JMS 2023; Vol 6 (3):246-250
UDC: 613.379-008.64-06:617.586-002.44
https://doi.org/10.55302/JMS2363246mt

BIOFILM IN CHRONIC DIABETIC FOOT ULCER-CASE REPORT

Julija Mitrova Telenta[1], Liljana Labacevska-Gjatovska[2], Marko Kostovski[2], Maja Dimova[1], Ivana Dohceva Karajovanov[1], Suzana Nikolovska[1], Silvija Duma[1],Hristina Breshkovska[1], Nikola Panovski[2]

[1]University Clinic of Dermatology, Faculty of Medicine, Ss. Cyril and Methodius University in Skopje, North Macedonia

[2]Institut of Microbiology and Parasitology, Faculty of Medicine, Ss.Cyril and Methodius University in Skopje, North Macedonia

Abstract

Diabetic foot ulcers(DFUs) as one of the most common complications in patients with diabetes mellitus are usually chronic wounds.The reason for its chronicity are infections and biofilm formation.

We present a patient with diabetic foot neuropathic ulcer on the right foot. Microbiological swab showed isolates of bacteria and fungi, *Candida albicans,Enterococcus* and *Acinetobacter* which were tested for biofilm formation with microtiter plate assay. Biofilm mass was evaluated spectrophotometrically by measuring the absorbence of crystal violet. *Enterococcus* was with strong potential of biofilm formation.

Wound surface was measured every week for a period of one month and it was reduced for 23.93%. Ulcer was treated with peroral antibiotic and antifungal medications and standard wound care was performed.

Microorganisms isolated from wound swabs showed mixed bacterial and fungal components. Current studies show that relation in between this biofilm is still unclear. All of this is a key role in treating chronic wounds, making it a challenge for everyone not only in the field of making diagnosis but also in the field of treatment.

Key words: diabetic foot ulcers(DFUs),bacteria, fungi, biofilm.

Introduction

Diabetic foot ulcers (DFUs) are one of the most common complications in patients with diabetes mellitus (DM). They have impact of the quality of life of this patients, because they are chronic wounds which are very complicated for treatment[1].

The most common reasons for chronicity of this ulcers are infections and biofilm formation. In DFUs there is colonisation with pathogenic bacteria and because of the immunological deficiencies related to diabetes infection is favoured [2,3]. In DFUs, the pathogens involved in infections vary from aerobic to anaerobic species, also these microorganisms can exist either in planktonic or sessile state [4,5].

When bacteria form biofilms, their cells produce extra cellular polymeric matrix in which they are encased and that confers them protection from the host's immune system and from antibiotics [6]. As a result, biofilms in DFUs may be the reason for the delayed healing and consequent infection chronicity [7,8,9], despite systemic antibiotic treatment [10].

We present a 60 years old patient with DM type 2 and chronic hypertrophic ulcer on the plantar site of the right foot.

Case report

A 60 years old male patient with history of DM type 2 presented at our clinic with non healing chronic hypertrophic neuropathic ulcer on the plantar site of the right foot which lasts for 6 months. The ulceration was treated with local antiseptic and homemade solutions based on different plants.

On the first admission color doppler diagnostic procedure was performed and it showed normal venous and arterial function of the lower extremites.

This patient was taking chronic therapy for DM (Metformin) and for hypertension HTA(Enalapril).His glucose level was iregulated with hyperglicemia above 9 mmol/l. Before starting with treatment, microbiological swab was taken from the wound bed. The results show isolates of *Candida albicans, Enterococcus and Acinetobacter*, so acording antibiogram the peroral antibiotic and

antifungal treatment with Ciprofloxacin and Fluconazole was started. The standard wound care was performed starting from the first admission at our clinic.

The wound surface was measured every week during one month of treatment.Digital photographs were taken and wound surface was measured with special software application *Sketch and Calc Elliot and Dobbs,2011.* Peroral antibiotic treatment was administered for 10 days. After 5 days of completed antibiotic treatment, control microbiological swab was taken. The results showed persistance of *Candida albicans, Enterococcus and Fusarium.* The isolates from microbiological swab were tested for their potential for biofilm formation.

