Original article

Plasma protein glycation status in Pakistan type 2 diabetic patients with or without nephropathy

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Abstract

Background: Amadori-modified glycated plasma proteins play an important role in diabetic microangiopathy. Many of the pathogenic changes that occur in diabetic nephropathy (DN) may be induced by non-enzymatic glycation (NEG). ***Objective:*** The aim of this study was to determine prevalence of DN and non-enzymatic glycation levels in diabetic population. ***Methodology*:** One hundred patients with type 2 diabetes and forty healthy control subjects were recruited after consent. Case participants were further divided into two groups as type 2 diabetics with nephropathy (n = 22) and type 2 diabetics without nephropathy (n = 78). Non-fasting plasma glucose (Trinder GOD-PAP method), total plasma proteins (biuret method), Erythrocyte sedimentation rate (Westergren's method), HbA_1c (glycohemoglobin spectrophotometry A_1c Kit) and non-enzymatic glycation (TBA colorimetric technique) were assayed. ***Results:*** Diabetic patients with nephropathy had higher ESR (55.33 ± 24.68 mm/1st hour *vs.* 46.88 ± 23.95 mm/1st hour *vs.*12.73 ± 2.34 mm/1st hour), total proteins (15.71 ± 4 g/dL *vs.*14.01 ± 4 g/dL *vs* 6.18 ± 1.16 g/dL) and non-enzymatic glycation (1.73 ± 0.48 mol./mol. *vs.*1.47 ± 0.58 mol./mol. *vs.* 0.48 ± 0.18 mol./mol.) measurements as compared to those without any similar renal complications and controls. Appreciable correlation existed between hyperglycemia and non-enzymatic glycation. ***Conclusion:*** Although the clinical consequences of NEG of circulating proteins remain ambiguous. In diabetic patients, however, extensively glycated species could exhibit significant alterations in function. Present study suggests DN as a frequently prevalent secondary complication of diabetes with a potential link with elevated NEG and glycemic control.

***Key words*:** Diabetic microangiopathy, nephropathy, glycated serum proteins.

Introduction

Diabetic nephropathy (DN) is a major cause of morbidity and is associated with increased cardiovascular mortality in type 2 diabetes mellitus. The specific pathological changes in the kidney, the clinical course, and the overall risk to develop nephropathy are quite similar in both types of diabetes.[1] An estimated one third of patients with type 1diabetes need renal dialysis after 15–20 years of the disease.[2] One quarter of all patients requiring renal transplants, are diabetics.[3] Nephropathy remains a significant cause of morbidity and mortality in the diabetic population and is the leading cause of end-stage renal failure in the Western World. As a result of the diabetic milieu, increased generation of reactive oxygen species thought to play a key role in the progression of DN.[4] Recent experimental studies have suggested that the receptor for advanced glycation end products (RAGE), which is central to the advanced glycation pathway, may mediate renal structural and

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functional damage via oxidative stress.[5] Tubulo-interstitial pathology in diabetic nephropathy is thought to be caused by cell injury that is induced by high ambient glucose levels and increased proportions of glycated proteins. Other mechanistic hypotheses engage glomerular ultrafiltration of proteins and bioactive growth factors and their effects on tubular cells. Some scholars promote tubular ischaemia due to reduced peritubular blood flow as a response to glomerular injury. All of these mechanisms contribute to renal tubulo-interstitial injury in DN. Dilatation of distal nephron segments is routinely seen in human biopsies or in histological sections from experimental diabetic nephropathy. It is these dilated tubules that are the primary source for pro-inflammatory and pro-fibrogenic cytokines and regulators.[6] As with retinopathy, nephropathy is strongly affected by the degree of hyperglycemia, hypertension and the duration of diabetes.[7] World-wide, today diabetes accounts for 20-50% of patients entering established renal failure programs and absolute numbers increase as greater longevity and western-style living has promoted an 'epidemic' of diabetes at all ages.[8] In addition to the recognized and powerful effects of environmental factors, there is abundant evidence in support of genetic susceptibility to the microvascular complication of nephropathy in individuals with both type 1 and type 2 diabetes. It seems likely that the risk for diabetes-associated kidney disease is magnified by inheriting risk alleles at several susceptibility loci, in the presence of hyperglycemia.[9]In the present study, we assessed the prevalence of DN and compared various diabetes related parameters, especially non-enzymatic glycation levels among type 2 diabetic patients with and without nephropathy as well as with normal controls.

