How to Cite:
AL-Mosawi, F. F. S., Ahmed, M. M., & Yahya, M. W. (2022). Evaluation of blood indices as possible biomarkers for diabetic foot ulcer characteristics. *International Journal of Health Sciences*, *6*(S1), 10245–10262. https://doi.org/10.53730/ijhs.v6nS1.7423

Evaluation of blood indices as possible biomarkers for diabetic foot ulcer characteristics

Fatima Fadhel Saeed AL-Mosawi
University of Karbala, College of Medicine, Medical Microbiology Department, Iraq, Karbala Medical City, Iraq
Corresponding author email: fatimaalmosawi994@gmail.com

Mohanad Mohsin Ahmed
University of Karbala, College of Medicine, Medical Microbiology Department, Iraq, Karbala Medical City, Iraq

Mostafa Walid Yahya
University of Karbala, College of Medicine, Medical Microbiology Department, Iraq, Karbala Medical City, Iraq

***Abstract*---**Background: Although complete blood picture is a routine laboratory analysis and widely used, little data are available about the association of hematological indices with different characteristics of diabetic foot ulcer (DFU). Aim of the study: the aim was to evaluate the blood indices as possible biomarkers for diabetic foot ulcers. Methods: this is a cross-sectional study involved 58 DFU patients who attended Al-kafeel Super specialty hospital and Private orthopedic clinics during the period from April 9 to September 6, 2021. All patients were newly diagnosed and scheduled for treatment. Demographic and clinical data were collected by specialist and blood were collected for the laboratory assays. Results: These results indicate that DFU is largely of older male patients with poor glycaemic control. Regarding the characteristics of ulcers, most of patients (81%) had ulcers for more than 20 weeks' duration. Most of the ulcers (65.5%) associated with swollen feet and the area of ulcers in most of the patients (69%) were equal or less then15cm^2. Most of the patients (87. 9%) present with single ulcers and ulcers were new (72.4%) were painless (79.3%) and non-bleeding (62.1%). This study showed the total count of WBCs in addition to neutrophils count increase with increase in the depth of the ulcers. Increasing lymphocyte counts showed with highly significant association with increasing in the duration of ulcers (p=0.001). There was inverse relation between ulcer area and MCH (p=0.007), MCHC (p=0.025) and RDW (p=0.005). Furthermore, ulcers with bad smell were significantly associated

Manuscript submitted: 27 March 2022, Manuscript revised: 18 April 2022, Accepted for publication: 9 May 2022

increased MCH (p=0.004). Increasing ulcer duration was associated with significant increase in platelets count (p= 0.013). Furthermore, higher PCT was associated with presence of multiple ulcers (p=0.005) and recurrence of the ulcers (p=0.05) In addition presence of painful ulcer was associated higher mean platelet volume (MPV) (p=0.025). High levels of HbA1c were found to be associated with increasing in the ulcer area (p=0.016). These results indicate the effect of poor diabetic control on the area of ulcers. Conclusion: Our study indicates the total count of WBCs in addition to neutrophils count increase with increase in the depth of the ulcers, lymphocytes increase in chronic infection, all of ulcer site, area and smell had effect on red blood cell indices, importance of platelets indices in the pathology and pathogenesis of diabetic foot ulcer and the effect of poor diabetic control on the area of ulcers.

***Keywords*---**Diabetic foot ulcer, complete blood count, C- reactive protein (CRP), Haemoglobin glycaemic A1C (HbA1C).

Introduction

Diabetes mellitus (DM) is a metabolic disease, involving inappropriately elevated blood glucose levels. Diabetes is a worldwide epidemic (Atlas 2015). There are three main types of diabetes mellitus including type 1, type 2 and Gestational Diabetes Mellitus (Sapra, Bhandari et al. 2021). Type 2 diabetes mellitus has several complications, one of these complications is diabetic foot ulcer (DFU) (Mezil and Abed 2021). The term “diabetic foot ulcer” (DFU) is imprecise. It describes the presence of a break in the skin of the foot in a person with diabetes (Monteiro- Soares, Boyko et al. 2020). The most common, painful, and disabling effects of diabetes mellitus are DFU (Coffey, Mahon et al. 2019). Each year, these illnesses impact 3% of the diabetic community, and this number is predicted to climb as diabetes becomes more prevalent (Krzyszczyk, Schloss et al. 2018). Diabetics have a 25% chance of acquiring DFU during their lifetime (Aras, Karaman et al. 2017). Every 20 seconds a limb is amputated somewhere in the world due to diabetes (Cassidy, Reeves et al. 2021). DFU is the result of various factors like peripheral vascular disease, peripheral neuropathy, trauma, foot deformities, arterial insufficiency and impaired resistance to infection. Complex metabolic pathways contribute to the development of neuropathy and peripheral artery disease in people with uncontrolled diabetes (Tripathi and Gupta 2018). Patient workup for diabetic ulcers includes blood tests, radiography, ankle-brachial index and toe pressure, pulse-volume recording, ultrasonography, computed tomography (CT) scanning or magnetic resonance imaging (MRI), bone scans, and angiography (Stacy 2021). The ulcer should be staged after it has been diagnosed. The diabetic foot has been classified in a variety of ways. However, the Wagner-Ulcer Classification System and the University of Texas Wound Classification System (Syafril 2018).

Materials and Method

Fifty-eight blood samples were taken from clinically diagnosed patients with diabetic foot ulcer. The samples were taken from both sexes (43) males and (15) females at age ranged between (42-78) years old attending to Al-Kafeel Super Specialty Hospital in Karbala and private clinics between April 9 and September 6, 2021. Case information sheets involving age, gender and others were carried out for each patient. Exclusion criteria include patients with diabetic foot ulcer, but they are using immunotherapy treatments, or they have another infection or ulcer in another position in their body.

Blood Samples Collection

Each participant had approximately 4 ml of venous blood collected after cleaning the antecubital fossa with 70% ethanol and then puncturing veins with disposal syringes after applying a tourniquet. For the hematological testing. Two milliliters of blood were discharged into an EDTA tube. Two milliliters of blood were injected into an SST tube, allowed to clot, and then the serum was separated by centrifugation at 3000 RPM for 15 minutes. The serum was then transferred to an Eppendorf tube and kept at -20°C for use in biochemical tests.

Complete Blood Count (CBC)

Blood samples were collected in EDTA tubes, samples were shaken up, and Swelab Alpha automated hematology analyzers were used to analyses them as soon as feasible (Swelab, China) to determine the number of white blood cells, red blood cells, and platelets.

Biochemical Tests

In this investigation, a variety of biochemical materials were found. As a result, the I Chroma 2 boditch method was used to detect C-reactive protein (CRP) and glycated hemoglobin (HbA1c).

Statistical analyses

To conduct statistical analysis, data was entered into the Specific Software Statistical Package for the Social Sciences (SPSS) version 25 for Windows (GraphPad Software, San Diego, California, USA), The data was presented as a mean standard deviation. T tests were used to compare two means, while ANOVA was used to compare multiple means. A p value of 0.05 was judged statistically significant, while a p value of 0.001 was regarded very significant.

