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Evaluation of Histopathological Features and Some Immunological Markers in Diabetic Foot Ulcer Patients

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Abstract. Background; diabetic foot patients are affected by complications with any injury, more than healthy people, as a result of the delay in discovering the problem, as the patient's lack of awareness of repeated injuries leads to complications with difficulty in treatment, these disorders are related to controlling the level of sugar in the blood. 39 samples of diabetic foot patients were collected from Al-Rifai General Hospital and the Diabetes Center in Nasiriyah city to conduct histological examinations. Aims of the study; the current study aims to characterize the histopathological features of diabetic foot ulcer cases. And assess immune and biochemical parameters associated with diabetic foot ulcer. In addition to evaluate the correlation between histopathological changes and immune responses. Methodology; Samples (blood, tissue specimen) were collected from diabetic patients with diabetic foot ulcer from Al-Rifai General Hospital and the Diabetic Center in in Nasiriyah city from April 2022 to september2022. Histopathological sections were prepared according to (Bancroft and Steven, 2008) , cytokines (TGF-β, IFN- γ and IL-17A) measured using the enzyme-linked immunosorbent technique ELISA. Result; the current study aims to demonstrate the histopathological changes associated with diabetic foot. results show purulent ulcer with a large gap between the edges of the lesion necrotic foci in the dermis tissue, Fibrin deposition with focal melanin pigmentation, degenerative changes with compaction of nuclei and extensive granulation formation in the dermis with inflammatory activity. The results showed a significant increase in the concentration of some cytokines (TGF- β, IFN- γ and IL-17A) and some Biochemical parameters such as (HbA1C and Triglycerides) in patients with diabetic foot compared to the healthy group. Conclusions; The present study concluded that the severity of diabetic foot ulcers is closely related to the high level of cytokines in diabetic patients with a preponderance in type 1 diabetes

Highlights:

1. tudy examines histopathological changes in diabetic foot ulcer patients.
2. Findings show necrosis, inflammation, elevated cytokines (TGF-β, IFN-γ, IL-17A).
3. Diabetic foot severity linked to cytokines and biochemical imbalances..

Keywords: Diabetic foot, gangrene, ulcer, TGF- β, IFN- γ and IL-17A

Introduction

Diabetes is a chronic metabolic disease resulting from a disorder in the metabolism of carbohydrates, proteins, and fats, characterized by a high level of glucose in the blood, resulting from a deficiency in the secretion of insulin, in whole or in part,

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as a result of a deficiency of insulin due to a defect in its secretion or action, or both, and this leads to an increase in the level of sugar in the blood, and weakness in the functions of some organs such as retinopathy, kidney disease, and cardiovascular disease [4]. In the extremities, sorbitol is the causative agent of diabetic neuropathy, and as a result, there is a loss of sensation in the extremities, and when combined with vascular weakness, it can lead to amputation of the extremities [2]. The foot is one of the least caring, caring and clean parts of the body, and the diabetic patient is generally more likely to suffer from foot problems because many diabetics do not feel the feet well as sugar affects the peripheral sensory nerves [5] In addition, many of these patients suffer from changes and deformities in the shape of the feet and toes due to the effect of sugar on the peripheral motor nerves, which exposes them to injuries more [4].

There are many factors that lead to diabetic foot, and these factors are divided into major and secondary factors, and one of the main factors is peripheral nerve inflammation, which usually reduces or loses sensation in patients with diabetes, and the disruption of the functions of the sympathetic and parasympathetic nervous system leads to the loss of the necessary protection To maintain the health and safety of the feet, especially the sensation of pain and the secretion of the amount of sweat needed to moisturize the skin also leads to the problem of diabetic foot narrowing or blockage of the peripheral arteries, which feed the legs below the knees, and the bacterial infection causes the tissues of the affected foot to wear out n [13], [15].

