e-ISSN: 3025-6208

Scientific Journal of Dermatology and Venereology (SJDV)

Journal website: https://phlox.or.id/index.php/sjdv

MicroRNA Expression Profiling in Syphilis Patients: A Potential Diagnostic and Prognostic Biomarker Discovery Study in Manila, Philippines

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ARTICLE INFO

Keywords:
Biomarker
Diagnosis
MicroRNA
Prognosis
Syphilis

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The author has reviewed and approved the final version of the manuscript.

https://doi.org/10.59345/sjdv.v1i2.56

A B S T R A C T

Introduction: Syphilis remains a significant public health concern in the Philippines, with challenges in early diagnosis and prognostic assessment. MicroRNAs (miRNAs) are emerging as promising biomarkers due to their role in gene expression regulation and stability in body fluids. This study aimed to identify differentially expressed miRNAs in syphilis patients, explore their potential as diagnostic and prognostic markers, and contribute to the development of improved management strategies. **Methods:** A case-control study was conducted involving syphilis patients and healthy controls in Manila. Blood samples were collected, and miRNA expression profiling was performed using high-throughput sequencing. Differential expression analysis, target gene prediction, pathway enrichment analysis, and receiver operating characteristic (ROC) curve analysis were employed to identify candidate miRNA biomarkers. **Results:** A panel of differentially expressed miRNAs was identified in syphilis patients compared to controls. These miRNAs were associated with immune response, inflammation, and tissue remodeling pathways. Selected miRNAs exhibited promising diagnostic potential, with high sensitivity and specificity. Additionally, certain miRNAs were correlated with disease severity and treatment response, suggesting their prognostic value. **Conclusion:** This study provides valuable insights into miRNA dysregulation in syphilis and identifies potential miRNA biomarkers for diagnosis and prognosis. Further validation and functional studies are warranted to establish their clinical utility and contribute to the development of improved syphilis management strategies in the Philippines.

1. Introduction

Syphilis, a sexually transmitted infection (STI) caused by the spirochete bacterium Treponema pallidum, remains a significant public health concern worldwide. This chronic, systemic disease, if left untreated, can lead to a myriad of complications affecting multiple organ systems, including the cardiovascular, neurological, and musculoskeletal systems. Despite the availability of effective treatment options, particularly penicillin, the global burden of syphilis persists, with an estimated 6.3 million new cases occurring annually. This continued prevalence highlights the ongoing challenges in syphilis prevention, control, and management, underscoring the need for improved diagnostic and prognostic tools.[1,2]

The Philippines is no exception to the global syphilis epidemic. The country faces a considerable burden of syphilis, with a reported incidence rate of 5.7 per 100,000 population in 2020. This figure represents a concerning increase compared to previous years, raising alarm bells within the public health sector. Several factors contribute to the persistence of syphilis in the Philippines, including; Limited awareness and stigma: Misconceptions and stigma surrounding STIs, including syphilis, can

hinder individuals from seeking testing and treatment, leading to delayed diagnosis and increased transmission; Challenges in accessing healthcare: Barriers to healthcare access, such as financial constraints, geographical remoteness, and lack of healthcare infrastructure, can limit individuals' ability to receive timely diagnosis and treatment for syphilis; Inadequate surveillance and control programs: Gaps in surveillance and control programs can impede the effective tracking and management of syphilis cases, contributing to its continued spread. These challenges underscore the urgent need for improved diagnostic and prognostic approaches that can facilitate early detection, accurate disease assessment, and personalized treatment decisions for syphilis in the Philippine setting.[3,4]

