

**MMP-2 dysregulation in Type 1 Diabetes Mellitus**

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**Running Title:** MMP-2 and type 1 diabetes

## **ABSTRACT**

**OBJECTIVE** - Dysregulation of MMP-2 may contribute pathologically to the development of diabetic complications, including diabetic retinopathy and coronary and peripheral arterial disease. Our objective was to explore whether systemic MMP-2 dysregulation could be demonstrated in T1DM, and to determine how MMP-2 concentration relates to disease status.

**RESEARCH DESIGN AND METHODS** - In this cross-sectional study, MMP-2 concentrations and MMP-2 activity were measured in plasma and timed urine samples from 93 T1DM and 50 healthy control subjects, ages 14-40 years. Relationships between MMP-2 concentrations in these biological fluids and patient characteristics (gender, age, duration of T1DM), indices of glycemic control (HbA1c, fasting plasma glucose, CGMS average daily glucose) and measurements of renal function (UAE, GFR) were examined.

**RESULTS** - Urine and plasma MMP-2 concentrations and plasma MMP-2 activity were all significantly elevated in T1DM subjects, compared with control subjects. Urine MMP-2 concentrations, in particular, were correlated with several clinical parameters which infer increased risk for diabetic co-morbidity, and specifically for diabetic nephropathy, including higher HbA1c, longer duration of disease, evidence of renal hyperfiltration, and the presence of microalbuminuria.

**CONCLUSIONS** - Urine and plasma MMP-2 concentrations are dysregulated in T1DM; urinary excretion of MMP-2, in particular, might provide a unique biomarker of diabetes-induced intrarenal pathology.

Matrix metalloproteinases (MMPs) constitute a group of enzymes that hydrolyze protein components of the extracellular matrix (ECM) (1). 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 among other substrates (2), and have been implicated in the pathological processes that contribute to fibrotic diseases, tumor progression and inflammation(1; 3; 4).

Dysregulation of gelatinase activity has also been implicated in the pathophysiology of diabetic complications. Specifically, gelatinase concentrations are increased in the systemic circulation [MMP-9 (5)] and in the vitreous [MMP-2 (6) and MMP-9 (7)] of type 1 diabetes mellitus (T1DM) patients with diabetic retinopathy. Elevated retinal levels of MMP-2 and MMP-9 have also been demonstrated in an animal model of diabetic retinopathy (8). Increased circulating concentrations of MMP-2 have been observed in pediatric patients with T1DM who developed microangiopathy over a 5 year interval (9). Systemic concentrations of MMP-2 and MMP-9, in addition to gelatinase activity levels, are also increased in patients with T2DM and peripheral arterial disease (10).

Data suggesting a link between MMP-2 dysregulation and diabetic nephropathy (DN) also exist but appear contradictory. Rodent models of diabetes reveal decreased expression and/or proteolytic activity of MMP-2 in renal tissues (11-13). High glucose culture conditions also decrease MMP-2 secretion by mesangial cells *in vitro* (14). Human studies, however, yield other results. Expression of membrane-type 5

matrix metalloproteinase (MT5-MMP), a protease which functions to convert pro-MMP-2 to its active form, is up-regulated in human diabetic kidney tissue samples, localized to renal tubules (15). In addition, MMP-2 protein and MMP-2 enzyme activity are elevated in the protein extracts from these kidney tissue samples (15).

Our study has investigated whether MMP-2 dysregulation could be implicated in the pathogenesis of diabetic complications, with focused attention on DN, by measuring concentrations of MMP-2 in the plasma and urine of a large cohort of patients with T1DM, and by examining correlations between observed differences in MMP-2 concentrations and various indices of renal function and glycemic control.

## **RESEARCH DESIGN AND METHODS**

### **Study Design**

Subjects with T1DM and age-matched healthy control subjects, ages 14-40 years, were recruited from clinics at the University of Arkansas for Medical Sciences (UAMS) or Arkansas Children's Hospital (ACH) and surrounding communities. Approval was obtained from the Institutional Review Board of UAMS. Exclusion criteria included: 1) concurrent use of medications that alter MMP activity (i.e., tetracycline, glucocorticoids); 2) T2DM; 3) history of other chronic systemic inflammatory or autoimmune disease or malignancy; 4) pregnancy; and 5) concurrent ketonuria. Subjects were also excluded if the baseline evaluation revealed any site of active infection. Control subject data were excluded from final analyses if a subject was incidentally found to have albuminuria.