There are a number of secondary factors, including not controlling the level of sugar in the blood for a long period of time, because this affects the peripheral nerves and leads to problems in the skin and arteries and weak immunity. Triglycerides, excessive obesity, smoking and alcohol consumption. Wearing tight and unsuitable shoes with excessive pressure on them from weight and walking barefoot leads to recurrence of foot injuries with neglect of foot hygiene and care. [17] Bruising, burning with hot water, walking on hot ground, as well as cleaning the foot using sharp materials, or cutting nails in the wrong way can contribute to this condition. Some patients may reach an advanced stage of diabetic foot without realizing it, and there are symptoms of diabetic foot that must be paid attention to. Diabetics suffer from it, including when the patient loses feeling in the foot, or it decreases with the presence of abnormal things such as cold or heat for no reason, or difficulty maintaining balance while standing or

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walking. The patient loses the ability to walk for a long time, so he can feel fatigue and stress in the muscles of the feet after a very short distance on Although he was able to walk long distances previously Doctors explain this stress due to the muscles' need for food and oxygen, which does not reach them as a result of damage to the arteries, and the elderly may not pay attention to this matter because they do not walk much, and then they do not discover this problem [10]. In addition to the previous factors, the role of pollution comes, where the opportunity is prepared for the microbes to attack the tissues that have lost their vitality as a result of the injury to the blood vessels and the nerves connected to them, and from here bacteria of various types begin to attack the tissues of the feet, and as a result many pus foci that increase in the body arise as a result of the lack of pain sensation. reach the bones of the feet [20].

Methods

Specimen collection

Samples (blood, tissue specimen) were collected from diabetic patients with diabetic foot ulcer from Al-Rifai General Hospital and the Diabetic Center in in Nasiriyah city from April 2022 to september2022.

Histopathological Analysis

Processing: Specimens were fixed in 10% formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin (H&E) according to (Bancroft and Steven, 2008)

Immune aspect

The levels of some cytokines (TGF- β, IFN- γ and IL-17A) in the studied samples were measured using the enzyme-linked immunosorbent technique ELISA

Biochemical aspect

The levels of some Biochemical parameters such as (HbA1C and Triglycerides) in the studied grouped were according to (Tietz, 1987).

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Result and Discussion

Histopathological features

The indicated results are shown in Figures 1, 2, and 3. purulent ulcer with a large gap between the edges of the lesion necrotic foci in the dermis tissue, Fibrin deposition with focal melanin pigmentation, degenerative changes with compaction of nuclei and extensive granulation formation in the dermis with inflammatory activity

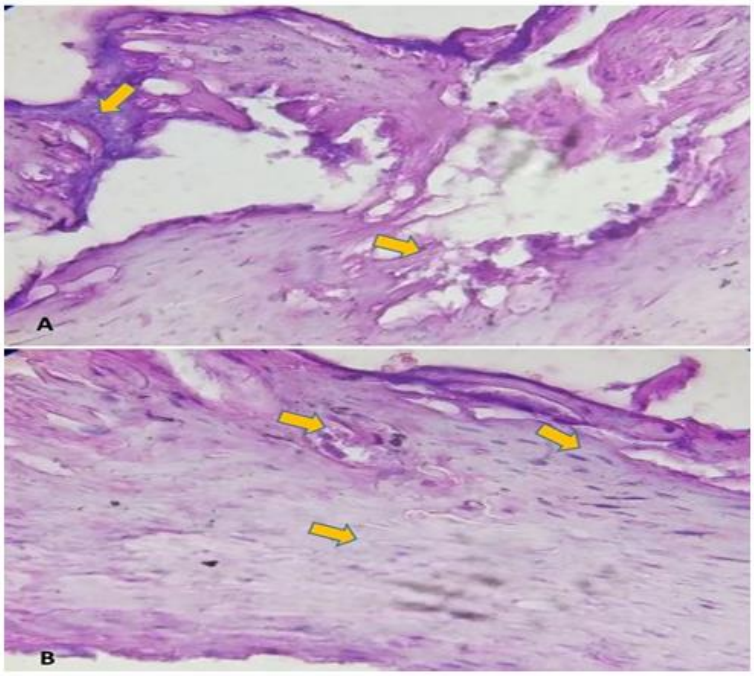

Figure 1: shows necrotic foci in the dermis tissue(A) , Fibrin deposition and inflammatory cell infiltration with focal melanin pigmentation(B)

