Matrix Metalloproteinases in Subjects With Type 2 Diabetes Mellitus: Pattern of MMP-2 and MMP-9 Profile in Diabetes Mellitus Type-2 Patients

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Abstract: Dysregulation of matrix metalloproteinases (MMPs) and tissue inhibitors of matrix metalloproteinases (TIMPs) may contribute to the development of Cardiovascular Diseases. The aim of this study was to investigate if the levels of MMPs in blood samples are potential markers of early development of diabetes mellitus type-2. The serum levels of both MMP-9 and MMP-2 were significantly higher in subjects with type 2 diabetes, compared to controls. These results show that those patients with DM-2 as the risk factor for generation as well as for cardiovascular complications (results were not shown). The MMP analysis of serum from a limited number of patients (n=10) with type-2 diabetes suggest that such analysis may be potentially useful as markers in studies of people at risk of progression to chronic cardiovascular diseases and may be a viable marker of the prognosis of the several pathological conditions associated with diabetes.

Keywords: Matrix metalloproteinase; Diabetes type-2; cardiovascular disease; blood

I. Introduction

Abnormal carbohydrate metabolism is an important and still growing social problem. For some years it has been recognized that cardiovascular complications are the leading cause of increasing premature mortality in patients with type 2 diabetes mellitus [1], [2], [3], [4]. Recently it has been proven that matrix metalloproteinases (MMPs) play an important role in atherosclerosis and rebuilding of the vascular wall [5]. The onset and progression of complications are delayed in patients with good glycemic control; hyperglycemia is thought to be an important regulator of vascular lesion development [3]. Recent studies indicate that elevated glucose concentrations can induce dysfunction of several intracellular signal transduction cascades, generation of reactive oxygen species (ROS), and accumulation of advanced glycation end products (AGEs) [4], [5]. However, the underlying mechanisms between hyperglycemia and vascular disease remain unclear.

Matrix metalloproteinases (MMPs) are members of a family of Zn²⁺- and Ca²⁺-dependent endopeptidases, which are essential for cellular migration and tissue remodeling in both physiological and pathological conditions [6]. MMPs are secreted by many types of cells as proenzymes. Increased matrix degradation by MMPs within the atherosclerotic plaque has been implicated as one of the key factors that leads to plaque instability, and consequently to cardiovascular events [6], [7], [8], [9], [10], [11], [12]. Furthermore, MMP activity has been correlated with clinical manifestations of unstable angina, plaque rupture, and the development of abdominal aortic aneurysms [13], [14], [15]. We studied the gelatinolytic activity of plasma in patients of DM. Our findings indicate that the activity of MMP-9 and MMP-2 is preferentially enhanced in blood plasma of hyperglycemic patients.

Hyperglycemia directly or indirectly (e.g., via oxidative stress or advanced glycation products) might increase MMP expression and activity in large vessels [16]. Within atherosclerotic plaques an imbalance between MMPs and TIMPs may induce matrix degradation, resulting in an increased risk of plaque rupture. Furthermore, because MMPs enhance blood coagulability they may play a role in acute thrombotic blockage of vessels and consequent cardiovascular complications [17], [18], [19], [20]. Despite these data, the role of MMPs in development of complications of type 2 diabetes (DM) is not fully understood [17], [18], [19], [20]. In our study, we have therefore endeavoured to compare the activities of selected matrix metalloproteinases (MMP-2, MMP-9) in subjects with diabetes and in non-diabetic controls of serum in chronic hyperglycemia.

II. Materials and Methods

A. Laboratory Analyses

All subjects were studied as part of an ongoing regular, investigation from a local based laboratory. Twelve (10 patients and 2 healthy persons) individuals were selected at random from a local general pathological laboratory.
Subjects with diabetes, hypercholesterolemia, renal disease (defined as a clinical history), a history of cardiovascular diseases (defined as a clinical history), as were subjects receiving any medication. Citrated venous blood was obtained and immediately centrifuged at 1,000g and 4°C for 20 min. Plasma was aliquoted and stored at -15°C for a batch analysis of MMPs by zymography and SDS-PAGE analysis. Total blood glucose was determined using standard methodology by the laboratory.

**B. Determination of Protein**
Protein concentrations were estimated by BCA protein assay kit using bovine serum albumin as the standard.

**C. Polyacrylamide Gel Electrophoresis**
SDS-polyacrylamide gel electrophoresis (SDS-PAGE) (12%) was performed according to the procedure of Laemmli [21] using a minigel system apparatus. Samples (50 μg of protein) were diluted in SDS-containing sample buffer without β-mercaptoethanol (under nonreducing condition) prior to being loaded. Electrophoresis was performed at room temperature at 16mA during stacking and 18mA per plate during resolving until the bromophenol blue dye reached the bottom of the gel. Protein containing bands were visualized by coomassie staining method.