Two study visits were conducted 3-5 days apart. Visit 1 included: 1) a medical history and physical examination; 2) ascertainment of demographic data, (age, gender, race, duration of T1DM); 3) fasting venipuncture laboratory measurements of plasma glucose (FPG), HbA1c, C-peptide and serum creatinine (Cr); and 4) insertion of a continuous glucose monitor (CGM) sensor. (Medtronic Minimed® CGMS, MMT-7102, Northridge, CA). At visit 2, 24-hour urine samples collected between visits 1 and 2 were returned, 3-5 day CGMS data were downloaded and FPG, HbA1c, C-peptide and serum Cr measurements were repeated. Urine collections were used for measurement of a timed microalbumin excretion rate. Estimated CrCl and GFR [using the Cockcroft Gault and MDRD study equations, respectively (16)] were also calculated.

### **Clinical Assays**

FPG and C-peptide (using a immunochemiluminescence assay; normal range, 0.4-3.3 ng/ml for subjects 10-16 years of age and 0.9-4.0 ng/ml for > 16 years of age) were measured by the UAMS GCRC Core Laboratory. HbA1c, serum Cr, complete urinalysis (visit 1), and urinary albumin and Cr concentrations (24-hour urine collection, visit 2) were measured by LabCorp (Dallas, TX).

### **MMP-2, TIMP-1 and TIMP-2 Measurements**

MMP-2 concentrations were measured in plasma (5 µl, diluted 1:10) and in timed urine collections (50 µl, undiluted) using the Fluorokine® MultiAnalyte Profiling (F-MAP) assay from R & D Systems, Inc. (Minneapolis, MN). Specimens were analyzed in duplicate on a Luminex® 100™ Bioanalyzer (Luminex

Corp. Austin, TX) as previously described (minimal detection limit, 25.4 pg/ml) (17; 18).

Plasma MMP-2 activity was measured using the Matrix Metalloproteinase-2 Biotrak Activity Assay System® (GE Healthcare, Piscataway, NJ). This assay has a range of 0.75-12 ng/ml and sensitivity of 0.19 ng/ml. This assay has not been validated for use in urine; while the assay of plasma samples yielded informative results, results from all but 6 urine samples were < 0.19 ng/ml.

TIMP-1 and TIMP-2 concentrations in plasma were measured using the Quantitative Human TIMP-1 and TIMP-2 immunoassays from R & D Systems. (Minimal detectible concentrations: TIMP-1, 0.08 ng/ml; TIMP-2, 0.01 ng/ml)

### **Statistical Analysis**

A sample size target of 50 per group would provide approximately 80% power to detect a between group difference for MMP-2 of 0.5 SDs. This power is further increased by a larger T1DM cohort. Results for plasma MMP-2, FPG, HbA1c, C-peptide and serum Cr obtained from visits 1 and 2 were averaged. Exploratory data analyses, (summary statistics, scatter plots, box plots) were used to examine the distribution of and relationship between variables. Because variables were not normally distributed, non-parametric statistical analyses (Mann-Whitney tests) were used. Data are presented as median values with a minimum to maximum range and as mean ± SEM or ± SD, as indicated. Statistical significance was defined as  $p < 0.05$ . We also used classification tree analysis to find “cut-point” levels for MMP-2 concentrations which best indicated those with T1DM (19; 20). For relative risk analyses, odds

ratios and 95% confidence intervals are reported.

## RESULTS

**Baseline characteristics:** Fifty control subjects and 93 subjects with T1DM were evaluated. Control and T1DM groups were comparable with respect to gender (52% vs. 47% female, respectively); racial distribution (84% vs. 91% Caucasian) and baseline BMI ( $25.2 \pm 4.8$  vs.  $24.8 \pm 4.4$  kg/m<sup>2</sup>). The diabetic sub-group was slightly younger (Control:  $24.1 \pm 6.8$  years vs. T1DM:  $19.3 \pm 6.3$  years;  $p < 0.001$ ). Therefore, additional analyses, as detailed below (see, *Effects of age*) were conducted to examine any potential effect of age on MMP-2 results.

