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Evaluating the clinical significance of insulin resistance, oxidant/ antioxidant status, some adipokines, and glycoproteins as monitoring indicators in Type 2 diabetic foot syndrome

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ABSTRACT

Diabetic foot syndrome (DFS) is a long-term consequence of diabetes caused by both micro and macrovascular issues. Due to the paucity of information and researches on DFS in Basrah, Iraq, in this study we examined and compared the role of insulin resistance (IR), oxidant/antioxidant status, some adipokines and glycoproteins in Type 2 diabetic patients with DFS. This was a case-control study involving 89 subjects, consisting of 45 DFS subjects and 44 healthy subjects. Their fasting Insulin, chemerin, Zonulin, ceruloplasmin, α2-macroglobulin, Malondialdehyde (MDA) and Total antioxidant capacity (TAC) were determined by ELISA methods. Demographics, glucose was assayed on UV-Vis Spectrophotometer, HbA1c measurement was determined using the Bio-Rad D-10® HPLC analyzer, and homeostasis model assessment for determined of insulin resistance (IR). When compared to controls, glucose, Insulin, HOMA-IR, HbA1c, Chemerin, Ceruplasmine, Zonulin, α-2Macroglobulin and Malondialdehyde levels were significantly elevated ($p<0.01$), while total antioxidant capacity was lowered ($p<0.01$). These measures could potentially be employed as prognostic biomarkers in both men and women with DFS, according to the area under the curve (AUC). The associations of insulin resistance (IR) with examined adipokines and glycoproteins were not significantly different, which may shed new light on the role of IR as an etiological cause of DFS and suggest that it could be a better monitoring index in women and men with DFS.

1. INTRODUCTION

DFS is a chronic complication that occurs as a result of both micro and macrovascular problems, and it is the leading cause of diabetes-related hospitalization [1]. The diabetes in adults (20-79 years) has increases from 151 million (4.6% of the global population at the time) to 463 million (9.3%) today. This number is predicted to rise to 578 million people (10.2%) by 2030 and will be jump to a staggering700 million (10.9%) by 2045 [2]. However, the prevalence of diabetic foot ulcers worldwide is higher in men (4.5%) than in women (3.5%) and in type 2 diabetic patients (6.4%) than in type 1 diabetics (95% confidence interval [CI]]: 4.6-8.1%)

(5.5%, 95% CI: 3.2-7.7%). The highest prevalence was found in North America (13.0%, 95% CI: 10.0-15.9%), while the lowest prevalence was found in Oceania (3.0%, 95% CI: 0.9-5.0%). Asia, Europe, and Africa had prevalence's of 5.5% (95% CI: 4.6-6.4%), 5.1% (95% CI: 4.1-6.0%), and 7.2 % (95% CI: 5.1-9.3%), respectively. Moreover, Australia had the lowest prevalence (1.5%, 95% CI: 0.7–2.4%), with Belgium has the highest prevalence (16.6%, 95% CI: 10.7–22.4%), followed by Canada (14.8%, 95% CI: 9.4–20.1%) and USA (13.0%, 95% CI: 8.3–17.7%) [3]. Furthermore, some reports notes that the mean prevalence of DFS in Saudi Arabia was 11.85% (4.7–19%), in Egypt is 4.2% (1–7.4%), in Jordan is 4.65% (4–5.3%), in Bahrain is 5.9% and in Iraq is 2.7% at the time of study [4]. In Iraq, there are several studies have been conducted in various province to explore the prevalence of DFS. For example, the results of the study conducted in Baghdad, Iraq found that a high percentage 61 (75.3%) of a total 81 type 2 diabetic patients suffer from DFS. The majority of DFS patients are men, including 43 (53.1%) men versus only 18 (22.2%) females, with a male to female ratio (M/F ratio = 2.4) [5]. Another study in three governorates of the Kurdistan Region of Iraq (Dohuk, Erbil and Sulaymaniyah) indicates that diabetic foot is sex-related, with DFU in women being found to be higher 26 (0.5%) than men 21 (0.4%) of the total 5186 diabetic patients [6]. Similarly, another study in Basra province, Iraq, found that two-thirds of 69 (57%) of the subjects women participating in the study were found 'at risk' of DFS [7]. However, as the global prevalence of type 2 diabetes mellitus rises, the burden of diabetic foot disease is predicted to rise (T2DM). Patients who acquire a diabetic foot ulcer (DFS) experience severe morbidity and mortality. Furthermore, a slew of risk factors are linked to poor glycemic control, including age, sex, family history, smoking history, hypertension, chronic conditions like ischemic heart disease, bronchial asthma, and thyroid disease, as well as a high BMI and waist circumference [8]. Furthermore, a study conducted in Basrah, Iraq, found that large metatarsal heads (36.2%), hammer toes (10.9%), and claw toes (36.2%) were among the foot deformities detected in diabetic patients who were prone to DFS (3.8 percent). These foot anomalies are thought to cause higher pressure in the patients' feet when they are all combined. Dry skin (17%), callosities (14.2%), tinea pedis (13.7%), and nail alterations (7.1%) were among the dermatological abnormalities observed in this study [9]. Similarly, a study in Iraq's Basra area discovered that diabetic foot condition affected over two-thirds of the 69 women who took part in the study (57 percent) (DFD). DFD was also substantially linked to having had diabetes for a long time and being a woman [7]. In the province of Basrah (southern Iraq), due to the paucity of information and research on diabetic foot syndrome. Towards this end, the role of insulin resistance, oxidant/antioxidant status, some adipokines (Chemerin and Zonulin), and glycoproteins (HbA1c, α-2-Macroglobulin, and Ceruloplasmin) in Type 2 diabetic patients with DFS were tested as potential biomarkers recommended for clinical use in the diagnosis, prognosis, and follow-up of patients with T2DM at high risk of developing diabetic foot syndrome.