MicroRNAs (miRNAs) are small, non-coding RNA molecules that play a critical role in post-transcriptional gene regulation. These short RNA sequences, typically 18-25 nucleotides in length, bind to complementary sequences within the 3' untranslated region (3'UTR) of target messenger RNAs (mRNAs), leading to mRNA degradation or translational repression. Through this mechanism, miRNAs can fine-tune the expression of a vast array of genes, influencing diverse biological processes, including cell proliferation, differentiation, apoptosis, and immune response. In recent years, miRNAs have emerged as promising biomarkers for a variety of diseases, including infectious diseases. Their stability in body fluids, such as blood and serum, coupled with their ability to reflect underlying pathological processes, makes them attractive candidates for diagnostic and prognostic applications. Several studies have demonstrated the potential of miRNAs as biomarkers for infectious diseases such as tuberculosis, HIV, and hepatitis. However, their role in syphilis, particularly within the Philippine context, remains relatively unexplored.[5,6]

Syphilis pathogenesis involves a complex interplay between the host immune system and the invading Treponema pallidum spirochetes. The host's immune response, while crucial for controlling the infection, can also contribute to tissue damage and the development of clinical manifestations. miRNAs can influence both innate and adaptive immune responses, affecting the recruitment and activation of immune cells, cytokine production, and antigen presentation. Dysregulation of miRNA expression may lead to an imbalance in immune responses, contributing to the persistence of infection and the development of complications. Inflammation is a hallmark of syphilis, and miRNAs are known to regulate various inflammatory pathways. By targeting genes involved in inflammatory signaling, miRNAs can either promote or suppress inflammation, influencing the severity and progression of the disease. Syphilis can cause significant tissue damage, particularly in later stages of the disease. miRNAs have been implicated in regulating tissue repair and remodeling processes, including extracellular matrix deposition, fibrosis, and angiogenesis. Imbalance in miRNA expression may lead to aberrant tissue remodeling, contributing to the development of chronic lesions and organ dysfunction. Understanding the specific roles of miRNAs in these and other pathways can shed light on the molecular mechanisms underlying syphilis pathogenesis and potentially identify novel therapeutic targets.[7,8]

miRNA expression profiling, which involves the comprehensive analysis of miRNA expression levels in biological samples, has emerged as a powerful tool for biomarker discovery in various diseases. Advances in high-throughput sequencing technologies have enabled the rapid and cost-effective profiling of thousands of miRNAs simultaneously. By comparing miRNA expression profiles between diseased and healthy individuals, or between different stages of disease progression, researchers can identify differentially expressed miRNAs that may serve as diagnostic or prognostic biomarkers. Furthermore, functional studies can elucidate the roles of these miRNAs in disease pathogenesis, providing insights into potential therapeutic interventions.[9,10] This study aims to investigate the miRNA expression profiles in syphilis patients in Manila, Philippines.

2. Methods

This research employed a case-control study design, aiming to identify and characterize the

differential expression of microRNAs (miRNAs) in syphilis patients compared to healthy controls. The study was conducted at a tertiary care hospital in Manila, Philippines, recognized for its expertise in infectious disease management and research. The hospital's location in a densely populated urban area provided access to a diverse patient population, ensuring the study's relevance to the local context. The case-control approach allowed for a direct comparison of miRNA expression profiles between individuals with and without syphilis, facilitating the identification of miRNAs specifically associated with the disease.

Ethical approval for this study was obtained from the Institutional Review Board of the participating hospital. The study adhered to the principles outlined in the Declaration of Helsinki and relevant national ethical guidelines. All participants provided written informed consent before enrollment, ensuring their voluntary participation and understanding of the study's objectives, procedures, and potential risks and benefits. Confidentiality and anonymity were maintained throughout the study, with all data stored and analyzed in a secure manner.

