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Original Article

# Association of matrix metalloproteinase-9 and tissue inhibitor of metalloproteinase-1 levels with diabetic foot ulcer in Egyptians with type 2 diabetes mellitus

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## Abstract

**Background**

The imbalance between matrix metalloproteinase-9 (MMP-9) and tissue inhibitor of metalloproteinase-1 (TIMP-1) may play an important role in impaired healing of diabetic foot ulcer.

**Aim of the work**

This study was designed to investigate the association of MMP-9 and TIMP-1 levels in diabetic foot ulcer among Egyptian patients with type 2 diabetes mellitus attending the outpatient clinic of National institute of diabetes and endocrinology.

**Patients and methods**

This study was conducted on a total number of 90 participants comprising three groups: group I included 35 type 2 diabetic patients without foot ulcer. Group II included 35 type 2 diabetic patients having various degrees of foot ulcer complications. Group III included 20 apparently healthy participants (as controls). In addition to the routine clinical and laboratory investigations, MMP-9 and TIMP-1 were measured. All patients were randomly taken from the outpatient clinic of National institute of diabetes and endocrinology.

**Results**

The study results showed that the higher levels of plasma MMP-9 and the lower levels of TIMP-1 were associated with patients with diabetic foot ulcer.

**Conclusion**

The measurement of plasma MMP-9 and TIMP-1 in type 2 diabetic patients may help us to know the patients at most risk to develop diabetic foot ulcer.

**Keywords:** Matrix metalloproteinase-9, tissue inhibitor of metalloproteinase-1, type 2diabetes mellitus

## Introduction

The diabetic foot syndrome is clearly one of the most important complications of diabetes. It not only occurs as a typical complication in the late stages of diabetes but also in patients with newly diagnosed diabetes [1]. Owing to the systemic effects of diabetes, not only do cellular abnormalities exist but interactions of growth factors and other mediators of wound healing are also impaired [2]. Thus, understanding the cellular and molecular abnormalities that contribute to the diabetic foot syndrome will enable the rational development of treatments that will reduce the incidence and severity of this major complication of diabetes. The complex and structured dynamics of wound healing involve several populations of cells (thrombocytes or platelets, neutrophile granulocytes, macrophages, fibroblasts, and

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keratinocytes), soluble factors (cytokines and growth factors), and proteases [e.g. matrix metalloproteinases (MMPs), plasmin, and elastase].

The MMP family comprises enzymes based on their substrate specificity; one of them is MMP-9, which is one of the gelatinase enzyme groups. MMP-9 is secreted mainly by leukocytes and to a lesser extent by keratinocytes [3]; MMP-9 is particularly involved in the wound healing process. It plays an essential role in initial wound debridement as well as in the phases of angiogenesis, epithelialization, and scar remodeling [4]. MMP-9 is needed to remove denatured fibrillar collagen and for the proper development of granulation tissue [5]. A balance between proteases and their inhibitors is necessary for correct wound healing. Several studies [6] have found elevated levels of proteases and reduced levels of inhibitors in chronic wounds. Tissue inhibitor of metalloproteinase (TIMP) interrupts the action of MMPs and regulates their action. There are four different subtypes (TIMP-1, TIMP-2, TIMP-3, and TIMP-4). The importance of MMPs in wound healing cannot be underestimated. They can negatively affect healing if not present in the correct levels [7]. Additionally, there is evidence that an imbalance between MMPs and TIMPs does significantly contribute to the pathogenesis of nonhealing chronic lesions [8]. Thus, it is reasonable to theorize that local (or systemic) treatment of chronic wounds with protease inhibitor(s) would promote healing, which may provide a novel target for the future therapy in diabetic ulcers.

## Patients and methods

This study was conducted on a total number of 90 participants which were subdivided into three groups: group I included 35 type 2 diabetic patients without foot ulcer, group II included 35 type 2 diabetic patients with foot ulcer, and group III included 20 apparently healthy participants (as controls). All patients were randomly taken from the outpatient clinics of National institute of diabetes and endocrinology, between September 2016 and October 2017. Type 2 diabetes mellitus (T2DM) was diagnosed according to the report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus [9]. The controls were clinically free from any recognizable disease. They were not receiving any medications. An informed consent was taken from each participant in the study, clarifying the aim of the study as well as all the required procedures. Subsequently, ethical approval was obtained from the research ethics committee of the General Organization of Teaching Hospitals and Institutes. Inclusion criteria included patients with T2DM with or without foot ulcers. Exclusion criteria included patients with type 1 diabetes mellitus.

