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ORIGINAL ARTICLE

Expression of Tissue Matrix Metalloproteinase-1 (MMP-1) In Neuropathic Diabetic Foot Ulcers with Delayed Healing: Cross-Sectional Study

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ABSTRACT

Background: Matrix metalloproteinases (MMPs) are characteristic of chronic wounds. That can affect the healing process. Therefore, we aimed to evaluate the immunoreactivity of tissue MMP-1 to histopathological analysis of delayed healing in neuropathic diabetic foot ulcers (DFUs).

Methods: This cross-sectional research included 21 diabetic patients with neuropathic foot ulcers who did not achieve at least a 50% decrease in the surface area of the ulcer after four weeks of standard treatment. Infected or ischemic ulcers were excluded. Following the debridement, two biopsies were collected from the base and the edge of the ulcer for histopathological assessment. The analysis focused on cellularity (involving fibroblasts and macrophages), vascular proliferation, inflammation, and collagen maturation. The evaluation of MMP-1 immunoreactivity was categorized as follows: 0 for no staining, 1 for mild staining, 2 for moderate staining, and 3 for strong staining.

Results: MMP-1 was found in 33.3% of ulcer bases and 52.4% at edges. A significant negative correlation was noted between ulcer surface area and MMP-1 expression in the base. Pathological evaluation indicated moderate cellularity in bases (85%) and edges (52%), with moderate vascular proliferation (71% for base, 52% for edge). Additionally, 38% of bases and 76% of edges lacked inflammatory cells, while mature collagen was present in 85% of bases and all edges.

Conclusion: Reduced tissue MMP-1 levels contribute to the slow healing of DFUs, which are characterized by high mature collagen, low cellularity, and chronic inflammation. Further trials are needed to explore MMP-1's potential as a treatment to enhance wound healing.

Keywords: MMP-1, Recalcitrant ulcer, Diabetic foot ulcer

INTRODUCTION

Nearly twenty-five percent of people with diabetes mellitus will develop diabetic foot ulcers (DFUs) at some point in their lives. DFUs are a serious health issue because they are frequently difficult to heal for a variety of internal and external reasons. **[1]** Wounds that do not heal in a timely and organized manner through the regular phases are classified as chronic wounds. The diabetic wound is an example of these wounds. **[2]**

Diabetic foot ulcers are the product of a complex interplay between biological risk factors (like peripheral neuropathy) and foot-related behaviors (such as wearing shoes that are too small for the wearer). High-quality preventive care might avert at least 75% of all instances. **[3]**

According to EL-Nahas et al., 1.2% of patients in Egypt had active foot ulcers (new onset ulcers). These ulcers lasted anywhere from four to twenty-four months. They also reported that 5.7% of people had previously developed an

ulcer, and the prevalence of diabetic foot ulcers was 6.9%. **[4]**

Matrix metalloproteinases (MMPs) are a group of endopeptidases that play a crucial role in various cellular processes, such as angiogenesis (the formation of new blood vessels) and vasodilation (the widening of blood vessels). They are also essential for the repair of injured skin. These zinc-dependent enzymes are found within the extracellular matrix (ECM).) **[5]**

MMPs exist in three distinct forms: pro-MMPs, active MMPs, and TIMPs (tissue inhibitors of metalloproteinases). Numerous studies have confirmed that active MMPs play a significant role in the healing process of wounds, contributing positively to wound recovery. However, quantitatively profiling specific active MMPs is essential for examining their roles during wound healing, particularly in the extracellular matrix remodeling phase. **[5]**

MMP-1 is the primary collagenase involved in wound healing; its specific proteolysis of type I collagen, a crucial component of the dermis, is essential for keratinocyte migration and re-epithelialization**.[6]**

In diabetic conditions, tissues remain in a prolonged inflammatory phase; the continuous stimulation of inflammatory cytokines leads to the dysregulation of matrix metalloproteinases (MMPs), which degrade growth factors and matrix proteins essential for wound healing, resulting in delayed healing.**[7]**

A higher level of MMP-1 among good healers is beneficial because it allows the proliferative phase of healing to be completed. Additionally, it has been suggested that proper regulation of MMP-1 is crucial for the healing process to progress correctly. The increase in TIMP-1 between week zero and week two indicates that regulating MMP-1 activity is essential for successful healing.**[8]**

MMP-1 expression peaks on the first day after wounding in the migrating basal keratinocytes at the edge of the wound. Following this peak, there is a gradual decrease in MMP-1 levels until re-epithelialization is complete. During the final stage of tissue remodeling, laminin isoforms expressed in keratinocytes signal the downregulation of MMP-1**. [9]**

To our knowledge, limited studies in literature have measured Matrix Metalloproteinase-1 expression in tissue biopsies from diabetic feet. So, we aimed to evaluate the immunoreactivity of tissue MMP-1 to histopathological analysis of delayed healing in neuropathic diabetic foot ulcers (DFUs)

METHODS

Patients

This observational cross-sectional study includes twenty-one diabetic patients with neuropathic foot ulcers who did not achieve a 50% reduction in ulcer surface area after eight weeks of standard treatment, which included sharp debridement and appropriate offloading. Ulcers that were infected or ischemic were not included in the study. Participants were selected from the diabetic foot clinic at the Specialized Medical Hospital of Mansoura University in Egypt for 24 months, from November 2020 to November 2022.