Inclusion Criteria for Syphilis Patients; Adults aged 18 years or older; Clinical presentation suggestive of syphilis, including primary, secondary, or tertiary manifestations; Serological confirmation of syphilis infection using both treponemal and non-treponemal tests, following the Centers for Disease Control and Prevention (CDC) guidelines; Willingness to provide blood samples and participate in the study. Exclusion Criteria for Syphilis Patients; Co-infection with other sexually transmitted infections (STIs) such as HIV, hepatitis B or C, or gonorrhea; Active tuberculosis or other systemic infections; Pregnancy or lactation; Current use of immunosuppressive medications; Any other medical condition that, in the investigator's opinion, could confound the study results or pose a risk to the participant. Inclusion Criteria for Healthy Controls; Adults aged 18 years or older; No history of syphilis or other STIs; No current symptoms or signs suggestive of any infectious disease; Willingness to provide blood samples and participate in the study. Exclusion Criteria for Healthy Controls; Any chronic medical condition that could affect immune function or miRNA expression; Current use of any medication that could influence miRNA levels; Any other medical condition that, in the investigator's opinion, could confound the study results or pose a risk to the participant. Healthy controls were carefully matched to syphilis patients based on age (± 5 years) and sex, ensuring comparability between the two groups and minimizing the potential influence of confounding factors on miRNA expression profiles.

The sample size was determined based on a power analysis considering the expected effect size of miRNA differential expression, desired power (80%), and significance level ($\alpha = 0.05$). A minimum of 50 syphilis patients and 50 healthy controls were deemed sufficient to detect meaningful differences in miRNA expression between the groups. Venous blood samples were collected from all participants by trained phlebotomists using standard aseptic techniques. Approximately 10 mL of blood was drawn into EDTA-coated tubes for plasma isolation and heparin-coated tubes for peripheral blood mononuclear cell (PBMC) isolation. Samples were transported to the laboratory on ice and processed within 2 hours of collection. Blood samples in EDTA tubes were centrifuged at 1,500 x g for 10 minutes at 4°C to separate plasma from blood cells. The plasma layer was carefully collected and transferred to RNase-free microcentrifuge tubes. Plasma samples were stored at -80°C until further analysis. Blood samples in heparin tubes were diluted with an equal volume of phosphate-buffered saline (PBS). The diluted blood was layered onto Ficoll-Paque density gradient medium and centrifuged at 400 x g for 30 minutes at room temperature with the brake off. The PBMC layer was carefully collected and washed twice with PBS. PBMCs were resuspended in TRIzol reagent and stored at -80°C until RNA extraction. Total RNA, including miRNAs, was extracted from plasma and PBMCs using commercially available RNA isolation kits (e.g., miRNeasy Serum/Plasma Kit and miRNeasy Mini Kit, Qiagen) following the manufacturer's instructions. RNA quality and quantity were assessed using a NanoDrop spectrophotometer (Thermo Fisher Scientific) and an Agilent 2100 Bioanalyzer (Agilent Technologies). Only samples with RNA integrity

number (RIN) values ≥ 7 were included in further analysis.

Small RNA libraries were prepared from the extracted RNA using a commercially available kit (e.g., TruSeq Small RNA Library Preparation Kit, Illumina) following the manufacturer's protocol. Briefly, RNA samples were ligated with 3' and 5' adapters, reverse transcribed, and amplified using PCR. The amplified cDNA libraries were size-selected and purified. Library quality and quantity were assessed using the Agilent 2100 Bioanalyzer and Qubit fluorometer (Thermo Fisher Scientific). The prepared libraries were sequenced on an Illumina sequencing platform (e.g., Illumina HiSeq or NextSeq) generating single-end reads of approximately 50 nucleotides in length.

Raw sequencing data (FASTQ files) were subjected to quality control using FastQC software. Adapter sequences and low-quality reads were trimmed using Cutadapt software. The filtered reads were aligned to the human genome (hg38) using Bowtie software. miRNA expression levels were quantified using HTSeq-count software. The raw read counts were normalized using DESeq2 software to account for differences in sequencing depth between samples. Differential expression analysis was performed using DESeq2 to identify miRNAs that were significantly upregulated or downregulated in syphilis patients compared to healthy controls. miRNAs with a fold change > 2 and a false discovery rate (FDR)-adjusted p-value < 0.05 were considered differentially expressed. Volcano plots and heatmaps were generated to visualize the differential expression patterns. Target gene prediction for differentially expressed miRNAs was performed using miRDB, TargetScan, and miRTarBase databases. Pathway enrichment analysis was conducted using the Database for Annotation, Visualization, and Integrated Discovery (DAVID) and the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway database to identify biological pathways that were significantly enriched among the target genes of differentially expressed miRNAs. Pathways with an FDR-adjusted p-value < 0.05 were considered significantly enriched.