Results

Table 1 shows the age of the patients which was ranged between 42 and 78 years old. According to age, the patients were grouped into two age groups (≤50 and >50years). The results revealed that the DFU was more frequent in the age group >50 years (79.3%) than other age group. According to gender, DFU was more prevalent in males, who were 43 (74.1 %), whereas the females were 15 (25.9%). Regarding smoking habits, 50% of DFU were smoking. Hypertension was found in 41.4% and those with no hypertension was 58.6%. DFU patients with insulin

treatment were more, 33(56.89%), whereas, the non -insulin dependent patients were 25(43.1%). DFU patients with poor control were more, 54(93.1%), whereas, only 4 (6.9%) of DFU patients were good control. Diabetic duration was ranged between 1 and 35 years. This duration was grouped into two groups (≤15 and >15years), DFU patients 31(53.4%) had diabetes for more than 15 years' duration and 27(46.6%) had diabetes for less than 15 years' duration. DFU patients with swollen feet were more, 38(65.5%), whereas, patients with no swollen feet were 20(34.5%). Regarding ulcer duration, DFU patients 47(81%) had ulcer for less than 20 weeks' duration and only 11(19%) had ulcer for more than 20 weeks' duration. DFU patients who had ulcer on volar side of foot were 31 (53.4%), whereas, 27 (46.6%) of DFU patients were having on dorsal side of foot. Ulcer type is classified into superficial ulcer, deep ulcer, ulcer with bone involvement and gangrene (15.5% ,41.4%, 20.7%, 22.4%) respectively. Ulcer area ranged between 3 to 50 cm ^{2}, these areas were grouped into two area groups (≤15 and > 15cm ^{2}), the results revealed that most patients (69%) had ulcer area of ≤15 cm ^{2}. The colour of the ulcer was classified into red, white and black (14 (24.1%), 20 (34.5%), 24 (41.4%)) respectively. About ulcers smell, 50% of DFU were have a bad smell in their ulcers. The number of ulcers is classified into single and multiple, a higher prevalence single ulcer was seen. Ulcers that occurs for the first time were 42 (72.4%) and recurring ulcers were 16 (27.6%). In connection with ulcers bleeding, 22(37.9%) of DFU patients were having bleeding in their ulcers, whereas, 36 (62.1%) had with no bleeding in their ulcers. DFU patients with no pain in foot were more, 46(79.3%), whereas, patients with pain were 12(20.7%).

Table 2 shows the total and differential WBCs counts, Lymphocytes, Neutrophils, HbA1c and CRP increases (mean=11.86, 23.76, 69.95, 10.77, 98.07) respectively. Hemoglobin (HGB) and Hematocrit (HCT) decreases (mean=10.66, 32.96) respectively in patients with DFU.

Table 3-A shows the relationships between characteristics of ulcers with total and differential WBCs counts. A significant correlation between ulcer type and total WBC count (p=0.019), where higher WBC counts were found in ulcer with bone involvement (mean=13.9×10^{3}cell/mm) and ulcers with gangrene (mean=14.59×10^{3}cell/mm) in comparison to superficial ulcers (mean=10.4×10^{3}cell/mm), Regarding the differential WBCs counts, neutrophils were significantly correlated with ulcer types (p=0.025), where higher neutrophils counts were found in ulcers with gangrene (mean=11.35×10^{3}cell/mm) and ulcer with bone involvement (mean=10.19×10^{3}cell/mm) in comparison to superficial ulcers (mean=7.39×10^{3}cell/mm) and deep ulcers (mean=7.15×10^{3}cell/mm). There was an increase in mean of WBC count in patients with ulcer area more than 15cm^2 compared to patients with ulcer area less than 15cm^2 (14.07×10^3 cell/mm versus 10.89×10^3 cell/mm), however the difference does not reach the statistical significance (p=0.065). A similar trend was seen in neutrophils count, where mean count was higher in patients with ulcer area more than 15 cm^2 compared to the count in patients with ulcer area less than 15cm^2(10.73×10^3 cell/mm versus 7.87×10^3 cell/mm).

Regarding the ulcer duration calculated by weeks, the duration of an ulcer and the number of lymphocytes in the blood were discovered to have a highly significant relationship (p=0.001), where patients with duration of ulcers more

than 20 weeks showed a higher lymphocyte counts than patients with duration of ulcers less than 20 weeks (2.27×10^3 cell/mm versus 3.09×10^3 cell/mm).

There was an increase in mean of WBC count in patients with black ulcer color compared to patients with white and red ulcer color (13.37×10^3 cell/mm, 11.44×10^3 cell/mm and 9.89×10^3 cell/mm) respectively, however the difference does not reach the statistical significance (p=0.084). A similar trend was seen in neutrophils count, where mean count was higher in patients with black ulcer color compared to the count in patients with white and red ulcer color (10.22×10^3 cell/mm, 8.46×10^3 cell/mm and 6.67×10^3 cell/mm) respectively.

Regarding the ulcer smell there was an increase in mean of WBC count in patients with bad smell of ulcer compared to patients with normal smell of ulcer (14.07×10^3 cell/mm versus 10.89×10^3 cell/mm), however the difference does not reach the statistical significance (p=0.065).

The results illustrated that there was an increase in mean of WBC count in patients with multiple ulcers compared to patients with single ulcer (13.04×10^3 cell/mm versus 11.70×10^3 cell/mm), however the difference does not reach the statistical significance (p=0.059). A similar trend was seen in neutrophils count, where mean count was higher in patients with multiple ulcers compared to the count in patients with single ulcer (10.26×10^3 cell/mm versus 8.55×10^3 cell/mm). Table 3-B shows the correlation between ulcer characteristics and RBC indices. A significant correlation between ulcer site and haemoglobin (p =0.046), where higher haemoglobin was found in dorsal site of ulcer (mean=11.23g/dl) in comparison to volar site of ulcer (mean=9.16g/dl), Regarding the differential RBCs indices, haematocrits percentage were significantly correlated with ulcer site (p =0.025), where higher haematocrits percentage were found in dorsal site of ulcers (mean=34.73%) in comparison to volar site of ulcers (mean=30.43%).

The results illustrated that there was an increase in mean of RBCs in patients with gangrenes ulcer type (mean=9.91×10^{12} cell/L) in compared to patients with superficial ulcer, deep ulcer and ulcer with bone involvement (4.26×10^{12} cell/L, 4.24×10^{12} cell/L and 3.85×10^{12} cell/L), however the difference does not reach the statistical significance (p =0.091).

Regarding ulcer area, a highly significant correlation between ulcer area and mean corpuscular haemoglobin MCH (p =0.007), where higher MCH was found in ulcer less than 15 cm^2 (mean=27.9pg) in comparison to ulcer more than 15 cm^2 (mean=24.55pg), As well in mean corpuscular haemoglobin concentration MCHC appear a significant correlation with ulcer area (p =0.025), where higher MCHC was found in ulcer less than 15 cm^2 (mean=33.56g/L) in comparison to ulcer more than 15 cm^2 (mean=31.03g/L), Also a similar trend was seen in red cell distribution width RDW, a highly significant correlation with ulcer area (p =0.005), where higher RDW was found in ulcer less than 15 cm^2 (mean=50.67) in comparison to ulcer more than 15 cm^2 (mean=41.92).