All patients were subjected to current history, past history, and full clinical evaluation. Laboratory investigations were as follows: 10 ml of venous blood was drawn from each patient in dry sterile vacutainers after overnight fasting. The first part of the collected blood was taken on EDTA tubes for determination of glycated hemoglobin level. The second part of collected blood was left to clot. Serum was rapidly separated by centrifugation. It was tested for fasting blood glucose and lipid profile (total cholesterol, triglycerides, high-density lipoprotein, and low-density lipoprotein) by using ARCHI TECT 8000 chemistry analyzer (Abbott, USA; supplied by Al Kamal Company, Cairo, Egypt), whereas MMP-9 and TIMP-1 levels were measured using enzyme-linked immunosorbent assay technique according to manufacturer's instructions (Nova Kits; Bioneovan Co. Ltd, Beijing, China).

### Statistical analyses

Statistical analysis was performed using the statistical package for the social sciences, version 13 (SPSS Inc., Chicago, Illinois, USA). Data are presented as mean ± SD for normal distribution samples, whereas the mean differences between two groups were compared by Student's *t* test, and one-way analysis of variance was applied to three groups. For non-normally distributed samples, data are represented as median (maximum–minimum). The median values between the two groups were compared by Mann–Whitney *U* test, whereas the Kruskal–Wallis *H* test was used for differences among the three groups. A *P* value less than 0.05 was considered statistically significant.

## Results

Comparison among the three main groups showed the MMP-9 mean values to be significantly higher in the diabetic patients with foot ulcer (group II) than the diabetic patients without foot ulcer (group I) ($P = 0.007$) as well as the control group (group III) ($P = 0.002$) (Table 1). Moreover, TIMP-1 mean values were found to be significantly lower in group II than group I ($P = 0.010$) as well as the control group (group III) ($P = 0.001$), which showed the highest values of TIMP-1 (Table 1). Comparison of MMP-9 based on extent of tissue involvement demonstrated that the foot ulcer group with bone or joint involvement showed a significantly higher value than the control group (group III) ($P = 0.001$), the diabetes mellitus (DM) group (group I) ($P = 0.001$) as well as foot ulcer group, with less deeper tissue involvement (i.e. only tendon/capsule and not extending to joint or bone) ($P = 0.001$) (Table 2).

Regarding TIMP-1, comparison in the different study groups demonstrated that the foot ulcer group with bone or joint involvement showed a significantly lower value than the control group (group III) ($P = 0.001$) as well as group I ($P = 0.015$). Moreover, the foot ulcer group with less deeper involvement (i.e. tendon or capsule only) showed lower values than group I ($P = 0.005$) in as well as group III (0.001) (Table 3). In addition, comparison between means of MMP-9 in the different groups demonstrated that the foot ulcer group with infection not associated with ischemia showed a significantly higher value as compared with the control group (group III) ($P = 0.004$). Similarly, the foot ulcer group with infection associated with ischemia showed higher MMP-9 values against the control group (group III) ($P = 0.001$) as

**Table 1: Comparison among the three study groups regarding various parameters with pairwise comparison among their values**

| Parameters | Control (*n*=20) | DM (*n*=35) | Foot ulcer (*n*=35) | Overall *P* | | Pairwise | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | C (*n*=20) vs. DM (*n*=35) | | C (*n*=20) vs. FU (*n*=35) | | DM (*n*=35) vs. FU (*n*=35) | |
| MMP-9 (ng/ml) | 13.3 (6.8-26.1) | 15 (10.9-31.3) | 22.8 (6.2-44.5) | 0.001** | a | 0.054 | c | 0.002* | c | 0.007* | c |
| TIMP-1 (ng/ml) | 1358 (721-1816) | 1069 (686-3784) | 865 (638-1212) | 0.001** | a | 0.124 | c | 0.001** | c | 0.010* | c |
| FBS (mg/dl) | 92 (68-156) | 206 (82-560) | 197 (104-502) | 0.001** | a | 0.001** | c | 0.001** | c | 0.904 | c |
| HbA1c (%) | 6±0.9 | 10±2.1 | 10±2.4 | 0.001** | b | 0.001** | c | 0.001** | d | 0.881 | d |
| Cholesterol (mg/dl) | 189 (34-252) | 189 (119-343) | 183 (113-287) | 0.269 | a | 0.149 | c | 0.582 | c | 0.222 | c |
| Triglycerides (mg/dl) | 134 (55-318) | 116 (43-351) | 107 (41-300) | 0.525 | a | 0.489 | c | 0.327 | c | 0.456 | c |
| HDL (mg/dl) | 49±6.1 | 38±5.6 | 34±7 | 0.001** | b | 0.001** | d | 0.001** | d | 0.041* | d |
| LDL (mg/dl) | 130 (13-178) | 141 (73-258) | 145 (90-262) | 0.268 | a | 0.151 | c | 0.139 | c | 0.860 | c |
| Age (years) | 47±8.6 | 47±10.3 | 51±9 | 0.191 | b | 0.961 | d | 0.156 | d | 0.189 | d |
| Duration (years) | - | 6 (4-22) | 10 (1-25) | 0.016* | a | - | c | - | c | 0.016* | c |