All patients provided written agreement and informed consent to participate in the trial and met the inclusion and exclusion criteria, ensuring that the study was ethically approved by the Mansoura Institutional Research Board (IRB), code number **MS/17.05.88.**

Inclusion criteria

1-Patients with diabetes, whether type 1 or type 2, who have a persistent neuropathic foot ulcer and have not seen a 50% decrease in ulcer surface area following 8 weeks of standard ulcer management treatment.

2-Ulcers with a surface area of more than 1 cm.

3-Ulcer classification according to University of Texas classification (1A) and (2A) (1A): Superficial wounds, not involving tendon, capsule, or bone and (2A): Wound penetrating to tendon or capsule**.[10]**

Exclusion criteria

- Patients taking immunosuppressive therapy.
- Smoking.
- Anemia (patients with Hb less than 10gm%)
- Chronic debilitating diseases.
- Infected foot ulcers.
- Peripheral arterial disease detected by Ankle

Brachial Index less than 0.9 or Monophasic waveform by **Handheld Doppler [11].**

Examination of the ulcer:

Assessment of the ulcer's site, surface area, base, edge, exudates, and the presence of infection (including warmth, redness, edema, and a polypoidal base) was categorized according to the Texas classification, which uses four grades combined with four stages, along with the offloading device. **[12].**

Assessment of the ulcer surface area

Wound surface area was calculated by multiplying the maximum length X maximum perpendicular width of the ulcer after full surgical debridement and removal of edge undermining **[13]**

Laboratory tests:

● Complete blood count (CBC) ● Serum cholesterol
● Fasting blood sugar ● HA1c
● Serum creatinine ● Albumin creatinine ratio

Tissue Biopsy:

In the initial debridement session, two samples were collected: one from the base and another from the ulcer's edge.

All specimens were fixed in a 10% neutral buffered formalin and embedded in paraffin. Four-millimeter-thick sections from each specimen were cut and stained with Hematoxylin and Eosin for histological examination. The pathologist evaluated the biopsy specimens in a blinded manner, scoring the following pathological parameters: cellularity, vascular proliferation, collagen, inflammation, and the presence or absence of pre-existing fibrosis, as illustrated in Figures 1 through 8.

❖ Cellularity (fibroblast, fibrocyte, and macrophage):
1) mild (<25cells/ high power field (HPF)
2) moderate (25_100 cells/HPF)
3) abundant(>100cells/HPF)

❖ Vascular proliferation
1) normal (<3 capillaries/ HPF)
2) moderate proliferation (3-10capillaries/HPF)
3) abundant proliferation (>10 capillaries/HPF)

❖ Collagen
1: **Mature collagen** (dense, acellular, eosinophilic, discrete fibers of collagen lying parallel with each other, with few fibrocytes interspersed)
2: **Immature collagen** (small, eosinophilic, wavy, indiscernible bands of collagen mixed with fibroblast and macrophage)

❖ Inflammation
1: **none** (<5 inflammatory cells)
2: **mild** (5-100 inflammatory cells)
3: **abundant** (>100 inflammatory cells)

❖ Pre-existing fibrosis: 1: **present** 2: **absent**

The highest combined score is **thirteen**, and the lowest score is **five**. The pathology score is determined for the ulcer's base and edge. The total pathology score is calculated by taking the average of the scores from the base and the edge of the ulcer.

The pathologist determined Immunoreactivity of Matrix metalloproteinase-1(MMP-1) on sections pre-treated via microwave (10 min in 0.01 M citric acid solution) and incubated overnight at 4C with human polyclonal antibody (Chongqing biopsied Co., Ltd., Chongqing China 1:100. The pathologist used Diaminobenzidine-hydrogen as chromogen, and counterstained the sections with diluted hematoxylin.

The positivity of MMP-1 was evaluated using the following scale:
0: No staining observed.
1: Slight staining noted.
2: Staining of moderate intensity.
3: Intense staining present

Statistical analysis and data interpretation:

The analysis of data was conducted utilizing IBM SPSS Statistics for Windows, Version 22.0. Qualitative data were presented as counts and percentages, whereas quantitative data were shown as median (minimum-maximum) for non-parametric data and mean (standard deviation) for parametric data, after normality assessments were carried out using the Kolmogorov-Smirnov test. A significant level

of 0.05 was established.