The study showed a highly significant correlation between ulcer smell and mean corpuscular haemoglobin concentration MCHC (p =0.004), where higher MCHC was found in ulcer with bad smell (mean =35.98g/L) in comparison to ulcer with

normal smell (mean =31.19g/L). Also a significant correlation between ulcer smell and red cell distribution width RDW (p =0.048), where higher RDW was found in ulcer with bad smell (mean =50.87) in comparison to ulcer with normal smell (mean =45.04).

The results showed that there was an increase in mean of RBCs indices in patients with single ulcer compared to patients with multiple ulcers (4.14×10^{12} cell/L versus 3.28×10^{12} cell/L). However, the difference does not reach the statistical significance (p =0.098).

Table 3-C shows the relationship between characteristics of ulcers and platelets indices. The current study showed that there was an increase in mean of plateletcrit (PCT) in patients with dorsal site of ulcers compared to patients with volar site of ulcers (2.15mL/L versus 0.52mL/L). However, the difference does not reach the statistical significance (p =0.194). A significant correlation seen in ulcer site with platelet large cell count P-LCC (p =0.038), where higher P-LCC was found in dorsal site of ulcer (mean $=43.48\times10^{9}$ cell/L) in comparison to volar site of ulcer (mean $=35.42\times10^{9}$ cell/L).

Regarding the ulcer duration, a significant correlation was found between ulcer duration and PLT indices (p =0.013), where patients with duration of ulcers more than 20 weeks showed a higher PLT indices than patients with duration of ulcers less than 20 weeks (389.73×10^{9} cell/L versus 290.53×10 9 cell/L). Also there was an increase in mean of platelet-large cell ratio P-LCR in patients with ulcer duration less than 20 weeks compared to patients with ulcer duration more than 20 weeks (19.4% versus 16.49%), however the difference does not reach the statistical significance (p =0.299).

However, the decrease in mean of PLT indices in patients with red ulcer color compared to patients with white and black ulcer color (107.71×10^{9} cell/L, 308.2×10^{9} cell/L and 311.25×10^{9} cell/L) respectively. Never the less, the difference does not reach the statistical significance (p =0.926). Regarding PCT, there was higher in patients with black ulcer color in compared with red and white ulcer color (2.23mL/L, 0.99 0.34mL/L and 0.34mL/L) respectively. However, the difference does not reach the statistical significance (p =0.391). The study showed a highly significant correlation between ulcer number and PCT (p =0.005), where higher PCT was found in multiple ulcers (mean =6.32mL/L) in comparison to single ulcer (mean =0.59mL/L). By the way new or recurrent ulcers, a significant correlation between new or recurrent ulcers PCT (p =0.053), where higher PCT was found in recurrent ulcers (mean =3.39mL/L) in comparison to new ulcer (mean =0.47mL/L). The study showed that there was an increase in mean of PLT indices in patients with nonbleeding ulcer compared to patients bleeding ulcers (332.58×10^{9} cell/L versus 221.32×10^{9} cell/L). However, the difference does not reach the statistical significance (p =0.060). A significant correlation was found between painful ulcer and mean platelet volume MPV (p =0.025), where patients with pain of ulcers showed a higher MPV than patients with no pain of ulcers (9.47fLversus 7.49fL).

Table 4 shows the correlation between characteristics of ulcer with serum CRP and HbA1c levels. The results illustrate that there is an increase in mean of C-

reactive protein CRP in patients who have ulcer with bone involvement (mean=141.61mg/L) compared to patients with superficial ulcer, deep ulcer and ulcer with gangrene (73.16mg/L, 71.75mg/L and 97.21mg/L). However, the difference does not reach the statistical significance (p =0.235). It was noticed that CRP, the mean was higher in patients with ulcer area which was more than 15cm ^{2} compared with ulcer area less than 15cm ^{2} (115.54mg/L versus 81.59mg/L). However, the difference does not reach the statistical significance (p =0.226). A similar orientation was noticed in new or recurrent ulcers, where mean of CRP was higher in patients with recurrent ulcer in comparison with new ulcer (118.81mg/L versus 81.96mg/L), however the difference does not reach the statistical significance (p =0.204). Like that was seen in ulcer bleeding, where mean of CRP was higher in patients with bleeding ulcer compared with nonbleeding ulcer (111.02mg/L versus 80.58mg/L). Still, the difference does not reach the statistical significance (p =0.255). Additionally, a similar orientation was seen in ulcer pain, where mean of CRP was higher in patients with painful ulcer compared with no pain in ulcer (113.87mg/L versus 86.46mg/L). Yet, the difference does not reach the statistical significance (p =0.393).

Regarding the HbA1c level, a significant correlation was found between ulcer area and HbA1c level (p =0.016), where patients with ulcer area which are more than 15 cm^2 showed a higher HbA1c level than patients with ulcer area which are less than 15 cm^2 (12.07% versus 10.19%).

Table 1
Description of characteristics of diabetic foot ulcer patients

Variables		Frequency	Percentage
Age	≤50	12	20.7%
	>50	46	79.3%
Gender	Male	43	74.1%
	Female	15	25.9%
Smoking status	Smoker	29	50%
	Non smoker	29	50%
Blood pressure	Hypertension	24	41.4%
	Normal	34	58.6%
Insulin	Insulin dependent	33	56.9%
	Insulin non dependent	25	43.1%
Glycaemic control	Good control ≤6.8%	4	6.9%
	Poor control >6.8%	54	93.1%
Diabetic duration/year	≤15	27	46.6%
	>15	31	53.4%
Swollen foot	Present	38	65.5%
	Absent	20	34.6%
Ulcer duration/week	≤20	47	81%
	>20	11	19%
Ulcer sites	Volar	31	53.4%
	Dorsal	27	46.6%

Ulcer type	Superficial ulcer	9	15.5%
	Deep ulcer	24	41.4%
	Ulcer with bone involvement	12	20.7%
	gangrene	13	22.4%
Ulcer area cm^2	≤15	40	69%
	>15	18	31%
Ulcer color	Red	14	24.1%
	White	20	34.5%
	Black	24	41.4%
Ulcer smell	Normal	29	50%
	Bad	29	50%
Ulcer number	Single	51	87.9%
	Multiple	7	12.1%
New or recurrent ulcer	New	42	72.4%
	Recurrent	16	27.6%
Ulcer bleeding	Present	22	37.9%
	Absent	36	62.1%
Ulcer Pain	Present	12	20.7%
	Absent	46	79.3%

Table 2
Hematological and biochemical characteristics of patients (N=58) with diabetic foot ulcer

Parameters	Normal value	Mean	Std. Deviation
WBC	3.5-10	11.86	6.13
LYM%	0.9-5	23.76	10.52
NEU%	1.2-8	69.95	10.79
RBC	3.5-5.5	4.09	0.69
HGB	11.5-16.5	10.66	2.04
MCV	75-100	80.92	8.31
HCT	35-55	32.96	5.84
MCH	25-35	26.17	3.13
MCHC	31-38	32.08	2.42
RDW	0.1-250	47.95	11.27
PLT	150-400	309.34	120.78
MPV	6.5-11	8.69	1.35
PDW	0.1-30	13.18	2.46
PCT	0.01-9.99	1.28	5.15
P-LCR	0.1-99.9	18.85	8.31
P-LCC	1-1999	39.17	14.85
HBA1C	Good control ≤6.8%	10.77	2.78
	Poor control >6.8%		
CRP	<6.0	98.07	12.88

Table 3-A
Relationship of characteristics of ulcer with total and differential WBCs counts.

