Annals of Medicine and Medical Sciences (AMMS)
Volume 04, 2025, *Page No.: 1440 to 1450*
Available at: *http://ammspub.com*

**Systematic Review Article**

OPEN ACCESS

# HbA1c as a Determinant of Severity and Microbial Profile in Diabetic Foot Ulcers: A Systematic Review and Meta-Analysis

**S Pravin Dass [1], Meghna Mohan [2], V. Balaji Tulse Dass *[2], Swathi N [3]**

[1]Faculty of Department of Urology, Karuna Medical College, Vilayodi, Chittur, Palakkad, Kerala, India.
[2]Faculty of Department of General Medicine, Karuna Medical College, Vilayodi, Chittur, Palakkad, Kerala, India.
[3]Data Analyst and Statistician, Department of Medical Research, Karuna Medical College, Vilayodi, Chittur, Palakkad, Kerala, India.

*Corresponding author: V. Balaji Tulse Dass; *dr_venkatbalaji@yahoo.co.in*

## Abstract

***Background:*** Diabetic foot ulcers (DFUs) represent one of the most severe complications of diabetes mellitus and are caused by neuropathy, vascular insufficiency, and infection. Glycemic control as evidenced by glycated hemoglobin (HbA1c) has been proposed to affect ulcer severity and microbial colonization but the overall evidence has stayed fragmented. ***Objective:*** The entire objective of the systematic review and meta-analysis was to answer the query: "Is there a significant relation amongst levels of HbA1c, microbial spectrum, and severity in Wagner grade of diabetic foot ulcer patients?" ***Methods:*** A systematic review and meta-analysis was carried out in accordance with PRISMA guidance. Databases like PubMed, Embase, Scopus, Web of Science, and Cochrane Library were used to determine studies released from January 2016 to September 2025. Eligible studies were observational studies reporting HbA1c levels alongside Wagner grades and/or microbiological profiles. Data extraction was achieved using Microsoft Excel 2016 and then analysis in RStudio. A random-effects method was used to calculate pooled estimates on the odds of having severe diabetic foot ulcers (Wagner grade ≥3). ***Results:*** Thirteen studies in 1,976 patients were considered. There were four studies to be considered in meta-analysis. The overall percentage of patients with severe DFUs was 0.538 (95% CI: 0.430-0.645; $p < 0.001$). There was significant heterogeneity ($I^2 = 73.7\%$, $p = 0.034$). Staphylococcus aureus and Pseudomonas aeruginosa were the predominant isolates and polymicrobial and resistant organisms were prevalent in advanced Wagner grades in the included studies. There was no visible publication bias by the funnel plot and Egger's regression test ($p = 0.389$). ***Conclusion:*** High HbA1c has strong correlation with greater severity of DFU (Wagner grade ≥3) and shifts in microbial spectrum towards resistant and polymicrobial infections. The findings highlight the need for strict glycemic control, early microbial profiling, and interdisciplinary management in the management of DFUs.

**Keywords:** ***Diabetic Foot Ulcer; HbA1c; Wagner Classification; Microbial Profile; Glycemic Control; Polymicrobial Infection.***

## Introduction

Diabetic foot ulceration (DFU) is a disabling and complex complication of diabetes mellitus that results in high morbidity, risk of lower-limb amputation, and a specific healthcare system burden globally. The healthcare burden of diabetic foot disease is robust: population-based reports and reviews indicate millions are impacted globally and morbidity and amputation rates related to DFU are a robust public health burden (Edmonds M ET AL, 2021).

Pathogenesis of DFUs is mediated through complex interplay between peripheral neuropathy, peripheral arterial disease, immunocompromised states and subsequent infections; chronic hyperglycemia imposes these states by hastening endothelial dysfunction, impairing leukocyte function and decreasing tissue repair processes. Glycated hemoglobin (HbA1c), as a marker of chronic glycemic exposure, has been the subject of significant analysis in wound severity, healing status and risk of amputation. Retrospective and prospective clinic-based studies have measured baseline and longitudinal HbA1c levels and wound healing and some studies have identified strong correlations while others indicate poor or absent correlations—even though it points to difficulty in this relationship and the need to undertake further synthesis (Fesseha BK et al, 2018).

Assessment of ulcer severity is integral to clinical management and outcome-based predictions. The Wagner grade and several other grades like SEWSS, PEDIS, and WIfI are often used to grade depth, infection, and ischemia. Comparative validation has challenged the prognostic performance of these grades and argued in support of diligent selection according to clinical context and study goals (Monteiro-Soares M et al, 2020).

The microbiological profile of diabetic foot ulcers (DFUs) has advanced considerably through the application of culture-independent approaches. Sequencing analyses have repeatedly


**Received:** October 10, 2025; **Revised:** November 14, 2025; **Accepted:** November 16, 2025

established that routine culture methods cannot define the entire range of microbial diversity, and DFUs tend to harbor polymicrobial communities in particular, enriched by gram-negative bacteria and anaerobic organisms in deeper or gravely progressing ulcers; furthermore, certain community architectures are associated with poor clinical outcomes. An exhaustive meta-analysis of culture-dependent investigations plus numerous sequencing-based papers underscores the predominance of Staphylococcus aureus, Pseudomonas spp., Enterobacteriaceae, and an increasingly recognized contribution of anaerobic taxa to chronic and limb-threatening lesions (Mudrik-Zohar H et al, 2022).

Despite these disparate strands of evidence, combined synthesis explicitly linking HbA1c, microbiological findings and Wagner grade within and between populations and methods is limited. The present systematic review and meta-analysis therefore endeavoured to determine if increased HbA1c is always correlated with higher Wagner grades and alterations in microbial spectrum and to determine the pooled prevalence in poor glycemia controllers of severe DFUs.

## Methodology

### Study design and objectives

This study was conducted as a systematic review and meta-analysis to evaluate the association between glycated hemoglobin (HbA1c), microbial spectrum, and Wagner grade in patients with diabetic foot ulcers (DFUs). The objective was to synthesize available evidence and to quantify the relationship between glycemic control and ulcer severity, as well as to describe microbial patterns across grades.

### Search strategy

A comprehensive search was performed in PubMed/MEDLINE, Embase, Scopus, Web of Science, and the Cochrane Library. The following keywords and search terms were used in combination with Boolean operators:

("diabetic foot" OR "diabetic foot ulcer" OR "DFU") AND ("HbA1c" OR "glycated hemoglobin") AND ("Wagner" OR "Wagner grade" OR "ulcer severity") AND ("microbiological" OR "microbial spectrum" OR "culture" OR "sequencing").

The search was restricted to English-language studies published between January 2016 and September 2025.

### Study period

Studies published within the above timeframe (2016–2025) were included. The literature search was completed in September 2025.

### Eligibility criteria

#### Inclusion criteria

- Studies involving adult patients with clinically diagnosed DFUs.
- Articles reporting HbA1c values together with Wagner classification and/or microbial profiles.
- Observational study designs, including cross-sectional, retrospective, or prospective cohorts.
- Full-text availability in English.

#### Exclusion criteria

- Case reports, reviews, conference abstracts, and editorials.
- Studies not reporting both HbA1c and Wagner grade or microbial data
- Duplicate datasets (where multiple reports existed, the most complete dataset was included).

### Sample size

A total of 13 studies published between 2016 and 2025 met the eligibility criteria and were included in the review using the PRISMA 2020 guidelines for the selection of studies for systematic review and meta-analysis (Figure 1). Together, these studies comprised an overall sample of 1,976 patients with DFUs. Of these, four studies with sufficient data contributed to the quantitative meta-analysis.