2. Materials and methods

2.1 Subjects

A case-control clinical trial is being conducted in this investigation. Between October 2021 and April 2022, samples were taken from a surgical ward at Al-Sadder teaching hospital in Basrah, Iraq, and the diabetes and endocrine glands center at Al-Mawany teaching hospital in Basrah, Iraq. In addition, some samples were taken from a private clinic administered by Al-Mawany teaching hospital consulting professor Dr. Abdul-Hadi. A total of 114 people took part in this study. Twenty-five volunteers (14 patients and 11 healthy controls) were withdrawn from the study due to their inability to continue. While 89 individuals, ranging in age from 40 to 65 years, were followed up for 6 months till end the study and classified into 2 main groups. The first group included 45 diabetic foot ulcer patients (24 men and 21 women). The second group was made up of 44 healthy participants (23 men and 21 women). Diagnosis of T2DF patients was based on the recommendation of American Diabetes Association [10]. The study received ethical approval (No.7/54/2533) from Basrah University, and each participant signed an informed consent form after hearing a detailed

description of the procedures. Informed consent and ethical guidelines were based on the Helsinki deceleration for the year 2000.

2.1.1 Inclusion Criteria

In the respective group, only the T2DF patients identified by doctors were chosen. They had a similar drug policy, which specified (Insulin). Healthy individuals who did not have T2DM, DFS, or a family history of either were included in the control group. They also did not take any medications that were thought to have an impact on serum glucose levels. For at least three months, all of the volunteers' clinical conditions remained constant.

2.1.2 Exclusion Criteria

Patients who were smokers, those with kidney and heart failure, hepatic diseases, thyroid disorders, malignancies, autoimmune diseases, inflammatory conditions, urinary tract infections, patients with polycystic kidney disease, patients with severe arthritis, pregnant women, people under the age of 35, and those with type 1 diabetes, were excluded from this study.

2.2 Sample Size Calculation and samples collection

The required sample size was determined using a single population proportion calculation with the following assumptions: 1.91% prevalence of diabetic foot ulcers, a 95% confidence level, and a 4 % margin of error (absolute level of precision).

$$\mathbf{n = [\ (z\alpha/2)^2 \times p\ (1 - p) / d^2\ ] = [\ (1.96)^2 \times 0.0191(\ 0.9809) / (0\ 04)^2\ ] = 45}, \quad \text{.....(11)}$$

Where: n is the necessary sample size, p is the adult diabetic foot ulcer prevalence rate (1.91%), Z is the standardized normal distribution value at the 95% confidence interval (1.96), and d is the 4% margin of error. The sample size was increased using the low design effect. Using the probability of a 10% nonresponse rate, the final sample size was modified, bringing the final sample size down to 49 participants [11]. All samples were withdrawn between 9:00 and 10:00 a.m. after a 12-hour fast and 30 minutes of relaxation in the supine posture. Fresh venous blood (5) mL were collected from patients (before the day of foot amputation surgery) and healthy volunteers by vein punch then divided into two parts, the first part (2 mL) was added into EDTA containing polypropylene tubes and shook gently to be utilized for the determination of the level of HbA1c. The second part (3 mL) was moved to gel tube which it was moved into a centrifuge at 402 x g for 20 minutes to get the serum. The collected serum immediately utilized in the estimation of variables in this study, while the rest were stored in plain tube at deep freezing at (-70) ºC until using.