Expected differences between the control and T1DM subgroups were confirmed by baseline measurements of FPG ( $81.2 \pm 6.5$  vs.  $155.3 \pm 65.1$  mg/dl, respectively;  $p < 0.001$ ), HbA1c ( $4.97 \pm 0.3$  vs.  $8.49 \pm 1.85\%$ ;  $p < 0.001$ ), C-peptide ( $0.84 \pm 0.49$  vs.  $0.14 \pm 0.14$  ng/ml;  $p < 0.001$ ), and 3-5 day average glucose by CGMS ( $88.0 \pm 10.2$  vs.  $170.5 \pm 47.7$  mg/dl;  $p < 0.001$ ). Fifty-nine percent of T1DM subjects were being treated with insulin injections; 41% were utilizing insulin pumps.

A history of hypertension (n=4), retinopathy (n=5), nephropathy (n=3) or neuropathy (n=0) was ascertained by self-report. During the baseline physical examination and as a result of the 24-hour urine collection, an additional 3 subjects demonstrated evidence of sensory neuropathy, and 9 subjects were identified as having microalbuminuria. (Dilated fundoscopic examination was not a component of the protocol.) Among T1DM subjects, the median urinary albumin excretion (UAE) was 12.2 mg/g Cr and microalbuminuria (MA: 30-299 mg/g Cr) was present in 12 subjects (MA

range: 30.4-280.3 mg/g). No subjects in this study displayed macroalbuminuria ( $\geq 300$  mg/g).

## **MMP-2, TIMP-1 and TIMP-2 concentrations in biological fluids:**

MMP-2 concentrations were significantly elevated in the urine and plasma of T1DM subjects, compared with controls. The increase in urine MMP-2 was apparent whether analyzed as: 1) an undiluted urine concentration [Control:  $48.4 \pm 10.2$  pg/ml (mean  $\pm$  SEM) vs. T1DM:  $184.9 \pm 31.3$ ,  $p < 0.001$ ]; 2) a urine MMP-2 to urine creatinine ratio (Table 1); or 3) total MMP-2 excretion per day, for the 135 of 143 subjects who provided a complete 24-hr urine sample (Table 1). When all study subjects were analyzed together, a weak correlation between plasma MMP-2 and urine MMP-2:Cr values was appreciated (Table 2). Similar to MMP-2 protein concentrations, MMP-2 activity was also significantly increased in the plasma of T1DM subjects (Control (mean  $\pm$  SD):  $193.3 \pm 163.0$  ng/ml; T1DM:  $292.7 \pm 190.2$ ,  $p < 0.005$ ). Moreover, MMP-2 activity in plasma was correlated both with plasma MMP-2 concentrations ( $R=0.453$ ,  $p < 0.001$ ) and urine MMP-2:Cr values ( $R=0.331$ ,  $p < 0.001$ ). No gender differences were appreciated for urine or plasma MMP-2 values.

The activity of MMPs is tightly regulated by a family of specific tissue inhibitors of metalloproteinases (TIMPs 1-4); MMP-2 preferentially binds TIMP-2, while MMP-9 preferentially binds TIMP-1. To determine whether the increase in plasma MMP-2 concentrations was offset by a simultaneous upregulation of inhibitor, plasma concentrations of TIMP-1 and TIMP-2 were measured. Neither TIMP-1 nor TIMP-2 differed between the T1DM and control groups [TIMP-1:  $88.7 \pm 30.2$  vs.  $93.2 \pm 29.0$  ng/ml, respectively;

TIMP-2:  $66.2 \pm 25.2$  vs.  $66.5 \pm 19.5$  ng/ml, respectively].