Data are presented as mean±SD for normal distribution samples, and as median (maximum-minimum) for non-normal distribution. C, control group (group III); DM, diabetics only group (group I); FU, diabetics with foot ulcer group (group II) HbA1c, glycated hemoglobin; FBS, fasting blood sugar; HDL, high-density lipoprotein; LDL, low-density lipoprotein; MMP-9, matrix metalloproteinase-9; TIMP-1, tissue inhibitor of metalloproteinase-1. [a]Kruskal-Wallis *H*. [b]One-way analysis of variance. [c]Mann-Whitney *U*. [d]*t* test. *Significant ($P$<0.05). **Highly significant ($P$<0.001).

**Table 2: Pairwise comparison between means of matrix metalloproteinase-9 in the different study groups, with the foot ulcer group subdivided into deep/superficial involvement of tissues**

| Groups | | Groups | | *P* | |
|---|---|---|---|---|---|
| C (*n*=20) | 13.3 (6.8-26.1) | DM (*n*=35) | 15 (10.9-31.3) | 0.540 | a |
| C (*n*=20) | 13.3 (6.8-26.1) | Tendon/caps (*n*=22) | 16.4 (6.2-36.7) | 0.078 | a |
| C (*n*=20) | 13.3 (6.8-26.1) | Bone/joint (*n*=13) | 26.6 (13.2-44.5) | 0.001** | a |
| DM (*n*=35) | 15 (10.9-31.3) | Tendon/caps (*n*=22) | 16.4 (6.2-36.7) | 0.287 | a |
| DM (*n*=35) | 15 (10.9-31.3) | Bone/joint (*n*=22) | 26.6 (13.2-44.5) | 0.001** | a |
| Tendon/caps (*n*=22) | 16.4 (6.2-36.7) | Bone/joint (*n*=13) | 26.6 (13.2-44.5) | 0.001** | a |

Data are presented as mean±SD for normal distribution samples, and as median (maximum-minimum) for non-normal distribution. C, control group (group III); DM, diabetics only group (group I); FU, diabetics with foot ulcer group (group II). [a]Mann-Whitney *U*. *Significant ($P$<0.05). **Highly significant ($P$<0.001).

**Table 3: Pairwise comparison between the means of tissue inhibitor of metalloproteinase-1 in the different study groups, with the foot ulcer group subdivided into deep/superficial involvement of tissues**

| Groups | | Groups | | *P* | |
|---|---|---|---|---|---|
| C (*n*=20) | 1358 (721-1816) | DM (*n*=35) | 1069 (686-3784) | 0.124 | a |
| C (*n*=20) | 1358 (721-1816) | Tendon/caps (*n*=22) | 866 (638-1212) | 0.001** | a |
| C (*n*=20) | 1358 (721-1816) | Bone/joint (*n*=13) | 861 (704-1171) | 0.001** | a |
| DM (*n*=35) | 1069 (686-3784) | Tendon/caps (*n*=22) | 866 (638-1212) | 0.005* | a |
| DM (*n*=35) | 1069 (686-3784) | Bone/joint (*n*=22) | 861 (704-1171) | 0.015* | a |
| Tendon/caps (*n*=22) | 866 (638-1212) | Bone/joint (*n*=13) | 861 (704-1171) | 0.775 | a |

Data are presented as mean±SD for normal distribution samples, and as median (maximum-minimum) for non-normal distribution. C, control group (group III); DM, diabetics only group (group I); FU, diabetics with foot ulcer group (group II). [a]Mann-Whitney *U*. *Significant ($P$<0.05). **Highly significant ($P$<0.001).

well as the noncomplicated diabetic DM group (group I) ($P$ = 0.001). Moreover, the infection without ischemia group showed significantly lower value than the infection associated with ischemia group ($P$ = 0.017) (Table 4).