2.3 Methods of Biochemical Estimation

The following procedures were used to analyzed blood samples for biochemical parameters: BMI was calculated as the following formula [BMI (kg/m^2) = Wt in kg/Ht in m^2] [12]. HbA1c measurements were determined using the Bio-Rad D-10® HPLC analyzer (Bio-Rad Laboratories, CA, USA). Serum glucose was assayed on UV-Vis Spectrophotometer (UV-EMC- LAB, Duisburg, Germany) by using the kit (Randox, County Antrim- GL2279/ UK). Insulin level was determined using the (BT-Lab, Shanghai- E0010Hu / China) kit which was a solid phase ELISA based on the sandwich principle. Serum Malondialdehyde (MDA) was estimated by kit (BT-Lab, Shanghai- E1371Hu / China), Total antioxidant capacity (TAC) was estimated by kit (BT-Lab, Shanghai- E2199Hu / China), Spexin was estimated by kit (BT-Lab, Shanghai- E3507Hu / China), Cerluplasmine (Cp) was estimated by ELISA kit (BT-Lab, Shanghai- E1229Hu / China), α-2-macroglobulin (A2MG) was estimated by ELISA kit (BT-Lab, Shanghai- E1097Hu / China), Chemerin was estimated by ELISA kit (BT-Lab, Shanghai- E1435Hu / China), Zonulin was estimated by ELISA kit (BT-

Lab, Shanghai- E1117Hu / China). IR was calculated by the Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) equation [HOMA-IR = Fasting insulin (μIU / mL) × Fasting glucose (mg / dL) / 405] [13].

2.4 Statistical Analysis

Statistical analysis was performed using SPSS software version 26 (IBM Corporation, New York, USA). The comparison between groups has been analyzed by using one way One-way analysis of variance (ANOVA) to find the statistical significance. The ROC curve, which is formed by graphing sensitivity (y axis) against 1-specificity (x axis) and calculating the area under the curve (AUC), was used to calculate the sensitivities and specificities, as well as the 95% confidence interval. $p< 0.05$ was considered statistically significant, $p< 0.01$ highly significant and an AUC value near 0 (or 1) implies a strong diagnostic value, the values of one group are mainly greater (or lower) than the values of the comparison group in this circumstance.

3. Results

The general characteristics of the subjects participated in the present study are presented in Table 1.

Table 1. The demographic characteristics of the present study (n=89)

The characteristics		DFS patients		Healthy controls	
		Women	Men	Women	Men
Total subject No.		21	24	21	23
Age (Years) (Mean ±SD)		51.58±3.00	50.75±1.10	50.20	49.10
T2DM Duration (Years) (Mean±SD)		10.09±2.00	9.80±0.10	0	0
DFS Duration (Years) (Mean±SD)		3.68±1.30	3.75±0.77	0	0
Demographic Area	Urban	19	21	20	21
	Rural	2	3	2	2
Educational Background	Learned	16	18	15	19
	Illiterate	5	6	6	4
Smoking Habits	Positive	1	3	0	2
	Negative	20	21	21	21
Food Habits	Vegetarian	3	2	3	3
	Non -Vegetarian	18	22	18	20
Employment Status	Employed	20	24	18	21
	Non - Employed	1	0	3	2

Compared with healthy control, the results revealed that patients with DFS had significantly increased (p<0.01) levels of HOMA-IR, Glucose, Insulin, HbA1c, Chemerin, Zonulin, Ceruloplasmin, α2-macroglobulin and Malondialdehyde, as shown in Table 2 and 3. Furthermore, same Tables 2 and 3 reflect that patients with DFS had significantly lower total antioxidant capacity ($p<0.01$), while BMI level was not significantly different ($p>0.05$) between the DFS patients and control groups. On the other hand, our data reported that there were a non-significant differences ($P>0.05$) in the values of the studied parameters between patients (men and women) with DFS.

Table 2: Levels of total parameters in men of healthy control and DFS patients. The values are the mean±SD.

Parameters	DFS Patients No = 24						Healthy Control No = 23
	Mean ± SD	Median	SE	Range	95% CI		Mean ± SD
					Lower	Upper	