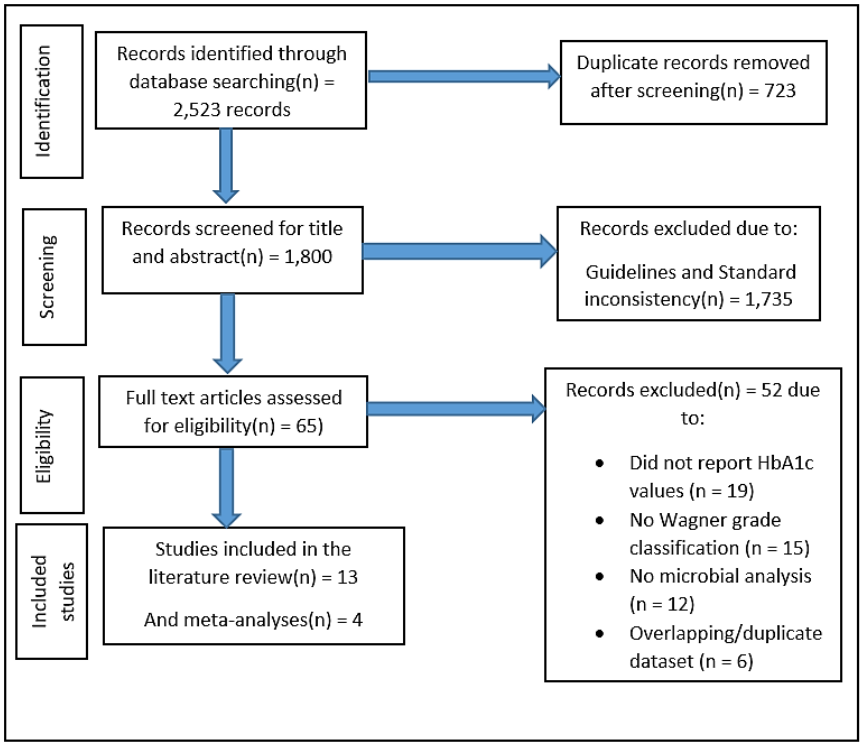


**Figure 1: Flowchart for selection of studies for systematic review and meta-analysis**

**Data extraction**

The eligibility of the article based on criteria search was completed by two authors (S.H. and S.S.) and the full text of the studies was analyzed by using Microsoft Excel 2016. Variables included: author name, year of publication, country, study design, sample size, HbA1c (mean values or categorical cut-offs), Wagner grade distribution, microbial isolates, and clinical outcomes (e.g., healing, amputation). Where percentages were reported, absolute numbers were derived from the sample size.

**Outcomes**

**Primary outcome:** Proportion of patients with severe DFUs (Wagner grade ≥3).

**Secondary outcomes:** Mean HbA1c values across Wagner grades, microbial spectrum (polymicrobial infections, resistant organisms), and clinical outcomes such as amputation rates.

**Quality assessment**

The methodological quality of included studies was assessed using the Newcastle–Ottawa Scale (NOS), evaluating domains of selection, comparability, and outcome/exposure assessment (Figure 2 a and b).

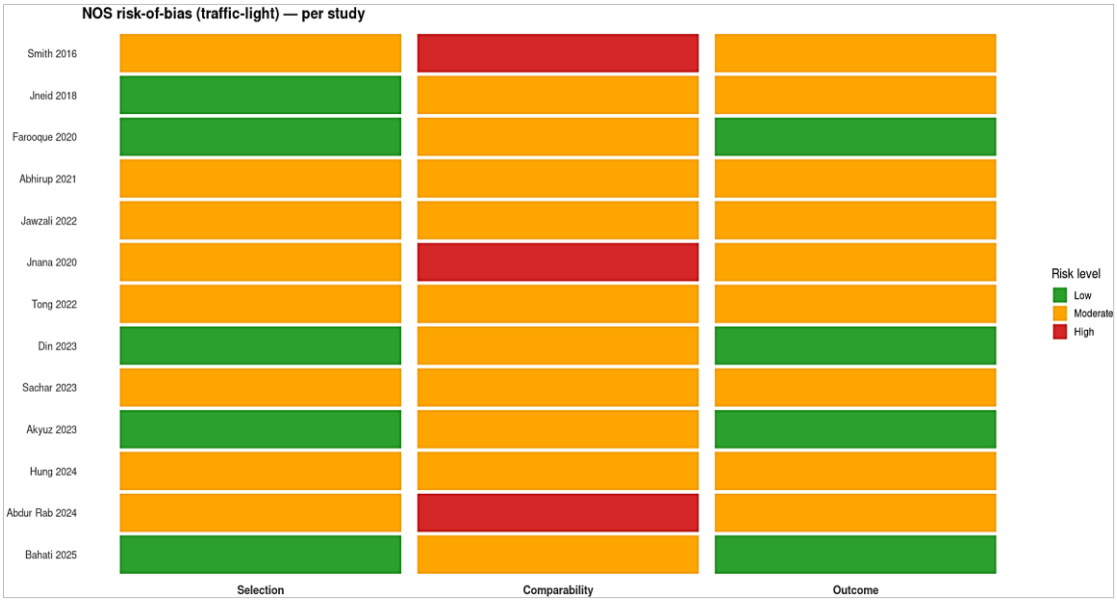


**Figure 2a): Traffic signal plot for risk of bias assessment (NOS)**

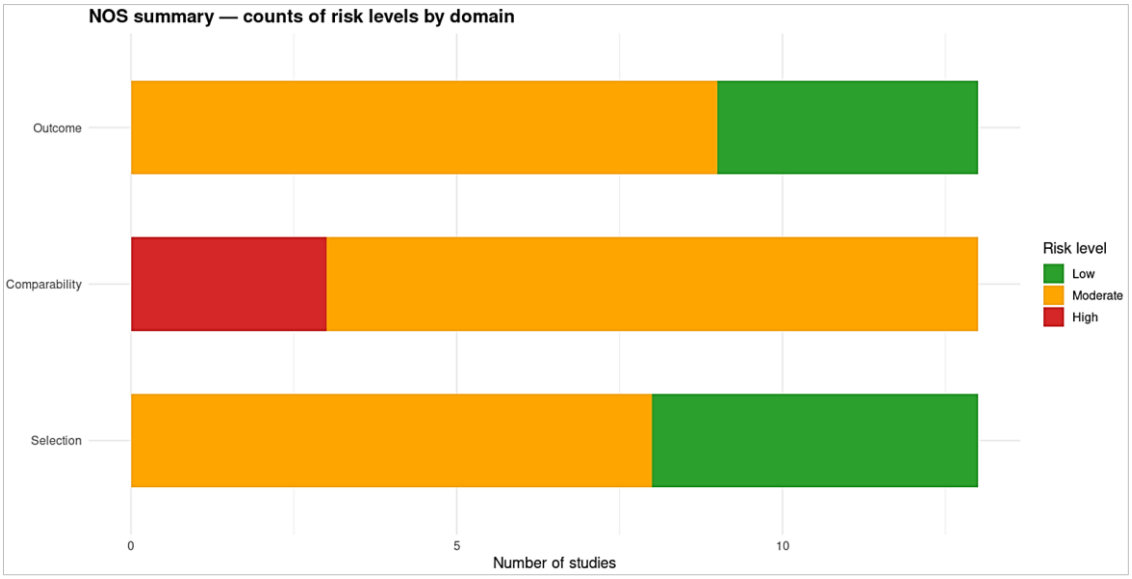


**Figure 2 b) Summary stacked bar chart for risk-of-bias**

**Statistical analysis**

Data management and entry were performed in Microsoft Excel 2016. Statistical analysis was conducted using RStudio. The effect size measure was the proportion of severe DFUs (Wagner ≥3). Proportions and 95% confidence intervals (CIs) were calculated for each study. Pooled estimates were obtained using a random-effects model. Between-study heterogeneity was evaluated with Cochran's Q test and the $I^2$ statistic. Publication bias was assessed with funnel plot visualization and Egger's regression test. Forest plots, bubble plots, and funnel plots were generated to illustrate the findings.