Furthermore, comparison between means of TIMP-1 in the foot ulcer group having infection without ischemia (i.e. infection only) showed significantly lower values of TIMP-1 compared with the control group (group III) ($P$ = 0.001) as well as group I ($P$ = 0.003). In cases having both infection and ischemia, levels of TIMP-1 were significantly lower than controls (group III) ($P$ = 0.004). No other significant finding was detected (Tables 5-7).

## Discussion

Diabetic foot ulceration is one of the serious complications of diabetes that may cause major extremity amputation with considerable economic and public health implications [10]. MMP-9 is one of the MMP families, which are known to be

**Table 4: Pairwise comparison between means of matrix metalloproteinase-9 in the different study groups, with the foot ulcer group subdivided into infection only and infection associated with ischemia**

| Groups | | Groups | | *P* | |
|---|---|---|---|---|---|
| C (*n*=20) | 13.3 (6.8-26.1) | No infection/no ischemia (*n*=35) | 15 (10.9-31.3) | 0.054 | [a] |
| C (*n*=20) | 13.3 (6.8-26.1) | Infection only (*n*=25) | 17.1 (6.2-36.7) | 0.004* | [a] |
| C (*n*=20) | 13.3 (6.8-26.1) | Both infection and ischemia (*n*=8) | 28.4 (13.2-44.5) | 0.001** | [a] |
| DM (*n*=35) | 15 (10.9-31.3) | Infection only (*n*=25) | 17.1 (6.2-36.7) | 0.061 | [a] |
| DM (*n*=35) | 15 (10.9-31.3) | Both infection and ischemia (*n*=8) | 28.4 (13.2-44.5) | 0.001** | [a] |
| Infection only (*n*=25) | 17.1 (6.2-36.7) | Both infection and ischemia (*n*=8) | 28.4 (13.2-44.5) | 0.017* | [a] |

Data are presented as mean±SD for normal distribution samples, and as median (maximum-minimum) for non-normal distribution. C, control group (group III); DM, diabetics only group (group I); FU, diabetics with foot ulcer group (group II). [a]Mann-Whitney *U*. *Significant ($P$<0.05). **Highly significant ($P$<0.001).

**Table 5: Pairwise comparison between means of tissue inhibitor of metalloproteinase-1 in the different study groups, with the foot ulcer group subdivided into infection only and infection associated with ischemia**

| Groups | | Groups | | *P* | |
|---|---|---|---|---|---|
| C (*n*=20) | 1358 (721-1816) | DM (*n*=35) | 1069 (686-3784) | 0.124 | [a] |
| C (*n*=20) | 1358 (721-1816) | Infection only (*n*=25) | 867 (638-1212) | 0.001** | [a] |
| C (*n*=20) | 1358 (721-1816) | Both infection and ischemia (*n*=8) | 876 (796-1171) | 0.004* | [a] |
| DM (*n*=35) | 1069 (686-3784) | Infection only (*n*=25) | 867 (638-1212) | 0.003* | [a] |
| DM (*n*=35) | 1069 (686-3784) | Both infection and ischemia (*n*=8) | 876 (796-1171) | 0.078 | [a] |
| Infection only (*n*=25) | 867 (638-1212) | Both infection and ischemia (*n*=8) | 876 (796-1171) | 0.853 | [a] |

Data are presented as mean±SD for normal distribution samples, and as median (maximum-minimum) for non-normal distribution. C, control group (group III); DM, diabetics only group (group I); FU, diabetics with foot ulcer group (group II). [a]Mann-Whitney *U*. *Significant ($P$<0.05). **Highly significant ($P$<0.001).

**Table 6: Absolute frequencies and relative percentages of study group according to presence of deep or superficial tissue involvement in patients with foot ulcer**

| Groups | Frequency | % |
|---|---|---|
| Control (group III) | 20 | 22.2 |
| Noncomplicated DM (group I) | 35 | 38.8 |
| Bone/joint (deep involvement) | 13 | 14.4 |
| Tendon/capsule (more superficial) | 22 | 24.4 |
| Total | 90 | 100 |

DM, diabetes mellitus.