BMI (kg/m²)	30.36±4.43	29.83	0.968	21.53-38.2	28.34	32.38	29.70±4.67
Glucose (mg/dl)	247.90±59.88**	320.00	13.067	225-38	220.64	275.16	98.52±7.67
Insulin (µIU/mL)	17.27±1.08**	17.13	0.237	15.81-19.95	16.78	17.77	5.92±1.25
HOMA-IR	10.59±2.74**	9.26	0.598	6.75-16.49	9.348	11.84	1.39±0.29
HbA1c (%)	9.47±1.13**	9.60	0.248	7.50-12.10	8.95	9.99	4.72±0.48
A2MG (mg/dL)	60.56±7.38**	58.84	1.611	42.57-73.43	57.20	63.92	17.28±2.89
Chemerin (ng/L)	898.44±237.66** *	860.60	50.66	532.1-1120	793.07	1003.82	622.08±124.31
CP (ng/mL)	592.06±49.71**	421.00	10.148	58-235	571.06	613.05	204.61±17.11
Zonulin (µg/mL)	176.99±38.65**	188.51	8.434	110-222.75	159.39	194.58	50.53±8.61
MAD (nmol/mL)	19.98±3.78**	19.88	0.825	5.01-23.59	18.26	21.70	7.65±1.74
TAC (U/mL)	1.71±0.41**	1.75	0.090	0.97-2.63	1.52	1.90	8.98±1.06

Data are presented as mean±standard deviation (SD); SE: standard errors; n: number of the subjects; range: difference between the highest and lowest values in the set; 95% CI: confidence interval/limits (lower and upper). *p*>0.05: *p*-value not significant, **p*<0.05: *p*-value significant; ***p*<0.01: *p*-value highly significant, indicating the level of significance in comparison with the corresponding control value.

Table 3: Levels of total parameters in women of healthy control and DFS patients. The values are the mean±SD.

Parameters	DFS Patients No = 21						Healthy Control No = 21
	Mean ± SD	Median	SE	Range	95% CI		Mean ± SD
					Lower	Upper	
BMI (kg/m²)	29.30±4.32	28.23	0.943	20.7 -39.54	27.33	31.26	28.81±4.15
Glucose (mg/dl)	318.80±42.53**	220.00	9.281	155-39	299.44	338.17	98.47±7.31
Insulin (µIU/mL)	16.90±1.17**	16.79	0.256	15.11-19.42	16.374	17.44	5.92±1.25
HOMA-IR	13.25±1.58**	13.39	0.345	9.93-16.27	12.534	13.97	1.43±0.29
HbA1c (%)	9.76±1.46**	9.90	0.319	7.40-12.70	9.09	10.43	4.8571±0.46
A2MG (mg/dL)	57.04±8.49**	57.53	1.854	43.43-70.18	53.18	60.91	16.66±2.53
Chemerin (ng/L)	749.80±156.40** *	735.18	33.34	509.4-1500	680.45	819.15	683.18±201.13
CP (ng/mL)	396.30±85.60**	394.51	18.25	180.95-551.48	358.34	434.25	260.05±41.31
Zonulin (µg/mL)	142.52±23.80**	137.00	5.194	100-183	131.68	153.35	47.34±9.23
MAD (nmol/mL)	19.79±1.40**	19.51	0.305	17.91-22.39	19.1578	20.43	7.33±2.02
TAC (U/mL)	1.72±0.62**	1.49	0.135	0.56-2.62	1.44	2.011	8.30±1.42

Data are presented as mean±standard deviation (SD); SE: standard errors; n: number of the subjects; range: difference between the highest and lowest values in the set; 95% CI: confidence interval/limits (lower and upper). p>0.05: p-value not significant, *p<0.05: p-value significant; **p<0.01: p-value highly significant, indicating the level of significance in comparison with the corresponding control value.

The AUC results show that glucose, Insulin, HOMA-IR, HbA1c, Zonulin, α-macroglobulin, chemerin, ceruloplasmin and malondialdehyde could potentially be used as better predictive biomarkers in patients men with DFS (AUC= 1.00, 1.00, 1.00, 1.00, 1.00, 1.00, 0.77, 1.00, 0.952, respectively) and in patients women

with DFS (AUC= 1.00, 1.00, 1.00, 1.00, 1.00, 1.00, 0.74, 0.92, 1.00, respectively). Total antioxidant capacity, on the other hand, might be employed as a prognostic biomarker in both men and women, with (AUC= -1.00) for both, as illustrated in Figure 1.