Subjects & methods

Physician-diagnosed one hundred type 2 diabetes mellitus (T2DM) patients attending District Head Quarter Hospital, Faisalabad, Pakistan were studied along with forty non-diabetic volunteers, taken as control. Inclusion criteria for recruitment were the following three conditions: 1) be suffering from type 2 diabetes, 2) be diagnosed with diabetes for at least 1 year, 3) none of the patients diagnosed with hepatitis B or C viral infection, known chronic liver disease or other diabetes complications except nephropathy. The Advanced Studies and Research Board of the University of Agriculture, Faisalabad provided ethical approval and informed consent was obtained from all the subjects. Baseline demographic information was obtained from medical records. The diagnostic criteria for nephropathy was positive dip stick test for protein or albumin excretion > 300 mg per 24 hrs).[10] Case subjects were further stratified based on the presence or absence of diabetes related renal comorbidities as 22 T2DM patients with nephropathy and 78 T2DM patients without nephropathy to compare various variables. In all subjects, blood samples were taken for the measurement of postprandial glucose (Trinder GOD-PAP method)[11], total proteins (biuret method),[12] ESR (Westergren's method),[13] glycated hemoglobin (glycohemoglobin spectrophotometry A_1c Kit) and non-enzymatic glycation (Thiobarbituric Acid (TBA) colorimetric technique).[14]

Results were expressed as means ± SD, number (n) or percent (%) as appropriate. Comparison and correlations between variables were done using student t-test and Pearson's correlation coefficient (r) with significance level set at $p \leq 0.05$. Multivariant regression by SPSS (version 14) was used to assess the relation between nephropathy and different biochemical parameters.

Results

The demographic characteristics of case and normal participants are presented in figures 1 and 2. Of one hundred diabetic

patients, 22 had renal impairment. This group had mean age 65.0 ± 8.45 years. Consisted of 10 women (45.45%) and 12 men (54.54%), age at onset of diabetes was 48.6 ± 5.76 years (duration of diabetes 16.3 ± 6.61 years).

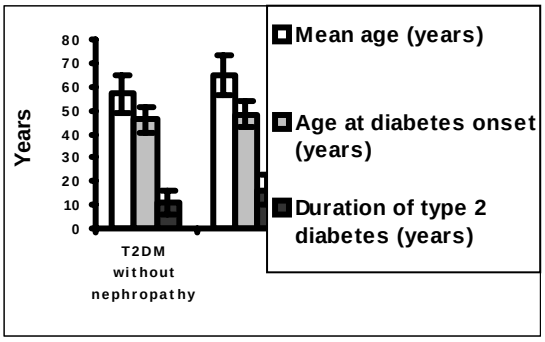

Figure 1: Mean age (years), age at diabetes onset (years) and diabetes duration (years) in type 2 diabetes mellitus patients (T2DM) with and without nephropathy and in normal subjects

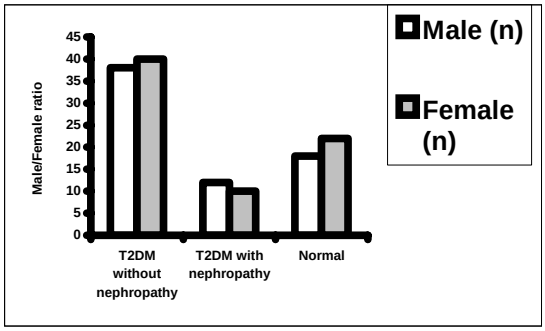

Figure 2: Male and female (n) in type 2 diabetes mellitus patients (T2DM) with and without nephropathy and in normal subjects

Thirty-eight men (48.71%) and forty women (51.28%) were type 2 diabetic persons without nephropathy with mean age of 57 ± 8.12 years. Age at onset of diabetes was 46 ± 5.19 years (duration of diabetes 10.8 ± 5.1 years). Third group of normal controls (52.6 ± 9.58 years mean age) had 45% men and 55% women.

Biochemical characteristics of study participants are summarized in table 1. All the subjects under study had mean age in the range of 50 -70 years. ESR values in our case patients with nephropathy and without nephropathy were statistically higher ($P < 0.05$) from normal controls. Diabetics with nephropathy also showed elevated ESR than diabetics without nephropathy ($P < 0.05$). Postprandial plasma glucose levels were analogous ($P > 0.05$) between both groups of case participants but appreciably higher ($P< 0.05$) than that of control participants.

