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The role of Vitamin D deficiency in patients with Diabetic Foot Ulcer

Sura Mustafa Qasim[1], Alaa Zanzal Ra'ad Al-dorri[2] , Mohanad Hasan Mahmood Al-Izzi[1]

[1] *Department of Biology, Science College, Tikrit University, Iraq.*

[2]*Department of Medical Microbiology, Tikrit University College of Medicine (TUCOM), Iraq*

Princesssoul92@yahoo.com

ABSTRACT

The aim of this study is identification the relationship between vitamin D deficiency in the diabetic foot ulcer patient and the aerobic bacterial types, because it's, with know their resistance to some antibiotics used. The present study was cross sectional type which carried out from 12th of August 2019 to 25th of February 2020 in Salah al-Din Governorate. Seventy patients suffered from DM presented with diabetic foot ulcer (41 male and 29 female), blood sample was drawn and taken swab from foot ulcer by swab transport media and send to the lab. We found the high significant ratio in decrease blood serum concentration ($p \leq 0.01$) of vitamin D in persons with diabetic foot and in persons with diabetes compared with the control group. Also, we found high significant level at ($p \leq 0.01$) in the number of patients with DM and DFU for males compared to females and (87%) patients with DFU had a bacterial infection, in addition, the poly-microbial infection was (60.7%) case more than single microbial infection (39.3%), plus showed that the infection with positive bacteria (58.7%) was more than negative bacteria (41%). The highest rate of bacterial infection in DFU was recorded for *S. aureus* (36.7%), while *S. ficaria* was the only cause of infection, and it was isolated (0.9%). Most of bacterial isolates that cause DFU were resistant to the antibiotics used except for Levofloxacin, which most bacteria were sensitive, while bacteria were resistance to carbincillin (100%).

Keywords: diabetic foot ulcer**,** diabetes mellitus**,** vitamin D

Introduction

Diabetes mellitus is common endocrine disease as a result of heterogeneous metabolic disorders, causes hyperglycemia due to deficiency in secretion and/or insulin action [(1)]. It include two main types: type 1, is resulting from destruction of insulin-producing pancreatic β-cells; while type 2, results from the peripheral resistance of insulin hormone [(2)]. The prevalence of diabetes has been increasing in epidemic proportions, with long-term complications [(3)]. DM have many complication, one of these complication is diabetic foot ulcer (DFU) has described as infection, ulceration and/or destruction of deep tissues associated with neurological abnormalities and various degrees of peripheral vascular disease[(3)], its commonly caused by repetitive stress in patients with peripheral neuropathy, or initial injury (trauma) that is not detected by the patient, together with a peripheral vascular disease, plus contributes to the development of foot ulcers [(4)]. An infection is a common origin of a DFU that can lead to a partial or complete lower limb amputation if not treated properly [(5)]. Some bacterial species considered as non-pathogenic, when alone or not capable of maintaining a chronic infection on their own, may co-aggregate symbiotically in a pathogenic biofilm and act synergistically to cause a chronic infection and causes delayed healing of DFU [(6)]. Generally, different microorganisms are found in infected DFUs, including: gram-positive aerobic *S. aureus*, *S. epidermidis*, *S. saprophytic* and *Streptococcus* spp, plus gram negative aerobes including *P. aeruginosa*, *P. mirabilis, and E .coli* spp. [(7)]. Vitamin D is steroid hormone fat-soluble [(8)]. Its synthetizing mainly made on the skin with the effect of ultraviolet light, and activated by two hydroxylation reactions in the liver and kidneys [(9)]. Hypovitaminosis D, will affect to the brain, heart, muscle, immune system, and bones, thus, leads to autoimmune diseases, infections, and neurological disorders [(10)], linked to the onset of diabetes by Vitamin D receptor (VDR) found on β-cell [(11)]. Low vitamin D causes block insulin secretion [(11)], via autoimmune destruction β-cell lead to T1DM or changes in tissue response to insulin causes T2DM [(12)].

