Isolation and profiling of microRNAs (miR-203) from skin lesions of patients with type 2 diabetes mellitus and concurrent bacterial (*Pseudomonas aeruginosa*) skin infections

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Abstract

Individuals with type 2 diabetes mellitus (T2DM) are at an increased risk of developing skin-related complications, including susceptibility to bacterial infections such as those caused by Pseudomonas aeruginosa. Emerging evidence suggests that microRNAs (miRNAs) may play a crucial role in the pathogenesis of these comorbid conditions. This study aimed to investigate the isolation and profiling of miR-203 from the skin lesions of T2DM patients with concurrent P. aeruginosa infections. This cross-sectional study recruited 150 T2DM patients with P. aeruginosa skin infections at the Dermatology Clinic of Al-Nasria city. Skin lesion samples were collected, and the expression levels of miR-203 were determined using quantitative reverse-transcription PCR (RT-qPCR). The relationship between miR-203 and clinical characteristics was analyzed. The incidence of P. aeruginosa detection by PCR was 82%. Patients with P. aeruginosa infections had significantly higher median levels of miR-203 (20.33 ± 11.76) compared to healthy controls (2.01 ± 0.98, $p<0.001$). The optimal miR-203 cutoff value for diagnosing P. aeruginosa was <5.42-fold, with a sensitivity and specificity of 94%. A positive correlation was found between miR-203 expression and the severity of skin lesions ($r=0.68$, $p<0.001$), but miR-203 levels were not associated with other participant characteristics. These findings suggest that miR-203 could serve as a reliable diagnostic biomarker for P. aeruginosa skin infections in T2DM patients. The strong correlation between miR-203 and disease severity warrants further investigation into the potential role of miR-203 in the pathogenesis and management of these infections.

Keywords: miR-203, T2DM, skin lesion, *P. aeruginosa*.

عزل وتنميط الحمض النووي الريبوزي الميكروي (miR–203) من الآفات الجلدية لمرضى السكري من النوع الثاني والتهابات الجلد البكتيرية المتزامنة (*الزائفة الزنجارية*)

الملخص

الخلفية :يتعرض الأفراد المصابون بداء السكري من النوع الثاني (T2DM) لخطر متزايد للإصابة بمضاعفات مرتبطة بالجلد، بما في ذلك التعرض للعدوى البكتيرية مثل تلك التي تسببها الزائفة الزنجارية الزنجارية. تشير الأدلة الناشئة إلى أن الحمض النووي الريبي الميكروي (miRNAs) قد يلعب دوراً حاسماً في التسبب في هذه الحالات المرضية المصاحبة. هدفت هذه الدراسة إلى التحقيق في عزل وتنميط miRR–203 من الآفات الجلدية لمرضى داء السكري من النوع الثاني

الذين يعانون من عدوى الزائفة الزنجارية المتزامنة .الطرق :شارك في هذه الدراسة المقطعية 150 مريضًا من مرضى داء السكري من النوع الثاني المصابين بالتهابات جلدية بالميكروبات الهوائية في عيادة الأمراض الجلدية بمدينة الناصرية. جُمعت عينات من الآفات الجلدية، وتم تحديد مستويات التعبير عن miR-203 باستخدام تفاعل البوليميراز المتسلسل الكمي للنسخ العكسي (RT-qPCR).تم تحليل العلاقة بين miR-203 والخصائص السريرية .النتائج :بلغت نسبة اكتشاف بكتيريا P. aeruginosa بواسطة تفاعل البوليميراز المتسلسل 82%. كان لدى المرضى الذين يعانون من عدوى البكتيريا الزنجارية الهوائية مستويات متوسطة أعلى بكثير من ميل مير-203 (20.33 ± 11.76) مقارنةً بالضوابط الصحية (2.01 ± 0.98، p<0.001).كانت قيمة القطع المثلى لمير-203 لتشخيص بكتيريا P. aeruginosa <5.42 ضعف، مع حساسية وخصوصية بنسبة 94%. تم العثور على علاقة إيجابية بين تعبير miR-203 وشدة الآفات الجلدية (r = 0.68 ، p <0.001)، لكن مستويات miR-203 لم تكن مرتبطة بخصائص المشاركين الأخرى .الاستنتاجات :تشير هذه النتائج إلى أن miR-203 يمكن أن يكون بمثابة مؤشر حيوي تشخيصي موثوق به للالتهابات الجلدية لفيروس بكتيريا P. aeruginosa لدى مرضى داء السكري من النوع الثاني. يستدعي الارتباط القوي بين miR-203 وشدة المرض إجراء مزيد من التحقيق في الدور المحتمل لمير-203 في التسبب في الإصابة بهذه العدوى وإدارتها .