People with diabetes whether suffering from nephropathy or not had noticeably higher plasma proteins concentrations ($P < 0.05$) than that of controls. Most interesting was the trend among case subjects; diabetics with nephropathy had considerably ($P < 0.05$) increased plasma protein concentrations as compared to those without nephropathy. Considerably elevated ($P < 0.05$) HbA_1c values among type 2 diabetic participants with and without renal complication existed than that of controls but these were insignificant among both diabetic groups ($P > 0.055$).

Diabetic patients exhibited higher NEG levels ($P < 0.05$) in comparison to healthy controls. Within the diabetic groups, those with nephropathy showed drastic increase in NEG ($P < 0.05$) than those without nephropathy.

Table 1: Biochemical characteristics of type 2 diabetics (with and without nephropathy) and normal participants

Biochemical Parameters	***T2DM with Nephropathy***	***T2DM without Nephropathy***	***Normal***
ESR (mm/1st hour)	55.33 ± 24.68	46.88 ± 23.95	12.73 ± 2.34
Post-prandial Glucose (mmol./L)	13.8 ± 3.17	12.91 ± 3.28	6.22 ± 0.71
Total plasma proteins (g/dL)	15.71 ± 4	14.01 ± 4	6.18 ± 1.16
HbA_1c (%)	11 ± 2	10 ± 2	6 ± 1.11
NEG (mol./mol.)	1.73 ± 0.48	1.47 ± 0.58	0.48 ± 0.18

Data are mean ± SD, n or % ± SD

Multivariate analyses revealed no association between nephropathy and various variables. Appreciable correlation (r = 0.735) existed between hyperglycemia and nonenzymatic glycation.

Discussion

Diabetic patients with and without nephropathy indicated almost comparable mean age, age at the onset of diabetes and male/female ratio. Given the limited generalizability of this hospital-based sampling, these results were found in a population diagnosed with type 2 diabetes and we do not know whether the proportion of undiagnosed diabetes varies for mean age, age at onset of diabetes and gender distribution or not. Postprandial plasma glucose, ESR, total proteins, HbA_1c and NEG showed variations among diabetic patients with and without nephropathy and controls. Regarding the prevalence of diabetes renal complications, our results were in compliance with the findings of Diabetic Association of Pakistan that indicated 20% prevalence of diabetic nephropathy.[15]

Duration of diabetes was significantly higher in T2DM group with nephropathy as compared to that without nephropathy. Duration of diabetes is a very important factor in the development of diabetic nephropathy and the cumulative risk of renal failure increase with diabetes duration as demonstrated in several studies, [16-20] This has also been confirmed in our study, that longer the duration of diabetes, higher the frequency of diabetic nephropathy.

The erythrocyte sedimentation rate has been used for predicting disease severity to assessing general sickness index. The erythrocyte sedimentation rate is a simple, inexpensive laboratory test that clinicians have used for decision-making. Many researchers criticize this test because of its lack of specificity and because the concept of erythrocyte sedimentation rate as a "sickness index" seems scientifically unsound. It is claimed that perceived utility of ESR test has been based on medical myths and its frequent use is based only on a consultant's demand or a shotgun approach to diagnosis. [21, 22]Despite all merits and demerits, ESR testing is still part of clinical chemistry in Pakistan. Existing data[23] suggest that an elevated ESR may occur in many different clinical settings such as serious underlying disease, most often infection, collagen vascular disease or metastatic malignancy and inflammation may play a role in the etiology of diabetes mellitus.[24] Our study provides limited support to the hypothesis that inflammation is an etiologic factor for diabetes. Nonetheless, higher measurements of ESR among type 2 diabetic patients in our study were extraneous as described elsewhere.[25]

Almost parallel mean postprandial plasma glucose levels between both groups of case participants were higher than that of control participants. Due to the tendency to rapid variations of hyperglycemia constant in the life of diabetic patients (above all in the postprandial phase), it is proper to think that postprandial glucose may exert an influence on the onset of complications. Postprandial glucose level is the major determinant of HbA_1c level after mean daily blood glucose.[26] One question that remains unanswered is whether postprandial hyperglycemia has a unique role in the pathogenesis of diabetic vascular complications or not and should be a specific target of therapy. Previously postprandial glucose has been linked to the progression of complications in type 2 diabetics.[27] But we did not find any such association of postprandial glucose with nephropathy in regression analysis.