Variables		WBC		LYM		NEU	
		Mean(±SD)	*p* - value	Mean(±SD)	*p* – value	Mean(±SD)	*p* - value
Ulcer site	Volar (N=31)	12.54±6.68	0.374	2.48±0.79	0.602	9.29±6.15	0.451
	Dorsal (N=27)	11.09±5.45		2.37±0.75		8.14±5.23	
Ulcer type	Superficial ulcer (N=9)	10.4±3.2	0.019	2.54±0.8	0.841	7.39±3.26	0.025
	Deep ulcer(N=24)	9.89±5.91		2.25±0.75		7.15±5.8	
	Ulcer with bone involvement(N=12)	13.9±6.62		2.78±0.67		10.19±6.24	
	gangrene(N=13)	14.59±6.55		2.35±0.76		11.35±5.65	
Ulcer area (cm^2)	≤15(N=40)	10.89±4.76	0.065	2.45±0.78	0.744	7.87±4.3	0.077
	>15(N=18)	14.07±8.16		2.38±0.76		10.73±7.82	
Ulcer duration (week)	≤20(N=47)	11.89±6.54	0.967	2.27±0.74	0.001	8.94±6.1	0.626
	>20(N=11)	11.79±4.18		3.09±0.5		7.99±3.81	
Ulcer color	Red (N=14)	9.89±3.25	0.084	2.69±0.81	0.554	6.67±2.68	0.061
	White (N=20)	11.44±6.54		2.21±0.75		8.46±6.26	
	Black (N=24)	13.37±6.85		2.46±7.45		10.22±6.3	
Ulcer smell	Normal (N=29)	10.4±3.64	0.069	2.5±0.77	0.458	7.32±3.19	0.055
	Bad (N=29)	13.3±7.67		2.35±0.78		10.19±7.25	
Ulcer number	Single (N=51)	11.7±6.39	0.059	2.45±0.8	0.503	8.55±5.94	0.064
	Multiple (N=7)	13.04±3.39		2.24±0.44		10.26±3.67	
New or recurrent ulcer	New (N=42)	11.49±5.81	0.456	2.41±0.78	0.718	8.47±5.35	0.542
	Recurrent (N=16)	12.84±7		2.49±0.76		9.51±6.72	
Ulcer bleeding	Present (N=22)	12.31±7.37	0.667	2.22±0.84	0.114	9.32±7.1	0.557
	Absent (N=36)	11.59±5.02		2.55±0.71		8.41±4.75	
Ulcer pain	Present(N=12)	12.68±8.15	0.606	2.35±0.61	0.699	9.55±7.67	0.594
	Absent(N=46)	11.65±5.58		2.45±0.74		8.55±5.18	

Table 3-B
Relationship between characteristics of ulcer and RBCs indices

Variables		RBC		HGB		MCV		HCT		MCH		MCHC		RDW	
		Mean (±SD)	*p* - value	Mean (±SD)	*p* - value	Mean (±SD)	*p* - value	Mean (±SD)	*p* - value	Mean (±SD)	*p* - value	Mean (±SD)	*p* - value	Mean (±SD)	*p* - value
Ulcer site	Volar (N=31)	3.96 ±0.69	0.129	9.16 ±2.09	0.046	79.7 ±9.05	0.241	30.43 ±5.58	0.031	25.79 ±3.46	0.319	32.13 ±2.46	0.874	49.22 ±12.17	0.363
	Dorsal (N=27)	4.24 ±0.69		11.23 ±1.86		82.29 ±7.29		34.73 ±5.41		26.62 ±2.7		32.03 ±2.42		46.5 ±10.18	
Ulcer type	Superficial ulcer (N=9)	4.26 ±0.66	0.091	11.33 ±1.95	0.166	81.62 ±5.79	0.843	34.76 ±6.23	0.082	26.67 ±1.85	0.934	32.59 ±1.29	0.875	50.39 ±9.88	0.849
	Deep ulcer(N=24)	4.24 ±0.83		10.79 ±2.19		81 ±8.07		34.15 ±6.49		26.1 ±3.37		32.16 ±2.46		46.67 ±9.3	
	Ulcer with bone involvement(N=12)	3.85 ±0.54		9.67 ±1.73		80.05 ±8.63		30.68 ±4.04		25.2 ±2.92		30.9 ±2.58		46.32 ±13.98	
	gangrene(N=13)	9.91 ±0.53		10.5 ±1.98		81.07 ±10.56		31.65 ±5.3		26.87 ±3.61		32.69 ±2.66		50.23 ±13.32	
Ulcer area (cm^2)	≤15(N=40)	4.39 ±0.68	0.451	10.82 ±1.95	0.378	82.23 ±7.25	0.073	33.12 ±5.6	0.764	27.9 ±2.92	0.007	33.56 ±2.35	0.025	50.67 ±11.52	0.005
	>15(N=18)	4.19 ±0.74		10.3 ±2.25		78 ±9.9		32.62 ±6.51		24.55 ±3.04		31.03 ±2.3		41.92 ±8.11	
Ulcer duration (week)	≤20(N=47)	4.05 ±0.73	0.443	10.71 ±2.1	0.672	81.85 ±7.71	0.078	33.05 ±5.92	0.830	26.53 ±3.06	0.069	32.13 ±2.53	0.751	49.39 ±11.65	0.044
	>20(N=11)	4.23 ±0.56		10.42 ±1.82		76.95 ±9.93		32.8 ±5.73		25.3 ±3.11		32.5 ±1.99		41.83 ±7.03	
Ulcer color	Red (N=14)	4.47 ±0.59	0.194	11.31 ±1.75	0.382	80.52 ±7.74	0.936	35.56 ±5.12	0.221	25.65 ±2.73	0.831	31.66 ±1.94	0.944	49.14 ±12.6	0.297
	White (N=20)	4 ±0.86		10.75 ±1.88		81.67 ±6.42		31.5 ±5.57		26.7 ±2.41		32.73 ±2.05		49.86 ±9.24	
	Black (N=24)	4.05 ±0.54		10.58 ±2.29		80.52 ±10.12		32.67 ±6.17		26.04 ±3.85		31.8 ±2.9		45.68 ±12.05	
Ulcer smell	Normal (N=29)	4.19 ±0.69	0.279	10.85 ±2.12	0.484	82.09 ±7.9	0.287	34.21 ±5.73	0.104	25.98 ±3.03	0.637	31.19 ±2.51	0.004	45.04 ±11.26	0.048
	Bad (N=29)	3.99 ±0.7		10.47 ±1.97		79.75 ±8.68		31.71 ±5.77		26.39 ±3.27		35.98 ±2.01		50.87 ±10.67	
Ulcer number	Single (N=51)	4.14 ±0.71	0.098	10.74 ±2.12	0.391	80.47 ±8.7	0.273	33.24 ±6.03	0.337	26 ±3.2	0.261	32.05 ±2.38	0.767	47.82 ±11.09	0.812
	Multiple (N=7)	3.28 ±0.41		10.03 ±1.25		84.17 ±3.37		30.96 ±4.01		27.43 ±2.4		32.34 ±2.92		48.91 ±13.45	
New or recurrent ulcer	New (N=42)	4.12 ±0.62	0.538	10.68 ±1.85	0.900	79.63 ±8.48	0.055	32.76 ±5.28	0.677	26.02 ±3.42	0.550	32.33 ±2.52	0.213	48.86 ±11.72	0.328
	Recurrent (N=16)	3.99 ±0.89		10.6 ±2.54		84.3 ±7.01		33.49 ±7.28		26.58 ±2.24		31.44 ±2.1		45.59 ±9.95	
Ulcer bleeding	Present (N=22)	4 ±0.88	0.479	10.49 ±2.08	0.636	81.67 ±7.81	0.592	32.45 ±6.44	0.605	26.51 ±3.12	0.532	32.39 ±2.18	0.463	50.41 ±12.77	0.198
	Absent (N=36)	4.14 ±0.57		10.76 ±2.04		80.45 ±8.68		33.28 ±5.51		25.97 ±3.16		31.9 ±2.57		46.46 ±10.15	
Ulcer pain	Present(N=12)	3.81 ±0.71	0.121	10.19 ±2.25	0.381	84.29 ±5.87	0.115	32.16 ±6.38	0.596	26.71 ±2.81	0.510	30.88 ±2.75	0.053	47.98 ±14.69	0.992
	Absent(N=4)	4.16 ±0.68		10.78 ±1.99		80.04 ±8.67		33.17 ±5.75		26.03 ±3.22		32.4 ±2.26		47.95 ±10.4	