RESULTS

The present study involved 21 cases, with a mean age of 54.64 years (SD ±7.49). The median duration of the disease was 17 years, ranging from 3 to 38 years. Nearly 90% of the participants received insulin treatment. Additionally, 29% of the cases exhibited retinopathy, while one-third of the participants were diagnosed with nephropathy. The median ulcer surface area among participants was 5.0 cm^2, ranging from 1.0 cm^2 to 10.5 cm^2. (Table 1)

The mean HbA1c among the studied cases was 8.45%, with a mean cholesterol level of 166.85 mg/dL. The median values for albumin-to-creatinine ratio (ACR), creatinine, and fasting blood glucose were 20.8, 0.9, and 152, respectively. Additionally, the mean hemoglobin was 11.44 g/dL, the mean white blood cell count (WBC) was 7.48 x 10^3, the mean albumin level was 3.87 g/mL, and the mean platelet count was 225.19 x 10^3. (Table 1).

The most frequently studied site of ulcers among cases was the metatarsal (57% of ulcers), followed by the midfoot (19%), big toe (14%), and toes (10%). The most commonly used offloading device was the forefoot wedge, which was utilized by 33% of the diabetic cases studied. (Table 2)

MMP-1 was detected in the base of seven ulcers (33.3%) and at the edge of eleven ulcers (52.4%). Most cases exhibited mild staining, with six cases in the base and nine cases in the edge, respectively

No instances demonstrated strong staining. A significant negative correlation was found between the area of the ulcer surface and the expression of tissue MMP-1 in the ulcer base among the studied cases (r=-0.506, p=0.02). (Figure 1).

Pathological evaluation revealed a moderate cellular density, with 25-100 cells per high-power field (HPF) observed in both the base and edge of the ulcer, corresponding to measurements of 85% and 52%, respectively. Vascular proliferation was mainly moderate, recorded at 71% in both the ulcer base and edge. Inflammatory cells were not detected in 38% of cases at the ulcer base and 76% at the edge, while the remaining ulcers showed a low prevalence. Mature collagen was the predominant feature at the base in 85% of cases and was observed in 100% of the edges of the ulcers. (Table 3,4)

There was no statistically significant association between MMP-1 expression at the base and the edge of the ulcers and the pathological findings of the ulcers (Tables 5,6)

Table (1): Clinical and laboratory characteristics of the cases studied

Total Number 21	
Age/years **Mean ± SD (min-max)**	54.64±7.49 (31.0-70.0)
Type of DM N (%) **Type 1** **Type 2**	 4 (19%) 17 (81%)
DM duration **Median (range)(IQR)**	17.0(3.0-38.0) (10.0-22.0)
Type of treatment N (%)	

Insulin **Oral hypoglycemic**	19 (90%) 2 (10%)
Retinopathy N (%)	6 (29%)
Nephropathy N (%)	7 (33%)
Ulcer Longitudinal diameter/ cm **Median (min-max) (IQR)**	2.0(1.3-4.0) (1.8-3.0)
Ulcer Transverse diameter/cm **Median (min-max) (IQR)**	1.7(0.7-3.0) (1.2-2.2)
Ulcer Surface Area/cm^2 **Median (min-max) (IQR)**	5.0(1.0-10.5) (2.37-6.84)
ACR (mg/gm) **Median (range)(IQR)**	20.8(14.0-49.0) (19.0-35.0)
Creatinine mg/dl **Median (range)(IQR)**	0.90(0.6-1.7) (0.8-1.2)
FBG (mg/dl) **Median (range)(IQR)**	152.0(72.0-226.0) (130.0-180.0)
HBA1C (%) **Mean ± SD (min-max)**	8.45±1.28(6.5-10.8)
Cholesterol (mg/dl) **Mean ± SD (min-max)**	166.85±39.67(136-191)
HB (gm/dl) **Mean ± SD**	11.44±0.99
WBCS x1000/ microliter of blood **Mean ± SD**	7.48±0.70
Albumin (gm/dl) **Mean ± SD**	3.87±0.33
Platelet x1000/microliter of blood **Mean ± SD**	226.19±34.5

N: number, SD: Standard deviation DM: Diabetes Mellitus, Cm$^{2:}$ square centimeter, min: minimum, max: maximum SD: standard deviation, mg: milligram, g: gram, dl: deciliter, HB: hemoglobin, WBC: white blood corpuscles, min: minimum, max: maximum, %: percentage ACR: albumin creatinine ratio

Table 2: Ulcer characteristics among studied cases

Ulcer site	N (%)	Offloading device	N (%)
Big toe	3(14)	Felt foam	2(10)
Metatarsal	12(57)	Forefoot wedge	7(33)
Midfoot	4(19)	Geisha	5(24)
Toes	2(10)	Heal wedge	1(5)
		Total contact cast	2(10)
		Removable cast walker (RCW)	4(19)

N: Number, %: percentage.