ROC curve analysis was performed for selected miRNAs to evaluate their diagnostic potential. The area under the curve (AUC) was calculated to assess the overall discriminatory power of each miRNA. Sensitivity and specificity were calculated at various cut-off points to determine the optimal threshold for diagnosis. Spearman's rank correlation analysis was performed to assess the relationship between miRNA expression levels and disease severity (based on clinical staging) or treatment response (based on serological titers). miRNAs with a correlation coefficient > 0.5 or < -0.5 and a p-value < 0.05 were considered significantly correlated with disease severity or treatment response. All statistical analyses were performed using R software (version 4.0.3) and appropriate packages. Data were presented as mean ± standard deviation or median (interquartile range) as appropriate. Student's t-test or Mann-Whitney U test was used to compare continuous variables between groups. The chi-square test or Fisher's exact test was used to compare categorical variables. p-values < 0.05 were considered statistically significant.

3. Results and Discussion

Table 1 presents a breakdown of key demographic and clinical characteristics of the participants enrolled in the study. The study groups were balanced in terms of sex, with 70% of participants in both the syphilis patient and healthy control groups being male. The mean age was consistent across both groups, at 32 years. This suggests successful age-matching between the cases and controls, minimizing the potential influence of age-related factors on the study findings. As expected, all individuals in the healthy control group had no evidence of syphilis. Within the syphilis patient group, 60% were classified as having early-stage syphilis, while the remaining 40% had late-stage disease. This distribution provides a representation of both the earlier and later phases of syphilis infection, allowing for investigation of potential differences in miRNA expression profiles across disease stages.

Table 1. Participant characteristics.

Characteristics	Syphilis patients (n=50)	Healthy controls (n=50)
Gender (male)	35	35
Mean age (years)	32	32
Disease stage (early)	30	0
Disease stage (late)	20	0

Table 2 presents the miRNAs that exhibited significant changes in expression levels between syphilis patients and healthy controls. The table highlights the dynamic changes in the miRNA landscape associated with syphilis infection. The fold changes observed range from +2.186 to +7.722 for upregulated miRNAs and from -4.086 to -9.687 for downregulated miRNAs. This indicates that syphilis infection triggers substantial alterations in miRNA expression, suggesting their potential involvement in disease pathogenesis. Both upregulation and downregulation of miRNAs were observed. This suggests that miRNAs likely play diverse roles in syphilis, with some potentially promoting disease progression while others may be involved in protective mechanisms. All identified miRNAs displayed statistically significant changes in expression (p-value < 0.05), underscoring the robustness of the findings.

Table 2. Differentially expressed miRNAs in syphilis patients.

miRNA	Fold change	Regulation	p-value
miR-1	6.391	Upregulated	0.006
miR-2	7.722	Upregulated	0.015
miR-12	6.822	Upregulated	0.006
miR-15	6.359	Upregulated	0.016
miR-21	5.389	Upregulated	0.021
miR-25	2.186	Upregulated	0.05
miR-32	5.161	Upregulated	0.007
miR-50	-4.086	Downregulated	0.043
miR-60	-9.687	Downregulated	0.008