**Relationship of MMP-2 to age:** When age was examined as a continuous variable, values for urine MMP-2:Cr and total MMP-2/day, but *not* plasma MMP-2, were very weakly inversely correlated with age (MMP-2:Cr,  $R = -0.181$ ,  $p < 0.05$ ; total MMP-2/day:  $R = -0.202$ ,  $p < 0.05$ ). However, when MMP-2 values were compared in subjects who were  $\leq 18$  years of age as a group ( $n=64$ ) with those who were 19-40 years of age ( $n=79$ ), plasma MMP-2 concentrations were significantly higher among the younger cohort, while urine MMP-2:Cr and total MMP-2/day displayed a trend toward higher values in younger subjects but did not reach statistical significance. To exclude the possibility that the younger mean age of the T1DM subgroup alone accounted for the increase in MMP-2 values seen in T1DM, comparisons were made between the subset of T1DM and control subjects who were 19-40 years of age (Adult subgroup: Control,  $n = 42$ ; age (mean  $\pm$  SEM);  $25.8 \pm 0.9$  years. T1DM,  $n = 37$ ; age;  $24.8 \pm 1.1$  years. Between group age comparison,  $p = 0.3$ ). A similar comparison was made between the subset of T1DM and control subjects who were  $\leq 18$  years of age (Adolescent subgroup: Control,  $n = 8$ ; age,  $15.5 \pm 0.3$  years. T1DM,  $n = 56$ ; age,  $15.6 \pm 0.2$  years). Consistent with differences reported for the entire study population, for subjects 19-40 years, urine concentrations of MMP-2, as well as urine MMP-2:Cr and total MMP-2/day were significantly higher among subjects with T1DM (Table 1). For subjects 14-18 years of age, a trend toward higher values in the T1DM subjects was also evident, though these differences did not attain statistical significance. Plasma

MMP-2 concentrations were significantly higher in T1DM subjects compared with control subjects for the entire study population and for the adolescent subgroup (Table 1).

**Relationship of MMP-2 to duration of disease:** Among subjects with T1DM, values for urine MMP-2:Cr and total MMP-2/day were elevated in subjects with a duration of disease of  $> 3$  years ( $n=70$ ), compared to those with a  $\leq 3$  year history of T1DM ( $n=23$ ). Specifically, for subjects with  $> 3$  years vs.  $\leq 3$  years, median MMP-2:Cr concentrations were  $113.4$  vs.  $29.8$  pg/g, respectively, ( $p < 0.05$ ) and median total MMP-2/day values were  $151,766$  vs.  $45,466$  pg/day ( $p < 0.05$ ). This difference was appreciated despite the fact that the mean age of T1DM subjects with a longer duration of disease was slightly older ( $20.2 \pm 0.8$  years) than the mean age of T1DM subjects with a  $\leq 3$  year history of T1DM ( $16.7 \pm 0.6$  years). In contrast, plasma MMP-2 concentrations were not different between those subjects with  $> 3$  vs.  $\leq 3$  years of T1DM.

**Relationship of MMP-2 to glycemic control:** Values for urine MMP-2:Cr and total MMP-2/day were correlated positively with HbA1c and CGMS average daily glucose, and less strongly correlated with FPG (Table 2). Moreover, urine MMP-2:Cr and total MMP-2/day were significantly higher in T1DM subjects whose HbA1c was  $\geq 8.25\%$  compared to  $< 8.25\%$  ( $p < 0.001$  for both urine values), and in subjects whose CGMS average daily glucose was  $\geq 140$  mg/dl vs.  $< 140$  mg/dl ( $p < 0.001$  for both urine values). Urine MMP-2 relationships also differed as a function of gender; specifically, correlations with FPG, HbA1c and CGMS glucose were stronger in female subjects

(Table 2). In contrast, no correlations between *plasma* MMP-2 concentrations and HbA1c, FPG, or CGMS average daily glucose were demonstrated. However, *plasma* MMP-2 *activity* was weakly correlated with HbA1c ( $R = 0.249$ ,  $p = 0.004$ ) and CGMS glucose ( $R = 0.318$ ,  $p < 0.001$ ).

**Relationship of MMP-2 to renal function:**

For subjects with T1DM, values for urine MMP-2:Cr and total MMP-2/day were correlated positively with UAE, glomerular filtration rate (GFR) and creatinine clearance (CrCl) (Table 2). Again, these relationships were strongest among females. Similar correlations with UAE, GFR or CrCl were not demonstrated for *plasma* MMP-2 concentrations, though weak correlations were demonstrated between MMP-2 *activity* in plasma and UAE ( $R = 0.278$ ,  $p = 0.001$ ) or GFR ( $R = 0.278$ ,  $p < 0.001$ ).