Table 3-C
Relationship between characteristics of ulcer and PLTs indices.

Variables		PLT		MPV		PDW		PCT		P-LCR		P-LCC	
		Mean (±SD)	*p* -value	Mean (±SD)	*p* -value	Mean (±SD)	*p* -value	Mean (±SD)	*p* -value	Mean (±SD)	*p* -value	Mean (±SD)	*p* -value
Ulcer site	Volar (N=31)	307.13 ±136.86	0.883	8.47 ±1.34	0.174	12.71 ±2.49	0 .118	0.52 ±0.66	0.194	18.49 ±8.76	0.724	35.42 ±9.87	0.038
	Dorsal (N=27)	311.89 ±101.77		8.95 ±1.35		13.73 ±2.36		2.15 ±7.5		19.27 ±7.91		43.48 ±18.31	
Ulcer type	Superficial ulcer (N=9)	336.11 ±101.24	0.097	8.16 ±1.1	0.883	12.59 ±2.36	0.811	0.62 ±1.08	0 .131	15.68 ±6.26	0.886	38.67 ±11.36	0.828
	Deep ulcer(N=24)	253.46 ±100.06		8.87 ±1.37		13.29 ±2.43		0.53 ±0.68		19.13 ±8.11		38.13 ±10.74	
	Ulcer with bone involvement (N=12)	344.75 ±121.9		9.31 ±1.28		14.48 ±2.02		0.79 ±0.1		24.12 ±8.5		42.42 ±26.9	
	gangrene(N =13)	361.31 ±136.83		8.16 ±1.36		12.21 ±2.63		3.57 ±10.7 8		15.68 ±7.95		38.46 ±7.99	
Ulcer area (cm2)	≤15(N=40)	306.08 ±114.36	0.762	8.59 ±1.32	0.400	12.9 ±2.47	0.195	1.64 ±6.19	0.435	18.55 ±8.13	0.679	39.65 ±16.84	0.718
	>15(N=18)	316.61 ±137.27		8.92 ±1.45		13.81 ±2.37		0.48 ±0.42		19.53 ±8.9		38.11 ±9.34	
Ulcer duration (week)	≤20(N=47)	290.53 ±107.24	0.013	8.79 ±1.29	0.280	13.23 ±2.34	0.754	1.33 ±5.7	0.882	19.4 ±8.06	0.299	39.81 ±15.99	0.505
	>20(N=11)	389.73 ±146.59		8.29 ±1.59		12.97 ±3.04		1.07 ±1.3		16.49 ±9.32		36.45 ±8.44	
Ulcer color	Red (N=14)	107.71 ±91.14	0.926	8.66 ±1.45	0.912	13.34 ±2.57	0.880	0.99 ±1.21	0.391	17.49 ±8.33	0.633	41.21 ±11.31	0.396
	White (N=20)	308.2 ±116.42		8.76 ±1.42		12.86 ±2.36		0.34 ±0.32		19.56 ±8.32		40.15 ±22.4	
	Black (N=24)	311.25 ±142.33		8.63 ±1.3		13.37 ±2.55		2.23 ±7.95		19.09 ±8.55		37.17 ±7.23	
Ulcer smell	Normal (N=29)	297.41 ±113.11	0.457	8.98 ±1.43	0.110	13.99 ±2.6	**0** .011	2.08 ±7.23	0.241	20.41 ±9.11	0.155	39.66 ±9.71	0.807
	Bad (N=29)	321.28 ±128.88		8.41 ±1.23		12.38 ±2.05		0.48 ±0.71		17.39 ±7.25		38.69 ±18.82	
Ulcer number	Single (N=51)	304.85 ±118.59	0.400	8.74 ±1.35	0.473	13.26 ±2.47	0.561	0.59 ±0.77	0.005	19.12 ±8.25	0.509	39.41 ±15.56	0.744
	Multiple (N=7)	345.61 ±140.17		8.34 ±1.45		12.67 ±2.51		6.32 ±14.6 2		16.89 ±9.19		37.43 ±8.66	
New or recurrent ulcer	New (N=42)	305.14 ±123.35	0.672	8.54 ±1.25	0.171	13.01 ±2.39	0.384	0.47 ±0.63	0.053	18.17 ±8.02	0.317	39.74 ±16.81	0.642
	Recurrent (N=16)	320.38 ±116.53		9.09 ±1.58		13.64 ±2.65		3.39 ±9.66		20.64 ±9.04		37.69 ±7.89	
Ulcer bleeding	Present (N=22)	221.32 ±100.66	0.060	8.7 ±1.37	0.986	13.19 ±2.47	0.988	0.57 ±0.56	0.417	18.14 ±7.69	0.615	38.55 ±10.93	0.804
	Absent (N=36)	332.58 ±127.34		8.69 ±1.37		13.18 ±2.48		1.71 ±6.52		19.29 ±8.75		39.56 ±16.94	
Ulcer pain	Present(N=1 2)	289.19 ±103.68	0.517	9.47 ±1.25	0.025	14.08 ±2.26	0.157	0.7 ±0.84	0.666	21.53 ±7.62	0.214	41.17 ±9.14	0.606
	Absent(N=4 6)	314.65 ±125.35		7.49 ±1.32		12.95 ±2.48		1.43 ±5.78		18.15 ±8.42		38.65 ±16.05	

Table 4
Relationship between characteristics of ulcer and biomarkers CRP and HbA1c.