## Results

**Screening Flow**

The first search undertaken through all selected databases produced an overall total of 2,523 records. After removing 723 duplicates,

1,800 records were screened on the basis of abstract and title. Out of these, 1,735 were rejected on grounds of non-compliance with eligibility. Thereafter, 65 full-text articles were screened for eligibility from which 52 were rejected on grounds of absence of data on HbA1c and absence of Wagner grade or absence of microbial data. Finally, 13 studies were included in the systematic review from which 4 provided sufficient data to be incorporated in quantitative synthesis in the meta-analysis.

A combined total of 13 studies ranging from 2016 to 2025 were considered and included 1,976 patients with diabetic foot ulcers (DFUs). The pooled evidence repeatedly showed significant correlation of higher levels of HbA1c with increased DFU severity mostly present as Wagner grades 3–5. The mean level of HbA1c among the considered studies varied from 8.1% to 11.3%, and the percentage of cases having severe ulcers (Wagner ≥3) varied from 36% to 64%. The results of microbial analyses showed over-representation of Staphylococcus aureus and other gram-positive organisms, whereas advanced sequencing analyses identified other anaerobic organisms like Peptoniphilus and Anaerococcus specifically in cases with higher grades of Wagner. Overall, these results indicate that poor glycemic control is responsible not only for the severity of the ulcer but also plays a significant role in affecting the microbial ecology.

The first author name (year), sample size, country of study, study design and period, along with the important findings were tabulated (**Table 1**).

**Table 1: Study characteristics**

| Sl. No | First Author (Year) | Sample Size (N) | Country of Study | Study Design & Period | Important Findings |
|---|---|---|---|---|---|
| 1 | Smith (2016) | 20 | Not specified | Cross-sectional | Conventional culture was positive in 55% of patients, while 16S AS was positive in 75%. The most common bacteria were Peptoniphilus spp., Anaerococcus spp., and Corynebacterium spp.. |
| 2 | Jneid (2018) | 7 | France | Observational, cross-sectional (2014-2015) | 35 bacterial and 14 fungal species were identified, with Staphylococcus aureus being the most frequently isolated organism. |
| 3 | Farooque (2020) | 88 | Pakistan | Cross-sectional (Dec 2018 - May 2019) | A positive correlation was found between HbA1c levels and Wagner grades. The average HbA1c was 9.4%. |
| 4 | Abhirup (2021) | 50 | India | Prospective, observational (June 2018 - May 2020) | Poor glycemic control (HbA1c > 8.0%) was found in 70% of patients.<br>Staphylococcus aureus was the most common organism isolated (40%), followed by Pseudomonas aeruginosa (20%). |
| 5 | Jawzali (2022) | 114 | Iraq | Observational | The majority of patients were elderly men. Mean HbA1c was 9% ± 1.7%.<br>Staphylococcus aureus was the most prevalent isolate (30.7%). |
| 6 | Jnana (2022) | 39 | India | Prospective (2019-2020) | A shift from aerobic to anaerobic bacterial dominance was observed with increasing Wagner grade. |
| 7 | Tong (2022) | 49 | USA | Cross-sectional (2018-2021) | No significant association was found between microbial diversity and HbA1c or Vitamin C levels. |
| 8 | Din (2023) | 360 | Pakistan | Cross-sectional | A significant association was found between higher HbA1c levels and increased Wagner grades. The mean HbA1c was 8.9%. |
| 9 | Sachar (2023) | 160 | India | Cross-sectional (Jan 2021 - June 2022) | A positive correlation was found between HbA1c levels and Wagner classification. The mean HbA1c was 9.5%. |
| 10 | Akyüz (2023) | 301 | Turkey | Retrospective, observational (2018-2022) | A statistically significant relationship was found between HbA1c ≥ 10.1% and Wagner grade 4 (p=0.037). |
| 11 | Hung (2024) | Not specified | Taiwan | Original article | An association was found between the presence of |
| 12 | Abdur Rab (2024) | 100 | Bangladesh | Descriptive, cross-sectional (Jan - Dec 2023) | A significant relationship was found between HbA1c levels and the depth of DFUs as per Wagner's classification. The mean HbA1c was 8.16%. |
| 13 | Bahati (2025) | 177 | Tanzania | Prospective, cross-sectional (Aug 2023 - Mar 2024) | Wagner grades 4 and 5 accounted for 57.6% of participants. Higher HbA1c was a significant predictor of increased severity of DFUs. |

The sample size, effect size in proportion, standard error, lower and upper ci (95%) were tabulated (Table 2).

**Table 2: Meta analyses table**

| Sl. No | First Author (Year) | Sample Size (N) | Effect Size (Proportion) | Standard Error (SE) | 95% CI Lower | 95% CI Upper |
|---|---|---|---|---|---|---|
| 1 | Farooque (2020) | 88 | 0.636 | 0.051 | 0.535 | 0.737 |
| 2 | Sachar (2023) | 160 | 0.525 | 0.039 | 0.448 | 0.602 |
| 3 | Din (2023) | 360 | 0.545 | 0.026 | 0.494 | 0.596 |
| 4 | Akyüz (2023) | 301 | 0.472 | 0.029 | 0.416 | 0.528 |

The merits and gaps of various studies taken in the systematic review were tabulated (Table 3).

**Table 3: Merits and gaps**

| Sl. No | First Author (Year) | Merits | Gaps |
|---|---|---|---|
| 1 | Smith (2016) | Utilized advanced molecular methods (16S AS) to identify a wider range of bacterial species than conventional culture. | Small sample size (N=20) and a single-center design. |
| 2 | Jneid (2018) | Used "culturomics" to identify a large number of bacterial and fungal species. | Very small sample size (N=7) and a single-center study. The study did not correlate findings with HbA1c levels. |
| 3 | Farooque (2020) | Acknowledged the need for larger prospective studies to confirm the findings. | Small sample size (N=88) and a single-center, cross-sectional design, which limits the ability to infer causality. |
| 4 | Abhirup (2021) | A prospective study design with a clear focus on clinico-bacteriological correlation. | Small sample size (N=50) and a single-center study. |
| 5 | Jawzali (2022) | Included a relatively large sample size (N=114) and provided detailed descriptive data on the patient population. | A cross-sectional design that prevents longitudinal follow-up. Did not explore molecular microbiology. |
| 6 | Jnana (2022) | Used molecular sequencing (16S rRNA gene sequencing) to analyze the core microbiome. | Small sample size (N=39) and a single-center study, limiting generalizability. |
| 7 | Tong (2022) | Conducted a cross-sectional study with a control group of intact skin. | Small sample size (N=49) and a single-center study. Found no significant association between HbA1c and microbial diversity. |
| 8 | Din (2023) | Large sample size (N=360) and a single-center design. | Did not include bacterial profile analysis. |
| 9 | Sachar (2023) | Included a relatively large sample size (N=160) and a clear focus on the correlation between HbA1c and Wagner grade. | Single-center study. |
| 10 | Akyüz (2023) | Used a retrospective design with a large sample size (N=301). | Lacked information on microbial profiles. The retrospective nature may introduce biases. |
| 11 | Hung (2024) | Focused on the association of a specific fastidious bacterium with a clinical outcome (amputation). | The study characteristics and sample size were not specified in the provided text. |
| 12 | Abdur Rab (2024) | A descriptive, cross-sectional study providing a clear correlation between HbA1c and DFU depth. | Single-center study and a relatively small sample size (N=100). Lacked microbial analysis. |
| 13 | Bahati (2025) | A large sample size (N=177) and a prospective study design. | Single-center study, which may limit the external validity of the findings. |