Material and Methods

A cross sectional study was carried out from 12th of August 2019 to 25th of February 2020 in Salah al-Din Governorate. Seventy patients with D.M had diabetic foot ulcer (41 male and 29 female), blood was drawn and taken swab from foot ulcer by swab transport

media, containing gel media to save the sample until transferred to the laboratory. Whereas 128 DM patients (65` male and 63 female) without diabetic foot. They had taken drawn blood from them. Blood sample (5 ml) collected from the vein by venipuncture in tube without having any anticoagulant. Serum were immediately separated by centrifugation and it was divided into two parts, one stored in Eppendorf Tubes at -80°C for ELISA test, and another for measurement level fasting blood glucose (FBG). After taken by swab transport media transported to the laboratory and transplanted to the appropriate culture media, namely Blood Agar, MacConkey Agar and Mannitol Salt Agar as a primary transplant, and incubated at 37 °C temperature for 18-24 hours [12], then implanted on culture media and chemical tests were made with confirmation of the diagnosis of some samples by Vitek, for the purpose of identifying the bacteria causing the ulceration, with a sensitivity test for 6 antibiotics to know how resistant bacteria [13].

Result and Discussion

The total number of patients with DM were 198 of clinical samples were collected from the general surgery wards, the internal resuscitation wards, and the internal consultation at General Hospitals in Salah al-Din Governorate. Out of 198 patients with DM were 70 (64.5 %) case suffered from DFU, while 128 (35.5 %) case were presented with DM only. They were compared with 80(100%) cases healthy individuals (control group) as in (Table 1).

Table 1: Distribution of patients (DM, D.F.U) and healthy people.

Diseases	Patient		Control	
	NO.	**%**	**NO.**	**%**
Diabetic mellitus	128	64.5 %	0	0
Diabetic foot ulcer	70	35.5 %	0	0
Total	198	71 %	80	100%

***X^2 = 50.086 *P≤ 0.01 *significant**

1. Levels of Vitamin D in patients with diabetic mellitus, diabetic foot ulcer and the control group.

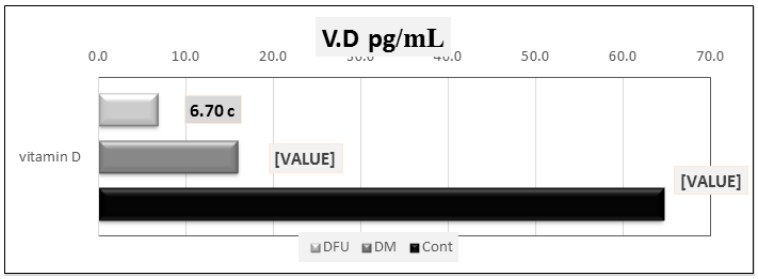


Figure 1: Levels of vitamin D in patients with DFU, DM and the control group.
***F. ratio=236.829 * P≤ 0.01 * Highly Significant (HS)**

The prevalence of this study shown in (figure 1), the mean of vitamin D level (6.70 pg. /ml) was occurred in patients with DFU who were infected with bacteria followed by patients with DM without ulcer infection (15.90 pg. /ml), while the highest mean was recorded in the control group (64.55 pg./ml). The relation was statistically highly significant. This study showed high significant decrease in Vitamin D levels in DFU and DM groups in comparison with control during the study period. These data Confirmed, that diabetic foot ulcer patients were greater vitamin D deficient and harmed, because they had significant lower serum vitamin D levels than diabetic patients without these DFU complications.

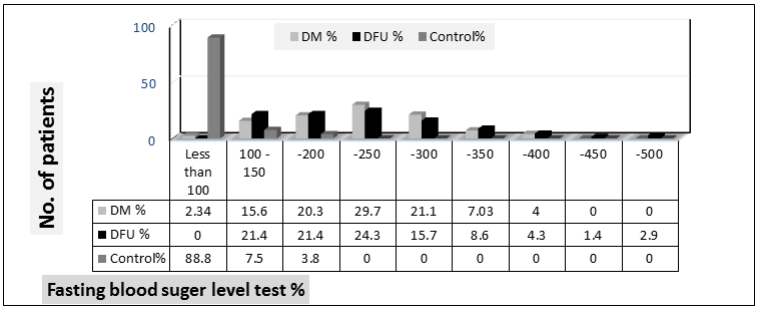
These result agree with the other studies, such as Tajik, E. (2019) [14]; Oraby *et al.* (2019) [15]; Patel and Pandya, (2020) [16]; and Dai *et al.* (2019) [17], that showed significantly reduced vitamin D levels in DM patients, severe with DFU, also some they reported that deficiency vitamin D an increased risk of DFU, and other complication T2DM. Also many studies such as Al-Rawaf *et al.* (2019) [18]; Hu *et al.* (2019) [19]; and Park *et al.* (2018) [20], they were noticing, improve level Vitamin D could significantly improvement in DFU healing and less the complication T2DM by using Vitamin D because ability to modulates impaired pancreatic b-cell function, insulin resistance, and systemic inflammation [21].