Table 3: Correlation between ulcer surface area and MMP-1 among studied cases.

		ulcer surface area longitudinal diameter	ulcer surface area transverse diameter	ulcer surface area
MMP-1 Base	Rs	-.247	-.267	-.506*
	P	.130	.101	.019
MMP-1 edge	Rs	.012	.112	-.009
	P	.941	.496	.968

Rs: Spearman correlation coefficient

Table 4 Pathological evaluation of ulcer base & edge among studied cases

	Base	%	Edge	%
Cellularity				
Mild	1	5	10	47
Moderate	18	85	11	53
Abundant	2	10	0	0
Vascular proliferation				
Normal	5	24	8	38
Moderate	15	71	11	52
Abundant	1	5	2	10
Collagen				
Immature	18	85	0	0
Mature	3	15	21	100
Inflammation				
None	8	38	16	76
Low	12	57	5	24
Abundant	1	5	0	0
Pre-existing fibrosis				
Absent	19	90	19	90
Present	2	10	2	10

Table 5: MMP-1 edge Expression and pathology scoring among studied cases.

MC: Monte Carlo test

	MMP edge			Test of significance
	No expression n=10(%)	Mild expression n=9(%)	Moderate Expression n=2(%)	
Cellularity				
Mild	**7(70.0)**	**3(30.0)**	**0(0.0)**	**MC**
Moderate	**3(30.0)**	**6(60.0)**	**2(100.0)**	**p=0.11**
Vascular proliferation				
Normal	**6(60.0)**	**4(44.4)**	**0(0.0)**	**MC**
Moderate	**4(40.0)**	**4(44.4)**	**2(100.0)**	**P=0.42**
Abundant	**0(0.0)**	**1(11.1)**	**0(0.0)**	
Collagen				
Mature	**10(100.0)**	**9(100.0)**	**2(100.0)**	
Inflammation				
None	**8(80.0)**	**7(77.8)**	**1(50.0)**	**MC**
Low	**2(20.0)**	**2(22.2)**	**1(50.)**	**P=0.65**
Pre-existing fibrosis				
Absent	**10(100.0)**	**7(77.8)**	**2(100.0)**	**MC**
Present	**0(0.0)**	**2(22.2)**	**0(0.0)**	**P=0.23**

Table 6: MMP-1 base expression and pathology scoring among studied cases.

	MMP Base			Test of significance
	No expression n=14(%)	Mild expression n=6(%)	Moderate Expression n=1(%)	
Cellularity				
Mild	1(7.1)	0(0.0)	0(0.0)	MC
Moderate	12(85.7)	5(83.3)	1(100.0)	P=0.91
Abundant	1(7.1)	1(16.7)	0(0.0)	
Vascular proliferation				
Normal	5(35.7)	0(0.0)	0(0.0)	MC
Moderate	8(57.1)	6(100.0)	1(100.0)	P=0.38
Abundant	1(7.1)	0(0.0)	0(0.0)	
Collagen				
Immature	2(14.3)	1(16.7)	0(0.0)	MC
Mature	12(85.7)	5(83.3)	1(100.0)	P=0.91
Inflammation				
None	5(35.7)	3(50.0)	0(0.0)	MC
Low	9(64.3)	2(33.3)	1(100.0)	P=0.38
Abundant	0(0.0)	1(16.7)	0(0.0)	
Pre-existing fibrosis				
Absent	13(92.9)	5(83.3)	1(100.0)	MC
Present	1(7.1)	1(16.7)	0(0.0)	P=0.76

MC: Monte Carlo test

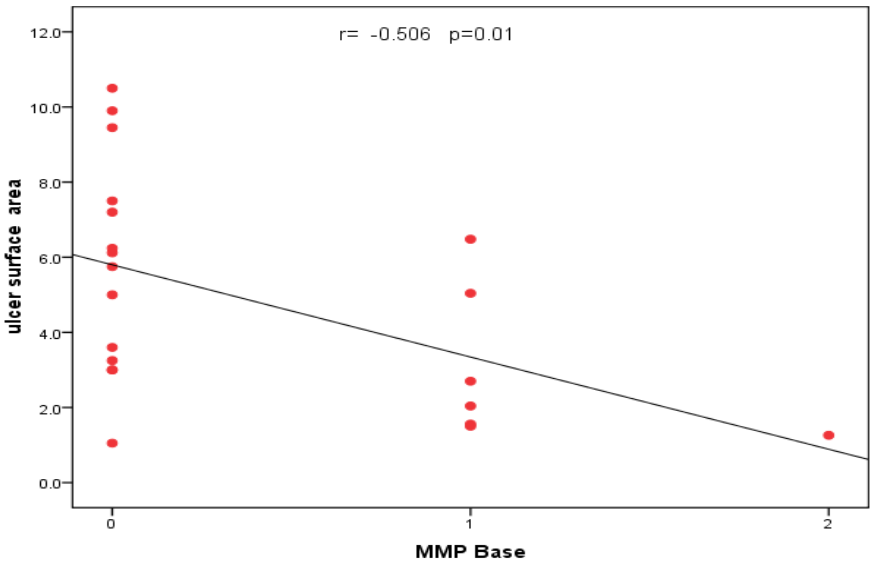

Figure (1): **Scatter diagram showing the correlation between ulcer surface area and MMP-1 base.**

Table: **MMP-1 expression based on pathology at both the base and edge among the studied cases.**

