

To Study Serum Mmp-9 Levels In Early Diabetic Nephropathy

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ABSTRACT : A key element in diabetic nephropathy (DN) is changes in the extracellular matrix (ECM) in several of the components in the kidney. Matrix metalloproteinases are a group of zinc endopeptidases secreted in variety of cells including kidney cells. MMPs play a central role in extracellular matrix (ECM) breakdown in various kidney diseases. Our aim was to compare the levels of serum MMP-9 in type 2 diabetes mellitus with microalbuminuria and without microalbuminuria. Blood samples were collected from 200 type 2 diabetic subjects out of which 100 subjects were without microalbuminuria and 100 subjects were with microalbuminuria. Serum MMP-9 levels were analysed by ELISA method. MMP-9 levels were significantly higher in patients with microalbuminuria (415.45 ± 175.8) ng/ml compared to patients without microalbuminuria (203.2 ± 26.47) ng/ml. The findings showed that increased levels of serum MMP-9 in type 2 diabetes mellitus may help in early detection of diabetic nephropathy.

KEY WORDS: Matrix metalloproteinase-9 (MMP-9), Diabetic nephropathy, extracellular matrix (ECM), microalbuminuria.

I. INTRODUCTION

The International Diabetes Federation's (IDF) fifth diabetes atlas has released the staggering figures. IDF says India's prevalence of diabetes among 20-79 year olds is 9.2%. India is home to over 61 million diabetic patients - an increase from 50.8 million in the year 2011. By 2030, India's diabetes burden is expected to cross the 100 million mark as against 87 million earlier estimated. The country is also the largest contributor to regional mortality with 983, 000 deaths caused due to diabetes this year [1]. However, due to the increase in type 2 diabetes, the absolute prevalence of DN has increased over the past two decades [2]. A key element in diabetic nephropathy (DN) is changes in the extracellular matrix (ECM) in different components of the kidney. When seen from clinical perspective, the changes in the ECM are important both in diagnostics and for prognostic and therapeutic purpose [3]. Matrix metalloproteinases (MMPs) have been linked to kidney changes in DN, and several MMPs are increased in plasma and urine from patients with diabetes [4]. Matrix metalloproteinases (MMPs) are a family of structurally related zinc-dependent endopeptidases which are capable of degrading all components of extracellular matrix (ECM). MMPs play an important role in the physiologic degradation of ECM, e.g., in tissue morphogenesis, tissue repair and angiogenesis. MMPs also have important functions in pathologic conditions characterised by excessive degradation of ECM [5, 6, and 7]. It has been argued that microalbuminuria becomes a predictor of advanced diabetic nephropathy when it coexists with rising blood pressure and/or falling GFR. And in diabetics it may not be a predictor but only an indicator [8]. In the present study we compared the levels of serum MMP-9 levels in patients with microalbuminuria and without microalbuminuria as serum MMP-9 enzymes play an important role in ECM breakdown in kidney.

II.

MATERIALS AND METHODS

The study group comprised of 200 type 2 diabetic subjects aged 35-70 years. One group comprised of 100 subjects diagnosed with type 2 diabetes mellitus with microalbuminuria and a second group of 100 subjects diagnosed with type 2 diabetes mellitus without microalbuminuria. Ethical clearance was obtained from Central Ethical Committee of the Institution. Patients with hypertension, Chronic renal failure with non-diabetic cause, cardiovascular disease, Liver disease and Infectious disease were excluded from the study. Fasting blood samples were collected from all patients and following biochemical parameters were analysed Fasting plasma glucose (FPG), Urea, Creatinine, HbA1c, total Cholesterol, Triglycerides, HDL cholesterol, LDL cholesterol and VLDL cholesterol. Biochemical analysis was done on Hitachi 902 analyser using kits supplied by Roche, Germany. Urine samples were analysed for microalbuminuria using commercially available immunoturbidometric method assay kits from Transasia. Levels of Matrix metalloproteinase-9 (MMP-9) were measured using commercially available enzyme-linked immunoabsorbent (ELISA) kit [Cell Sciences, USA].

Statistical analysis Data was analysed using Statistical Package for Social Science(SPSS).Results are expressed as mean±SD for normally distributed data and as median and range for skewed data.t-test was done for FBS, Creatinine Cholesterol, Triglycerides, LDL, VLDL, HbA1c and Mann-Whitney test was done for Urea, Matrix metalloproteinase-9(MMP-9), HDL and duration of diabetes. A p-value less than 0.05 was considered to be statistically significant.