Vitamin D deficiency could be associated to the pathophysiology [22], by existence 36 different tissues in the body able to interact with the physiology of vitamin D, like the kidneys, bones, intestines, the heart, liver, pancreatic beta cells, immunologic system cells pathophysiology [23]. Vitamin D deficiency contributes to both the initial insulin resistance and the subsequent onset of diabetes caused by β-cell death [24]. Vitamin D deficiency has been linked to the onset of diabetic, by the role of Vitamin D in maintaining the normal release of insulin by the pancreatic beta cells (β-cells) and overcome this resistance by releasing more insulin, thus preventing hyperglycemia [25]. Poor glycemic control causes increased fatty acid and protein kinase, which reduces insulin signals due to a change in adipo-kinase secretion and phosphorylation of substance insulin receptor[26], ditto glucose toxicity bring about Increased (calcium and AGEP) deposition thus, insulin resistance and ischemia and vascular nerve damage result of stimulation of cellular immunity, in addition[27], deposition of immune complex in the blood vessels a result of the activation monocyte, release of pro-inflammatory cytokine and stimulation migration neutrophils [28].

Likewise Vitamin D deficiency with glucose toxicity, activation of oxidative stress and pro-inflammatory reactions, formation of non-enzymatic proteins, hypoxia and insulin resistance in type 2 diabetes, have a role in the formation and progression of diabetic peripheral neuropathy[29]. Moreover Vitamin D has play an important role in increasing the expression of insulin receptors, furthermore effect induces antimicrobial peptides production in keratinocytes from diabetic foot ulcers hence, and healing was delayed [30]. Furthermate, Vitamin D regulate the function of both innate and adoptive immune systems.

2. Blood Serum Glucose level for D.M, D.F.U and control.

From the frequency of the fasting blood glucose level testing in this study showed a significant increase in the concentration of blood glucose compared to the control group. The measurement blood level glucose for control group was ≥100 mg/dl in 71 (88.8%) persons which meant they do not have diabetes, while there was found DM 3(2.34) cases, but lack of case recorded for DFU patients in this level. Increase FBS level in DFU that exceeded the 450 mg/dl in some patients.



	Less than 100	100 - 150	-200	-250	-300	-350	-400	-450	-500
DM %	2.34	15.6	20.3	29.7	21.1	7.03	4	0	0
DFU %	0	21.4	21.4	24.3	15.7	8.6	4.3	1.4	2.9
Control%	88.8	7.5	3.8	0	0	0	0	0	0

Figure 2: Distribution of D.M, D.F.U and control according to the Sugar test.
***X^2 =236.851 * P ≤ 0.001 *Significant.**

The current study showed high significance increase in blood glucose concentration (P≤ 0.001) in DM and DFU as compared with control group. These results corresponding with the studies of Asfandiyarova, (2015) [31] who saw that diabetes is made when the fasting blood glucose level is 126 mg/dL or higher on at least two tests. Also Alshayban and Joseph, (2020) [32]; Wallia (2019) [33] and Vatansever *et al.* (2020) [34], who reported that the incidence of diabetic mellitus with high blood glucose level in diabetes drastically lowers insulin's effects on body by pancreas is unable to produce insulin, or by resistant tissue to the effects of insulin or doesn't produce enough insulin to maintain a normal glucose level. As a result, glucose tends to build up in your bloodstream (hyperglycemia) and may reach dangerously high levels if not treated properly. Insulin or other drugs are used to lower blood sugar levels [35]. The presence of hyperglycemia and diabetes in elderly patients is associated with increased risk of complications, include damage to the eye, kidneys, nerves, heart, and the peripheral vascular system, and increased mortality compared with subjects with normoglycemia [36] result of accumulation cytotoxic, free radicals resulted from initial inflammation, followed by infiltration of activated macrophages and lymphocyte in the inflammatory DFU; this leads to a reduction in plasma insulin concentration, and leading chronic hyperglycemia state [37].

3. Isolation of Bacteria from patients with DFU

After taking a swab for each patient and cultured on the culture media for aerobic bacterial, found that 61 cases (87%) positive for microbial infection and 9 patient (13%) were no microbial growth. These findings are in agreement with those Gupta et al. (2019) [38], who reported that bacterial growth in DFU was (92.31%) cases. It is likely that no bacterial growth will take place for patients to take antibiotics, or the causative may be a virus, fungus, or anaerobic bacteria that cannot be isolated in the same way that used in this study, because they need special media and special implant methods. As it is expected the results revealed a significant difference at 1% probability level (P = 0) in the recurrence D.F.U patient according to the growth bacteria in D.F.U swab as shown in (Figure 3).