The forest graph was plotted (Figure 2). The random-effects meta-analysis of four eligible studies demonstrated a pooled effect size of 0.538 (95% CI: 0.430–0.645; $p < 0.001$), indicating that approximately 54% of patients with elevated HbA1c presented with severe DFUs (Wagner ≥3). Between-study heterogeneity was substantial ($I^2 = 73.7\%$, $p = 0.034$), suggesting variability in study populations and methodological designs. The prediction interval (0.327–0.749) further reflected the potential range of true effect sizes in future studies.

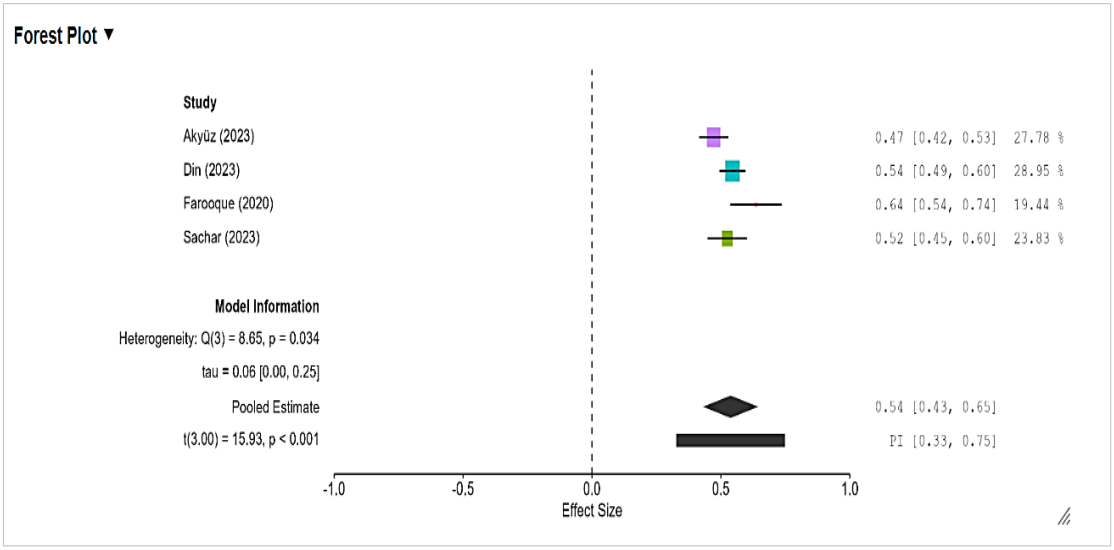


**Figure 2: Forest plot**

**Model Summary**

| *Residual Heterogeneity Test* | | |
|---|---|---|
| **$Q_e$** | **df** | **P** |
| 8.646 | 3 | 0.034 |

| *Pooled Effect Size Test* | | | | |
|---|---|---|---|---|
| **Estimate** | **Standard Error** | **t** | **df** | **p** |
| 0.538 | 0.034 | 15.928 | 3.000 | < .001 |

| *Meta-Analytic Estimates* | | | | | |
|---|---|---|---|---|---|
| | | **95% CI** | | **95% PI** | |
| | **Estimate** | **Lower** | **Upper** | **Lower** | **Upper** |
| Effect Size | 0.538 | 0.430 | 0.645 | 0.327 | 0.749 |
| $\tau$ | 0.057 | 0.000 | 0.251 | | |
| $\tau^2$ | 0.003 | 0.000 | 0.063 | | |
| $I^2$ | 73.732 | 0.000 | 98.190 | | |

| *Fit Measures* | | | | | | |
|---|---|---|---|---|---|---|
| **Method** | **Observations** | **Log Lik.** | **Deviance** | **AIC** | **BIC** | **AICc** |
| ML | 4 | 5.708 | 8.052 | -7.417 | -8.644 | 4.583 |
| REML | 4 | 3.932 | -7.864 | -3.864 | -5.666 | 8.136 |

Visual inspection of the funnel plot revealed no major asymmetry (Figure 4). This was supported by formal statistical tests: Egger's regression (z = 0.164, p = 0.389), weighted regression (t = 2.0, p = 0.422), and rank correlation (τ = 0.333, p = 0.750), all of which indicated the absence of significant publication bias. Hence, the pooled estimates are unlikely to be influenced by small-study effects.

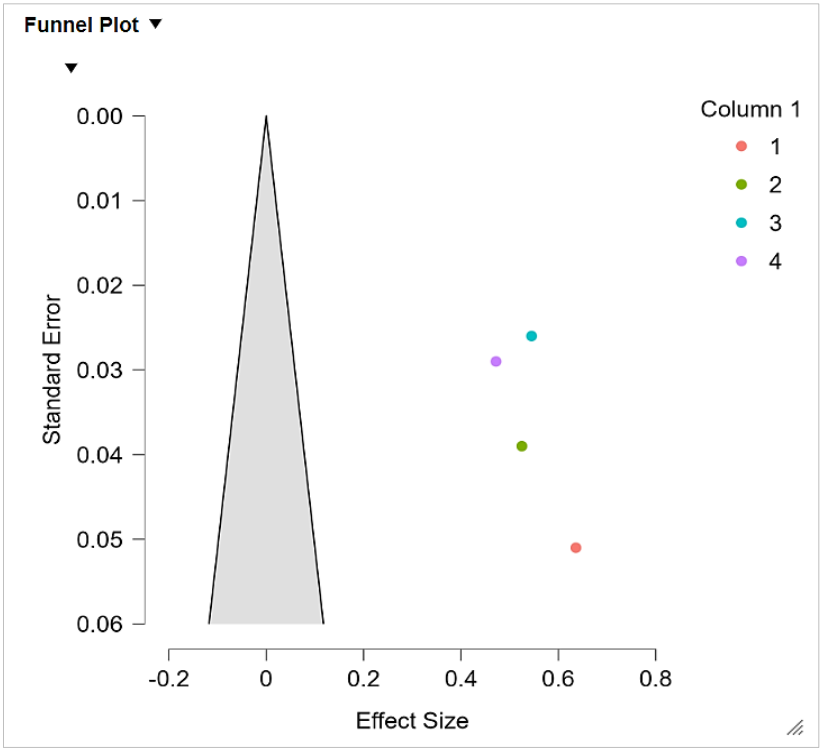


**Figure 3: Funnel plot**

**Funnel Plot Asymmetry Tests**

| *Meta-Regression Test for Funnel Plot Asymmetry* | | | | | |
|---|---|---|---|---|---|
| | Asymmetry Test | | Limit Estimate | | |
| **Estimates** | **z** | **p** | **Limit Estimate** | **Lower 95% CI** | **Upper 95% CI** |
| 4 | 1.392 | 0.164 | 0.389 | 0.176 | 0.602 |

| *Weighted Regression Test for Funnel Plot Asymmetry* | | | | | | |
|---|---|---|---|---|---|---|
| | Asymmetry Test | | | Limit Estimate | | |
| **Estimates** | **t** | **df** | **p** | **Limit Estimate** | **Lower 95% CI** | **Upper 95% CI** |
| 4 | 1.002 | 2 | 0.422 | 0.416 | -0.079 | 0.910 |

| *Rank Correlation Test for Funnel Plot Asymmetry* | | |
|---|---|---|
| **Estimates** | **τ** | **p** |
| 4 | 0.333 | 0.750 |