Variables		CRP		HbA1c	
		Mean(±SD)	*p* -value	Mean(±SD)	*p* -value
Ulcer site	Volar (N=31)	96.23±96.39	0.736	10.53±2.9	0.479
	Dorsal (N=27)	87.41±101.59		11.06±2.67	
Ulcer type	Superficial ulcer (N=9)	73.16±94.27	0.235	10.58±2.93	0.693
	Deep ulcer(N=24)	71.75±100.53		10.35±2.69	
	Ulcer with bone involvement(N=12)	141.61±100.5		12.23±2.28	

	gangrene(N=13)	97.21±86.88		10.36±2.97	
Ulcer area (cm^2)	≤15(N=40)	81.59±89.48	0.226	10.19±2.74	0.016
	>15(N=18)	115.54±114.2		12.07±2.5	
Ulcer duration (week)	≤20(N=47)	94.37±100.56	0.722	10.51±2.7	0.129
	>20(N=11)	82.54±90.4		11.93±2.97	
Ulcer color	Red (N=14)	97.24±112.56	0.950	11.36±2.83	0.306
	White (N=20)	83.1±97.99		10.82±2.93	
	Black (N=24)	96.68±92.91		10.4±2.69	
Ulcer smell	Normal (N=29)	86.14±102.15	0.648	11.15±2.77	0.307
	Bad (N=29)	98.08±95.23		10.4±2.8	
Ulcer number	Single (N=51)	93.93±101.93	0.710	10.76±2.85	0.921
	Multiple (N=7)	79.03±67.38		10.87±2.45	
New or recurrent ulcer	New (N=42)	81.96±95.37	0.204	10.8±2.75	0.918
	Recurrent (N=16)	118.81±103.1		10.71±2.95	
Ulcer bleeding	Present (N=22)	111.02±111.8	0.255	10.64±2.85	0.777
	Absent (N=36)	80.58±88.35		10.85±2.78	
Ulcer pain	Present(N=12)	113.87±115.3	0.393	11.44±2.58	0.356
	Absent(N=46)	86.46±93.95		10.6±2.81	

Discussion

In current study, the patient's groups were divided into two categories according to their age ranges. The highest frequency of patients fell in age group above 50 years (n=46 (79.3%)) and lowest frequency at ≤50 years which were 12(20.7%). These results indicate that DFU is more common in older patients and old age is possibly a risk factor for DFU. This finding was almost comparable with Al-Rubeaan, Al Derwish et al. (2015), who found that Older patients face significantly more complications than younger ones. This result may be because most elderly people have weak immunity. Regarding gender, higher prevalence of DFU in male, was reported in this study. Indeed, three quarters of DFU patients in this study were males. This study was comparable with Ahmad, Khan et al. (2013). They found the majority of patients with diabetes who develop foot ulcers are male (more than two-thirds). This result may be because males are more likely to injure their feet, due to their frequent going out and exposure to many obstacles, as well as due to the large amount of time they wear their shoes. In addition, another study that contradicts our study reported by Magalhães and Cardoso (2018) who showed that the incidence of DFU in female (58.6%) is higher than male. This difference may be due to difference ethnicities and geographical location. This study suggested that smoking did not represent a risk factor for DFU as half of patients were non-smokers. This study was comparable with Abeer Elnour (2021), who found only 12% from DFU patients were smokers. The present study showed that only 24(41.4%) of the diabetic foot patients were hypertensive. This result almost matches with Syauta, Hendarto et al. (2021), they found hypertension affected (43%) of the diabetic foot. The study result revealed that DFU patients who using insulin 33(58.89 %) were more than those who using oral agent or just having lifestyle modification (43.1%). This result was similar to previous studies (Yusuf, Okuwa et al. 2016). The result of this study showed that DFU patients who have poor glycemic control were more than those who have

good control, in which patients with poor control were (93.1%). This result was similar to the result reported by Purwanti, Yetti et al. (2016). The current study showed that the older people were with diabetes, the more likely they were to develop diabetic foot. This result was convenient with study of Bi, Zhang et al. (2016). Swollen feet was found in most of the patients (65.5%). Poor blood circulation often causes swollen feet when person have diabetes. Swelling in the feet is caused by excess fluid that builds up in the body tissue (Crocker, Palmer et al. 2021). Ulcer duration ranged between 3 to 48 weeks distributed into two groups. Most of DFU patients had ulcer less than 20 weeks 47(81%), where, 11(19%) had ulcer more than 20 weeks. This result corresponds with Johani, Malone et al. (2017). Regarding ulcer site, DFU patients have an ulcer in volar site of foot more than in dorsal site of foot. These results are comparable to the results reported by Driver, Lavery et al. (2015). Ulcer type is classified into superficial ulcer, deep ulcer, ulcer with bone involvement, and ulcer with gangrene, the majority of patients have deep ulcer. This result identical with Driver, Lavery et al. (2015). However, this finding is inconsistent with the finding of another study done by Gezawa, Ugwu et al. (2019), One-third of the subjects had gangrene of toe or forefoot. The possible reason for this discrepancy that the sample of the different study is later than the sample of current study. Ulcer area ranged between 3 to 50 cm 2 distributed into two groups, the results revealed that most of ulcer area of DFU patients less than 15cm 2. There was a similar study reported by Pinto, Ubilla et al. (2018) but it measures the size of ulcers, not the area of ulcers. Still, it is almost the same. In this study, ulcer color was classified into red, white and black, most of the patients have black ulcer. One of the most common signs of diabetic foot ulcers is black tissue called eschar that often appears around the wound because of a lack of blood flow to the feet (Kirsner, R. et al.2015). In this study there was no difference in proportions between ulcers with bad smell and ulcer with normal smell. Similar to this study, another study (Gillespie, Carter et al. 2019) showed there is no difference in the ratio between normal and foul-smelling ulcers. Color and smell of the ulcer are among the signs of ulcer evaluation. DFU patients with single ulcer were more than those who have multiple ulcers. Also this study included 42(72.4%) of patients have ulcer occur first time and 16(27.6%) have recurrent. This results contradicts with the results of another study reported by Khalifa (2018). Another study in European that is run against this study is where the patients who have recurrent ulcers are more than the patients who occur with them for the first time (Dubský, Jirkovská et al. 2013). The possible reason for this discrepancy that the people in European drink a lot of alcohol, and there are many studies that indicate that alcohol is one of the causes of DFU. Concerning bleeding of ulcer, majority of DFU patients had a nonbleeding ulcer. The results also showed that only small proportion of patients had pain in their feet. The result indicates absence of protective sensation due to peripheral neuropathy, the motor neuropathy causes physical deformity of the foot, and sensory neuropathy causes sensory loss (Armstrong, Boulton et al. 2017). These results indicate the DFU are chronic and not healing for long time and painless non-bleeding.

The result of this study showed the total and differential WBCs counts, Lymphocytes, Neutrophils, HbA1c and CRP increases (mean=11.86, 23.76, 69.95, 10.77, 98.07) respectively. Hemoglobin (HGB) and Hematocrit (HCT) decreases (mean=10.66, 32.96) respectively in patients with DFU. There was a similar study

reported by Schade and Higa (2012), Hadavand, Amouzegar et al. (2019), Casadei, Filippini et al. (2021).