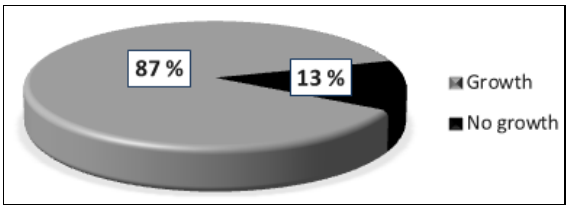


Figure 3: Distribution of D.F.U patient according to the growth of bacteria in D.F.U.
***X^2=38.629 * P≤ 0.01 *significant.**

The growth of bacterial isolates was classified into two types: The first case was culture samples had infection derived from a single microorganism, as a pathogen for DFU in 24 cases (39.3%), the second case was mixed growth includes more than one type of bacteria that causative diabetic foot ulcers in 37 cases (60.7 %). This study revealed non-significant difference at (P = 0.121) in according to the single or mixed bacteria growth, showing in (Figure 4).

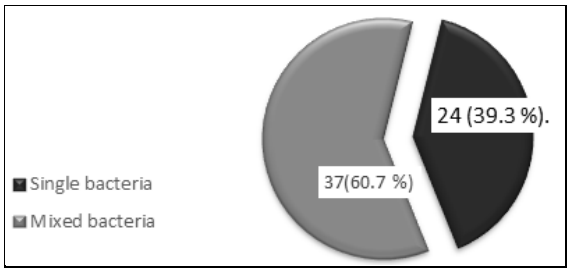


Figure 4: Distribution of D.F.U patient according to the growth bacteria.
***X^2=2.400 *P = 0.121 *non-significant**

From the positive cases of microbial growth, 109 isolates were collected. First diagnosis by using Gram stain, because it's one of the most rapid, reliable, and inexpensive methods for estimating bacteria, showed aerobic bacteria isolates were including 64 positive isolates (58.7%) and 45 negative isolates (41.3%) from diabetic foot ulcer. This study showed significance increase in gram positive bacteria (P =0.069), in diabetic foot ulcer as compared with gram negative bacteria during the experiment period, showing in (Figure 5).

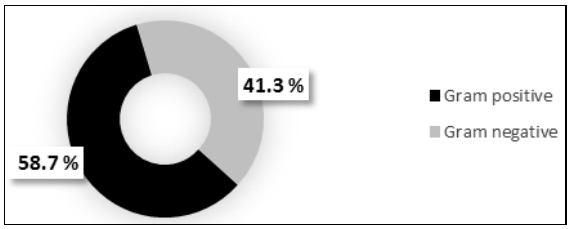


Figure 5: Distribution of D.F.U bacteria according to the gram stain.
***X^2=3.312 * P≤ 0.01 *Non-significant.**

These results agree with other study such as Ogba *et al.* (2019) [39]; and Radji *et al.* (2014) [40] who indicated that (62.8%) were poly-microbial in DFI, also study of Barman and Jain, (2017) [41]; reported a preponderance of mono-microbial culture growth (64%). Poly-microbial growths were seen in 29% of patients whereas 7% of the specimens were sterile. Also in agreement with these findings, several studies have reported a high prevalence rate of poly-microbial infections 55.7%, than mono-microbial infections (Saseedharan et al., 2018). [42] Also Perim et aL. (2015) [43] reported that infections were mostly due to Gram-positive bacteria and poly-microbial bacteremia, Ogba et al. (2019) [39]; the poly-microbial infection rate in this study was 36 (72.0%) while the mono-microbial rate was 14 (28.0%). Our findings is higher than the 67.2% reported by Shanmugam et al. (2013) [44] but lower than the 87.2% poly-microbial infection rates reported by Altrichter et al. (2015) [45]. The high poly-microbial infection rate reported in this study point to the fact that most of our subjects suffered severe diabetic foot infections. M et al. (2018) [46] were reported that mono-microbial infections were less common (n = 81; 31.2%) than poly-microbial infections (n = 132; 50.7%), while Nelson, A et al. (2018) [47], whose reported that the groups of pathogens were: Gram-positive cocci (70.6%); Gram-negative bacilli (36.7%). The presence of bacteria as poly-microbial flora isolated from a DFU does not reveal which microorganisms are pathogens, determining the role of specific bacteria is all the more difficult because poly-microbial interactions may modulate the pathogenic potential of one or the other [48], and ordinary non-pathogenic species are able to collaborate and interact together with synchronization to create a functionally equivalent path group (FEP) responsible for the infection chronicity and the maintenance of the pathogenic biofilm, therefore, some bacterial species considered as non-pathogenic, when alone or not capable of maintaining a chronic infection on their own, may co-aggregate symbiotically in pathogenic biofilm and act synergistically to cause a chronic infection, like S. aureus exposed to other bacteria shifts towards commensalism with the attenuation of virulence characteristics that concept may explain the delayed healing of chronic wounds [49], therefore cultures from chronically infected DFUs are often poly-microbial.