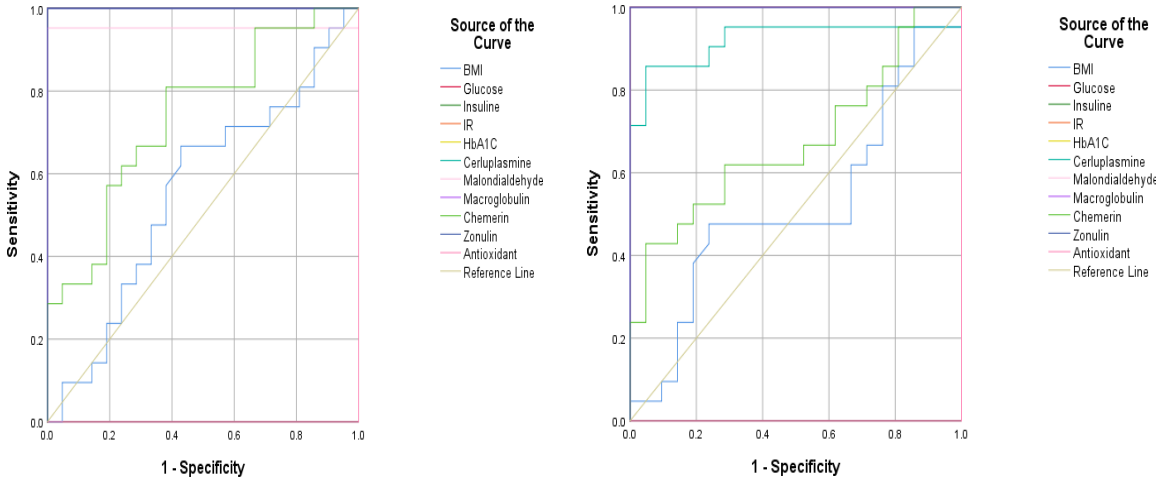

Figure 1: Receiver operating characteristic curve (ROC) for the levels of various clinical parameters in men (A) and women (B) of DFS patients.

4. Discussion

The majority of patients and healthy control people in this study were nonsmokers, according to the findings. Furthermore, the most of both patients and healthy controls were from urban areas, and the majority of them were educated and employed. Differences in habitats, pollution, social, mental, genetic, food habits, and other factors distinguish urban and rural settings, and these differences are growing rapidly in urban areas [14]. Furthermore, all of the diabetic patients with or without diabetic foot ulcers, as well as healthy controls, were from the province of Basrah. As a result, due to the small number of patients who attend Al-Sadder teaching hospital, our findings cannot reflect the actual situation of all patient groups in Iraq. Instead, we rely on the cooperation of patients and their willingness to participate in the current study [13]. In DFUs, the acute phase response is mostly determined by limb ischaemia, infection severity, and the presence of osteomyelitis. Diabetic people with a higher BMI have a higher risk of developing foot ulcers [15]. In this setting, visceral fat thickness is more harmful than subcutaneous fat thickness, possibly due to higher production of nonesterification fatty acids (which reach the liver directly through the portal system). Surprisingly, truncal obesity has been linked to a higher percentage of fast twitch (white) to red fibers in skeletal muscle, as well as lower insulin binding and insulin sensitivity. Truncal obesity is caused by a combination of genetics and a sedentary lifestyle [16]. Several metabolic enzymes that may contribute to insulin resistance in skeletal muscle, such as glycogen synthetase and insulin receptor substrate 1, as well as genes for 3 adrenoreceptors and the leptin receptor, have been identified as putative obesity genes. Such genes may impart the "thrifty genotype" by predisposing to fat storage, which in times of scarcity provided a survival benefit, but in modern civilization resulted in obesity and type 2 diabetes, which is highly associated with diabetic complications such as DFS [17]. Hyperinsulinemia combined with a lack of insulin action in diabetic foot syndrome can result in a number of negative physiologic outcomes, including the following: First, an increase in local wound blood glucose levels, which increases the damage caused by high amounts of glucose metabolic intermediates accumulating [18], [19]. Second, it may activate nuclear factor-B, activator protein-1, and early growth response-1, three important pro-inflammatory transcription factors (EGR-1). Intercellular adhesion molecule-1, matrix metalloproteinase (MMP-2 and MMP-9), tissue factor, and plasminogen activator inhibitor-1 are all expressed in response to these three transcription factors. These proteins are critical for

NADPH oxidase enzymes, which generate powerful oxidative free radicals that harm tissue cells. As a result, hyperglycemia may increase the transcription of three important pro-inflammatory factors, triggering the inflammatory response and causing local oxidative stress [20]. Third, it may cause a rise in inflammatory cell factor levels and inflammatory cytokine levels in the aftermath of trauma, resulting in an increased inflammatory response and an excessive inflammatory response. Additionally, immune cell protein breakdown may be accelerated, reducing immune function [17].