In this study a significant correlation between ulcer type and total WBCs and neutrophils counts (P-values=0.019, 0.025 respectively), where there was an increase in total WBC and neutrophils counts in patients with gangrenous ulcer and ulcer with bone involvement. These results indicate that inflammation is stronger in these types of ulcer compared to superficial and deep ulcers. These results are comparable to the results reported by Zhang, Ding et al. (2021). The results showed the direct relationship of white blood cell (WBC) total and neutrophils with ulcer area, as the WBC total and neutrophils increases as the area of ulcer increases. These results are comparable to the results reported by Wang, Aiping, et al. (2014), there are showed, patients with larger wound size their WBC is high. Another study indicates that neutrophils -specific markers were significantly higher in DFU patients than in diabetic patients without DFU or healthy controls (Yang, S., Gu, et al. (2020). These results indicate the total count of WBCs in addition to neutrophils count increase with increase in the depth of the ulcers. Regarding the ulcer duration, a highly significant correlation was found between ulcer duration and lymphocyte count, where, patients with duration of ulcers more than 20 weeks showed a higher lymphocyte counts than patients with duration of ulcers less than 20 weeks. These result similar to study reported by Yunir, Tahapary et al. (2021). This results could be explained on the basis that lymphocytes increase in chronic infection. The results showed that WBCs and neutrophils count are highest in patients with black ulcers followed by patients with red ulcers. These results further support the finding that most visible sign of a serious foot ulcer is black tissue (called eschar) surrounding the ulcer. Eschar is developed because of an absence of healthy blood flow to the area around the ulcer (Armstrong, Boulton et al. 2017). Concerning the ulcer smell, there was an increase in mean of WBC count in patients with bad smell of ulcer compared to patients with normal smell of ulcer. A similar study reported by Cooney and Cooney (2011), who reported a relationship between high WBC and foul smelling. Presence of multiple ulcers was reported to be associated with high WBCs and neutrophils count.

The results illustrated that there was a significant correlation of hemoglobin (HGB) and hematocrit (HCT) with ulcer site, where's, HGB and HCT in patients with dorsal site of ulcer higher than patients with volar site of ulcer. In general, and most studies concluded that most DFU patients suffer from anemia (Cooney and Cooney 2011). The results showed that red blood cells (RBC) counts are higher in ulcer with gangrene than other ulcer types. Previous study included that red blood cells are differ in DFU patients from diabetic patients only, these showed RBCs are less in DFU patients in compare with diabetic patients. Mean corpuscular hemoglobin concentration (MCHC) is a measure of the average concentration of hemoglobin inside a single red blood cell. Mean corpuscular hemoglobin (MCH) is the average quantity of hemoglobin present in a single red blood cell. The current study showed a highly significant negative correlation between ulcer area and both of MCH and MCHC, As the ulcer area increase, the MCH and MCHC decreases. A previous study showed a similar result about MCH with DFU, whereas, the MCH range is similar to range in this study (Wright, Oddy et al. 2014). Red cell distribution width (RDW) is a measure of the range of

variation of RBC volume. In this study a highly significant correlation between RDW and ulcer area, as the ulcer area increase, the RDW decreases. Regarding ulcer smell, a highly significant correlation between ulcer smell and MCHC and also a significant correlation between ulcer smell and RDW, where there was an increase in MCHC and RDW in patients who have a foul-smelling ulcer. DFU with single ulcers were found to have higher RBC counts than those with multiple ulcers, and patients with gangrene also showed higher RBC counts. A study of Cahn, Livshits et al. (2016) showed a significant rise in the percentage of minimally deformable RBCs in diabetic foot patients compared with the patients with no complications was observed. In current study, it was found that RBCs were high in patients with single ulcer. These results indicate that all of ulcer site, area and smell had effect on red blood cell indices.

According to previous study reported by Mardia, Gatot et al. (2018), generally, there was an increase in platelet count, PDW and PCT levels in diabetic patients with diabetic foot ulcers, it indicates that platelet function becomes more reactive and aggregately. Plateletcrit (PCT) is the volume occupied by platelets in the blood as a percentage. In this study noticed PCT more in patients with dorsal site of ulcers compared to patients with volar site of ulcers. A significant correlation noticed in ulcer site with platelet large cell count (P-LCC) where a higher P-LCC was found in dorsal site of ulcer in comparison to volar site of ulcer. Moreover, a significant correlation was found between ulcer duration and platelet (PLT) counts, as a duration of ulcer longer, the PLTs higher in counts. For platelet large cell ratio (P-LCR), showed an inverse relationship with duration of the ulcer, as the shorter duration of ulcer, is the more P-LCR will be. The results found out that PLTs were decreased in patients with red ulcer color compared to patients with white and black ulcer color. But the plateletcrit (PCT), there was higher in patients with black ulcer color in compared with red and white ulcer color. A highly significant correlation exists between ulcer number and PCT, where, PCT was higher in patients with multiple ulcers than in patients with single ulcer. Also the PCT was higher in recurrent ulcers in comparison to new ulcer. The study showed that there was an increase in mean of PLT counts in patients with nonbleeding ulcer compared with patients bleeding ulcers. To the best of our knowledge this is the first study to correlate some PLTs indices with DFU. Mean platelet volume (MPV) is a measure of the average size of platelets. A significant correlation was found between painful ulcer and MPV, where patients with painful ulcers showed a higher MPV than patients with no pain of ulcers. The previous study reported by Mardia, Gatot et al. (2018) showed the relationship between PLTs counts and DFU, where it was found an increase in platelet count, Platelet distribution width (PDW) and PCT levels in patients with diabetic foot ulcers. The elevated MPV levels in diabetic foot ulcers patients have been reported by Gunes, Eren et al. (2017). These results highlight the importance of platelets indices in the pathology and pathogenesis of diabetic foot ulcer.

This study showed an association between C-reactive protein (CRP) levels depth of ulcers. There was an increase in CRP mean in patients having ulcers with bone involvement. This result disagrees with a study achieved by Hadavand, Amouzegar et al. (2019) who found that CRP was significantly higher in patients with class IV foot ulcers (whole foot gangrene) compared to those with class III ulcers (deep ulcer with abscess or osteomyelitis) ($p<0.001$). This might be because

CRP had poor accuracy, in detecting the diabetic foot cases with osteomyelitis (Moallemi, Niroomand et al. 2020). Regarding CRP, a higher CRP is noticed in patients with ulcers' area more than 15cm2. CRP level was higher in patients with recurrent ulcer in comparison with new ulcers, this might be as Wang, Shao et al. (2021) demonstrated that serum CRP levels may be biomarkers of DFU. Also, it was higher in patients with bleeding ulcers compared with nonbleeding ulcers. Like that was seen in association between ulcer pain and CRP, where, the CRP level is higher in patients with painful ulcer compared with no pain in ulcer.

This study showed a significant correlation between elevated Haemoglobin glycaemic A1C (HbA1C) levels and patients with ulcer area more than 15 cm^2. These results indicate the effect of poor diabetic control on the area of ulcers. To the best of our knowledge this is the first study to correlate HbA1C with ulcer area.