4. Sensitivity test of bacterial isolates to some antibiotics

Table 2: Diameter of antibiotic inhibition zone of gram positive bacteria isolation from D.F.U.

Bacteria	Carbincillin (py)		Penicillin (G)		Vancomycin (VN)		Levofloxacin (Lev)		Ceftriaxone (CRO)		Augmentin (Au)	
	R%	S %	R%	S %	R %	S %	R %	S %	R %	S %	R %	S %
S. aureus	100	0	100	0	52.5	47.5	0	100	100	0	85.7	14.28
S. epidermidis	100	0	82	12	66.7	33.3	33	77	66.7	33.3	100	0
S. saprophytic	100	0	71.4	28.6	57.2	42.8	28.5	71.4	100	0	92.8	7.2
S. pyogenes	100	0	75	25	100	0	25	75	100	0	25	75
Total antibiotic R\S	100	0	82.1	17.9	69.1	30.9	34.6	89.2	91.7	8.3	75.9	24.1

***X =110.123 * P≤ 0.01 *Significant.**

The results showed that all Gram positive and negative bacteria were showed high resistance (100%) for Carbincillin, followed by penicillin G. While all bacteria isolate were sensitive to Levofloxacin, these result converge with I and Ma (2017) [50], which showed the almost bacteria isolated

from DFU were sensitive to Levofloxacin. Also all Gram negative isolates and Staphylococcus spp., were appearance resistance to Vancomycin, these result differentia with Shareef et al (2018) [51], adduce that maximum susceptibility coagulase negative *Staphylococcus aureus* to Vancomycin (73.34%), and *Staphylococcus aureus* (86.67%), but Patil et al. (2017) [52], reported that all gram positive aerobic organisms were sensitive for vancomycin. In addition, Augmentin was very effective against *S. pyogenes* (75%), which agree with Hasan (2017) [53], but resistance to gram negative isolate (65.24%), these Contradicts Ogba et al (2019) [39], showed that while gram-negative bacteria were more susceptible to Augmentin (87.5%). Due to the small number of *S. ficaria* and first isolated from DFU, so it was found to be sensitive (100%) to Levofloxacin and resistant (100%) to all other antibiotics, addendum Dorgham (2019) [54], reported that the bacteria resistance levofloxacin were *P. aeruginosa* (76%), *K. pneumoniae* (76 %), *P. mirabilis* (63.6%), *E. coli* (87.5%) and *S. aureus* (33.3%). In another way, results of the present study harmonize with Sarla (2019) [55], that reported *S. aureus* sensitive to Levofloxacin (84.21%), *Pseudomonas* resistance to Ceftriaxone (100%), and resistance *S. aureus* and *E.coli* to Augmentin (80.39%),(83.33%) respectively, where Ji et al. (2014) [56], showed Augmentin, Ceftriaxone and Levofloxacin resistance *P. aeruginosa* (66.7%),(50.0%),(25.0%) *E. coli* (75.0%), (0 %), (25.0%) and *P. mirabilis* (50.0%), (33.3%), (16.7%) respectively, but Dai, J., et al. (2020) [17], reported that the *S. aureus, S. epidermidis* and *S. pyogenes* resistance to Penicillin (84.6%), (90.9%), (75 %) and Levofloxacin (0%), (36.4%), (25.0%) respectively. Also Kang et al. (2018) [57], reported that long-term use of antibiotics makes the patients with diabetic foot ulcers resistant to the antibiotics

Table (3): Diameter of antibiotic inhibition zone of gram negative bacteria from D.F.U.