Insulin resistance (IR) can be seen in three primary tissues, according to the majority of studies: The first is the liver, which plays a key part in metabolism processes; thus, inflammation and fat accumulation may play a substantial role in liver dysfunction, leading to hepatic insulin resistance. IR and liver lipid content may be linked to an increase in the size and number of triglyceride-rich very low density lipoproteins (VLDLs), which play a role in protein and lipid metabolism as well as detoxification processes [21]. Obesity and overweight also increase fat synthesis and uptake, as well as the gene expression of molecules involved in fatty acid metabolism and storage, such as PPAR-, lipoprotein lipase, and fatty acid binding proteins. Mitochondrial dysfunction is another major trigger for the activation of stress pathways in hepatocytes, which precedes insulin resistance [22]. Muscle tissue is the second site, as it has been discovered that muscle tissue is responsible for the majority of insulin-stimulated glucose elimination, and so any decrease in skeletal muscle would result in insulin resistance. Furthermore, IR in the skeletal muscle is mostly caused by lipids and the resulting skeletal muscle inflammation [23]. Because insulin signaling is blocked in cultured myocytes and skeletal muscle, overexposure to non-esterified fatty acids (NEFA) is a significant predictor of insulin resistance in skeletal muscle. Furthermore, decreased insulin sensitivity to insulin receptor substrate/phosphoinositide 3-kinase (IRS/PI3-K) molecules may be due to increased levels of free fatty acids, as well as an increase in intramyocellular fat and fatty acid transporters, which occurs before skeletal muscle insulin resistance [17]. The third site is adipose tissue, which plays a key role in releasing FFAs into circulation gradually during fasting stages and facilitating the rapid draining of free fatty acids into triglyceride reserves during the postprandial state. Changes in the functional condition of adipose tissue can impede the conversion of FFAs to lipids directly [24]. Furthermore, adipose tissue function modulation may have an impact on the regulation of circulating glucose levels and free fatty acids. As a result, the homeostatic cycle of adipose tissue works in tandem with the energy balance of the entire body. Resistance to insulin's lipogenic effect could be considered as a homeostatic adaptation, and such complex and intertwined regulation cycles make it difficult to distinguish the functions of individual systems. However, abnormalities in adipose tissue self-regulation may have negative consequences for overall body homeostasis [12]. Finally, a few signs of IR are glucose intolerance, dyslipidemia, endothelial dysfunction, raised pro-coagulant factors, hemodynamic abnormalities, elevated inflammatory markers, aberrant uric acid metabolism, elevated ovarian testosterone release, and sleep apnea. As a result, pathophysiological investigations suggest that IR promotes a pro-inflammatory state and dyslipidemia, which may be substantially responsible for microvascular consequences such as kidney failure, foot ulceration and amputation, and diabetes-related acquired blindness [22]. High levels of HbA1c have been proven in studies to be a key biomarker for the diagnosis of diabetic peripheral neuropathy. In reality, glycemic management and tight control of HbA1c levels are linked to a reduction in diabetic complications: a 7% drop in HbA1c is linked to a 60% reduction in occurrences of peripheral neuropathy [25]. Diabetes mellitus is a multisystemic disease characterized by poor glucose, lipid, and protein metabolism, as well as a weakened immune system. Lower extremity ulcers, which are essentially an endocrine pathology, are one of the most serious consequences of diabetes and may necessitate a multidisciplinary and methodical treatment [26]. On the other hand, a disruption in peripheral microvascular blood circulation might affect tissue perfusion, resulting in amputation and delayed recovery, as well as increased infection susceptibility due to the formation of resistant germs. In places where tissue exudation is worsened, exposure to repetitive traumas and moderate trauma may cause ulcers more quickly than predicted,