MMP-1 base	**n=21**	**%**
No expression	**14**	**66**
Mild expression	**6**	**29**
Moderate	**1**	**5**
MMP-1 edge	**n=21**	**%**
No expression	**10**	**47**
Mild expression	**9**	**43**
Moderate expression	**2**	**10**

N: number, percentage

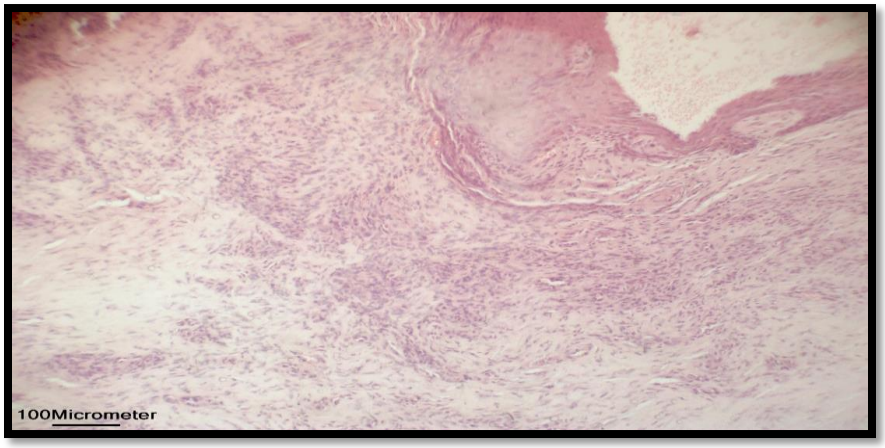

Figure 2: Edge of ulcer increased vascularity (H&E staining 100x)

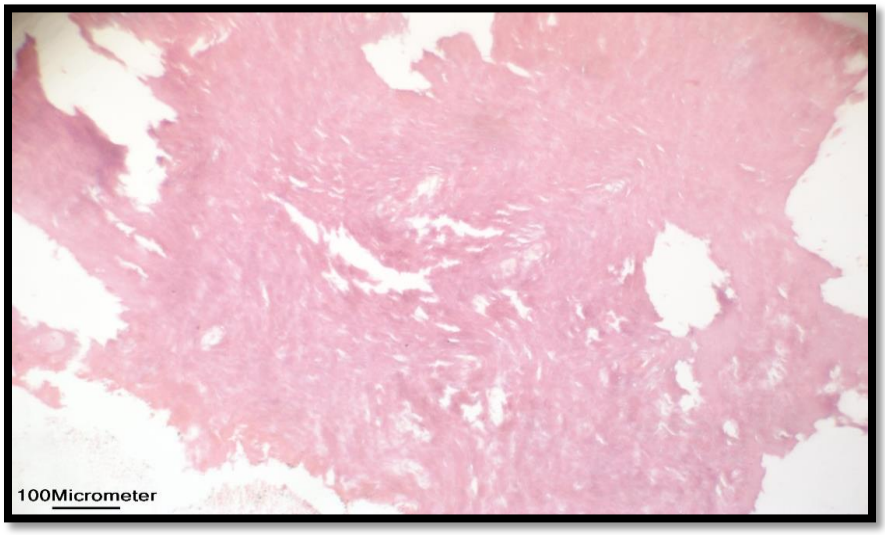

Figure 3: Dense collagen at the base of the ulcer (H&E staining 100x

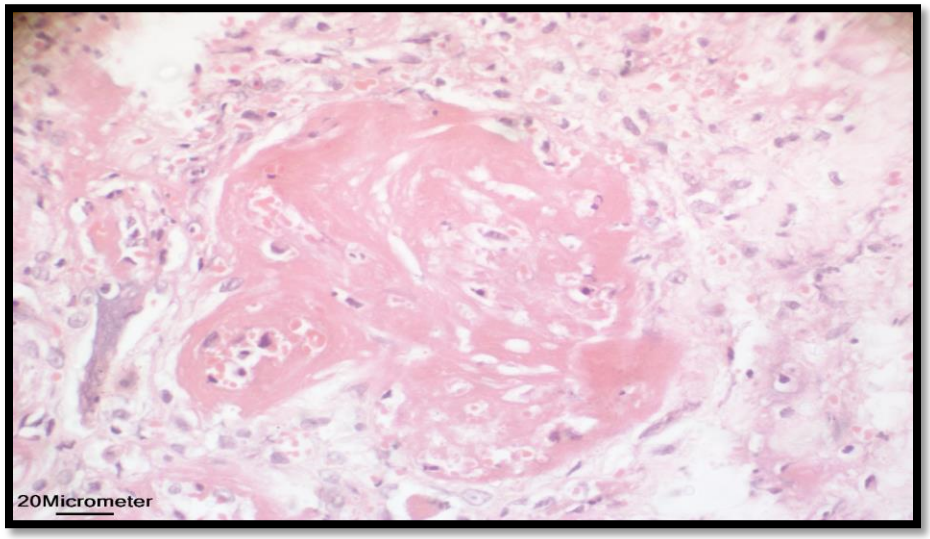

Figure 4: Immature collagen at the base of the ulcer (H&E staining 400x)

