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Association between Matrix metalloproteinase -13 and Bacteria Isolated From Diabetic Foot Ulcers

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ABSTRACT

This study was conducted to ascertain the existence of an association between Matrix metalloproteinase-13 (Mmp-13) and bacteria isolated from diabetic foot ulcers. The bacteria were isolated by sterile swabs to be identified according to morphology and culture, and identified by the Vitek-2 system. Venous blood samples were also taken from diabetic ulcers and diabetics without ulcers. Finally, the biofilm formation was measured using methods such as Congo Red agar and tissue culture microplate. Out of the 60 clinical specimens only (80%) specimens gave bacterial growth. Biofilm formation found that 37 (77%) of bacterial isolates can produce biofilm by Congo Red agar, while only 33 (66.7 %) from bacterial isolates have the ability to produce strong biofilm by titer plate method. The present study also observed that the concentration of Mmp-13 in diabetic ulcer infection increased significantly ($P < 0.05$) than in diabetic patients without foot ulcer infection.

Keywords: Biofilm, Matrix metalloproteinase, Congo agar

1-Introduction

Ulcers on the feet caused by diabetes are one of the most significant issues affecting public health, and they place a significant socioeconomic burden on those who are afflicted. Chronic ulcers are a dangerous illness that may lead to amputation, which can lower the quality of life of diabetes patients. The ulceration of the diabetic foot does not happen by itself; rather, it is often the result of some kind of trauma, which the patient may not even be aware of at the time. A wound is regarded to be infected when a pathogenic bacterium seriously colonizes and the human immune system is unable to eliminate it [1,2]. This damage may be caused by footwear that does not fit well, walking barefoot, foreign objects, or scalding from hot water [1]. The microbial role in infection is dependent on their concentration, the species composition, and the host's response [3]. MMPs are a family of zinc endopeptidases that can degrade all of the components of the extracellular matrix. [4] Matrix metalloproteinases are involved in the degradation of the extracellular matrix. They remove damaged protein, degrade the temporary extracellular matrix, stimulate migration to the center of the wound, remodel the granulation tissue, and possibly govern angiogenesis [4]. They are crucial participants in every step of the healing process. The purpose of this research was to determine whether or not there is a connection between Mmp-13 and the bacteria that are often found in diabetic foot ulcers.

2. Materials and Methods

The study included 30 blood samples from diabetics without foot ulcers and 60 blood and swab samples from diabetic foot infection ulcers collected during the period from the beginning of September 2021 to March 2022 who attended to the private laboratory in Baghdad city, Iraq.

The blood samples were put into a Gel tube to separate the serum. The blood was left at room temperature for 30 minutes to clot, and then it was spun at 3000 g for 2 minutes. The serum was then put into three sterile Eppendorf tubes and frozen at -20 C until the time of use.

2.1. Identification of bacteria

A single colony was taken from each primary positive culture that was grown on MacConkey agar, blood agar, and nutrient agar. This colony was then grown in pure culture and analyzed for its morphological and cultural characteristics (blood hemolysis, lactose fermentation, colonial form, size, color, frontiers, and texture). Finally, the organism was identified using the Vitek-2 system [5].

2.3. Detection of biofilm formation

1- Qualitative method by Congo Red Agar(CRA):- this accomplished by (6).

2- Quantitative method by Titer plate method :- this accomplished by (7).

2.3.Estimation of serum Mmp-13: This accomplished according to the ELISA kit [8].

4. Results & Discussion

This study was conducted with a total of (90) samples divided into two groups: (60) suffered from diabetic foot ulcers (DFU) and (30) suffered from diabetes without foot ulcers. Patients who attended the private laboratory in Baghdad city, Iraq, collected during the period from the beginning of September 2021 to March 2022, during the time. The 48 (80%) were given positive culture, and another 12 (20%) samples were also considered positive (caused by other causative agents).

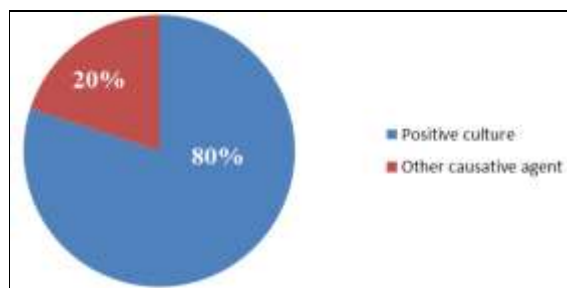


Fig. 1: The occurrence of bacteria isolated from 60 DFU patients

4.1. Identification of bacteria

It was observed that gram-negative bacteria were present in 76% of DFU patients, but gram-positive bacteria were detected in only 24% of DFU patients, according to the data that were published by Bansal et al. [9]. The result of this study revealed that gram-negative bacteria were higher than gram positive bacteria.