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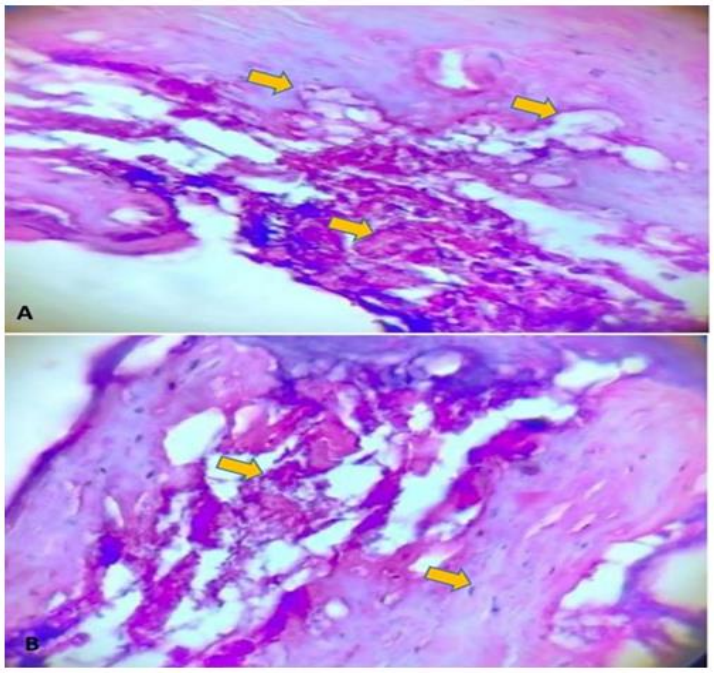

Figure 2: Necrosis of the tissues of the dermis with foci of purulent inflammation(A) , Tissue necrosis with fibrin deposits and inflammatory infiltrate(B)

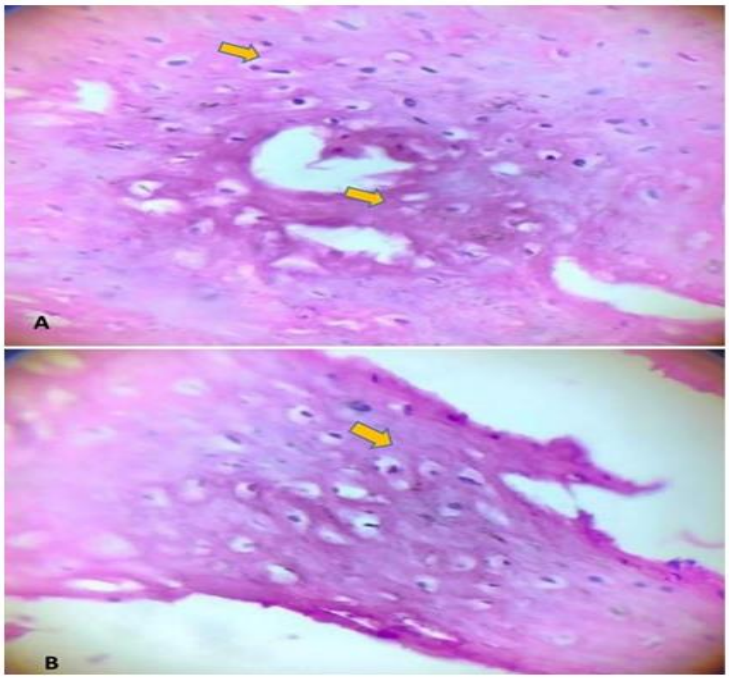

Figure (3) Skin ulcer with a large gap between the edges of the lesion(A), They show degenerative changes with compaction of nuclei and extensive granulation formation in the dermis with inflammatory activity(B)