Biofilm formation was examined by the microtiter plate assay (MP) described by Christensen et al(1985) with modification [11].

Biofilm mass was evaluated spectrofotometrically by measuring the absorbence of crystal violet, a cationic dye that quantitatively stains non specifically negatively charged biofilm constituents based on ionic interactions in 96 well microtiter plates [12].

The absorbence of each well was measured at 570 nm using a microplate spectrophotometer (ELISA microplate reader). Optical density-OD for each isolate and the negative control was calculated as an arithmetical mean of the absorbencies of the three wells(positive control, negative control and isolate). This value was compared with mean OD of the negative control.

The following international reference strains were used as positive controls for biofilm production: the biofilm producers *Staphylococcus aureus* ATCC 29213 (for Gram positive bacteria) and *E.coli* ATCC 25922 (for Gram negative bacteria) as recommended by the National Committee for Clinical Laboratory Standards.

For interpretation of the results optical density cut-off value (ODc) calculated from the average OD values of the negative controls (ODn) was used.

ODn=0.1170

ODc= 0.4682

The results for biofilm production were interpreted as follows:

- absence of biofilm formation (OD strain < ODc) (OD strain < 0.468)
- weak biofilm formation (ODc<ODstrain <2xODc) (0.468<ODstrain < 0.936),
- moderate biofilm formation (2xODc <ODstrain<4xODc) (0.936 <ODstrain< 1.872)
- strong biofilm formation (4xODc <ODstrain) (1.872 <ODstrain)

The results showed that only *Enterococcus* has strong potential for biofilm formation with OD=1.989. Wound surface was measured every week and the results were : wound surface at week 0= 1,88cm^2, wound surface at week 1= 1,64 cm^2, wound surface at week 2=1,53 cm^2, wound surface at week 3 =1,43 cm^2.

The percentage of wound healing after one month of treatment was 23.93% compared to the wound surface from week 0 (the first examination at our clinic).[Fig 1,2,3,4].

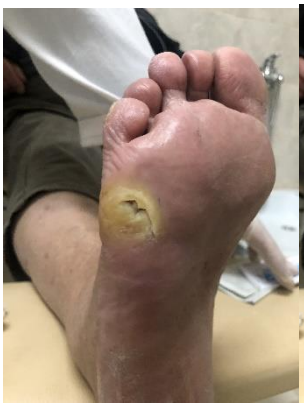

Figure 1(week 0)

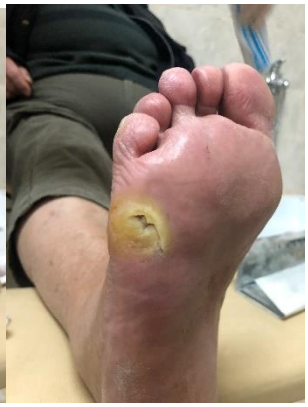

Figure 2(week 1)

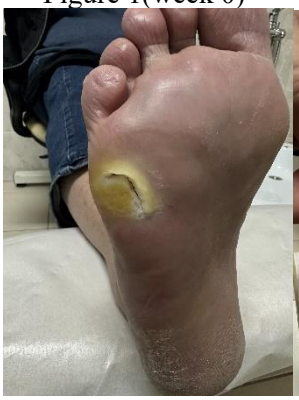

Figure 3 (week 2)

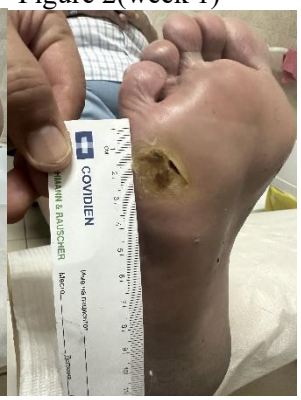

Figure 4(week 3)

Discussion

Chronic wounds are wounds with impaired healing process that lasts more than 3-6 weeks[13,14]. They result from wound infection or biofilm formation.