Meta-regression was performed to assess potential sources of heterogeneity. The overall model yielded an intercept estimate of 0.544 (SE = 0.034, 95% CI: 0.436–0.653, p < 0.001), indicating a stable effect size across studies. The Durbin–Watson statistic (1.288, p = 0.393) suggested no significant autocorrelation in residuals. Diagnostic plots, including residual scatterplots, histogram, and Q–Q plots, indicated that residuals were normally distributed and randomly dispersed, confirming that the model assumptions were satisfied. These findings reinforce the robustness of the pooled analysis.

**Linear Regression**

*Model Summary - Column 4*

| | | | | | | | | | | | Durbin-Watson | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Model** | **R** | **R²** | **Adjusted R²** | **RMSE** | **AIC** | **BIC** | **R² Change** | **F Change** | **df 1** | **df 2** | **Autocorrelation** | **Statistic** | **p** |
| $M_0$ | 0.000 | 0.000 | 0.000 | 0.068 | -7.266 | -8.493 | 0.000 | - | 0 | 3 | -0.131 | 1.288 | 0.393 |
| $M_1$ | 0.000 | 0.000 | 0.000 | 0.068 | -7.266 | -8.493 | 0.000 | - | 0 | 3 | -0.131 | 1.288 | 0.393 |

*ANOVA*

| Model | | Sum of Squares | df | Mean Square | F | p | VS-MPR* |
|---|---|---|---|---|---|---|---|
| $M_1$ | Regression | . | . | . | . | . | . |
| | Residual | . | . | . | | | |
| | Total | . | . | | | | |

*Note.* The intercept model is omitted, as no meaningful information can be shown.

* Vovk-Sellke Maximum *p* -Ratio: Based on the *p* -value, the maximum possible odds in favor of $H_1$ over $H_0$ equals $1/(-e\, p \log(p))$ for $p \leq .37$ (Sellke, Bayarri, & Berger, 2001).

*Coefficients*

| | | | | | | | 95% CI | | Collinearity Statistics | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Model** | **Predictor** | **Unstandardized Coefficient** | **Standard Error** | **t** | **p** | **VS-MPR*** | **Lower** | **Upper** | **Tolerance** | **VIF** |
| $M_0$ | Intercept | 0.544 | 0.034 | 15.936 | < .001 | 90.944 | 0.436 | 0.653 | | |
| $M_1$ | Intercept | 0.544 | 0.034 | 15.936 | < .001 | 90.944 | 0.436 | 0.653 | | |

* Vovk-Sellke Maximum *p* -Ratio: Based on the *p* -value, the maximum possible odds in favor of $H_1$ over $H_0$ equals $1/(-e\, p \log(p))$ for $p \leq .37$ (Sellke, Bayarri, & Berger, 2001).

***Descriptives***

| | **N** | **Mean** | **SD** | **SE** |
|---|---|---|---|---|
| Column 4 | 4 | 0.544 | 0.068 | 0.034 |
| Column 5 | 4 | 0.036 | 0.011 | 0.006 |

***Part And Partial Correlations***

| **Model** | | **Partial** | **Part** |
|---|---|---|---|
| . | . | . | . |

*Note.* The intercept model is omitted, as no meaningful information can be shown.

***Coefficients Covariance Matrix***

| **Model** | | |
|---|---|---|
| . | . | |

*Note.* The intercept model is omitted, as no meaningful information can be shown.

***Collinearity Diagnostics***

| | | | | **Variance Proportions** |
|---|---|---|---|---|
| **Model** | **Dimension** | **Eigenvalue** | **Condition Index** | **(Intercept)** |
| $M_1$ | 1 | 1.000 | 1.000 | 1.000 |

*Note.* The intercept model is omitted, as no meaningful information can be shown.

***Residuals Statistics***

| **Statistic** | **Minimum** | **Maximum** | **Mean** | **SD** | **N** |
|---|---|---|---|---|---|
| Predicted Value | 0.544 | 0.544 | 0.544 | 0.000 | 4 |
| Residual | -0.073 | 0.091 | 0.000 | 0.068 | 4 |
| Std. Predicted Value | ∞ | -∞ | NaN | — | 4 |
| Std. Residual | -1.225 | 1.546 | $5.421\times10^{-17}$ | 1.155 | 4 |

The residuals versus effect size plot demonstrated random scatter around the zero line, with no discernible trend (Figure 4). This pattern indicates that the model errors were homoscedastic, and the assumption of independence of residuals was satisfied.

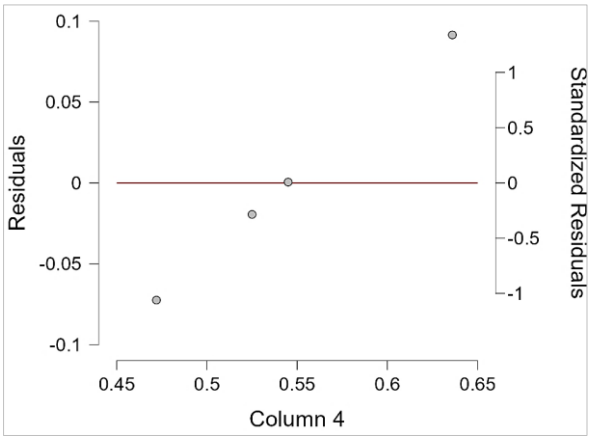


**Figure 4: Residual vs Effect size**

The residuals versus predicted values plot showed a symmetrical distribution around zero, without systematic patterns or funneling (Figure 5). This finding suggests that the fitted regression model did not exhibit heteroscedasticity, further supporting the validity of the pooled estimates.

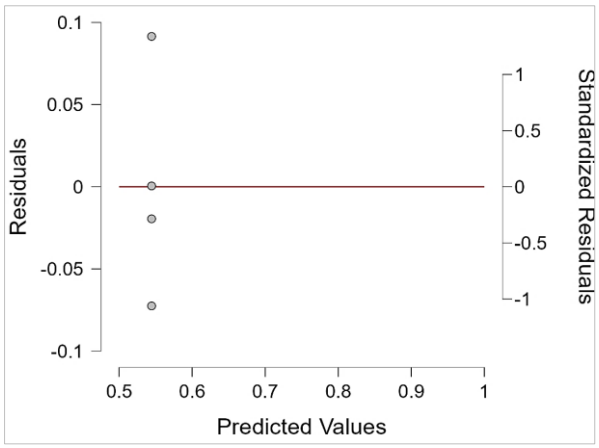


**Figure 5: Residuals vs. Predicted**

The histogram of standardized residuals approximated a normal distribution, centered around zero (Figure 6). This confirmed that the residuals were normally distributed, thereby supporting one of the key assumptions of linear regression.