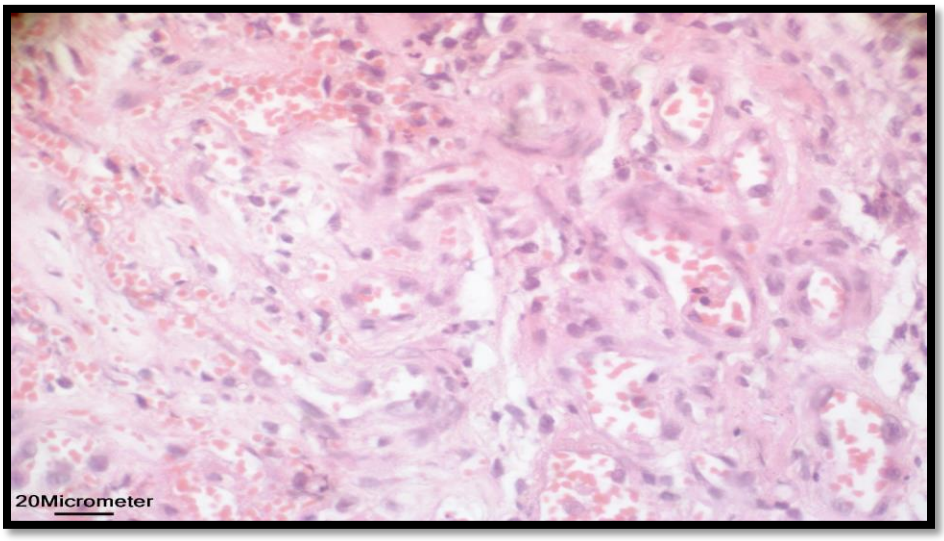

Figure 5: Increased vascularity at the base of the ulcer (H&E staining 400x)

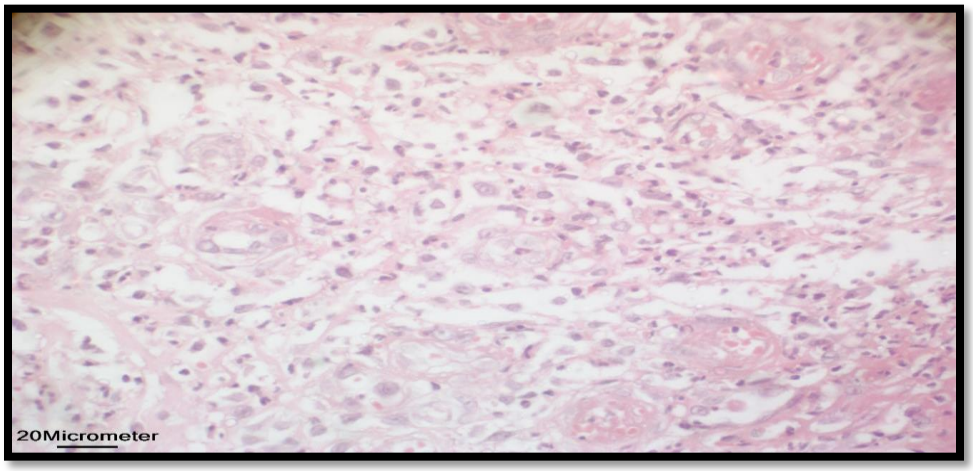

Figure 6: Inflammatory cells at the base of the ulcer (H&E staining 400x)

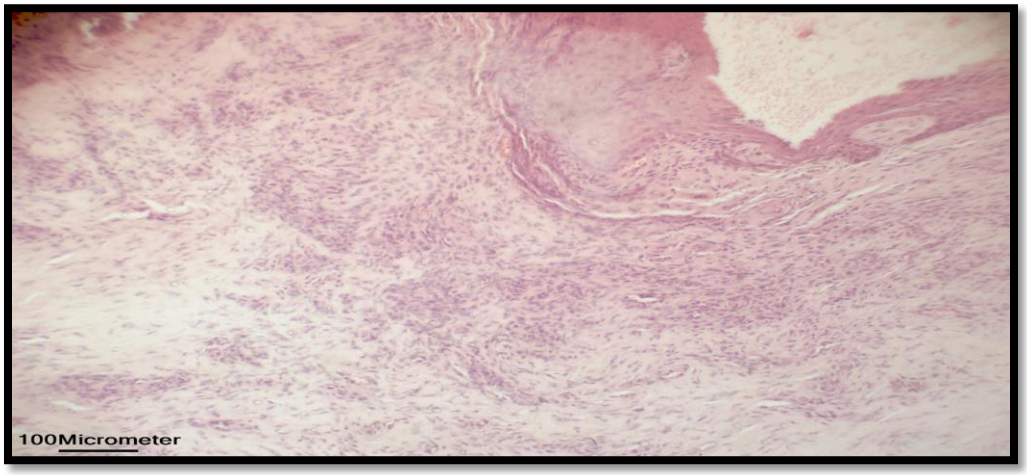

Figure 7: Edge of ulcer increased cellularity (H&E staining 100x)