**Table 7: Absolute frequencies and relative percentages of study group according to presence of infection with or without ischemia of in patients with foot ulcer**

| Groups | Frequency | % |
|---|---|---|
| Control (group III) | 20 | 22.7 |
| Noncomplicated DM (group I) | 35 | 39.8 |
| Infection only | 25 | 28.4 |
| Infection and ischemia | 8 | 9.1 |
| Total | 88 | 100 |

DM, diabetes mellitus.

involved in leukocytes influx, angiogenesis, re-epithelialization, and extracellular matrix remodeling that is necessary in wound healing [11]. Owing to this role, the MMP activities need to be tightly regulated at levels of mRNA transcription and stability control, at the protein level via activators and inhibitors, including TIMP [12]. If the balance between MMPs and TIMPs becomes disrupted, wound healing is delayed [13]. Some researchers found high values of MMP-9/TIMP-1 ratio were associated with poor ulcer healing [14].

This study was designed to investigate the association of MMP-9 and TIMP-1 levels with diabetic foot ulcer in Egyptian patients with T2DM. Comparison was done between mean values of MMP-9 and TIMP-1 in the three groups under study: the noncomplicated diabetic group (DM) (group I) ($N$ = 35), the diabetic foot ulcer group (group II) ($N$ = 35), and the control group (group III) ($N$ = 20). The results of this study have shown higher MMP-9 values in the diabetic patients with foot ulcer (group II) than the diabetic patients without foot ulcer (group I), as well as the control group (group III), with the last group showing lower values of MMP-9 than both other groups.

Moreover, TIMP-1 showed lower values in the diabetic patients with foot ulcer (group II) than the diabetic patients without foot ulcer (group I), and the control group (group III) showed higher values of TIMP-1 than either of the two other groups.

In agreement with the results of this study, Wang *et al.* [15] found that the mean plasma concentrations of MMP-9 of patients with T2DM were significantly higher than that of controls. They also found that the plasma levels of MMP-9 were high in diabetic patients with macroangiopathy than in patients without macroangiopathy. The investigators further postulated that the presence of SNP-1562C>T affecting MMP-9 expression is associated with T2DM and diabetic foot ulcer [15]. Several research studies have directed their

investigation to the analysis of wound fluid [14]. Liu and colleagues have shown that there was high concentration of MMP-9 levels and high MMP-9 to TIMP-1 ratios in diabetic wounds, thought by the investigators to be the cause leading to poor wound healing in patients with diabetic foot ulcer. Moreover, in a study by Mclennan *et al*. [16] that was done on wound fluid taken from diabetic 'Baboon,' the researchers found increased amount of active form of MMP-9, and they suspected this to be the cause for the persistence of the inflammatory wound.

In the current study, comparison between means of MMP-9 in the group having infected foot ulcer (without ischemia) showed a significantly higher value as compared with the control group (group III) ($P = 0.004$). Moreover, the infection and ischemia group showed higher MMP-9 values against the control group ($P = 0.001$) as well as the noncomplicated diabetic group (group I) ($P = 0.001$). In addition, the foot ulcer with infection only group showed significantly lower values than the infection and ischemia group ($P = 0.017$). Comparison between means of TIMP-1 in the foot ulcer with infection only group showed significantly lower values of TIMP-1 compared with the control group ($P = 0.001$) and group I ($P = 0.003$). Patients with both infection and ischemia showed lower values than the controls (group III) ($P = 0.004$). No other significant finding was detected.

The results of this study are in accordance with the findings of Li *et al*. [17]. These investigators detected the expression of MMP-9 and TIMP-1 in wounds from different diabetic patients, and they found that the ratio MMP-9/TIMP-1 was significantly increased in diabetic wounds and increased even more with higher degrees of infection.

## Conclusion

The results of this study show the higher levels of plasma MMP-9 and the lower levels of TIMP-1 to be associated with patients with diabetic foot ulcer, especially when wounds show deeper involvement of tissues (i.e. bone and/or joint). Thus, we suggest that the determination of such parameters in type 2 diabetic patients may be used as markers to help differentiate those patients who are at risk of developing persistent diabetic foot ulcer showing poor healing. However, in view of the relatively limited number of patients included in this study, we recommend that large-scale studies be performed, preferably possessing a longitudinal design, to provide clearer evaluation of the advantage to be gained by determining levels of MMP-9 and TIMP-1 regarding the assessment of wound healing.

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### Conflicts of interest

There are no conflicts of interest.

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