III. RESULTS

Our study included a total number of 200 type 2 diabetic subjects. 100 subjects without microalbuminuria 58% were male and 42% were females and 100 subjects with microalbuminuria comprised of 57 % males and 43% females. The mean age of the subjects with microalbuminuria was 55.28±8.10 years and the mean age in subjects without microalbuminuria was 49.47±10.21 years. The mean duration of diabetes in subjects with microalbuminuria was 8.81±5.61 years whereas the duration of diabetes in subjects without microalbuminuria was 2.17±1.81 years which showed a significant long duration of diabetes in subjects with microalbuminuria compared to subjects without microalbuminuria.The mean fasting blood glucose and HbA1c in subjects without microalbuminuria were 182.86 ± 58.07 mg/dl and 8.99 ± 1.33% and with microalbuminuria were 203.14 ± 57.13 mg/dl and 9.22 ± 1.53% respectively. The mean serum urea and creatinine were 24.50 ±5.53 and 0.78 ± 0.16 mg/dl in diabetic subjects without microalbuminuria and 28.15 ± 65 and 0.88 ±0.24mg/dl with microalbuminuria respectively. The above mentioned biochemical parameters were found to be more significantly altered in diabetic subjects with microalbuminuria compared to subjects without microalbuminuria and control subjects. Triglycerides and VLDL cholesterol were also significantly higher in diabetic subjects with microalbuminuria compared to diabetic subjects without microalbuminuria and control subjects. On the other hand HDL cholesterol was found to be significantly lower in subjects with microalbuminuria (39.93±6.946) mg/dl when compared to subjects without microalbuminuria (43.72± 9.043) mg/dl. Total cholesterol and LDL cholesterol did not show any significance between two groups. The serum Matrix metalloproteinase -9 (MMP-9) levels were significantly higher in subjects with microalbuminuria (399.56±149.85) ng/ml compared to those without microalbuminuria (232.27±84.29) ng/ml and control subjects (157.00± 73.55) ng/ml.

Table 1: Comparison of age, biochemical parameters and serum matrix metalloproteinase-9 (MMP-9) levels between control and type 2 diabetic subjects.

| PARAMETER | CONTROL | DIABETIC | |
|----------------------------------|---------------|------------------------------|---|
| | | Without Microalbuminuria | With microalbuminuria |
| n | 50 | 100 | 100 |
| Age (years) | 29.74±8.94 | 49.47±10.21 ^{a**} | 55.28±8.10 ^{(b, c)**} |
| FBS (mg/dl) | 77.08±4.46 | 182.86± 58.07 ^{a**} | 203.14± 57.13 ^{(b, c)**} |
| Urea (mg/dl) | 22.58±3.16 | 24.50 ±5.53 ^{a**} | 28.15 ± 65. ^{(b, c)**} |
| Creatinine (mg/dl) | 0.54±0.14 | 0.78 ±0.16 ^{a**} | 0.88 ± 0.24 ^{(b, c)**} |
| Total Cholesterol (mg/dl) | 195.52±35.71 | 191.94± 47.30 | 195.11±48.63 |
| Triglycerides (mg/dl) | 86.78±32.71 | 137.55± 47.54 ^{a**} | 151.07± 55.43 ^{b**} |
| HDL Cholesterol (mg/dl) | 48.62±9.86 | 43.72± 9.04 ^{a**} | 39.93± 6.94 ^{(b, c)**} |
| LDL Cholesterol (mg/dl) | 128.42±31.45 | 120.37±41.26 | 123.34± 41.91 |
| VLDL Cholesterol (mg/dl) | 17.56±6.44 | 27.10± 9.55 ^{a**} | 30.17± 10.96 ^{b**} , ^{c*} |
| HbA1c (%) | NA | 8.99±1.33 | 9.22± 1.53 ^{c*} |
| MMP-9 (ng/ml) | 157.00± 73.55 | 232.27±84.29 ^{a**} | 399.56±149.85 ^{(b, c)**} |

Data are mean ± SD, **p<0.001, *p<0.05

a= comparison between normal and type2 diabetic patients without microalbuminuria

b=comparison between normal and type2 diabetic patients with microalbuminuria

c=comparison between type2 diabetic patients with and without microalbuminuria

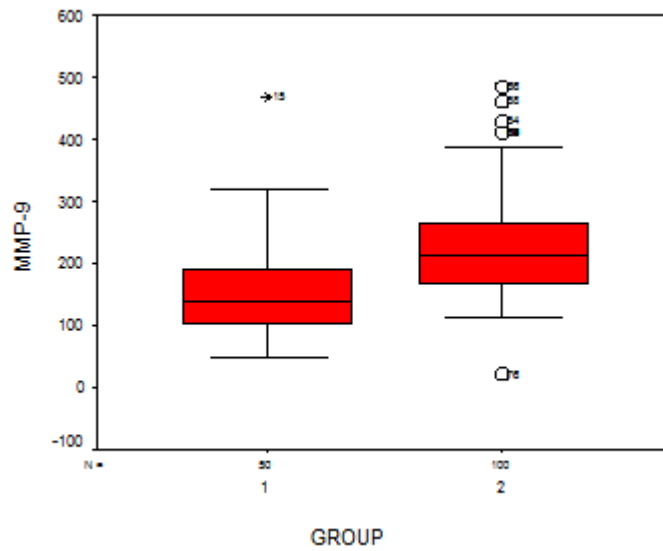


Figure 1: Matrix metalloproteinase-9 (MMP-9) concentration in control (group-1) and in diabetic patients without microalbuminuria (group-2).

