Alteration on MMP-2 Levels in Women Diabetes Patients in Basrah

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Abstract. Diabetes nephropathy (DN) is characterized by gathering of extracellular matrix (ECM) in the kidney. Extracellular matrix (ECM) degradation could influence by high glucose concentration through the activities of MMPs. ECM gathering is engaged in the pathogenesis of diabetic nephropathy. Matrix metalloproteinases (MMPs) are types of enzymes, which are mostly parted in ECM homeostasis. In normal kidneys, Gelatinase A (MMP-2) are the most important MMPs, so they may be studied as earlier and more specific markers for DN. This study aimed to evaluate the diagnostic value of MMP-2 as new indicators in diabetic patients. The study included 20 normal people as a control group and 67 samples collected from Al-Faihaa center for diabetes. Lipids profile were measured and MMP-2 levels detected by ELISA technique. The results showed cholesterol levels were at normal rates in control group, and at low risk levels in diabetes groups. Also the low risk levels of triglycerides were detected in diabetes groups, Furthermore, the risk values of HDL-Cholesterol was detected in group 2 significant increase in the MMP-2 concentration in patients with age 30 years or older which could be as an early sign of diabetes disease.

Keywords: Women Diabetes; MMP-2 alteration; Extracellular matrix, lipid profiles, MMP-2 values

1. Introduction

Matrix metalloproteinases (MMPs) are calcium- and zinc dependent endoproteinases of nonspecific affinity to numerous compounds of extracellular matrix (ECM). Once synthesized, MMPs are secreted as inactive proenzymes that are activated by other MMPs or plasmin [1] it is a very important one kind of antibodies. MMP-2 (72 kDa) is a constitutively synthesized by fibroblasts, macrophages, epithelial and endothelial cells of mesenchymal origin [1]. MMPs are zinc-containing endopeptidases that are involved in remodeling the ECM and are crucial for tissue development and homeostasis [2]. The gelatinases, MMP-2 and MMP-9, are critical for normal vascular development, functioning, and remodeling. These particular MMPs regulate inflammation, with MMP-2 having anti-inflammatory actions and MMP-9 thought to be pro-inflammatory [3]. The activity of MMP-2 is also regulated by specific tissue inhibitors of MMPs (TIMP-1 and TIMP-2) TIMP-1 is a potent inhibitor of activated MMPs. It is a multifunctional protein, which is found in most tissues and body fluids, TIMP-1 appears as a key regulator of extracellular matrix degradation. Moreover, TIMP-1 up-regulates signaling
pathways to promote cell cycle progression and in addition to inhibiting MMP-2 and MMP-9 activity, it may inhibit apoptosis in various cell lines [4]. MMP-2 is expressed in the collecting duct in the rabbit [5], the glomerulus and proximal tubules of rats [6], and in the proximal and distal tubules in the monkey [2]. The expression of MMP-9 appears to be mainly confined to the glomerulus [7]; although there are reports of expression in the rabbit collecting duct [5]. Gelatinase A (MMP-2) and gelatinase B (MMP-9) are the most important MMPs in normal kidneys and are therefore assumed to play major roles in basement membrane homeostasis [8]. Diabetic kidney disease or DN is a well described complication of diabetes that results from glomerular (as well as tubular and interstitial) damage secondary to continued hyperglycemia [9]. 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 [1-2, 6,8]. Despite considerable progress in elucidation of the mechanisms leading to development of diabetic angiopathy, our understanding of the precise events involved in this process, including abnormalities in vascular remodeling, is still far from complete. Recently it has been proven that matrix metalloproteinases (MMPs) play an important role in atherosclerosis and rebuilding of the vascular wall [10]. Microalbuminuria (MA) is considered to be a marker of risk factor for DN and progressive renal insufficiency in diabetes [14]. However MA may not be as sensitive and specific a predictor of the DN as previously suggested [15]. Other markers of risk for DN are needed for optimal clinical management so we conducted this study to investigate the level of MMP-2 as a potential marker of early nephropathy in diabetes patients.

2. Patients and methods

2.1. Study design

This case–control study was carried out in the science of college in Basrah university, where the samples collected between February 2018 and march 2018 from the women diabetes patients at AL-Faihha endocrine center, the work was done with the help from cell and biotechnology research unit; The aim of the study was explained to all participants and all of them gave informed women diabetes patients. Inclusion criterion was patients with diabetes and patients were excluded from the study if they suffered from other chronic systemic inflammatory or autoimmune disease or malignancy or concurrent use of medications other than insulin (e.g. corticosteroids). Demographic information was collected; the total number of the study was 64 participants, all of the female diabetic patients. The samples divided into 4 groups; group 1 included the control samples (n=20) with age (28-65); group 2 included the patients with age 10-30 (n:7); group3 patients with age 30-60 (n:37); group 4 patients with 60-80 (n:20)

2.2. Clinical information

All participants in this study were subjected to complete clinical examination and all type 1 diabetic patients participating in this study treated with insulin therapy. In addition, all participants were asked about their age, length, weight, and diabetes type.

2.3. Biochemical Analysis

Lipid profile included total cholesterol, high-density lipoprotein (HDL)-cholesterol, triglycerides were measured in plasma samples by using laboratory kits (BIOLABO, France). According to kits manuals the values of total cholesterol, (HDL)- cholesterol, triglycerides in samples were estimated in term of risk as in table (1).

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Table (1) the risk values of total cholesterol, high-density lipoprotein (HDL), triglycerides.

| Levels      | cholesterol | HDL Cholesterol | triglycerides |
|-------------|-------------|-----------------|---------------|
| Normal      | <200        | -               | 108           |
| Low risk    | 200-239     | <40             | >160          |
| High risk   | >240        | ≥60             | >221          |

2.4. Measurement of Matrix Metalloproteinase-2

The levels of Metalloproteinase-2 (MMP-2) in plasma was measured by enzyme-linked immunosorbent assay (ELISA) kit (Cloud-Clone corp, USA). The values were detected by using ELSA analysis software online.