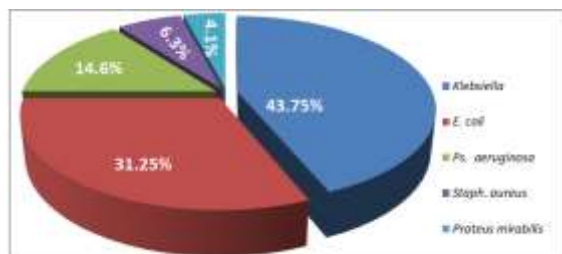


Fig. 2: The percentage of bacteria isolated from 48 clinical specimens.

The research conducted by Bansal et al.[9] revealed that gram-negative bacteria were detected in 76% of DFU patients, but gram-positive bacteria were discovered in only 24% of DFU patients. According to the findings of this inquiry, gram-negative bacteria were discovered to be the most common kind of bacteria to be detected most of the time[10].

The findings of this study were comparable to those found in [11], which discovered that gram-negative organisms were isolated from 57.6% of DFU patients at a higher frequency than gram-positive organisms were recovered from 42.3% of DFU patients. The findings of this study were similar to those found in [11].

This variance in frequency of type and number of isolates has been attributed to the differences in sample collection and research methods, as well as the length of the ulcer wound and the depth of the

ulcer wound, as well as the glycemic state of the patient [12].

4.2. Biofilm formation

4.2.1. Qualitative method for biofilm Detection by Congo Red Agar

A total number 48 isolates found that 37 (77%) isolates from different types of bacteria able to produce biofilm by Congo Red agar , results showed that *Klebsiella* spp was 15/21(43.75%), *E. coli* was 11/15(31.25%), *Pseudomonas aeruginosa* was 7/7(100%), *S. aureus* was 3/3(100%) ,and *P. mirabilis* was 1/2(50%) ,this results shown in (Figure 3).



Fig. 3: The biofilm formation by Congo red assay (CRA)

The result in [13] noted that out of 147 isolates of bacterial isolated, biofilm detected by CRA in 64 (43.5%) isolates and dissimilar with the CRA method, 11 were found to be biofilm-producing bacteria and 99 as non-biofilm producers. The results that were obtained during this study were in disagreement with [13]. In the research conducted by Knobloch et al. [14], which does not advocate using the CRA approach for the identification of biofilm, 128 isolates of *S. aureus* were tested, CRA only found 3.8% of them to be biofilm producers, whereas TCP found 57.1% of them to be biofilm-forming bacteria.

4.2.2. Quantitative method for biofilm Detection by Titer microplate method: The production of biofilm on polymeric surfaces was evaluated using a semi-quantitative microtiter plate technique (biofilm assay). To improve the reliability of the results, this test was carried out three times for each sample. only 14/21 (66.7%) *Klebsiella* spp, 9/15(60%) *E. coli*, 7/7 (100%) *Ps. aurogenosa*, 2/3(66.7%) *Staph. auerus* and 1/2(50%) *P. mirabilis* were strong biofilm former and only 2/21 (9.5%) *Klebsiella* spp and 1/15(6.7%) *E. coli* were moderate biofilm former while, 5/21 (23.8%) *Klebsiella* spp, 5/15(33.3%) *E. coli*, 1/3(33.3%) *Staph. auerus* and 1/2(50%) *P. mirabilis* were non-biofilm former as shown in (Table 1).