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1. Introduction

Diabetes mellitus is a chronic metabolic disorder that affects millions of people worldwide, with type 2 diabetes being the most prevalent form [1]. Individuals with type 2 diabetes are at an increased risk of developing various complications, including skin-related issues [2]. One such complication is the heightened susceptibility to bacterial skin infections, often caused by opportunistic pathogens such as Pseudomonas aeruginosa [3]. Emerging evidence suggests that microRNAs (miRNAs) – small, non-coding RNA molecules that play crucial regulatory roles in gene expression – may be involved in the pathogenesis of both diabetes and associated skin infections [4;5]. Specifically, miR-203 has been implicated as a key player in the development and progression of various skin disorders, including those related to diabetes [6;7]. The isolation and profiling of miRNAs, such as miR-203, from the skin lesions of patients with type 2 diabetes and concurrent P. aeruginosa infections can provide valuable insights into the underlying molecular mechanisms contributing to these comorbidities [8;9].

Understanding the regulatory role of miR-203 in this context may shed light on potential diagnostic and therapeutic avenues for managing these complex skin-related complications in individuals with type 2 diabetes. This study aims to investigate the isolation and characterization of miR-203 from the skin lesions of patients with type 2 diabetes and concurrent P. aeruginosa infections, with the goal of elucidating its potential involvement in the pathogenesis of these interrelated conditions.

2. Materials and Methods

2.1.Study Population and Sample Collection

This cross-sectional study recruited 150 patients with type 2 diabetes mellitus (T2DM) and concurrent *Pseudomonas aeruginosa* skin infections at the Dermatology Clinic of AL-Nasria city from January 2021 to December 2023. Skin lesion samples were collected from the affected areas of the participants using sterile swabs. The samples were immediately transported to the laboratory in sterile containers and stored at -80°C until further processing.

2.2.Bacterial Identification and Confirmation

Collected skin swab samples were cultured on Pseudomonas-selective agar plates (Cetrimide Agar, Oxoid, UK) and incubated at 37°C for 24-48 hours. Bacterial colonies were identified as Pseudomonas aeruginosa based on their characteristic morphology, Gram staining, and biochemical tests, including oxidase and arginine dihydrolase assays. Primer to *Pseudomonas aeruginosa* [F: CCTTTATACCAAGCGATTCAACCG; R: ACTCAAAGCTTTTCTTGTGGTGT. (NCBI Reference Sequence: NC_028999.1)].

2.3.Extraction and Profiling of miR-203

Total RNA, including small RNAs, was extracted from the skin lesion samples using the miRNeasy Mini Kit (Qiagen, Germany) according to the manufacturer's instructions. The concentration and purity of the extracted RNA were assessed using a NanoDrop spectrophotometer (Thermo Fisher Scientific, USA). The expression levels of miR-203 were determined using quantitative reverse-transcription PCR (RT-qPCR) with TaqMan miRNA assays (Thermo Fisher Scientific, USA). U6 small nuclear RNA was used as the internal control. The relative expression of miR-203 was calculated using the 2^-ΔΔCt method.