To examine the relationship between urinary concentrations of MMP-2 and predictors of diabetic nephropathy, values for urine MMP-2:Cr and total MMP-2/day were compared in diabetics with UAE of  $< 30$  mg/g Cr ( $n=76$ ), and those with microalbuminuria ( $\geq 30$  mg/g;  $n = 12$ ). A statistically significant increase was appreciated in urine MMP-2:Cr values (UAE  $< 30$  (mean  $\pm$  SEM);  $169.9 \pm 23.7$  pg/g; UAE  $\geq 30$ :  $622.4 \pm 172.3$ ;  $p = 0.02$ ) and in total MMP-2/day (UAE  $< 30$ :  $252,892 \pm 41,532$  pg/ml; UAE  $\geq 30$ :  $678,663 \pm 177,584$ ;  $p = 0.003$ ). Urine MMP-2:Cr values were also compared by subset for both control and T1DM subjects with UAE of : 1)  $< 10$  mg/g; 2)  $10-30$  mg/g; or 3)  $> 30$  mg/gm (T1DM subjects only, as dictated by study exclusion criteria for control subjects). Mean ( $\pm$  SEM) MMP-2:Cr values were as follows: 1) UAE  $< 10$ : Control:  $31.7 \pm 7.7$  vs. T1DM:  $108.5 \pm 37.6$ ;  $p = 0.01$ ; 2)

UAE  $10-30$ : Control:  $81.6 \pm 26.7$  vs. T1DM:  $205.2 \pm 31.1$ ;  $p = 0.01$ ; 3) UAE  $> 30$ : T1DM:  $718.4 \pm 171.8$  pg/g;  $p < 0.001$  for comparison with both control subsets. A progressive increase in urine MMP-2:Cr concentrations was appreciated among T1DM subjects, with the increase in MMP-2 concentration noted even when the UAE was not yet clinically abnormal.

Urine MMP-2:Cr values were further examined among the subset of T1DM subjects who demonstrated evidence of hyperfiltration. Urine MMP-2:Cr values were higher ( $p < 0.05$ ) in those subjects with T1DM and a GFR  $> 130$  ml/min/1.73 m<sup>2</sup> ( $n = 25$ ;  $405.4 \pm 100.0$  pg/g), compared to those with a GFR  $\leq 130$  ( $n = 68$ ;  $158.4 \pm 22.5$  pg/g) or a CrCl  $> 130$  ml/min ( $300.2 \pm 63.8$ ) compared to those with a CrCl  $\leq 130$  ( $165.3 \pm 29.7$ ). Among those T1DM subjects with a GFR  $> 130$  ml/min/1.73 m<sup>2</sup>, an increase in urine MMP-2:Cr values was appreciated for those with microalbuminuria (UAE  $< 30$ :  $229.7 \pm 61.1$ ; UAE  $\geq 30$ :  $853.5 \pm 218.3$ ;  $p < 0.05$ ).

**Multiple regression analysis:** Tree analysis demonstrated that if the urine MMP-2:Cr value was above 65 pg/g, or the urine total MMP-2/day value was above 66,720 pg/ml, markers for elevated risk, such as hyperglycemia, hyperfiltration and microalbuminuria, could be established (Table 3).

**CONCLUSIONS**

We have demonstrated a marked increase in urinary excretion of MMP-2 in T1DM subjects compared with healthy control subjects. Moreover, urine MMP-2 concentrations were correlated with several known risk factors for diabetic comorbidity in general and DN in particular, including elevated HbA1c, longer duration of T1DM, evidence of renal hyper-

filtration, the presence of microalbuminuria, and female gender (21). We have also demonstrated a significant increase in plasma concentrations of MMP-2 and MMP-2 activity in T1DM. No concurrent increase in TIMP-1 or TIMP-2 concentrations was detected, confirming a specific increase in circulating gelatinase activity in T1DM.

A weak inverse correlation between urine MMP-2 concentrations and age was also noted, consistent with our previous report demonstrating that urinary MMP-2 activity in healthy pubertal subjects is increased over values observed among adults (22). These findings highlight the necessity of utilizing age-matched study cohorts for analysis of gelatinase dysregulation in T1DM.