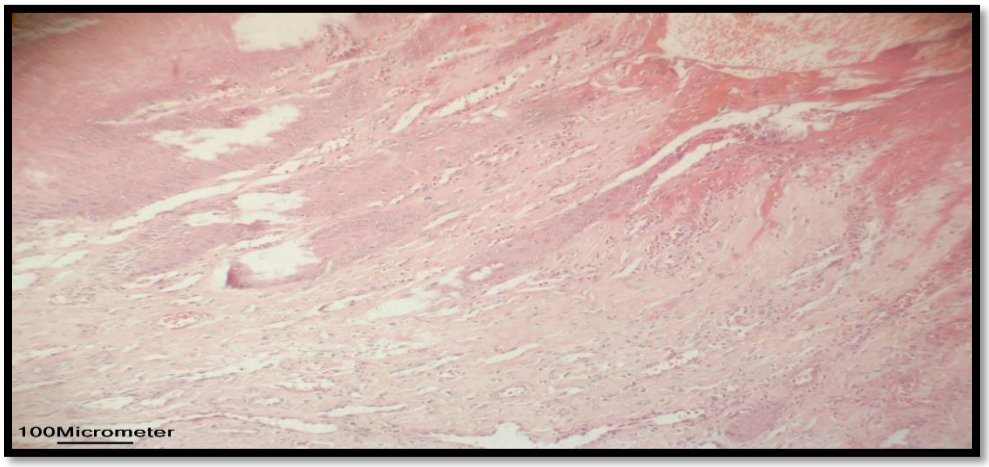

Figure 8: Edge of ulcer increased collagen (H&E staining 100x)

The positivity of MMP-1 was evaluated using the following scale:
0: No staining observed.
1: Slight staining noted.
2: Staining of moderate intensity.
3: Intense staining present

DISCUSION

Diabetic Foot Ulcers (DFUs) are serious complications of diabetes mellitus that lead to costly treatments and negative outcomes. Additionally, various types of foot ulcers significantly impact patients' quality of life (QoL). Patients with diabetes are often hospitalized due to these foot wounds.**[14]**

Coordinated interactions between the biological and immune systems play a crucial role in the complex process of wound healing. This process involves a series of precisely timed and regulated actions that correspond to the appearance of various cell types in the wound bed at different stages. The conventional model of wound healing includes several progressive phases—hemostasis, inflammation, angiogenesis, proliferation, re-epithelialization, and remodeling—all of which overlap with one another. **[15]**

Because matrix metalloproteinases (MMPs) can break down every extracellular matrix component, they are essential to the healing process of wounds. There is an overabundance of MMPs and a decrease in MMP tissue inhibitors (TIMPs) in diabetic foot ulcers. Impaired healing is most likely a result of this imbalance. On the other hand, nothing is understood about how MMPs alter when wounds heal. The proteolytic activity of MMPs regulates the wound's collagen breakdown. **[16].** This shows the fundamental role of MMPs in the process of wound healing.

Numerous studies have investigated circulating MMPs in diabetic foot ulcers, both in wound fluid and serum.**[17]**

The current study involved twenty-one diabetic patients from the diabetic foot clinic at Mansoura Specialized Medical Hospital. All participants had chronic neuropathic foot ulcers and did not achieve at least a 50% reduction in ulcer surface area after an 8-week treatment period of standard ulcer management. Many parameters were evaluated among these patients including laboratory findings, ulcer site, surface area, ulcer edge and base pathology, and MMP-1 at base and edge.

Among the studied cases, the metatarsal was the most frequently studied site of ulcers, accounting for 57% of cases. This was followed

by the midfoot at 19%, the big toe at 14%, and the toes at 10%. This indicates that forefoot ulcers are present in 81% of the ulcers included in the study, which already had proper offloading modality. Research conducted in the United Kingdom in 2001 found that 77.8%, 11.9%, and 10.3% of patients had foot ulcers on their forefoot, midfoot, and hindfoot, respectively. **[18]**

The data from this study differ from those found in research conducted on 127 diabetic patients in Norway. Ribu and his colleagues discovered that ulcer locations were as follows: toes (46%), metatarsals (20%), mid-foot/hind-foot (18%), and multiple locations (17%). **[19].** This difference may be attributed to various cultural factors, such as differences in footwear and walking bare feet and the presence of foot deformities related to the ulcer.

The laboratory findings from the studied cases revealed the following results: the mean HbA1c was 8.45%, the mean cholesterol was 166.85%, the median albumin-to-creatinine ratio (ACR) was 20.8%, the mean creatinine level was 0.9 mg/dL, and the mean fasting blood glucose level was 152 mg/dL. Additionally, the complete blood count (CBC) showed a mean hemoglobin level of 11.44 g/dL, mean white blood cell count (WBC) of 7.48 x 10^9/L, mean albumin level of 3.87 g/dL, and mean platelet count of 225.19 x 10^9/L.