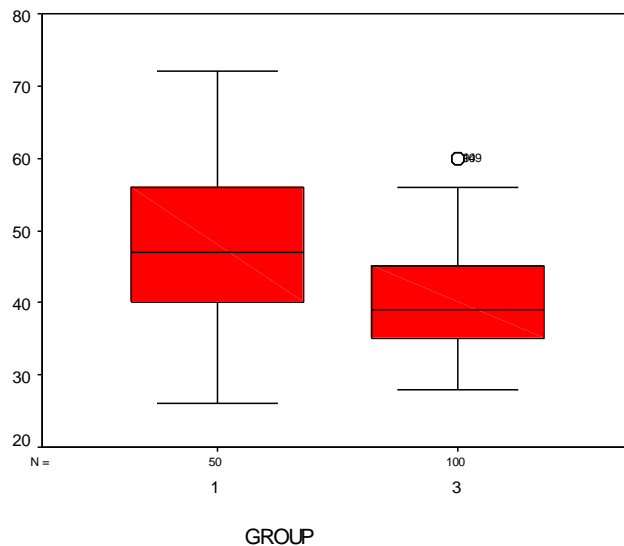


Figure 2: HDL cholesterol concentrations in control (group-1) and in diabetic patients without microalbuminuria (group-2).

IV. DISCUSSION

In the present study serum from subjects with type 2 diabetes was analysed for Matrix metalloproteinase (MMP-9). The results showed that the concentration of Matrix metalloproteinase (MMP-9) in serum was elevated in the subjects with microalbuminuria. Similar studies were done in Taiwanese population and the results showed an increase in plasma MMPs levels and but also suggested that MMP expression and activity were elevated before the onset of complications in diabetic patients. The ECM consists of basement membrane (BM), collagen, elastin, proteoglycans (glycosaminoglycans – GAGs) and hyaluronan, and structural – adhesive glycoproteins. The BM is important for the physical support and cellular attachment of cells and maintenance of their structural integrity, thus allowing cells to create and maintain their own special environment and provides a filtering – sieving mechanism due to the strong anionic charges of its matrix [9]. The major physiologic regulators of ECM degradation in the glomerulus are MMP [10,11]. MMPs are multidomain proteins, consisting typically of a prodomain, a catalytic domain, a hinge region, and in the case of collagenases, gelatinases, and MT-MMPs, a hemopexin domain. Most MMPs are secreted as inactive proMMPs that can be activated by cleavage of the prodomain by proteinases such as plasmin and MT-MMPs, or by oxidation of a

reactive cysteine within the prodomain[5]. Matrix metalloproteinases (MMPs) constitute a group of enzymes that hydrolyze protein components of the extracellular matrix, the subgroup of MMPs known as gelatinases, specifically gelatinase A (MMP-2) and gelatinase B (MMP-9) digest collagen, denatured collagens (i.e., gelatins), laminin, elastin, and fibronectin. They are involved in pathological processes that contribute to fibrotic diseases, tumor progression, and inflammation [12,13and14].Increased MMP-9 activity has been seen in the pathophysiology of diabetes complications. MMP-9 (gelatinase) concentrations are increased in the systemic circulation in type -1 diabetic patients [15]and also in the vitreous of eyes of type 1 diabetic patients with diabetic retinopathy[16]. Elevated retinal levels of MMP-2 and MMP-9 have also been demonstrated in an animal model of diabetic retinopathy [17, 18].Recent animal studies have shown that the expression of gelatinase MMP-9 is associated with activated macrophages[19] and macrophage accumulation and activation in the kidney have been shown to correlate with the onset of diabetic nephropathy[20,21].Studies have shown increased monocyte activation and differentiation to activated macrophages which can further induce inflammatory cytokines, resulting in increased activation and expression of matrix metalloproteinase (MMPs), particularlyMMP-9 [22,23 and 24].A balance between ECM synthesis and degradation is required for maintaining the structural and functional integrity of the glomerulus. Any changes in MMP expression or activity will directly alter the ECM turnover, which may lead to glomerular scarring and a decline in renal function [25, 26].Hyperglycemia has an important role in the initiation of the pathological process; excess glucose intermediaries enter pathways such as the polyol pathway, lead to the activation of protein kinase C (PKC), and result in the formation of advanced glycation end products (AGEs). The primary injury is believed to take place in the glomerular tuft, disrupting the integrity of the basement membrane and subsequently expanding the mesangial volume, leading to glomerular leakage of albumin and an eventual decline in renal function.Since all components of the kidney are exposed to hyperglycemia, changes in tubular and interstitial structures are also seen [27].The reduced binding of heparin sulphate proteoglycan matrix to AGE-modified collagen, laminin, and fibronectin significantly alters the polyanionic nature of BM, affecting the charge-mediated properties of basement membrane (BM) which is a component of ECM [28].ECM markers are not in standard clinical use but are used in clinical trials and in experimental animal studies. We used serum MMP-9 as a marker for our study which may throw new light on the complexity of DN development and may provide us with new tools in the future for early detection of kidney complications in patients with type 1 and type 2 diabetes. If these markers levels show changes with treatment, they may be a useful tool to evaluate the therapeutic interventions.

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