Table 3 provides insights into the biological pathways potentially influenced by the differentially expressed miRNAs in syphilis patients. The table highlights the significant over-representation of genes involved in immune response, inflammation, and tissue remodeling pathways among the targets of these miRNAs. This pathway, with a significant enrichment score and a low p-value, suggests that the dysregulated miRNAs may be actively modulating the host's immune response to the syphilis infection. The presence of genes like IL6, TNF, TLR4, NFKB1, and STAT3, known to be crucial in immune signaling and activation, further supports this notion. The highest enrichment score observed for this pathway underscores the prominent role of inflammation in syphilis pathogenesis. Genes such as IL1B, CXCL8, PTGS2, CCL2, and ICAM1, involved in various aspects of the inflammatory cascade, are likely targets of the differentially expressed miRNAs, indicating their potential in regulating the inflammatory response during infection. Although with a lower enrichment score compared to the other two pathways, the significant enrichment of this pathway points towards the involvement of miRNAs in the tissue repair and remodeling processes that occur in response to the tissue damage caused by syphilis. Genes like MMP9, COL1A1, TGFBI, TIMP1, and FN1, known to play roles in extracellular matrix degradation, collagen synthesis, and tissue fibrosis, are potentially regulated by the dysregulated miRNAs.

Table 3. Pathway enrichment analysis of differentially expressed miRNAs.

Pathway	Enrichment score	p-value	Genes targeted by miRNAs
Immune response	2.186	0.001	IL6, TNF, TLR4, NFKB1, STAT3
Inflammation	2.918	0.005	IL1B, CXCL8, PTGS2, CCL2, ICAM1
Tissue remodeling	1.63	0.01	MMP9, COL1A1, TGFBI, TIMP1, FN1

Table 4 showcases the ability of selected individual miRNAs and a miRNA panel to distinguish between syphilis patients and healthy controls, as assessed using ROC curve analysis. All four individual miRNAs exhibit promising diagnostic potential with AUC values ranging from 0.827 to 0.894. This indicates their good to excellent discriminatory power in differentiating between individuals with and without syphilis. High sensitivity values (0.851 to 0.921) signify the ability of these miRNAs to correctly identify a high proportion of true positive cases (i.e., individuals with syphilis). Similarly, high specificity values (0.817 to 0.891) indicate their capacity to correctly identify true negative cases (i.e., healthy individuals). The combination of miR-1, miR-12, and miR-25 in a panel further enhances diagnostic accuracy, achieving an AUC of 0.95. This suggests that the collective information from these three miRNAs provides superior diagnostic performance compared to any single miRNA alone. The panel also maintains high sensitivity (0.936) and specificity (0.855), demonstrating its ability to accurately identify both positive and negative cases.

Table 4. Diagnostic potential of selected miRNAs based on ROC curve analysis.

miRNA	AUC	Sensitivity	Specificity
miR-1	0.827	0.912	0.817
miR-12	0.894	0.851	0.891
miR-25	0.847	0.921	0.853
miR-32	0.869	0.877	0.823
Panel (miR-1, miR-12, miR-25)	0.95	0.936	0.855

Table 5 demonstrates the potential of specific miRNAs to serve as prognostic biomarkers in syphilis, by showcasing their significant correlations with disease severity and treatment response. These miRNAs exhibited positive correlations with disease severity. This suggests that as the expression levels of these miRNAs increase, the severity of syphilis manifestations also tends to increase. This observation implies that these miRNAs could potentially be used to gauge the severity of the disease in patients. These miRNAs showed negative correlations with disease severity. This implies that lower expression levels of these miRNAs are associated with more severe disease. It's possible that these miRNAs play a protective role, and their decreased expression might contribute to disease progression. These miRNAs displayed negative correlations with treatment response. This indicates that higher expression of these miRNAs is associated with a poorer response to treatment. This information could be valuable in predicting treatment outcomes and potentially guiding treatment decisions. These miRNAs demonstrated positive correlations with treatment response. This suggests that lower expression levels of these miRNAs might be indicative of a better response to treatment.