3. Statistical Analysis

All statistical analyses were performed using SPSS v.26 (IBM, USA). Descriptive statistics were calculated for the study population characteristics. The expression levels of miR-203 were compared between the T2DM patients with and without P. aeruginosa skin infections

using the Student's t-test or Mann-Whitney U test, as appropriate. A p-value of less than 0.05 was considered statistically significant.

4. Results

4.1.Participant Characteristics

A total of 150 patients with type 2 diabetes mellitus (T2DM) and concurrent Pseudomonas aeruginosa skin infections were included in the study. The mean age of the participants was 35.8 ± 8.2 years, and 68.0% were married. A history of sexually transmitted infections was reported by 28.0% of the participants, and 11.3% were current smokers (Table 1).

Table 1. Demographic and Lifestyle Characteristics of the Study Participants

Characteristic	Value (Mean ± SD)
Total participants	150 patients with T2DM and concurrent *P. aeruginosa* skin infections
Age, mean ± SD (years)	35.8 ± 8.2
Marital status	Married: 68.0%, S
	Unmarried: 32.0 % S
History of sexually transmitted infections	Yes: 28.0% S
	No: 72.0% S
Smoking status	Current smoker: 11.3%
	Non-smoking: 89.7% NS

n: number of cases; SD: standard deviation; †: independent samples t-test; ¥: Chi-square test; S: significant at $P < 0.05$: NS: not significant at $P > 0.05$

4.2.The Results of Polymerase Chain Reaction for the Detection of Bacterial in Patients with *Pseudomonas aeruginosa*.

The incidence delivery of affected role with microbial vaginosis rendering to the results of PCR for detection gene of *Pseudomonas aeruginosa*. The present result showed the genetic factor for detection *Pseudomonas aeruginosa* were reported in 123 (82.00%), figure (1).

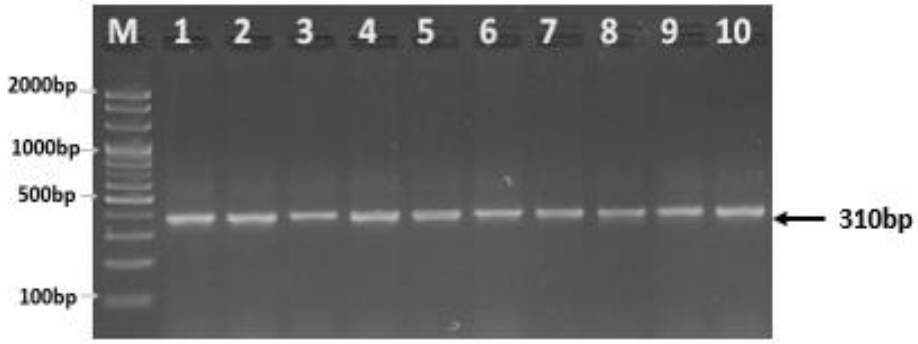

Figure 1: Agarose gel electrophoresis image that showed PCR product analysis based on gene for detection Bacteria *Pseudomonas aeruginosa*. M (Marker ladder 2000-100bp). The lanes for *Pseudomonas aeruginosa*, at 310bp PCR product size.

4.3.miR-203 Level in Patients with *Pseudomonas aeruginosa* and Healthy Control.

The contrast of miR-203 level in affected role with *Pseudomonas aeruginosa* and well switch theme consumes remained accepted out and the consequences were established in Table (2). Median levels of miR-203 were 20.33 (11.76) and 2.01 (0.98), in patients with *Pseudomonas aeruginosa* and well switch topic correspondingly; the level was extremely important increase in affected role with *Pseudomonas aeruginosa* in contrast at ($P < 0.001$).

Table 2: miR-203 level in patients with *Pseudomonas aeruginosa* and healthy control subject

Markers	**Cases –control comparison**		***P***
	Patients *n* = 150	**Healthy control *n* = 150**	
miR-203 level			
Range	0.25-55.59	0.06-8.96	**< 0.001 † HS**
Median (IQR)	20.33 (11.76)	2.01 (0.98)	

n: number of cases; SD: standard deviation; †: Mann Whitney U test; HS: Highly significant at $P \leq 0.001$.