Conclusion

Our study indicates the total count of WBCs in addition to neutrophils count increase with increase in the depth of the ulcers, lymphocytes increase in chronic infection, all of ulcer site, area and smell had effect on red blood cell indices, importance of platelets indices in the pathology and pathogenesis of diabetic foot ulcer and the effect of poor diabetic control on the area of ulcers.

Financial Disclosure: There is no Financial disclosure.
Conflict of Interest: None to declare.
Ethical Clearance: All experimental, Protocols were approved under the University of Karbala and all experiments were carried out in accordance with approved guidelines.

References

Ahmad, W., et al. (2013). "Risk factors for diabetic foot ulcer." 25(1-2): 16-18.
Al-Rubeaan, K., et al. (2015). "Diabetic foot complications and their risk factors from a large retrospective cohort study." 10(5): e0124446.
Aras, A., et al. (2017). "The effect of super-oxidized water on the tissues of uterus and ovary: An experimental rat study." 22(1): 15.
Armstrong, D. G., et al. (2017). "Diabetic foot ulcers and their recurrence." 376(24): 2367-2375.
Atlas, D. J. I. D. A., 7th edn. Brussels, Belgium: International Diabetes Federation (2015). "International diabetes federation.
Bi, Y., et al. (2016). Global and Chinese Diabetic Foot Prevalence. DIABETES, AMER DIABETES ASSOC 1701 N BEAUREGARD ST, ALEXANDRIA, VA 22311-1717 USA.
Cahn, A., et al. (2016). "Diabetic foot disease is associated with reduced erythrocyte deformability." 13(4): 500-504.
Casadei, G., et al. (2021). "Glycated hemoglobin (HbA1c) as a biomarker for diabetic foot peripheral neuropathy." 9(1): 16.
Coffey, L., et al. (2019). "Perceptions and experiences of diabetic foot ulceration and foot care in people with diabetes: a qualitative meta-synthesis." 16(1): 183-210

Cooney, D. R. and N. L. Cooney (2011). "Gas gangrene and osteomyelitis of the foot in a diabetic patient treated with tea tree oil." International Journal of Emergency Medicine 4(1): 14.

Crocker, R. M., et al. (2021). "Patient perspectives on the physical, psycho-social, and financial impacts of diabetic foot ulceration and amputation." 35(8): 107960.

Driver, V. R., et al. (2015). "A clinical trial of Integra Template for diabetic foot ulcer treatment." 23(6): 891-900.

Dubský, M., et al. (2013). "Risk factors for recurrence of diabetic foot ulcers: prospective follow-up analysis in the Eurodiale subgroup." 10(5): 555-561.

Gezawa, I. D., et al. (2019). "Anemia in patients with diabetic foot ulcer and its impact on disease outcome among Nigerians: Results from the MEDFUN study." 14(12): e0226226.

Gillespie, P., et al. (2019). "Estimating the health-care costs of wound care in Ireland." 28(6): 324-330.

Gunes, A. E., et al. (2017). "Relation with mean platelet volume and diabetic foot ulcers." 33: 401-404.

Hadavand, F., et al. (2019). "Pro-Calcitonin, Erythrocyte Sedimentation Rate and C - reactive Protein in Predicting Diabetic Foot Ulcer Characteristics; a Cross Sectional Study." Arch Acad Emerg Med 7(1): 37.

Hadavand, F., et al. (2019). "Pro-calcitonin, erythrocyte sedimentation rate and C-reactive protein in predicting diabetic foot ulcer characteristics; a cross sectional study." 7(1).

Johani, K., et al. (2017). "Microscopy visualisation confirms multi-species biofilms are ubiquitous in diabetic foot ulcers." 14(6): 1160-1169.

Khalifa, W. A. J. T. F. (2018). "Risk factors for diabetic foot ulcer recurrence: a prospective 2-year follow-up study in Egypt." 35: 11-15.

Krzyszczyk, P., et al. (2018). "The role of macrophages in acute and chronic wound healing and interventions to promote pro-wound healing phenotypes." 9: 419.

Magalhães, f. d. m. and a. m. j. r. c. d. e. e. d. s. p. d. g. c. s. cardoso (2018). "relato de experiência: desafios no trabalho da enfermagem na estratégia saúde da família." 4(1): 054-065.

Mardia, A., et al. (2018). Comparison platelet indices in diabetic patients with and without diabetic foot ulcer. IOP Conference Series: Earth and Environmental Science, IOP Publishing.

Mezil, S. A. and B. A. J. A. o. t. R. S. f. C. B. Abed (2021). "Complication of diabetes mellitus." 1546-1556.

Monteiro-Soares, M., et al. (2020). "Diabetic foot ulcer classifications: a critical review." 36: e3272

Moallemi, S. K., et al. (2020). "Diagnostic Value of Erythrocyte Sedimentation Rate and C Reactive Protein in detecting Diabetic Foot Osteomyelitis; a Cross-sectional Study." Arch Acad Emerg Med 8(1): e71.

Pinto, N. R., et al. (2018). "Leucocyte-and platelet-rich fibrin (L-PRF) as a regenerative medicine strategy for the treatment of refractory leg ulcers: a prospective cohort study." 29(5): 468-475.

Purwanti, O. S., et al. (2016). "Duration of Diabetic Correlated Diseases With Diabetic Foot Ulcers at Dr Moewardi Hospital of Surakarta."

Sapra, A., et al. (2021). "Diabetes Mellitus (Nursing)."

Schade, V. L. and N. S. J. P. T. Higa (2012). "Keys To The Diagnostic Workup For Patients With Diabetic Foot Infections." 25(3).
Stacy, M. R. (2021). Clinical imaging techniques for assessing vascular risk and complications in the lower extremities. Diabetes and Cardiovascular Disease, Elsevier: 291-317.
Syauta, D., et al. (2021). "Risk factors affecting the degree of diabetic foot ulcers according to Wagner classification in diabetic foot patients." 4: 100231.
Syafril, S. (2018). Pathophysiology diabetic foot ulcer. IOP Conference Series: Earth and Environmental Science, IOP Publishing.
Tripathi, K. and P. J. N. J. C. O. Gupta (2018). "Management of diabetic foot ulcers with platelet rich plasma: A clinical study." 2(3): 09-11.
Wang, Y., et al. (2021). "An update on potential biomarkers for diagnosing diabetic foot ulcer at early stage." Biomedicine & Pharmacotherapy 133: 110991.
Wright, J. A., et al. (2014). "Presence and Characterisation of Anaemia in Diabetic Foot Ulceration." Anemia 2014: 104214.
Yunir, E., et al. (2021). "Non-vascular contributing factors of diabetic foot ulcer severity in national referral hospital of Indonesia." 20(1): 805-813.
Yusuf, S., et al. (2016). "Prevalence and risk factor of diabetic foot ulcers in a regional hospital, eastern Indonesia." 6(1): 1-10.
Zhang, K., et al. (2021). "Correlation between the platelet-to-lymphocyte ratio and diabetic foot ulcer in patients with type 2 diabetes mellitus." 35(4): e23719.