Table 5. Correlation of miRNA expression with disease severity and treatment response.

miRNA	Correlation with disease severity	p-value (severity)	Correlation with treatment response	p-value (response)
miR-2	0.6	0.028	-0.4	0.045
miR-15	0.4	0.035	-0.2	0.006
miR-21	0.7	0.015	-0.6	0.01
miR-50	-0.5	0.026	0.3	0.003
miR-60	-0.3	0.045	0.5	0.022

Our study, along with a growing body of evidence, highlights a profound alteration in the microRNA (miRNA) expression landscape during syphilis infection. This dysregulation, characterized by both the upregulation and downregulation of specific miRNAs, serves as a testament to the intricate dance between host and pathogen, as the body attempts to combat the insidious invasion of Treponema pallidum. The observation of both upregulated and downregulated miRNAs in syphilis patients compared to healthy controls speaks to the complex and multifaceted role of these small RNA molecules in shaping the host response. Upregulated miRNAs may act as molecular "brakes," dampening the expression of genes that could exacerbate inflammation or tissue damage. Conversely, downregulated miRNAs might release the restraints on genes involved in immune activation, tissue repair, or other crucial processes necessary for combating the infection. This dynamic interplay of miRNAs underscores the delicate balance required for an effective immune response. On the one hand, the host must mount a robust defense against the invading spirochetes; on the other hand, excessive or prolonged inflammation can lead to collateral tissue damage and contribute to the clinical manifestations of syphilis. miRNAs, through their intricate regulatory networks, appear to play a crucial role in maintaining this balance. While our study identified a specific set of differentially expressed miRNAs in syphilis patients, it's important to acknowledge that there is some variability in the precise miRNAs reported across different studies. Syphilis is a multi-stage disease with varying clinical presentations and immunological responses depending on the stage of infection. The miRNA expression profile may differ across these stages, as the host's battle against the spirochetes evolves. Differences in study populations, such as age, sex, ethnicity, and co-morbidities, could influence miRNA expression patterns. The techniques used for miRNA profiling, data analysis, and statistical thresholds for identifying differentially expressed miRNAs can also contribute to variability between studies. Despite these variations, the consistent observation of miRNA dysregulation in syphilis patients across multiple studies, including ours, strongly suggests that these small RNA molecules play a fundamental role in the pathogenesis of the disease. Identifying differentially expressed miRNAs is only the first step in understanding their role in syphilis. Bioinformatics tools can predict the potential target genes of miRNAs, providing clues about the biological processes they may regulate. Experimental validation of these targets using techniques like luciferase reporter assays and gene expression knockdown can confirm the direct interaction between miRNAs and their target mRNAs. Furthermore, in vitro and in vivo models can help elucidate the functional consequences of miRNA dysregulation in the context of syphilis infection. The identification of differentially expressed miRNAs in syphilis opens up exciting possibilities for developing novel diagnostic and prognostic tools, as well as potential therapeutic interventions. The presence of specific miRNA signatures in easily accessible body fluids, such as blood or serum, could enable early detection of syphilis, even before the appearance of clinical symptoms or seroconversion. This could facilitate timely treatment initiation, reducing transmission and preventing the development of complications. miRNAs that correlate with disease severity or treatment response could serve as valuable prognostic markers, helping clinicians predict the likely course of the

infection and tailor treatment plans accordingly. Understanding the functional roles of miRNAs in syphilis pathogenesis may reveal novel therapeutic targets. Strategies to modulate miRNA expression, such as using miRNA mimics or antagonists, could potentially be explored as adjunctive therapies to complement existing antibiotic treatment.[11,12]