Bacteria	CarbincilliN (py)		Penicillin (G)		Vancomycin (VN)		Levofloxacin (Levo)		Ceftriaxone (CRO)		Augmentin (Au)	
	R%	S%	R%	S%	R%	S%	R%	S%	R%	S%	R%	S%
P .mirabilis	100	0	100	0	100	0	20	80	66	34	66.5	33.5
Ps. Aeruginosa	100	0	100	0	100	0	12.5	87.5	69	31	87.5	12.5
E. coli	100	0	100	0	81.2	18.8	16.7	83.3	61	39	88.9	11.1
S. ficaria	100	0	100	0	100	0	0	100	100	0	100	0
K. pneumoniae	100	0	100	0	100	0	16.7	83.3	100	0	83.3	16.7
Total antibiotic R\S	100	0	100	0	96.2	3.8	13.2	86.8	59	41	65.2	34.8

***X =26.908, * P≤ 0.01,*Significant.**

The higher prevalence of multidrug-resistant organisms was also observed in study of Stacy et al. (2014). The higher antibiotic resistance in tertiary care hospitals is because, widespread use of broad-spectrum antibiotics results in selective survival of drug-resistant organisms In the present study, univariate analysis showed that, poor glycemic control, previous hospitalization, previous history of amputation, previous antibiotic usage, size of the ulcer, necrotic ulcer, recurrent ulcers, higher grade of ulcer, presence of osteomyelitis, peripheral vascular disease, neuropathy, and ischemic causes microcirculation , which may lead to poor penetration of antibiotics into the deep site of the ulcer and creates conditions that increase resistance diabetic foot bacterial infection [60]. Also DFU infections were predominantly poly-microbial with the ability to form biofilm, and the transfer of resistance genes by transport means which is an important virulence factor to make it multidrug-resistant [61]. Moreover resistance in bacteria develops because of mutations or horizontal gene transfer from increased antibiotic exposure causing selection pressure that provides a competitive advantage for mutated strains for better survival in changed host environment also Gram negative organisms more liable to become drug resistant due to the presence of a more mature multilayered cell membrane structure and the elaboration of many different plasmids and other mobile genetic elements responsible for horizontal gene transfer between bacteria [62].

Conclusion

We can conclude the vitamin D-level decrease in DFU more than DM patients, plus vitamin D plays an important role in activating immune cells and enhancing their work in addition to regulating insulin secretion while reducing cell resistance to insulin. *Staphylococcus aureus* and *E. coli*, were higher rates in infection of DFU insulation have been shown and play an important role in the development of DFU disease. Also *S. ficaria* can infect DFU as a pathogen. Levofloxacin was the most effective antibiotic in bacterial inhibition, but Carbincillin didn't effective against bacteria.

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الملخص

تهدف هذه الدراسة المايكروبايلوجية الى معرفة العلاقة بين نقص فيتامين (د) في مرضى قرح القدم السكرية وتشخيص أنواع البكتيريا الهوائية المسببة لها، مع معرفة مقاومتها لبعض المضادات الحيوية المستخدمة. الدراسة الحالية هي دراسة مقطعية. اجريت الدراسة في محافظة صلاح الدين، من 12 اب 2019 إلى 25 شباط 2020. سبعون مريضا عانوا من قرحة القدم السكرية (41 ذكر و 29 أنثى) اخذت لهم مسحة من مكان التقرح بواسطة سواب معقم ونقلت إلى المختبر. اظهرت النتائج ارتفاع معنوي عالي عند مستوى ($p\leq0.01$) في تركيز مصل الدم لفيتامين د لدى الاشخاص المصابين بالقدم السكرية والاشخاص المصابين بالسكري مقارنة مع المجموعة الضابطة. كما وجدنا ارتفاع معنوي عند مستوى ($p\leq0.01$)في عدد مرضى الذكور المصابين مقارنة بالإناث. كما بينت النتائج ان (87٪) مرضى DFU لديهم اصابة بكتيرية ، كما سجلت الإصابة التي كان سببها عدة انواع من البكتريا (60.7٪) نسبة أكثر من إصابة بجرثومة مفردة (39.3٪) ، بالإضافة إلى أن نسبة الإصابة بالبكتيريا الموجبة (58.7٪) كانت اعلى من البكتيريا السالبة (41٪). اضهرت الدراسة ان اعلى نسبة بكتريا عزلت من القرح القدم السكرية هي S.aureus (36.7٪)، بينما كانت S. ficaria المسبب الوحيد للعدوى وقد تم عزلها بنسبة (0.9٪). كانت معظم العزلات البكتيرية المسببة DFU مقاومة للمضادات الحيوية المستخدمة ما عدا الليفوفلوكساسين حيث كانت معظم العزلات حساسة، في الوقت ذاته، كانت البكتيريا شديدة المقاومة للكاربنسلين (100٪).