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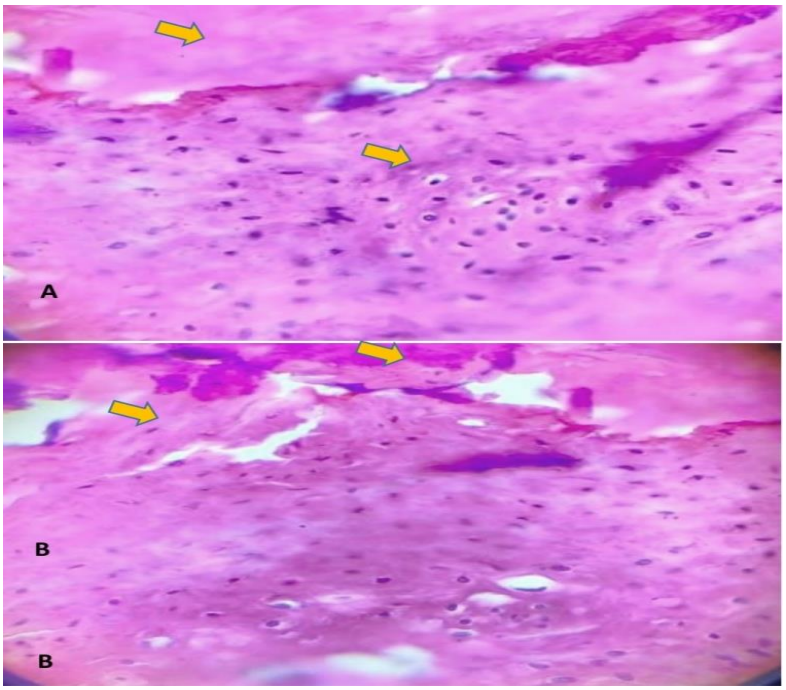

Figure 4: degenerative changes of dermis tissue with inflammatory activity are shown Area of rich infiltrated area with round mononuclear cells (A&B)

Immune and biochemical aspect

Table 1: shows the levels of cellular cytokines (TGF- β, IFN- γ and IL-17A), biochemical parameters (HbA1C and triglycerides) between the two types of diabetes and healthy people.

gropes / Variable	Diabetic patient grope		Control grope	p-value	LSD
	Type1(n.20)	Type2(n.20)	Healthy(n.20)		
TGF- β (pg/mL)	3.676^{a}±0.1631	4.026^{a}±0.2027	1.330^{b}±0.07801	<0.0001	0.38
IFN- γ (pg/mL)	10.76^{a}±0.1457	16.12^{b}±0.2925	7.902^{c}±0.1443	<0.0001	0.50
IL-17A (pg/mL)	8.281^{a}±0.2200	7.018^{b}±0.1610	2.720^{c}±0.1606	<0.0001	0.40
HbA1C (%)	11.21^{b}±0.3189	10.29^{a}±0.1722	6.926^{c}±0.1560	<0.0001	0.49
Triglycerides (mg/dL)	212^{a}.1±5.202	196.3^{a}±2.899	139.9^{b}±6.981	<0.0001	9.77

The different letters refer to a significant difference, The same letters refer to no significant differences at the 0.05 level

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Table (2) shows the Correlations between study variables.

	Correlations				
Variable	TGF- β	IFN- γ	IL-17A	HbA1C	Triglycerides
TGF- β	1				
IFN- γ	0.656**	1			
IL-17A	0.694**	0.382**	1		
HbA1C	0.737**	0.364*	0.807**	1	
Triglycerides	0.716**	0.406**	0.807**	0.822**	1

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed)

Discussion

In our study, necrotic foci in the dermis tissue with purulent ulcer and fibrin deposition with severe inflammatory activity were diagnosed. most cells identified in the inflammatory infiltrate in patients with diabetic foot were the macrophages. The presence of these cells in a high number is due to microorganisms that infected the lesion, and also to cellular debris resulted from the convergent action ischemia an infection, Especially with the presence of a significant increase in inflammatory mediators such as cytokines and interferon.