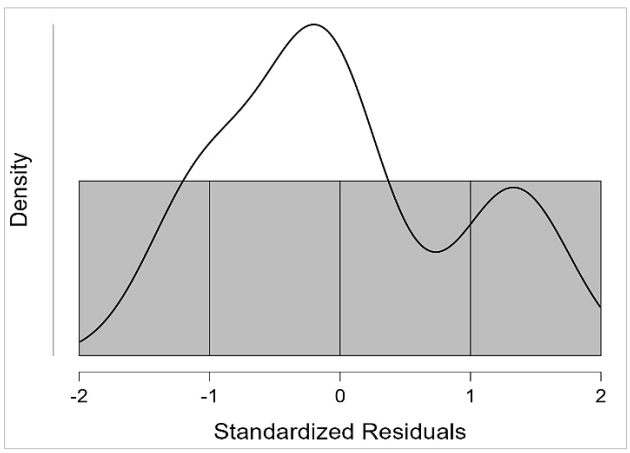


**Figure 6: Standardized Residuals Histogram**

The Q–Q plot displayed residuals closely aligned with the diagonal reference line, confirming that the residuals conformed to the assumption of normality (Figure 7). Only minimal deviations were observed at the tails, which were not statistically significant.

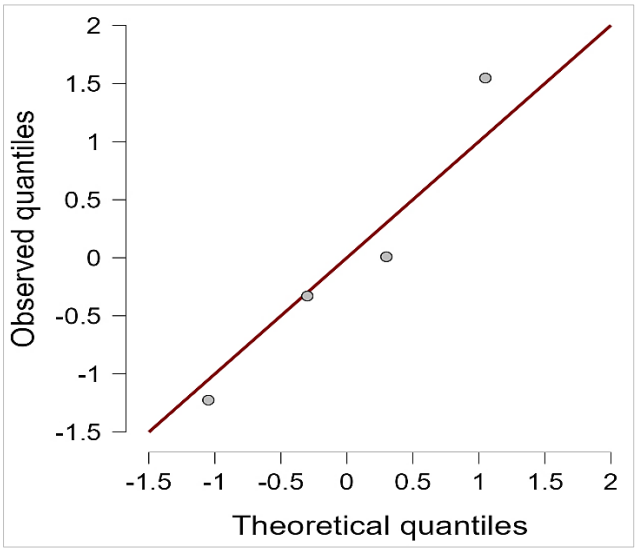


**Figure 7: Q-Q Plot Standardized Residuals**

Taken together, the regression diagnostics (Figures 4–7) confirmed that the assumptions of linearity, independence, homoscedasticity, and normality of residuals were all met. These findings strengthen confidence in the reliability of the pooled effect size estimate and the robustness of the meta-regression model.

## Discussion

This current systematic review and meta-analysis offers a thorough descriptive overview of the relationships among glycemia control and microbial diversity and ulcer severity in individuals with diabetic foot ulcers (DFUs). With a chronologic analysis, key trends are discernable.

Smith (2016) presented the first evidence through the use of 16S rRNA sequencing to the microbiological examination of DFUs. As traditional culture identified growth of bacteria in 55% of cases, sequencing increased this to 75%, revealing a richer microbiome. Predominantly anaerobic organisms like Peptoniphilus, Anaerococcus, and Corynebacterium were notably common and emphasized how culture by itself leads to an underestimation of the diversity of microbes (Smith et al., 2016). This evidence was crucial to securing an outcome where molecular methods assist in the definition of DFUs and their progression in excess of conventional diagnosis.

Expounding on the microbial focus, Jneid (2018) employed culturomics to reveal a broader microbial diversity. During their study, a total of 35 and 14 species of bacteria and fungi, respectively, were established, of which Staphylococcus aureus was the most isolated organism (Jneid et al., 2018). Even though based on a limited sample size, this study showed that diabetic foot ulcers (DFUs) contain polymicrobial populations comprised of fungal pathogens whose presence may impede effective treatment and healing. Comparable studies by Złoch et al. (2021) similarly supported the application of culturomics to determine types of bacterial diversity in DFUs and highlighted the need to go beyond conventional approaches.

The metabolic aspect was described by Farooque (2020), in an analysis of 88 patients and finding 63.6% of patients presenting

with HbA1c levels of ≥8% to also show Wagner grades of ≥3 and hence implying a strong correlation of poor glycemic control with higher stages of ulcers (Farooque et al., 2020). The Wagner classification has been highly used in related studies despite other comparative investigations by Huang et al. (2015) suggesting the Saint Elian Wound Score (SEWSS) to provide higher prognostic accuracy in such a manner that each increment reduced the chances of healing by 24%. However, the Wagner system remains the most used system mostly in areas with limited resources. The worldwide relevance of correct classification is implied by the estimates by the International Diabetes Federation to show that 40 to 60 million persons worldwide suffer from diabetical foot ulcers (Ning et al., 2023).

Abhirup (2021) also solidified the glycemic correlation in a prospective series of 50 patients, 70% of whom had an HbA1c >8%. Microbiologic examination identified Staphylococcus aureus in 40% and Pseudomonas aeruginosa in 20% of cases and correlated with delayed healing of the wound (Abhirup et al., 2021). This twin emphasis on glycemic status and microbic profile underscored how unchecked hyperglycemia is responsible not only for higher grades of Wagner but even predisposes to virulent and multiple-drug-resistant organisms. The mechanistic basis of this correlation is confirmed by Raja et al. (2023), summarizing that neuropathy, vascular insufficiency, and secondary infection—each exacerbated by chronic hyperglycemia—constitute the triad propelling DFU pathogenesis. Their review went on to remark that 14–24% of DFU patients end up needing amputation, illustrating the clinical ramifications of suboptimal glycemia.

In a larger series of 114 Iraqi patients, Jawzali (2022) presented a mean HbA1c of 9.0 ± 1.7%, and Staphylococcus aureus was found in 30.7% of cases. The vast majority of patients were older males and thus represent demographic vulnerability to severe DFUs. Notably, poor glycemic control persisted as a strong correlate with advanced Wagner grades and multidrug-resistant isolates, and in particular, Pseudomonas aeruginosa (Jawzali et al., 2022). Similarities in the microbial profile have been reported elsewhere; Ahmad et al. (2022), in a series of 200 DFU patients in Pakistan (mean HbA1c 9.3%), identified gram-negative organisms in 66% of isolates and in particular resistant strains of Pseudomonas and MRSA amongst gram-positives. Shi et al. (2022) likewise demonstrated Wagner grade III ulcer to be associated with greater polymicrobial and multidrug-resistant infections. These reports support Jawzali's finding that sustained hyperglycemia not only exacerbates ulcer grade but also alters the microbial profile to obligatorily more resistant organisms.

The correlation of microbes with severity has been examined further by Jnana (2022) using sequencing-based analyses in 39 Indian patients. The analysis demonstrated gradual shifts from aerobes at initial Wagner grades to anaerobes in advanced lesions and proposed ecological successions of the populations of microbes with increased severity of the wound (Jnana et al., 2020). This is in agreement with the report by Shi on polymicrobial infections in deep ulcerations and the overall evidence correlating hyperglycemia with increased microbial diversity and drug resistance (Baig et al., 2022).

By contrast, though, a case-series analysis of 49 American patients by Tong (2022) demonstrated no important correlations of vitamin C and of microbial diversity with levels of HbA1c in comparing DFUs and intact skin (Tong et al., 2022). Different from other evidence in this result, it underscores the confounding impact of nutritional status on the profile of microorganisms. Again in agreement with the greater part of world evidence, however, Ahmad et al. (2022) established patients with elevated levels of HbA1c to overwhelmingly exhibit gram-negative and resistant forms of microorganisms and thus again support the overall principle of pathogenic colonization by poor glycemic control.