Table 1: Frequency of the biofilm formation among bacterial isolates

Types of bacteria	Strong biofilm formation NO.(%)	Moderate biofilm formation NO.(%)	Non-biofilm formation NO.(%)
<i>Klebsiella spp</i> (21)	14(66.7%)	2(9.5%)	5(23.8%)
<i>E. coli</i> (15)	9(60%)	1(6.7%)	5(33.3%)
<i>Ps. aeruginosa</i> (7)	7(100%)	0(0%)	0(0%)
<i>S. aureus</i> (3)	2(66.7%)	0(0%)	1(33.3%)
<i>P. mirabilis</i> (2)	1(50%)	0(0%)	1(50%)

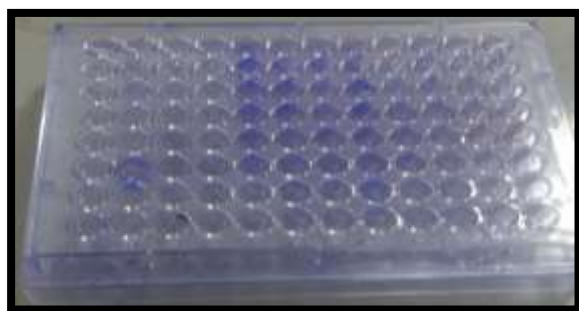


Fig. 4: The biofilm production by the Titer plate method

The capacity to create biofilm allows bacteria to protect themselves against a variety of stressors, such as immunological reactions and antimicrobial treatments. The ability of bacteria to form biofilms has been linked to an increase in antibiotic resistance as well as chronic recurrent infections, particularly in diabetics. Different types of microorganisms can exist independently or combine to form micro-communities within a matrix of extracellular polymeric substances that is called biofilm [15].

4.3. Evaluation of Matrix metalloproteinase -13 (Mmp-13) in DFU

The present study also observed that the concentration of Mmp-13 (pg/ml) in DFU infection

was 1299 ± 37.43 pg/ml is increased significantly than, diabetic patients without FU infection was 232.8 ± 16.61 pg/ as shown in figure (5) .

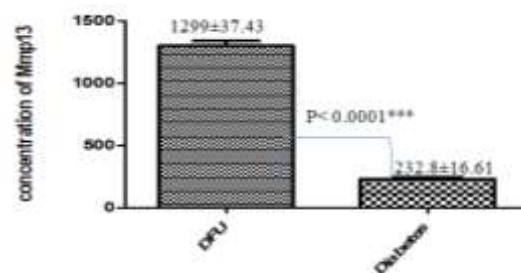


Fig. 5: Mmp-13 serum level in DFU patients and D without FU.

The result of our study that demonstrated serum Mmp-13 was increased in diabetic foot ulcers than in diabetes patients. This result agrees with [16] who found the level of serum Mmp-13 was significantly increased in patients suffering from DFU compared to the T2DM group.

MMPs seem to have a role in a variety of different processes throughout the natural process of wound healing. MMPs take part in the elimination of tissue that has lost its vitality during the early phase of the wound-healing process. During the phase of the healing process known as angiogenesis, MMP activities are required for the contraction of the wound matrix, the migration of fibroblasts, the migration of keratinocytes, and the epithelialization of keratinocytes. MMPs have a role in the remodeling of newly formed connective tissue during the last phase of the wound healing process. In conclusion, proper wound healing and tissue repair cannot occur without the spatiotemporal regulation of the expression of a number of different matrix metalloproteinases (MMPs) [17, 18].

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العلاقة بين matrix metalloproteinase-13 والبكتيريا المعزولة من قرحة القدم السكري

الملخص

أجريت هذه الدراسة بهدف التأكد من وجود علاقة بين Mmp-13 والبكتيريا المعزولة من قرحة القدم السكري. تم عزل البكتيريا بواسطة مسحات معقمة لتحديد هويتها وفقاً للصفات المظهرية وعلى الأوساط الزرعية ، تم تحديدها بواسطة نظام VITEK-2 ، كما تم أخذ عينات الدم الوريدي من قرحة القدم السكري والسكري بدون قرحة. أخيراً ، تم قياس تكوين الأغشية الحيوية باستخدام طرق مثل أجار الكونغو الأحمر ولوحة زراعة الأنسجة. حيث وجدت النتائج من بين 60 عينة سريرية كانت 48 (80%) عينة أعطت نمو بكتيري و 12 (20%) أخرى لم تعطي أي نمو. وجد تكوين البيوفيلم أن 37 (77%) عينة بكتيرية لديها القدرة على إنتاج غشاء حيوي بواسطة أجار الكونغو الأحمر ، بينما 33 فقط (66.7%) من العزلات البكتيرية لديها القدرة على إنتاج غشاء حيوي قوي بطريقة لوحة زراعة الأنسجة. لاحظت الدراسة الحالية أيضاً أن تركيز Matrix metalloproteinase-13 في الإصابة بقرحة السكري يزيد بشكل ملحوظ ($P > 0.05$) من مرضى السكري بدون قرحة.