despite the fact that diabetes-related neuropathy enhances pain and heat feeling [23]. In this study, significantly higher levels of HbA1c (>7%) in the blood of DFS patients compared to healthy controls may indicate poor glycemic or diabetes control, which is linked to lower survival in the general population of diabetic patients, resulting in higher morbidity, and a strong link between high-risk foot and the development of progressive neuropathy [13]. HbA1c is a more comprehensive marker of total glycemic control than plasma glucose, possibly because it represents blood glucose in both postprandial and fasting phases. HbA1c is a non-enzymatic glycosylation of the terminal valine unit of haemoglobin's -chain after exposure to plasma glucose that happens over the course of 90-120 red blood cell days and shows up as an average present in the blood glucose for 3-4 months [23]. The hemoglobin molecule remains glycosylated once it has been glycosylated, therefore the accumulation of glycated hemoglobin within the red cell reflects the average level of glucose to which the cell has been exposed over its life cycle. On the other hand, the HbA1c cut-point is still debatable from a diagnostic standpoint. Hyperglycemia causes increased glucose-haemoglobin binding (glycation response) in diabetic foot syndrome patients, resulting in a greater value of glycosylated haemoglobin (HbA1c) in a concentration-dependent way [26]. The increased amounts of malondialdehyde in the serum of diabetic foot participants in this investigation could be explained by the enhanced elimination of serum lipid peroxide by the aldehyde dehydrogenase enzyme in liver mitochondria. The enzyme aldehyde dehydrogenase destroys harmful aldehyde and protects tissue against aldehyde buildup. In addition, increasing urine MDA breakdown can help to reduce serum MDA. These findings showed that diabetes patients were more likely to accumulate potentially hazardous oxidative stress, which could be one of the key causes underlying chronic diabetic foot syndrome [27]. Uncontrolled and excessive oxidative stress has been shown in certain studies to contribute to the maintenance and development of the inflammatory process, which plays a significant role in the etiology of chronic non-healing wounds. Furthermore, increased ROS formation in diabetic wounds via multiple ROS-generating enzymes may result in impaired wound healing processes due to increased cell apoptosis and aging, as well as higher oxidative stress, lipid peroxidation, protein modification, and DNA damage [28]. The release of vascular relaxing factor, on the other hand, is inhibited, and the current hyperglycemia causes the generation of reactive oxygen species (ROS) in both endothelial and smooth muscle cells. Furthermore, insulin resistance causes an increase in the generation of reactive oxygen species (ROS) by inhibiting the phosphatidylinositol-3 kinase (IP-3) pathway and activating the signaling of protein kinase C [29]. Furthermore, diabetic foot syndrome may promote vascular smooth muscle hypertrophy by causing the production of numerous vasoconstrictors and vasoactive chemicals. Hyperglycemia also promotes atheromatous plaque development by increasing ROS production and dyslipidemia [30]. TAC serve a critical function in quenching ROS generation, resulting in multi-compartmental defense against cellular molecular damage. In addition, the intricate interactions that occur in vivo between individual antioxidants can be considered [31]. As a result, increased oxidative stress via lipid peroxidation, glucose auto-oxidation, increased glucose flux through the polyol pathway, non-enzymatic and progressive protein glycation, and the formation of advanced glycosylation end products could be attributed to a significant decrease in TAO levels among DFS patients in this study (AGEs). Furthermore, increased polyol pathway activity may result in a depletion of NADPH molecules, which are used by the enzyme aldose reductase to convert glucose to sorbitol. In addition, lower TAC levels compared to greater ROS levels indicate depleted antioxidant defenses and an inability to scavenge and neutralize ROS' harmful effects [14]. Depletion of antioxidant capacity, whether due to a lack of enzymatic or non-enzymatic antioxidants, makes the cell vulnerable to oxidative attack, even in physiological settings where redox state is maintained through a precise balance of low ROS generation and cellular defense pathways [30]. This defiance mechanism may be altered in diabetes mellitus and its complications, as well as other pathological conditions, and thus the ineffective scavenging mechanisms of free radicals may play a crucial role in causing tissue damage in diabetic patients, particularly those with foot ulcer syndrome. Furthermore, greater levels of ROS and RNS may activate stress-signaling pathways and deplete both enzymatic and non- enzymatic antioxidants,

negatively impacting the quality of life and lifespan of DFS patients [28]. The healing process, on the other hand, may necessitate the recruitment of inflammatory cells and the release of different mediators. Meanwhile, a drop in TAC and an increase in oxidative stress induced by wounds in diabetes patients with continuous hyperglycemia may play a key role in prolonging the inflammatory response and causing an ulcer by delaying the transition to the next step [30]. As a result, controlling oxidative stress and the inflammatory response may be a crucial step in hastening wound closure. Finally, antioxidants' combinatorial impact in the human body may provide more protection against oxidative stress than any single antioxidant alone [28]. Although the precise mechanism is yet unknown, patients with diabetic foot have greater serum levels of ceruloplasmin, which may be a marker of oxidative stress. It is an inflammation-sensitive protein and an acute-phase reactant. It has been shown that Cp contains pro-oxidant activity, which causes low-density lipoprotein to oxidize (LDL). Several studies postulated that DFS is a state of chronic inflammation with increased oxidative stress [32]. So, increased levels of ROS induce lower levels of endothelial activation promoter (nitric oxide), which can lead to leucocyte adhesion, impaired barrier function, and micro-angiopathy, resulting in the DFS alterations. Ceruloplasmin has a copper- dependent oxidase activity, acting as a ferroxidase enzyme that catalyzes the oxidation of ferrous iron (Fe^{2+}) to ferric iron (Fe^{3+}). It is also capable of incorporating iron into transferrin without the creation of hazardous iron compounds [27]. The capacity of ferrous iron (Fe^{2+}) to participate in the Fenton reaction, which produces oxidizing species such as superoxide anion ($O2^{\bullet-}$) and hydroxyl radicals ($^{\bullet}OH$), which can cause oxidative damage to biomolecules and promote oxidative stress in the body, is extremely harmful. Additionally, iron buildup and oxidative stress have been linked to the pathogenic evolution of neurodegenerative illnesses like DFS [21]. Therefore, this antioxidant (Cp) may be able to allow the incorporation of iron into transferrin without the generation of harmful iron compounds in DFS. Furthermore, in this redox reaction, the oxygen molecule is directly reduced to water, which may provide insight into the mechanism by which ceruloplasmin inhibits superoxide-induced lipid peroxidation [33].