Our patient had chronic DFU which lasted more than 24 weeks.The reason for its chronicity was biofilm formation , which was confirmed with microtiter plate assay(MP) described from Christensen et al.The results showed that *Enterococcus* was with high potential for biofilm formation using spectrophotometrical method for evaluation of the biofilm mass. It was isolated with swabs before and after the antibiotic treatment as was also *Candida albicans.*

Enterococcus is a facultative anaerobe.Acording the literature presence of *Candida albicans* was responsible for making network in which bacterial species *Enterococcus* was captured.T

his strong conection was also one of the reasons for antimicrobial resistance making a physical barrier which can't let antimicrobials to get directly to the pathogens in the biofilm structure.

The wound surface was reduced for 23.93% in the peirod of 4 weeks treatment.Ericsson et al. confirmed that if during 4 weeks of standard care, the surface of the wound is reduced by 50%, it is likely that healing will occur with the same treatment in 12 weeks. If less than 50% reduction occurs, it is unlikely to heal with this treatment and a change in treatment and reassessment is necessary[15].

Verbanic et al. in their study conclude that there was no significant difference in the microbiome composition of wounds, compairing wound swabs before and after debridement of wounds[16]. But the results were not the same with healed vs. unhealed wounds, because there was the over – representation of facultative anaerobes in the microbiome of non healing wounds.

Chellan et al. in their study identified that 30% of cases with diabetic foot ulcers are with *Candida spp.* isolates being the most prevalent, and the presence of A*spergillus spp*. and *Trichosporon spp*. was shown also[17].

These studies confirm that not only the bacterial microbiota, where increased bacterial diversity is associated with delayed wound healing process, but also the mycobiome have similar impact on wound healing[18].

Because fungi is an opportunistic pathogen, when wound is treated with antibiotics than fungi are colonising the surrounding skin as an ideal environment created for fungal infection.Higher levels

of blood glucose proportionaly makes *Candida* isolates to display higher activity of enzyme which results in higher virulence, so *Candida spp.* from commensal become pathogen species[18].The use of antibiotics targeting bacteria may create an environment favourable to fungal colonization and expansion[19].

Polymicrobial nature of biofilm makes wound treatment more difficult, increasing antibiotic resistance and providimg expansion of fungal infections. Current literature suggests use of antifungal drugs such as fluconazole, amphotericin B and antibacterial therapy, or use of a broad- spectrum topical antimicrobial that targets both[20].In our case we used fluconazole as treatment option.

Conclusion

We presented a case with diabetic foot ulcer and the reason for its chronicity was biofilm formation. Current studies show that relation in between this biofilm is still unclear.

The results showed that *Enterococcus* was with strong potential for biofilm formation. *Candida albicans* also was isolated before and after the antibiotic and antifungal treatment.It is one of the most prevalent fungal isolates in DFUs , so it is associated with delayed wound healing.

The strong conection between these microorganisms also sugests the reason for antimicrobial resistance. All of this is a key role in treating chronic wounds, making it a challenge for everyone not only in the field of diagnostic , but also in the field of treatment.