**D. Zymogram of Protease Activity**
Polyacrylamide minigels (12%) were cast containing 0.1% gelatin. Gelatin solution was made up as 2% stock in distilled water and dissolved by heating. Samples (50 μg of protein) was applied to the gel in standard SDS loading buffer containing 0.1% SDS but lacking β-mercaptoethanol, it was not boiled before loading [22]. The gels were run in 4°C at 16mA per gel during stacking and 18mA per gel during resolving until the dye front reaches at the end of the gels. Then soaked the gels in 200 ml of 2.5% (v/v) Triton X-100 in distilled water in shaker for 1 hour with one change after 30 minute at 20°C to remove SDS. Next the gels were soaked in the assay buffer (50mM Tris, 200mM NaCl, 10mM CaCl₂, 0.05% Brij 35, pH 7.5) for 12 hours at 37°C and then stained with Coomassie Brilliant Blue-R 250 in 50% methanol and 10% acetic acid and this was followed by washing with distilled water for one minute. The clear zone of lysis against a dark background indicates enzyme activity. The zones of gelatin lysis increased with increasing dose of enzyme-containing samples and time of gel incubation.

**E. Activation of Progelatinase Studies**
These were carried out as described by Murphy *et al* [23]. Progelatinase incubated with HgCl₂ (2mM) was analyzed for activity and molecular mass change. The enzyme was activated at 37°C with a final concentration of 2mM HgCl₂ from a 10X stock solution in 50mM NaOH in calcium assay buffer (CAB) composed of 50mM Tris-HCl, pH 7.5, 200mM NaCl, 10mM CaCl₂, 0.05% Brij 35 for 2 hours. Control enzyme received an equal amount of NaOH without HgCl₂. These studies were done in zymogram.

**F. Inhibition Studies**
The inhibitors EDTA (20mM) and PMSF (1mM) were added to the triton X-100 soaked gels for 1 hour, then incubated with reaction buffer CAB (50mM Tris, 200mM NaCl, 10mM CaCl₂, 0.05% Brij 35 pH 7.5) containing these inhibitors for 12 hours at 37°C. These were then stained with Coomassie Brilliant Blue R-250 followed by washing with distilled water for one minute as above. The clear zone of lysis against the dark Coomassie background indicates protease activity.

### III. Results

**Table 1. Shows the correlations of Serum Glucose Levels and MMP Levels in Diabetes Compared to Healthy Person**

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<th>P-1</th>
<th>P-2</th>
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<th>P-8</th>
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</thead>
<tbody>
<tr>
<td><strong>Fasting Blood Sugar (FBS) (mg/dl)</strong></td>
<td>85</td>
<td>86</td>
<td>150</td>
<td>148</td>
<td>156</td>
<td>162</td>
<td>175</td>
<td>155</td>
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<td>155</td>
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<td>165</td>
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<tr>
<td><strong>2-hr Postprandial Blood Sugar (mg/dl)</strong></td>
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<td>112</td>
<td>217</td>
<td>214</td>
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<td>234</td>
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<td>S</td>
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*N: Normal/Healthy Person; P: Diabetic Patient
NS: Not Significant; S: Significant

**Figure 1. 12% SDS-PAGE profile of serum samples of normal (N) individuals and diabetic patients (P)**

92 kDa Gelatinase (MMP-9)
72 kDa Gelatinase (MMP-2)
IV. Discussion

The blood glucose level of serum samples from normal and diabetic patients were compared in Table 1 both in fasting and two hrs after meals. We have also given a SDS-PAGE profile of the same samples (Fig. 1). Furthermore we have seen the gelatinase activities (Figs. 2, 3 & 4) in the serum samples of diabetic patients compared to normal healthy individuals at 92kDa and 72 kDa regions which are specifically inhibited by the metalloproteinase inhibitors EDTA (Fig. 3). From this results it may be concluded that the gelatinase activities which we have seen are nothing but of different MMPs (MMP-2 & MMP-9). When we compared the gelatinase activities (Figs. 2,3, & 4) and the different patients of sugars levels (Table-1), it has been seen that there is correlation between the MMPs level and of the blood glucose level of serum samples of diabetic patients. MMPs activities are significantly higher in the serum samples of diabetic patients rather than the non-diabetic individuals (Figs. 2,3,4). The serum levels of both MMP-9 and MMP-2 were significantly higher in subjects with type 2 diabetes, compared to controls (Figs. 2,3,4). These results show that those patients with DM-2 as the risk factor for generation as well as for cardiovascular complications (results were not shown). The MMP analysis of serum from a limited number of patients (n=10) with type-2 diabetes suggest that such analysis may be useful as markers in studies of people at risk of progression of several pathological conditions associated with diabetes. The MMP analysis of serum from a limited number of patients (n=10) with type-2 diabetes suggest that such analysis may be potentially useful as markers in studies of people at risk of progression to chronic
cardiovascular diseases and may be a viable marker of the prognosis of the several pathological conditions associated with diabetes.

V. References