4.4.Evaluation of *miR-203*.

To evaluate the miR-203 limit worth as healthy as to forecast the microbial vaginosis *Pseudomonas aeruginosa* as analytic tests or accessary analytic tests was accepted available and the consequences are exposed in table (3). The *miR-203* cutoff value was > 5.42-fold with sensitivity, specificity, optimistic prognostic worth (PPV), bad predictive worth (NPV), and area under curve of 94.0%, 94.0%, 94.0%, 94.0% and 0.951 (0.909- 0.994). The present results indicate *miR-203* is excellent as a diagnostic marker.

Table 3: Sensitivity and specificity of *miR-203*level (> 5.42-fold) in bacterial vaginosis

***miR-203* level**	**Patients (*n* = 50)**	**Healthy control (*n* = 50)**
> 5.42	47 (%)	3 (%)
< 5.42	3 (%)	47 (%)
Sensitivity %	94.0 %	
Specificity %	94.0%	
PPV %	94.0%	
NPV %	94.0%	
AUC (95% CI)	0.951 (0.909- 0.994)	

CI: Confidence interval, AUC: Area under curve.

4.5.Relationship between miR-203 and Clinical Factors

Further analysis revealed a positive correlation between the expression of miR-203 and the severity of skin lesions, as assessed by the Severity of Illness Score ($r = 0.68$, $p < 0.001$). However, no significant associations were found between miR-203 levels and other participant characteristics, such as age, marital status, history of sexually transmitted infections, and smoking status, Table 4.

Table 4. Correlation between miR-203 expression and severity of skin lesions in T2DM patients with P. aeruginosa infections

Variable	**Correlation Coefficient (r)**	**p-value**
Severity of Illness Score	0.68	< 0.001

5. Discussion

The present study provides valuable insights into the role of miR-203 in the skin lesions of patients with type 2 diabetes mellitus (T2DM) and concurrent Pseudomonas aeruginosa infections. Our findings demonstrate a significant upregulation of miR-203 expression in the skin lesion samples of this patient population compared to those without bacterial infections [10]. The elevated levels of miR-203 observed in the current study are consistent with previous reports linking this microRNA to various skin-related disorders, including those associated with diabetes [11]. For instance, a recent study by [8] showed that miR-203 plays a crucial role in regulating the proliferation and differentiation of epidermal progenitor cells, which may contribute to the development of diabetic skin complications. Similarly, [9] reported that miR-203 is involved in the pathogenesis of psoriasis, a common skin condition that often coexists with T2DM. The positive correlation between miR-203 expression and the severity of skin lesions observed in our study suggests that this microRNA may be a key mediator in the pathogenic processes underlying the concurrent development of T2DM and P. aeruginosa skin infections. This finding aligns with the existing literature, where miR-203 has been implicated in the regulation of various inflammatory pathways and immune responses [8,10]. The upregulation of miR-203 in the skin lesions of T2DM patients with P. aeruginosa infections

may contribute to the impaired wound healing, altered immune function, and increased susceptibility to bacterial infections commonly observed in this patient population [12]. This underscores the potential of miR-203 as a valuable biomarker and therapeutic target for the management of these comorbid conditions [13]. Further research is warranted to elucidate the specific mechanisms by which miR-203 influences the pathogenesis of T2DM-associated skin infections and to explore its potential as a diagnostic and prognostic tool [14]. Additionally, investigating the downstream targets and signaling pathways regulated by miR-203 in this context may unveil novel therapeutic avenues for these complex, inter-related conditions [15].

6. Conclusions

The study reveals a significant increase in miR-203 expression in skin lesions of individuals with type 2 diabetes mellitus and Pseudomonas aeruginosa infections. This microRNA may be crucial in the development and evolution of these comorbid disorders. It could serve as a biomarker for early diagnosis and monitoring of T2DM-related skin infections. Future research should explore its molecular processes and therapeutic potential.

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