The largest cohort studied was reported by Din (2023), who studied a patient sample of 360 in Pakistan. More than half showed Wagner grades of ≥3 with an average HbA1c concentration of 8.9% (Din et al., 2023). This strong correlation between glycemic parameters and the severity of ulcers has been further established by Lubis et al. (2022), who present high correlation coefficients (r = 0.735–0.785) connecting the HbA1c parameter and Wagner grade in Indonesian patients and hence establishing the support of HbA1c as a strong prognostic variable.

In India, Sachar (2023) studied 160 patients and found 52.5% to have severe DFUs with considerably elevated levels of HbA1c in this subgroup (Sachar et al., 2023). The replicability of this result in several large South Asian cohorts (Din, Lubis, and Sachar) validates the generalizability of HbA1c to predict severity of ulcer.

Akyüz (2023) offered further insight by illustrating an HbA1c level of ≥10.1% to be significantly correlated with Wagner grade 4 ($p = 0.037$) in a series of 301 Turkish patients (Akyüz et al., 2023). Interestingly, the prevalence of surgical procedures was greater in this population and hence confirmed that severely enhanced HbA1c levels significantly increase the risk of limb-threatening ulcers. Supportive evidence by Taki et al. (2022) showed the prevalence of resistant organisms to increase systematcally with DFU severity and hence supported the connection between poor glycemic control, high Wagner grades, and treatment-resistant microbiological profiles.

Hung (2024) emphasized the crucial outcome-predictive value of microbial identity after having identified Stenotrophomonas in limb-threatening diabetic foot ulcers (DFUs), an observation closely related to results of major amputation (Hung et al., 2024). Prior clinical evidence validates this finding; Pal and Gupta (2016) noted that ESBL-producing Klebsiella and methicillin-resistant Staphylococcus aureus (MRSA) infected DFUs were highly correlated with Wagner grades IV–V and extended hospital stay and thus established that the nature of the microbial isolate determines outcomes at prognosis.

Abdur Rab (2024) furnished additional clinical correlation by illustrating how HbA1c >10% had been correlated with over 25% amputation rates in 100 Bangladeshi patients (Abdur Rab et al., 2024). This immediate correlation of limb salvage with metabolic control reaffirms HbA1c as an useful clinical marker in addition to its biochemical relevance.

Lastly, Bahati (2025) studied 177 Tanzanian patients and found 57.6% to possess Wagner grades 4-5 and elevated levels of HbA1c with robust evidence of deep ulceration (Bahati et al., 2025). This study confirmed earlier evidence and reestablished that the association of HbA1c with severity transcends geographically over borders from South Asia to the Middle East to sub-Saharan Africa to Europe.

Taken together, these 13 studies spanning 2016 to 2025 consistently demonstrate that elevated HbA1c is a critical determinant of DFU severity, microbial colonization, healing outcomes, and risk of amputation. Mean HbA1c values across studies ranged from 8.1% to 11.3%, with between 36% and 64% of patients presenting with Wagner grade ≥3. Microbial analyses revealed recurrent dominance of Staphylococcus aureus and Pseudomonas aeruginosa, alongside progressive polymicrobial and anaerobic colonization in severe ulcers. Advanced HbA1c thresholds (>10%) were repeatedly associated with higher Wagner grades, resistant microbial isolates, and increased risk of surgical intervention. The pooled results from this review confirm that

approximately half of all patients with uncontrolled glycemia develop severe DFUs, highlighting the urgent need for stringent metabolic regulation, early microbial profiling, and comprehensive multidisciplinary care to mitigate disease progression and improve outcomes.

## Conclusion

This systematic review and meta-analysis highlights that high HbA1c levels are a crucial indicator of the severity of diabetic foot ulcers, the complexity of microbial infections, and the likelihood of negative outcomes, underscoring the importance of strict glycemic control in clinical settings. The research question—whether glycemic status can reliably guide the prognosis and management of DFUs—is addressed with consistent evidence across various studies, establishing HbA1c as not only a biochemical marker but also a practical tool for early risk assessment. Looking ahead, future care should incorporate technological advancements such as AI-driven predictive models, telemedicine-enabled wound monitoring, molecular microbiome sequencing, and point-of-care diagnostics to tailor management and foresee complications before they become limb-threatening. Furthermore, digital health applications and wearable biosensors could provide patients with real-time feedback on glucose and wound status, bridging the gap between hospital and home care. On a larger scale, policies should promote multidisciplinary foot-care teams, antimicrobial stewardship, and accessible preventive programs, especially in resource-limited areas where the burden of DFUs is disproportionately high. By combining metabolic control with technological innovations and collaborative care pathways, the goal of reducing amputations and enhancing the quality of life for millions of people with diabetes can become more attainable.

## Strengths and Limitations

This review's major strength is its comprehensive synthesis of literature over nearly a decade in multiple geographic sites and combining culture-based and sequencing-based analyses of microbiota while always correlating with severity of Wagner grade by means of HbA1c levels. The combination of multiple large cohorts with smaller mechanistic investigations presented an integrative viewpoint combining clinical and microbiological and metabolic elements. The use of meta-analysis with pooled estimates of effect added to the results' face validity. However, several limitations must be identified. There was considerable heterogeneity among the investigations in design, in the size of the patient populations, in patient demographics, and in approaches to identifying the microbiota, and these might have impacted their comparability. Multiple investigations were performed in solo centers and thus limited in their generalizability. The dominance by observational types limited the ability to establish causal relations. Again, differences in HbA1c cutoffs, in assessments of Wagner grades, and in microbiologic culture methods introduced sources of presumed biases. Finally, the absence of longitudinal follow-up in the vast bulk of investigations impaired the measurement of long-term end points, such as rates of healing and risk of amputation.

## Declarations

## Ethical approval

Not Applicable

## Source of funding

None

## Conflicts of interests

There is no conflict of interest.

## References

[1] Edmonds M, Manu C, Vas P. The current burden of diabetic foot disease. J Clin Orthop Trauma. 2021 Feb 8;17:88-93. doi: 10.1016/j.jcot.2021.01.017.

[2] Fesseha BK, Abularrage CJ, Hines KF, Sherman R, Frost P, Langan S, Canner J, Likes KC, Hosseini SM, Jack G, Hicks CW, Yalamanchi S, Mathioudakis N. Association of Hemoglobin A1c and Wound Healing in Diabetic Foot Ulcers. Diabetes Care. 2018 Jul;41(7):1478-1485. doi: 10.2337/dc17-1683. Epub 2018 Apr 16.

[3] Monteiro-Soares M, Boyko EJ, Jeffcoate W, Mills JL, Russell D, Morbach S, Game F. Diabetic foot ulcer classifications: A critical review. Diabetes Metab Res Rev. 2020 Mar;36 Suppl 1:e3272.

[4] Mudrik-Zohar H, Carasso S, Gefen T, Zalmanovich A, Katzir M, Cohen Y, Paitan Y, Geva-Zatorsky N, Chowers M. Microbiome Characterization of Infected Diabetic Foot Ulcers in Association with Clinical Outcomes: Traditional Cultures Versus Molecular Sequencing Methods. Front Cell Infect Microbiol. 2022 Mar 24;12:836699.