2.5. Statistical Methods

Data were analyzed with an SPSS version 20. Analysis of variance (ANOVA or F-test). Predictive values were assessed by the area under the receiver operator characteristic curve. For these tests, a probability (p-value) < 0.05 was considered significant.

3. Results

3.1. Clinical Information

Data collected from the patients about their age, weight and length showed in table (2).

Table (2) Age range, length and weights of all groups.

| Groups     | Control           | Diabetes Patients |
|------------|-------------------|-------------------|
| Age        | 20-70 (n=20)      | 10-30(n=7)        | 30-60(n=38) | 60-80(n=27) |
| Length (cm)| 169.42±4.795      | 160.33±5.686      | 157.125±2.587 | 160.3±5.696 |
| Weight (kg)| 71.54±9.80        | 75.67±5.508       | 66.25±12.464 | 80.7±13.267 |
| BMI*       | 24.911            | 29.176            | 16.733       | 31.405      |

Values = mean ± Sd

*BMI= Body Mass Index

3.2. Lipid profile analysis

The results showed no significant (p<0.05) different in both cholesterol and triglycerides while there was a significant (p<0.05) different in HDL-Cholesterol comparing with control groups. However, levels of cholesterol was at normal rates in control group, while in diabetes groups at low risk levels. Furthermore, the risk values of HDL-Cholesterol was detected in group 2, also the low risk levels of triglycerides were detected in diabetes groups (table 3).

3.3. Correlates of MMP-2

MMP-2 concentrations of control and diabetes patients were calculated by using standards curve (fig.1). The results revealed there was a significant (p<0.05) increasing in the MMP-2 concentrations in diabetes patients groups (3,4) comparing with control while no significant different between control and patient groups (2). In addition, there was a significant (p<0.05) between the groups 2 with other patients groups (3,4). Table (3)
4. Discussion

The results showed that lipid profile was at risk levels in DN patients compared with the control groups can be due to the damage in the renal tissue. The quantity of triglycerides in the liver is significantly raised in uncontrolled diabetes mellitus, which results from increased free fatty acids levels in plasma. Lipid abnormalities in diabetes may be due to essential abnormality of the disease process, induced by difficulties of diabetes like nephropathy or genetically determined [13]. In fact, pre-diabetic individuals often exhibit an atherogenic pattern of risk factors that includes higher levels of total cholesterol, LDL cholesterol, and triglycerides and lower levels of HDL cholesterol than individuals who do not develop diabetes (14,15).

The main finding of this study is that plasms from women patients with Type 1 diabetes and without clinical coronary artery disease was differ in MMP-2 levels in groups (3,4) while no differ in group (2) compared to controls. The impact and contribution of MMPs to the onset and progression of DN may be most critical in the earlier phases of the disease process, at a time in which enhanced matrix turnover, release of pro-fibrotic growth factors and altered cell motility may damage the glomerular apparatus and tubular architecture. It was stated that several MMPs have been shown to play a role in renal pathology, enlargement of the kidney mesangium due to ECM over-accumulation is a major characteristic of DN, preceding the onset of albuminuria. Diabetic nephropathy is described by gathering of mesangium matrix and condensing of basement membrane inside the glomeruli. These changes are the result of an unequal in the synthesis and degradation of ECM components, in particular collagen, fibronectin, and laminin, moreover MMP-2 is controlled the degradation of type IV collagen and laminin, components of ECM proteins [9]. Early detection of diabetic microvascular complication, particularly nephropathy, has a pivotal role in prevention of end-stage renal disease in children and
adolescents with diabetes [7]. On the other hand, diseases duration positively correlated with MMP-2 levels and that discovery seemed to be logical as duration of diabetes, independently predicts diabetes severity and complications [12]. But Zelmanovitz et al. were failed to find any correlation between age and MMP-2 [7].

The raised levels of MMP-9 in plasma are a feature of Type 2 diabetes with microalbuminuria [16], and in Type 1 patients [17] although it is unclear what relevance plasma MMP concentrations have to any tissue biological process [18,19]. The current study revealed that the levels of MMP-2 significantly increased in diabetic groups with respect to control group. These findings were in compatible with Derosa and his colleagues [20] who measured MMP-2 and -9 in children and adolescents with and without macroangiopathy and they found that MMP-2,-9 levels were significantly increased in diabetic groups

Kathryn et al. have demonstrated a marked increase in urinary excretion of MMP-2 in type 1 diabetic subjects compared with healthy control subjects. Moreover, urine MMP-2 concentrations were correlated with several known risk factors for diabetic comorbidity in general and diabetic nephropathy in particular [21].

Ali et al. suggest that MMP-9 is more sensitive and earlier marker than MMP-2 in differentiation of DN type 2 [22]. Additional wound fluid and ulcer punch biopsies derived from diabetes subjects show increased expression of MMP-2, MMP-1, MMP-8 and MMP-9 [23,24]. However, other groups have described down regulation of an MMP induction and activation system in internal mammary arteries derived from subjects with diabetes [25] the MMP9, MMP2, MMP8, and MMP10 genes may participate in the pathogenesis of genetic risk factors for DN [26].

5. Conclusions

By doing the research, we conclude that the MMP-2 concentration within the healthy people is much less than it’s concentration within the diabetes patients. According to the results there was observed that the concentration of the MMP-2 increase from 30 years old and more; so it can be a symptom some type abnormality if it changes in concentration from the normal range; the correlation between the age and the MMP-2 concentration.

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7. References

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