The inflammatory phase relies on an orchestrated sequence of the immune response within the wound, with neutrophils arriving first followed by pro-inflammatory M1 macrophages and pro-reparative M2 macrophages after phenotypic shift, and then finally T-lymphocytes [6], [17], [19]. Growth factors, including PDGF and TGF-β and inflammatory cytokines such as TNF-α are secreted by infiltrating neutrophils and monocytes in the early stage of the inflammatory phase for wound cleansing, and wound contraction is facilitated by myofibroblasts. Low capacity for wound contraction and low generation of granulation tissue have been observed in abnormal wound healing which is associated with an antagonistic effect of TNF-α on TGF-β. Increased levels of TNF-α in DWs have been reported [7]. as well as increased levels of other proinflammatory

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cytokines like (IL)-1β, IL-6, IL-8, in more recent studies [7], [9]. The elevated amount of ROS and cellular debris as well as presence of lipopolysaccharides (LPS) from biofilm can trigger and maintain abnormally increased levels of local and circulating inflammatory mediators [16].

The results showed a significant increase in the concentration of TGF- β in the serum of patients with diabetic foot compared to the healthy group, as the highest concentration (4.026±0.2027) was reached in the serum of patients with type 2 diabetes while the concentration of the standard sample was (1.330±0.07801). This result is largely consistent with the results of (Tesch,2007) which indicate that TGF concentrations are noticeable in diabetic patients, but their increase is steadily with type 1. This is explained by the fact that hyperglycemia in the body stimulates the expression of TGF in different types of body cells, such as macrophages, which are the main mediator in the inflammation process. Some studies have also indicated that the role of TGF in patients with type 1 diabetes is regulatory and works to inhibit the immune pathology of self-antigens without affecting the immune response [8].

The results showed a significant increase in the concentration of IFN- γ in the serum of patients compared to the healthy group. The highest concentration was (16.12±0.2925) in the serum of patients with type 2 diabetes, while the concentration of the standard sample was (7.902±0.1443). There are many studies that support these results and show a clear concept that "the destruction of beta cells is closely associated with the increased expression of the proinflammatory kinases" IFN [11]. The mechanism of direct destruction of kinases induces increased infiltration of macrophages in peripheral tissues and pancreatic cells and the release of nitric oxide and free oxygen radicals. This explains the infiltration of many phagocytic cells in the area of tissue damage.

The results indicate a significant increase in the concentration of IL-17A in the blood serum of patients compared to the control sample (healthy people). The highest concentration was (8.281±0.2200) in the blood serum of patients with type 1 diabetes, while the concentration of the control group of healthy people was (2.720±0.1606). These results are consistent with the study conducted by [14].

The results indicated statistically significant differences in HBA1C values in patients with diabetic foot compared to the healthy group, the highest percentage

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(11.21±0.3189) was in patients with type 1 diabetes, while the percentage in the healthy group was (6.926±0.1560). This result is completely consistent with results of (Kaleli et al., 2019). High HbA1c is a strong indicator of diabetic foot complications due to its contribution to the development of peripheral neuropathy and vascular complications, [1], [20].

The results showed a significant increase in the levels of triglycerides in the blood serum of patients with diabetic foot compared to the healthy group. The highest concentration of triglycerides reached (212.1±5.202) in patients with type 2 diabetes compared to healthy people, where it reached (139.9±6.981), which contributes to its accumulation on the walls of blood vessels, causing programmed cell death and activating tumor necrosis factor, which increases cell destruction and the exudation of their cellular components [18].

References

[1] N. A. E. F. Al Kafrawy, E. A. A. E. A. Mustafa, A. E. D. Abd El-Salam, O. M. Ebaid, and O. M. A. Zidane, "Study of risk factors of diabetic foot ulcers," Menoufia Medical Journal, vol. 27, no. 1, p. 28, 2014.

[2] S. Bain and S. Bay, "At a glance factsheet: GLP-1 receptor agonists and diabetic retinopathy," Diabetes & Primary Care, vol. 23, pp. 103–104, 2021.

[3] J. D. Bancroft and A. Steven, Theory and Practices of Histological Technique, 2nd ed. London: Churchill Livingstone, 2008, p. 662.

[4] J. B. Buse et al., "2019 update to: management of hyperglycemia in type 2 diabetes, 2018. A consensus report by the American Diabetes," 2020.