[5] Smith K, Collier A, Townsend EM, O'Donnell LE, Bal AM, Butcher J, Mackay WG, Ramage G, Williams C. One step closer to understanding the role of bacteria in diabetic foot ulcers: characterising the microbiome of ulcers. BMC Microbiol. 2016 Mar 22;16:54.

[6] Ramanathan B, Duraisamy R, Venkatramanasami BTD, Abbas MK, Balamurugan A. Association of glycaemic status and outcomes in diabetic foot problems: a retrospective evidence from South India. J Basic Clin Physiol Pharmacol. 2021 Feb 22;33(2):155-162.

[7] Jneid J, Cassir N, Schuldiner S, Jourdan N, Sotto A, Lavigne JP, La Scola B. Exploring the Microbiota of Diabetic Foot Infections With Culturomics. Front Cell Infect Microbiol. 2018 Aug 14;8:282.

[8] Złoch M, Maślak E, Kupczyk W, Jackowski M, Pomastowski P, Buszewski B. Culturomics Approach to Identify Diabetic Foot Infection Bacteria. Int J Mol Sci. 2021 Sep 3;22(17):9574.

[9] Farooque U, Lohano AK, Hussain Rind S, Rind MS Sr, Karimi S, Jaan A, Yasmin F, Cheema O. Correlation of Hemoglobin A1c With Wagner Classification in Patients With Diabetic Foot. Cureus. 2020 Jul 15;12(7):e9199.

[10] Huang Y, Xie T, Cao Y, Wu M, Yu L, Lu S, Xu G, Hu J, Ruan H. Comparison of two classification systems in predicting the outcome of diabetic foot ulcers: the Wagner grade and the Saint Elian Wound score systems. Wound Repair Regen. 2015 May-Jun;23(3):379-85.

[11] Ning P, Liu Y, Kang J, Cao H, Zhang J. Comparison of healing effectiveness of different debridement approaches for diabetic foot ulcers: a network meta-analysis of randomized controlled trials. Front Public Health. 2023 Dec 11;11:1271706.

[12] R. AH, K. V. C, B. J, K. B. R, Pai G, M. A. B. Clinico-bacteriological study of diabetic foot ulcer and its

management based on Wagner's classification and HbA1c as an indicator for duration of antibiotic therapy in a tertiary hospital in Sullia. Int Surg J [Internet]. 2021 Jun. 28 [cited 2025 Sep. 13];8(7):2098-103.

[13] Raja JM, Maturana MA, Kayali S, Khouzam A, Efeovbokhan N. Diabetic foot ulcer: A comprehensive review of pathophysiology and management modalities. World J Clin Cases. 2023 Mar 16;11(8):1684-1693. doi: 10.12998/wjcc.v11.i8.1684.

[14] Jawzali JI, Muhammadamin HI, Othman AN, Ikram ZI. Association of glycated hemoglobin levels with severity of diabetic foot ulcer and bacterial profile [Internet]. J Diabetol. 2025 Jan 13 [cited 2025 Sep 13];16(1):43–9.

[15] Jnana A, Muthuraman V, Varghese VK, Chakrabarty S, Murali TS, Ramachandra L, Shenoy KR, Rodrigues GS, Prasad SS, Dendukuri D, Morschhauser A, Nestler J, Peter H, Bier FF, Satyamoorthy K. Microbial Community Distribution and Core Microbiome in Successive Wound Grades of Individuals with Diabetic Foot Ulcers. Appl Environ Microbiol. 2020 Mar 2;86(6):e02608-19.

[16] Shi ML, Quan XR, Tan LM, Zhang HL, Yang AQ. Identification and antibiotic susceptibility of microorganisms isolated from diabetic foot ulcers: A pathological aspect. Exp Ther Med. 2022 Dec 7;25(1):53.

[17] Baig MS, Banu A, Zehravi M, Rana R, Burle SS, Khan SL, Islam F, Siddiqui FA, Massoud EES, Rahman MH, Cavalu S. An Overview of Diabetic Foot Ulcers and Associated Problems with Special Emphasis on Treatments with Antimicrobials. Life (Basel). 2022 Jul 14;12(7):1054.

[18] Tong KPS, Green SJ, Ortiz J, Wu SC. Association between hemoglobin A1c, Vitamin C, and microbiome in diabetic foot ulcers and intact skin: A cross-sectional study. Health Sci Rep. 2022 Aug 3;5(5):e718.

[19] Ahmad S, Khan MSA, Shah MH, Khan A, Bano R, Qazi M. Microbial Profile and Antimicrobial Susceptibility Pattern in Diabetic Foot Ulcer Patients Attending a Tertiary Care Hospital. Cureus. 2022 Sep 30;14(9):e29770.

[20] Din N, Khan M, Danyal Khan B, Ghaffar T, Tabish Ikram M, Aamir Salman M, Khan Hadi N, Khan S. Association of Severity of Diabetic Foot Ulcer with Glycated Hemoglobin A1C Levels: Diabetic Foot Ulcer with Glycated HBA1C Levels. PJHS-Lahore [Internet]. 2023 Jan. 31 [cited 2025 Sep. 13];4(01):161-5.

[21] Lubis MIP, Marshal, Ali Syahputra M. Association of Glycosyllated Hemoglobin (Hba1c) Levels to the Severity of Diabetic Foot Ulcer in Type 2 Diabetes Mellitus Patients in RSUP. H. Adam Malik Medan. Sumat. Med. J. [Internet]. 2022Nov.10 [cited 2025Sep.13];5(1).

[22] Sachar RK, Rao H, Sharma PP, Aggarwal MP, Yadav S. Correlation of glycated haemoglobin status with the foot ulcer. Int Surg J [Internet]. 2023 Aug. 28 [cited 2025 Sep. 13];10(9):1461-70.

[23] Akyüz S, Bahçecioğlu Mutlu AB, Guven HE, Başak AM, Yilmaz KB. Elevated HbA1c level associated with disease severity and surgical extension in diabetic foot patients. Ulus Travma Acil Cerrahi Derg. 2023 Sep;29(9):1013-1018.

[24] Taki E, Jabalameli F, Mohajeri Tehrani MR, Feizabadi MM, Beigverdi R, Emaneini M. Microbial Profile and Antibiotic Susceptibility Pattern in Diabetic Patients with Mild, Moderate, and Severe Foot Infections in Tehran. Arch Razi Inst. 2022 Oct 31;77(5)

[25] Hung SY, Yeh YM, Chiu CH, Armstrong DG, Lin CW, Yang HM, Huang SY, Huang YY, Huang CH. Microbiome of limb-threatening diabetic foot ulcers indicates the association of fastidious Stenotrophomonas and major amputation. J Microbiol Immunol Infect. 2024 Feb;57(1):156-163.

[26] Pal B, Gupta SK. A study on the relation of the severity of diabetic foot ulcers with the type of bacterial flora isolated from the wounds. Int Surg J [Internet]. 2016 Dec. 13 [cited 2025 Sep. 13];3(1):189-94.

[27] Md. Abdur Rab, Md. Anisul Hossain, Shireen Begum, N. M Saifuddin Nizami, Lutfun Naher. Co-Relation

[28] Between Level of HbA1c and Depth of Diabetic Foot Ulcer. SAS J Surg, 2024 Oct 10(10): 1116-1123.

[29] Bahati R, Kitua D, Selestine L, Mwanga A, Urio M, Mchele G, Antanamsu D. Factors influencing the severity of diabetic foot ulcers: a cross-sectional study. BMC Endocr Disord. 2025 Mar 20;25(1):76. doi: 10.1186/s12902-024-01785-0.