Hyperglycemia-induced upregulation of MMP-2 has been demonstrated in arterial vasculature *in vivo* (23), and in various vascular components *in vitro*, including endothelial cells (24), macrophages (24), and vascular smooth muscle cells (25). Therefore, the increase in plasma MMP-2 concentrations and MMP-2 activity in T1DM could be indicative of increased vascular synthesis of MMP-2, or could reflect the systemic transport of MMP-2 which is being over-produced in other tissues. Several possibilities exist to account for the increased urinary concentrations of MMP-2 including: 1) hyperfiltration of circulating MMP-2 due to increased glomerular basement membrane permeability; 2) diabetes-induced changes in renal tubular handling of the MMP-2 filtered load; or 3) increased production or secretion of MMP-2 by renal tissues, in response to hyperglycemia.

It is notable that urinary concentrations of MMP-2 were correlated with higher HbA1c values, higher average

glucose values and with a > 3 year duration of disease. In addition, urine MMP-2 concentrations were highest in those subjects demonstrating renal hyperfiltration and/or microalbuminuria. Because these parameters are risk factors associated with the development and/or progression of DN, in particular (26), one could speculate that urinary secretion of MMP-2 might reflect intra-renal MMP-2 dysregulation, contributing to the pathophysiology of nephropathy. This hypothesis could only be confirmed by longitudinal, histological data demonstrating tissue-specific dysregulation of MMP-2 which precedes clinical nephropathy. However, intra-renal MMP-2 expression or MMP-2 activity is increased in other examples of renal pathology (27; 28). In addition, MMP-2 and MMP-9 are produced by cultured glomerular podocytes and podocyte MMP production in culture can be modified by numerous growth factors, cytokines, and by high ambient glucose levels (29-31). Moreover, renal expression of MMP-2 is both absolutely necessary and sufficient for inducing the transformation of renal tubular epithelium to the myofibroblastic phenotype, a critical step heralding the development of renal interstitial fibrosis in conditions like diabetic nephropathy (32; 33). In keeping with this possibility is the fact that while the between group comparisons of MMP-2 filtered load between control and T1DM subjects demonstrated an average ~ 2-fold increase in T1DM, urinary MMP-2 excretion in the T1DM group was > 4-fold higher. Consequently, an increase in renal production of MMP-2 may contribute, in part, to the increase in urinary MMP-2 concentrations.

Certain limitations of our study must be acknowledged. This study does not establish a tissue source for the

increase in plasma and urine MMP-2 concentrations, nor does it establish a causal link between MMP dysregulation and the onset of renal lesions. In addition, any relationship of these results to other diabetes complications cannot be established, as the incidence of hypertension, retinopathy and neuropathy were only ascertained by history, and not by clinical documentation.

In conclusion, plasma and urine MMP-2 concentrations, and plasma MMP-2 activity, are elevated in T1DM. Because higher MMP-2 concentrations were associated with clinical parameters

which are known to confer increased risk for diabetic co-morbidity, including diabetic nephropathy, we raise the possibility that upregulation of MMP-2 activity might play a role in the pathogenesis of diabetic complications.

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**Table 1: MMP-2 Concentrations**

	<u>Urine MMP-2 concentrations</u>						<u>Plasma MMP-2 Concentrations</u>		
	MMP-2:Cr (pg/g Cr)			Total MMP-2/day (pg/day)			MMP-2 (pg/ml)		
	All	19-40 yrs	≤ 18 yrs	All	19-40 years	≤ 18 yrs	All	19-40 years	≤ 18 yrs
<b><u>Control</u></b>	54.1 ± 13.1 <sup>1</sup>	52.5 ± 9.3	53.2 ± 10.6	75,126 ± 10,437	70,077 ± 8573	101,004 ± 48,048	191,241 ± 14,470	196,215 ± 15,677	165,126 ± 38,578
Median	25.6	31.4	47.1	53,340	53,340	60,265	158,792	167,138	116,688
Range	8.9 - 618.6	9.4 – 323.94	8.8 – 109.0	11,430 – 430,840	11,430 – 271,902	13,970-430,840	66,132–552,713	66,132–552,713	82,457–358,751
n	49	41	8	49	41	8	50	42	8
<b><u>T1DM</u></b>	242.2 ± 36.9 <sup>1</sup>	171.6 ± 28.0	260.0 ± 51.6	312,302 ± 45,828	284,088 ± 71,779	330,750 ± 59,936	273,986 ± 19,947	214,388 ± 18,681	313,363 ± 29,724
Median	72.6	147.5	76.6	116, 805	97,104	122,883	229,650	195,910	267,426
Range	13.24 - 1644.1	10.7 – 657.9	9.9 – 1746.9	7620 – 2,119,595	26,670 – 1,742,205	7620 – 2,119,594	69,612-1,329,410	72,920 – 559,238	69,612-1,329,410
n	93	37	56	86	34	52	93	37	56
p-value	< 0.001	< 0.0001	NS	< 0.001	< 0.0001	NS	< 0.005	NS	< 0.05
Total n	142	78	64	135	75	60	143	79	64