Hyperglycemia, a greater level of HbA1c, poor blood sugar control, and complications of foot ulcer syndrome may all contribute to an increased level of -Macroglobulin (A2MG) in diabetic foot patients. Meanwhile, some recent studies have found that A2MG binds to blood insulin hormone or influences insulin internalization by the target cell in patients with DFA [34]. On the other hand, it's possible that a higher amount of serum A2MG will lower insulin bioavailability and compromise blood sugar management. Furthermore, up-regulated acute and chronic inflammatory cytokines, which rise in inflammatory situations such as DFS, may stimulate A2MG gene synthesis in diabetic patients [35]. Indeed, it performs a number of critical tasks, including inhibiting proteinases produced during inflammation and delivering them to an endocytotic clearance pathway. On the other hand, it is not "fail safe" and can be harmed by reactive oxygen species produced endogenously or exogenously, resulting in a variety of pathological diseases. As a result, it can be used to diagnose and predict the prognosis of a variety of disorders, including inflammatory bowel disease, liver fibrosis, acute pancreatitis, diabetes, and cardiovascular diseases [34]. Chemerin levels in DFS patients' blood may be elevated for a variety of reasons: First, it could be the result of increasing body weight and insulin resistance, which produce dysregulation of most adipokines, including chemerin, and a long-term proinflammatory effect that could link it to obesity-related insulin resistance [36]. Second, as an autocrine, paracrine, and endocrine signaling molecule, it may play a role in controlling local immune responses and inflammation of tissue injury, adipose tissue development, glucose homeostasis, and may contribute to the metabolic derangement characteristic of obesity and obesity-related diseases. Chemerin may act as a potent chemoattractant of immature dendritic cells and macrophages when acting as a ligand on cells expressing chemerin receptors in human inflammatory fluids, according to scientific investigations [28]. Third, diabetic complications may cause a polymorphism in the chemerin gene, which has been linked to glucose intolerance, decreased glucose-stimulated insulin secretion, and decreased glucose uptake in skeletal muscle and white

adipose tissue, resulting in a negative relationship between obesity and insulin sensitivity. As a result, an increase in serum chemerin levels can be used as an independent predictor of diabetes complications like DFS [37]. In our study, DFS patients had higher amounts of serum zonulin (ZO) in their blood, which could be linked to poor glycemic control, insulin resistance, and enhanced inflammation processes. Subclinical inflammation appears to have a key role in diabetes and related complications, including DFS, in addition to the known risk factors. Type 2 diabetes affects immune system components, with the most noticeable changes occurring in adipose tissue, liver, pancreatic islets, vasculature, and circulating leukocytes [38]. Furthermore, a ZO-dependent increase in intestinal permeability has been linked to a variety of diseases, including diabetes. Furthermore, triggering events (gluten, elevated fatty acid, etc.) cause tight junction disassembly, alterations in intestinal permeability and unregulated antigen trafficking from the intestinal lumen to the submucosa cause chronic inflammatory diseases [39]. Increased intestinal permeability is linked to increased inflammation and insulin resistance (IR), which may impact the formation of diabetic foot ulcers. Hyperglycemia, on the other hand, can induce tissue damage and promote the development of endothelial dysfunction, which is linked to insulin resistance and inflammation [40]. Furthermore, increased levels of ZO have been linked to higher glucose levels, dyslipidemia, inflammation, and insulin resistance in type 2 diabetes (T2DM) and obesity, suggesting that it may have a role in T2DM and obesity pathophysiology. Thus, elevated zonulin may suggest a reaction in DFS that is related to inflammation, rather than only intestinal permeability [41].

5. Conclusion

DFS is caused by a different number of pathophysiologic factors (e.g. obesity, predominant insulin resistance, insulin secretion, or a combination of factors). This study indicated that insulin, HOMA-IR, HbA1c, chemerin, zonulin, ceruloplasmin, α2-macroglobulin total antioxidants, and malondialdehyde possesses clinical efficacy as good predictive biomarkers in the diagnosis, prognosis, and follow-up of patients with T2DM at high risk of developing diabetic foot syndrome in order to reduce the number of diabetic foot syndrome patients who need an amputation.

6. References

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