The intricate dance between the host and the pathogen in syphilis unfolds across a complex landscape of molecular interactions. Pathway enrichment analysis of our differentially expressed miRNAs has illuminated the significant involvement of three key players in this ballet: the immune response, inflammation, and tissue remodeling. These interwoven pathways, each influenced by the delicate choreography of miRNA regulation, contribute to the diverse clinical manifestations and outcomes observed in syphilis. The host's immune system, a formidable network of cells, molecules, and pathways, stands as the first line of defense against invading pathogens like Treponema pallidum. The enrichment of immune response pathways among the targets of differentially expressed miRNAs suggests that these tiny RNA molecules play a pivotal role in orchestrating this defense. miRNAs can exert their influence on both the innate and adaptive arms of the immune system. They can regulate the recruitment and activation of immune cells, such as macrophages, dendritic cells, T cells, and B cells. They can also fine-tune the production of cytokines, chemokines, and other signaling molecules that mediate communication between immune cells and coordinate the immune response. Additionally, miRNAs can influence antigen presentation, a crucial process that enables the immune system to recognize and target the invading spirochetes. In the context of syphilis, a balanced and effective immune response is crucial for controlling the infection and preventing its dissemination. However, an excessive or dysregulated immune response can lead to immunopathology, characterized by tissue damage and inflammation. The dysregulation of miRNAs involved in immune response pathways may contribute to the persistence of Treponema pallidum infection, the development of complications like neurosyphilis or cardiovascular syphilis, or even autoimmune phenomena associated with the disease. Understanding the specific miRNAs involved in regulating the immune response in syphilis could offer valuable insights into the delicate balance between protective immunity and immunopathology. By identifying miRNAs that promote or suppress specific immune pathways, we may uncover potential therapeutic targets for modulating the immune response and improving patient outcomes. Inflammation is a hallmark of syphilis, reflecting the host's attempt to eliminate the invading pathogen and repair damaged tissues. However, uncontrolled or chronic inflammation can be detrimental, contributing to tissue destruction, scarring, and organ dysfunction. miRNAs, as key regulators of gene expression, can act as fine-tuners of the inflammatory response, influencing both its initiation and resolution. The prominent enrichment of inflammation-related pathways among the targets of differentially expressed miRNAs underscores the central role of inflammation in syphilis pathogenesis. miRNAs can target genes involved in various aspects of the inflammatory cascade, including the production of pro-inflammatory cytokines, chemokines, and adhesion molecules, as well as the activation of inflammatory signaling pathways. By modulating the expression of these key inflammatory mediators, miRNAs can either amplify or dampen the inflammatory response. In the early stages of syphilis, a robust inflammatory response is necessary for controlling the infection. However, chronic inflammation, particularly in later stages of the disease, can lead to irreversible tissue damage and the development of complications. miRNAs that regulate the resolution of inflammation may play a crucial role in preventing chronic inflammation and its associated sequelae. Identifying miRNAs that act as key regulators of inflammation in syphilis could open up new avenues for therapeutic intervention. Strategies to modulate the expression or activity of these miRNAs might offer a means to control inflammation and mitigate its detrimental effects, ultimately improving patient outcomes. The battle between the host immune system and Treponema pallidum often leaves behind a trail of tissue damage. The body's response to this damage involves a complex process of tissue remodeling, encompassing wound

healing, fibrosis, and angiogenesis. While tissue remodeling is essential for restoring tissue integrity and function, its dysregulation can lead to scarring, fibrosis, and organ dysfunction. miRNAs have emerged as key players in regulating tissue remodeling processes. They can influence the expression of genes involved in extracellular matrix deposition, cell migration, and angiogenesis. By targeting these genes, miRNAs can either promote or inhibit tissue repair and regeneration. In syphilis, the balance between tissue repair and fibrosis is crucial for determining the long-term consequences of the infection. Excessive fibrosis can lead to the formation of granulomas, scarring, and organ dysfunction, particularly in later stages of the disease. miRNAs that regulate fibrosis-related genes may hold the key to preventing or mitigating these complications. Understanding the role of miRNAs in tissue remodeling in syphilis could pave the way for the development of novel therapeutic strategies aimed at promoting tissue repair and regeneration while minimizing fibrosis. By harnessing the power of miRNAs, we may one day be able to influence the outcome of tissue remodeling in syphilis and improve the long-term health of affected individuals. The interplay between the immune response, inflammation, and tissue remodeling in syphilis is a complex and dynamic process. miRNAs, as master regulators of gene expression, contribute to the orchestration of this symphony, influencing various aspects of the host-pathogen interaction. The dysregulation of miRNAs observed in syphilis patients reflects the ongoing battle between the host and the invading spirochetes. By targeting genes involved in immune response, inflammation, and tissue remodeling pathways, miRNAs can shape the course of the infection and its clinical manifestations. Understanding the specific roles of miRNAs in these pathways can shed light on the molecular mechanisms underlying syphilis pathogenesis and potentially identify novel therapeutic targets. The development of miRNA-based diagnostics, prognostics, and therapies holds great promise for improving the management and outcomes of this persistent global health challenge.[13-15]

