dismutase to produce hydrogen peroxide (H2O2), which is subsequently further reduced by catalase and glutathione peroxidase.

The current investigation shows that, in comparison to controls, patients' SOD activity has dramatically decreased. SOD is rendered inactive by the H2O2 generated during the auto-oxidation of glucose. SOD glycation may rise in DM patients as a result of decreasing SOD activity brought on by ageing. Table 02 displays the results of the current investigation, which indicate a substantial association between myeloperoxidase and SOD (MPO vs. SOD, r=-.461*). Using NADPH and GSH, the other enzyme, catalase (CAT), expresses and detoxifies H2O2. Researchers have shown in their studies [25] that CAT activity increases in response to a decrease in glutathione peroxidase activity. Aging led to an increase in H2O2 production. CAT activity was similarly observed to be elevated by Inal et al. Plasma proteins are especially susceptible to ROS oxidation because extracellular fluids usually contain very few antioxidant enzymes. Consequently, plasma from dialysis patients often contains elevated amounts of advanced oxidation protein products (AOPPs), also called oxidized protein products. It was discovered that albumin, the most common plasma protein, was the main source of AOPPs in plasma [26]. The high molecular weight AOPPs were mostly formed by albumin aggregates, which were probably the product of di-tyrosine crosslinking or disulfide bridges. Because of the low molecular weight of AOPPs, albumin was present in its monomeric form.

Arteriolosclerosis and kidney disease are largely caused by proinflammatory cytokines. Diabetic nephropathy and other diabetic microvascular complications are the outcome of them. It also has an impact on some inflammatory illnesses and the regulation of immune responses. Diabetic nephropathy shares features with several chronic inflammatory diseases, according to prior research, and patients with this condition have also been found to have higher than normal levels of classical inflammatory mediators, such as tumor necrosis factor (TNF), interleukin-1 (IL-1), and interleukin-6 (IL-6) [27]. The onset and progression of this disease have also been connected to proinflammatory cytokines and hyperactive immunological responses. It follows that polymorphism is likely to alter the state of inflammation if it can alter the protein structure or gene expression of cytokines [28]. Table 02 indicates that a negative connection (r= -0.416*) was found in this study between IL-6 and Vitamin D.

**TABLE-02:** THE PEARSON'S CORRELATION BETWEEN THE CHARACTERISTICS OF INDIVIDUALS WITH DIABETIC NEUROPATHY

<table>
<thead>
<tr>
<th>PARAMETERS</th>
<th>CORRELATION COEFFICIENT</th>
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<tbody>
<tr>
<td>MDA vs TNF-α</td>
<td>.146*</td>
</tr>
<tr>
<td>MDA vs Vit D</td>
<td>-.443*</td>
</tr>
<tr>
<td>MDA vs NO</td>
<td>.542*</td>
</tr>
<tr>
<td>MPO vs MDA</td>
<td>.223*</td>
</tr>
<tr>
<td>MPO vs SOD</td>
<td>-.350*</td>
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<tr>
<td>MPO vs GSH</td>
<td>-.340**</td>
</tr>
<tr>
<td>MPO vs Catalase</td>
<td>-.118**</td>
</tr>
<tr>
<td>IL-6 vs Vit D</td>
<td>-.305*</td>
</tr>
<tr>
<td>TNF-α vs Vit D</td>
<td>-.081*</td>
</tr>
<tr>
<td>TNF-α vs IL-6</td>
<td>.268*</td>
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</table>

**CONCLUSION**

In conclusion, oxidative stress-induced mitochondrial damage is a key component in the etiology of diabetic nephropathy. The goal of clinical management for diabetic nephropathy may be to limit the
generation of reactive oxygen species (ROS) or block the mechanisms that lead to oxidative stress. IL-6 has a wide range of actions related to diabetic nephropathy, from the start of diabetes to the development of renal failure.

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FUNDING The writers have provided their funding for this project.

STATEMENT OF INFORMED CONSENT Every study participant gave their informed consent.

DATA AVAILABILITY STATEMENT On-demand access to raw data will be provided.

CONFLICTS OF INTEREST None of the writers have a conflict of interest.

REFERENCES