References

1. Afonso AC, Oliveira D, Saavedra MJ, Borges A, Simões M. Biofilms in Diabetic Foot Ulcers: Impact, Risk Factors and Control Strategies. *International Journal of Molecular Sciences*. 2021; 22(15):8278. https://doi.org/10.3390/ijms22158278
2. Geerlings SE. Immune dysfunction in patients with diabetes mellitus (DM). *FEMS Immunol. Med. Microbiol.*1999;26:259–265.
3. Lipsky BA, Berendt AR, Deery HG, Embil JM, Joseph WS, Karchmer AW, Lefrock JL, Lew DP, Mader JT, Norden C, et al. Diagnosis and Treatment of Diabetic Foot Infections. *Clin. Infect. Dis.* 2004;39: 885–910.
4. Noor S, Zubair M, Ahmad J. Diabetic foot ulcer—A review on pathophysiology, classification and microbial etiology. *Diabetes Metab. Syndr. Clin. Res. Rev.* 2015; 9: 192–199.
5. Pouget C, Dunyach-Remy C, Pantel A, Schuldiner S, Sotto A, Lavigne JP. Biofilms in Diabetic Foot Ulcers: Significance and Clinical Relevance. *Microorganisms* 2020;8: 1580.
6. Versey Z, Nizer WSDC, Russell E, Zigic S, DeZeeuw KG, Marek JE, Overhage J, Cassol E. Biofilm-Innate Immune Interface: Contribution to Chronic Wound Formation. *Front. Immunol.* 2021;*12*.
7. Stewart PS, Costerton JW. Antibiotic resistance of bacteria in biofilms. *Lancet.* 2001; 358: 135–138.
8. Stewart PS. Mechanisms of antibiotic resistance in bacterial biofilms. *Int. J. Med. Microbiol.* 2002;292: 107–113.
9. Fux C, Costerton J, Stewart P, Stoodley P. Survival strategies of infectious biofilms. *Trends Microbiol.*2005;13: 34–40.
10. Price BL, Morley R, Bowling FL, Lovering AM, Dobson C. Susceptibility of monomicrobial or polymicrobial biofilms derived from infected diabetic foot ulcers to topical or systemic antibiotics in vitro. *PLoS ONE.*2020; 15: e0228704.
11. Christensen GD, Simpson WA, Bismo AL, Beachery EH. The adherence of the slime-producing strains of Staphylococcus epidermidis to smooth surfaces. Infect immune. 1982;37:318–26
12. Stepanovic S, VukovicD, HolaV, Bonaventura G, Djukic S, Irkovic IC, RuzickaF. Quantification of biofilm in microtiter plates: overview of testing conditions and practical recommendations for assessment of biofilm production by staphylococci. APMIS. 2007; 71(5), 687-690.
13. Olsson M, et al.The humanistic and economic burden of chronic wound:a systematic review.Wound Repair regen.2019;27:114-125.
14. Artinengo L, et al.Prevalence of chronic wounds in the general population: systematic review and meta-analysis of observational studies.Ann.Epidemiol.2019;29:8-15

15. Eriksson E, Liu PY, Schultz GS, et al. Chronic wounds: Treatment consensus. Wound Rep Reg. 2022; 30(2): 156- 171. doi:10.1111/wrr.12994
16. Verbanic S, Shen Y, Lee J, Deacon J, Chen I. Microbial predictors of healing and
17. short-term effect of debridement on the microbiome of chronic wounds. Biofilms Microbiomes 2020; 6: 21. https://doi.org/10.1038/s41522-020-0130-5.
18. Kalan L, Loesche M, Hodkinson BP, Heilmann K, Ruthel G, Gardner SE, et al. Redefining the chronic-wound microbiome: fungal communities are prevalent, dynamic, and associated with delayed healing. mBio 2016; 7(5): e01058-16. doi: 10.1128/ mBio.01058-16.
19. Chellan G, Shivaprakash S, Ramaiyar SK, Varma AK, Varma N, Sukumaran MT, et al. Spectrum and prevalence of fungi infecting deep tissues of lower-limb wounds in patients with type 2 diabetes. J Clin Microbiol 2010; 48(6): 2097-2102. doi: 10.1128/ JCM.02035-09.
20. Kalan L, Grice EA. Fungi in the Wound Microbiome. Adv Wound Care (New Rochelle) 2018; 7(7): 247-255. doi: 10.1089/wound.2017.0756.
21. Townsend EM, Sherry L, Kean R, Hansom D, Mackay WG, Williams C, et al. Implications of antimicrobial combinations in complex wound biofilms containing fungi. Antimicrob Agents Chemother 2017; 61(9): e00672. doi: 10.1128/AAC.00672-17.