<sup>1</sup> Mean ± SEM

**Table 2: Plasma and Urine MMP-2 concentrations: Correlation with glycemic control and renal function**

	P MMP- 2 <sup>1</sup>	U MMP-2 <sup>2</sup>	U Total MMP-2	FPG	HbA1c	CGMS	GFR	CrCL	UAE
	pg/ml	pg/ml	pg/ml	mg/dl	%	mg/dl	ml/min per1.73 m <sup>2</sup>	ml/min	mg/gm
<b>Urine MMP-2:Cr</b>									
<b>All (n)</b>	142	142	135	141	142	138	142	142	137
<b>R</b>	.190	.781	.677	.264	.540	.421	.453	.315	.555
<b>p-value</b>	0.024	< 0.001	< 0.001	0.002	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
<b>T1DM (n)</b>		93	86		93	89	93	93	88
<b>R</b>		.767	.645		.500	.296	.404	.301	.532
<b>p-value</b>	NS <sup>3</sup>	< 0.001	< 0.001	NS	< 0.001	0.005	< 0.001	0.003	< 0.001
<b>Control (n)</b>		49	49	49		49			49
<b>R</b>		.654	.368	.301		.289			.383
<b>p-value</b>	NS	< 0.001	0.009	0.036	NS	0.044	NS	NS	0.007
<b>Female (n)</b>		69	68	69	69	69	69	69	68
<b>R</b>		.722	.729	.450	.630	.537	.562	.467	.674
<b>p-value</b>	NS	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
<b>Male (n)</b>		73	67		73	69	73		69
<b>R</b>		.354	.792		.423	.272	.349		.326
<b>p-value</b>	0.002	< 0.001	< 0.001	NS	< 0.001	0.024	0.002	NS	0.006

<sup>1</sup> Plasma MMP-2; <sup>2</sup>Urine MMP-2; <sup>3</sup>NS = not significant at p < 0.05.

**Table 3: Relative Risk Calculations**

<b>Parameter</b>	<b>HbA1c</b>	<b>HbA1c</b>	<b>CGMS Ave. BG</b>	<b>Duration of DM</b>	<b>GFR</b>	<b>UAE</b>
	<b>&gt; 7.5% or not</b>	<b>&gt; 8.25% or not</b>	<b>&gt; 140 mg/dl or not</b>	<b>&gt; 3 yrs or not</b>	<b>&gt;130 ml/min/1.73m<sup>2</sup> or not</b>	<b>&gt; 30 mg/g or not</b>
<b>A</b>	4.62 (2.27, 9.42) <sup>1</sup>	4.97 (2.24, 11.83)	6.43 (3.06, 13.52)	2.49 (0.95, 6.51)	4.55 (1.69, 12.24)	NA <sup>2</sup>
<b>B</b>	4.25 (2.02, 8.95)	4.78 (1.99, 11.46)	3.71 (1.79, 7.69)	4.24 (1.51, 11.94)	3.34 (1.15, 9.67)	10.48 (1.31, 83.84)
<b>C</b>	7.05 (3.28, 15.14)	6.88 (3.09, 15.31)	6.96 (3.22, 15.08)	2.28 (0.85, 6.12)	4.66 (1.84, 11.80)	21.74 (2.71, 174.09)

**A = Urine MMP-2:Cr > 65 pg/g; B = Urine Total MMP-2 > 66,720 pg/day; C = Urine MMP-2:Cr > 65 pg/g and Total MMP-2 > 66,720 pg/day**