[5] H. C. Cardoso et al., "Risk factors and diagnosis of diabetic foot ulceration in users of the Brazilian Public Health System," Journal of Diabetes Research, vol. 2019, 2019.

[6] S. A. Eming, P. Martin, and M. Tomic-Canic, "Wound repair and regeneration: Mechanisms, signaling, and translation," Sci. Transl. Med., vol. 6, p. 265sr6, 2014.

[7] M. T. Goldberg, Y.-P. Han, C. Yan, M. C. Shaw, and W. L. Garner, "TNF-α suppresses α-smooth muscle actin expression in human dermal fibroblasts: An

ISSN 3063-8186. Published by Universitas Muhamamdiyah Sidoarjo

https://doi.org/10.21070/ijhsm.v2i1.49

implication for abnormal wound healing," J. Investig. Dermatol., vol. 127, pp. 2645–2655, 2007.

[8] K. B. Gomes, K. F. Rodrigues, and A. P. Fernander, "Role of transforming growth factor-β in diabetes nephropathy," Hindawi Publishing Corporation, pp. 1–6, 2014.

[9] G. Han and R. Ceilley, "Chronic wound healing: A review of current management and treatments," Adv. Ther., vol. 34, pp. 599–610, 2017.

[10] A. Hingorani et al., "The management of diabetic foot: A clinical practice guideline by the Society for Vascular Surgery in collaboration with the American Podiatric Medical Association and the Society for Vascular Medicine," Journal of Vascular Surgery, vol. 63, no. 2, pp. 3S–21S, 2016.

[11] M. J. Hussain, M. Peakman, and H. Gallat, "Elevated serum levels of macrophage-derived cytokines precede and accompany the onset of IDDM," Diabetologia, vol. 39, p. 60–69, 1996.

[12] S. Kaleli, C. Varım, A. Nalbant, and M. Akdoğan, "Interleukins as a marker of inflammation in diabetic foot syndrome and type 2 diabetes mellitus," 2019.

[13] M. R. Kaminski et al., "Risk factors for foot ulceration in adults with end-stage renal disease on dialysis: A prospective observational cohort study," BMC Nephrology, vol. 20, no. 1, p. 1–11, 2019.

[14] N. Kikodze, I. Pantsulaia, K. Rekhviashvili, M. Iobadze, and N. Jakhutashvili, "Cytokines and T regulatory cell in the pathogenesis of type 1 diabetes," Georgian Medical News Journal, vol. 222, pp. 29–35, 2014.

[15] J. Liu et al., "Risk factors for diabetic peripheral neuropathy, peripheral artery disease, and foot deformity among the population with diabetes in Beijing, China: A multicenter, cross-sectional study," Frontiers in Endocrinology, vol. 13, 2022.

[16] Y. Mendoza-Marí et al., "Epidermal growth factor effect on lipopolysaccharide-induced inflammation in fibroblasts derived from diabetic foot ulcer," Scars, Burns & Healing, vol. 8, 2022, Art. no. 20595131211067380.

[17] I. Petrakis, I. J. Kyriopoulos, A. Ginis, and K. Athanasakis, "Losing a foot versus losing a dollar; a systematic review of cost studies in diabetic foot complications," Expert Review of Pharmacoeconomics & Outcomes Research, vol. 17, no. 2, pp. 165–180, 2017.

ISSN 3063-8186. Published by Universitas Muhamamdiyah Sidoarjo

https://doi.org/10.21070/ijhsm.v2i1.49

[18] M. S. Phipps and C. A. Cronin, "Management of acute ischemic stroke," BMJ, vol. 368, 2020.

[19] M. Portou, D. Baker, D. Abraham, and J. Tsui, "The innate immune system, toll-like receptors, and dermal wound healing: A review," Vascular Pharmacology, vol. 71, pp. 31–36, 2015.

[20] P. Schilrreff and U. Alexiev, "Chronic inflammation in non-healing skin wounds and promising natural bioactive compounds treatment," International Journal of Molecular Sciences, vol. 23, no. 9, p. 4928, 2022.