Fesseha BK et al. (2018) concluded that there does not appear to be a clinically meaningful association between baseline or prospective A1C levels and wound healing in patients with diabetic foot ulcers (DFUs). The paradoxical finding of accelerated wound healing alongside an increase in A1C levels in participants with better baseline glycemic control needs to be confirmed in further studies.**[16].** Xiang et al. (2019) found no link between ulcer healing and baseline HbA1c levels. However, for patients with diabetic foot ulcers, HbA1c levels between 7.0% and 8.0% after therapy were associated with a greater likelihood of ulcer healing. **[18]**

In the current study, MMP-1 expression was observed at the base of seven ulcers (33.3%) and the edge of eleven ulcers (52.4%). Most cases exhibited only mild staining, with six instances at the base and nine at the edge. In none of the cases, the staining was strong.

According to Muller et al. (2008), a high level of MMP-1 in the wound fluid of 16 ulcers is essential for wound healing. In contrast, an excess of MMP-8 and MMP-9 is harmful and could be targeted for new topical treatments. Additionally, the MMP-1/TIMP-1 ratio serves as a predictor of wound healing in diabetic foot ulcers.**[19].** Another meta-analysis showed that high levels of MMP-1, 2, and 9 delayed the healing of diabetic foot ulcers, while high expression of MMP-8 in tissues improved wound healing.**[20]**

Suhodolčan et al. (2021) found that a reduction in MMP-1 and MMP-2 levels in wound biopsy samples from venous ulcers, after four weeks of observation, can predict improved healing of chronic venous ulcers**.[21]**

We believed that there was a delay between the occurrence of the ulcer and the patient's first visit, which may cause variability in MMP-1 levels.

In this research, a statistically significant inverse relationship was observed between the ulcer surface area and the expression of tissue MMP-1 in the base of the ulcers examined (r=-0.506, p=0.019).

Out of 4,832 consecutive patients with diabetic foot ulcers (DFUs) who presented for their first visit to one of 65 secondary or tertiary diabetic foot services across 15 of the 17 regions in Queensland, Australia, between July 2011 and December 2017, it was found that ulcers with a surface area greater than 3 cm^2 were associated with poorer healing compared to smaller ulcers, without identifying the underlying causes **[22]**.

In the present study, the pathological evaluation indicated a moderate level of cellularity, showing between 25 to 100 cells per high power field (HPF) in both the ulcer base and margins (85% and 52%, respectively). Vascular proliferation was also primarily moderate, recorded at 71% for both the ulcer base and

margins. Furthermore, inflammatory cells were not present in 38% of the ulcer base and 76% of the margins, with a low occurrence in the remaining ulcers. Mature collagen was the most prominent finding, noted in 85% of the base and 100% of the margins of the ulcers.

Diabetic wounds are characterized by delayed healing, affecting acute and chronic wounds. They often have a prolonged inflammatory phase, which impedes the formation of mature granulation tissue and decreases tensile strength, likely due to vascular damage and ischemia**.[23].** There are also physiological aspects such as increased serum matrix metalloproteinase-9, impaired collagen accumulation, variations in the ratio of collagen types, dysregulation of neuropeptide expression in the skin, and a suppressed inflammatory response **[24].**

The imbalance between the accumulation of ECM components and their remodeling by matrix metalloproteinases is responsible for the slow healing process in diabetic patients **[25].**

The current study showed a non-statistically significant association between MMP-1 expression at the base and the edge of the ulcers and the pathology findings of the ulcers. We have a lack of studies on diabetic foot ulcer histopathology and its relation to matrix metalloproteinases.

Studies on various types of matrix metalloproteinases (MMPs) have been conducted concerning diabetic foot ulcers. A Chinese study found that effective healers experienced nearly a fivefold decrease in MMP-9 concentration by week four after enrollment, whereas poor healers did not show this reduction. The decline in MMP-9 produced by inflammatory cells suggests a decrease in the inflammatory phase of healing, allowing the process to advance to the subsequent proliferation phase. Conversely, the higher levels of activated MMP-9 in the poor healer group support the theory that certain MMPs released by inflammatory cells can hinder the healing process **[26].**

Further, longitudinal studies are needed to assess the effect of debridement on these pathological data and achieve a better correlation with healing. Additional large-scale studies involving a significant number of individuals are crucial for confirming these findings and clarifying the relationship between MMP1 and the prediction of healing for diabetic foot ulcers.

Conclusion

Reduced levels of tissue MMP-1 directly contribute to the delayed healing of diabetic foot ulcers. These ulcers are pathologically characterized by elevated mature collagen, decreased cellularity, and persistent inflammation. It is essential to conduct further trials to investigate the role of MMP-1 and its potential as a topical or systemic treatment to enhance wound healing.

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- All authors reviewed the manuscript and accepted it for publication.
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Ethics approval and consent to participate

The study was approved by the Mansoura Institutional Research Board (IRB), code number MS/17.05.88. Written and informed consents were taken from all participants

Availability of data and material

All data are available at the corresponding author: Ahmed Albehairy

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