Diabetic subjects whether suffering from nephropathy or not had noticeably higher plasma proteins concentrations as compared to controls. Diabetics with nephropathy had considerably increased plasma protein concentrations as compared

to those without nephropathy. This is attributed mainly to intracellular protein degradation in target tissues of insulin in diabetes mellitus. Degradation of serum proteins is also affected in diabetes and starvation. In normal conditions, a general correlation exists between isoelectric points of serum proteins and their degradative rates. This relationship is abolished in diabetes.[28] Anabolic processes like protein synthesis are sacrificed to catabolic activity such as gluconeogenesis. [29, 30] Factor of proven or suspected efficacy in attenuating renal disease progression is hyperglycemia.[31] Plasma haemoglobin A_1c reflects ambient mean glycaemia over a 2–3 months period.[32] Considerably elevated HbA_1c values among type 2 diabetic participants with and without renal complication existed than that of controls were in agreement with Kalia *et al*.[33] Huraib *et al*.[34] observed elevated concentrations of HbA_1c (10.3 ± 2.6%) in their diabetic patients with nephropathy as mentioned in our study.

It was demonstrated by Piwowar *et al*.[35] that diabetic patients had significantly higher levels of glycation products in comparison to healthy people. AGEs were increasing progressively from normoalbuminuria, through microalbuminuria to macroalbuminuria. Plasma AGE correlated significantly with urinary albumin/creatinine ratio. Glycation occurs with age and in certain pathological conditions like diabetes. Currently there is no commonly accepted or widely used method to detect glycation. Commercially available kits are not economical. There is no internationally accepted standard for glycation. The lack of internal standards leaves assays open to error, which require high degree of accuracy and reproducibility for each sample run. Currently most common methods used for detection are HPLC, ELISA and immunohistochemistry.[36] The thiobarbituric acid method, used for the estimation of non-enzymatic glycation in present study is less frequently used in diagnostic laboratories in Pakistan. It is quite economical to replace costly chromatographical techniques. International studies using TBA method have indicated that glycated serum proteins are sensitive indicator of the degree of hyperglycemia in diabetes. [37, 38] Rationale to estimate NEG in present study is the globally accepted vision that nonenzymatic glycation of proteins and subsequent formation of advanced glycation end products (AGEs) is one of the pathogenetic mechanisms thought to link hyperglycemia to diabetic retinopathy and nephropathy.[39]

Considerably higher NEG levels in diabetic participants with and without nephropathy compared to non-diabetic subjects were not surprising as Kathryne *et al*.[40, 41] indicated that type 2 diabetic patients had higher serum glycation levels than that of normal subjects (4.24 ± 0.88 vs. 3.15 ± 0.81 unit/ml and 4.6 ± 0.7 vs. 3.1 ± 0.8 unit/ml respectively). Kidney is a target for AGE-mediated damage and is also a contributor to circulating AGE concentration as seen in diabetes being major site of clearance of AGEs. Animal studied have clearly demonstrated a pathogenic role for AGEs in diabetic nephropathy. [42]

Diabetic nephropathy is a leading cause of end-stage renal failure, which could account for disabilities and high mortality rates in patients with diabetes. Recent large landmark clinical studies have shown that intensive glucose control reduces the risk of the development and progression of diabetic nephropathy.[43, 44]

In diabetes mellitus, hyperglycaemia accelerates non-enzymatic glycation and oxidative stress leading to damage of macromolecules, among others proteins. This manifests in the increased levels of advanced glycation end products (AGE) and advanced oxidation protein products (AOPP). The chronic hyperglycemic status

also favors glycation reactions (irreversible glucose binding on protein amino groups), thereby leading to advanced glycation endproducts. Through their recognition by cell receptors, advanced glycation endproducts also participate in the development of oxidative stress and the inflammatory status.[45] Glycated plasma proteins are regarded as an intermediate index of diabetic control, showing rapid response to the short-term improvement of blood sugar as compared to glycated hemoglobin.[46]

Conclusion

The clinical consequences of NEG of circulating proteins remain ambiguous. In diabetic patients, however, extensively glycated species could exhibit significant alterations in function. Present study suggests DN as a frequently prevalent secondary complication of diabetes with a potential link with elevated NEG and glycemic control.

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