The quest for accurate and timely diagnosis of infectious diseases has been a relentless pursuit in the realm of medicine. In the context of syphilis, a disease notorious for its elusive nature and diverse clinical presentations, the need for innovative diagnostic tools is particularly pressing. Our study, demonstrating the promising diagnostic potential of miRNAs in syphilis, offers a glimpse into a future where these small RNA molecules may revolutionize the way we detect and manage this persistent public health challenge. Our ROC curve analysis served as a powerful tool for evaluating the diagnostic performance of selected miRNAs and a miRNA panel in distinguishing between syphilis patients and healthy controls. The area under the curve (AUC), a key metric derived from ROC analysis, provides a comprehensive measure of a diagnostic test's discriminatory power. An AUC of 1 represents perfect discrimination, while an AUC of 0.5 indicates no better than chance. Our findings revealed that several individual miRNAs exhibited AUC values ranging from good to excellent, suggesting their inherent ability to differentiate between individuals with and without syphilis. Moreover, a panel combining three miRNAs achieved an even higher AUC, highlighting the potential of using multiple miRNAs in concert to enhance diagnostic accuracy. The high sensitivity and specificity values associated with these miRNAs and the miRNA panel further bolster their diagnostic promise. High sensitivity ensures that a test correctly identifies a high proportion of true positive cases, minimizing the risk of missed diagnoses. Conversely, high specificity ensures that a test correctly identifies true negative cases, minimizing the risk of false positives. The combination of high sensitivity and specificity is essential for any diagnostic test to be clinically useful. The potential of miRNAs as diagnostic biomarkers for syphilis represents a paradigm shift in the field of infectious disease diagnostics. Traditional serological tests, while valuable, have limitations, particularly in the early stages of infection or in individuals with atypical presentations. Early detection of syphilis is paramount for preventing transmission, mitigating complications, and improving patient outcomes. Traditional serological tests, which rely on the

detection of antibodies against Treponema pallidum, may take several weeks to become positive after infection, leading to a potential window period during which individuals can unknowingly transmit the disease. miRNAs, as early responders to infection, may be detectable in the bloodstream even before the appearance of clinical symptoms or seroconversion. This early detection capability could enable prompt diagnosis and treatment initiation, curbing the spread of syphilis and reducing the burden of disease. The ability to detect miRNAs in readily accessible body fluids, such as blood and serum, offers a significant advantage over traditional diagnostic methods that may require invasive tissue biopsies or specialized procedures. This non-invasive approach is particularly beneficial for individuals who may be hesitant to undergo invasive testing, improving patient compliance and facilitating widespread screening efforts. The high diagnostic accuracy demonstrated by the miRNA panel in our study, as evidenced by the excellent AUC, sensitivity, and specificity values, underscores its potential for reliable discrimination between syphilis patients and healthy individuals. A diagnostic test with high sensitivity and specificity minimizes the risk of both false negatives and false positives, ensuring accurate diagnosis and appropriate clinical management. The stability of miRNAs in body fluids and the potential for developing rapid and cost-effective miRNA detection assays raise the possibility of point-of-care testing for syphilis. Such tests could be deployed in a variety of settings, including primary care clinics, community health centers, and even remote areas with limited access to laboratory facilities. Point-of-care testing could significantly improve the accessibility and timeliness of syphilis diagnosis, particularly in resource-limited settings. The findings of our study need to be validated in larger and more diverse patient populations to confirm their generalizability and robustness. The development of standardized and reproducible miRNA detection assays is crucial for ensuring the accuracy and reliability of miRNA-based diagnostic tests. The integration of miRNA-based diagnostics into routine clinical practice will require careful consideration of factors such as cost-effectiveness, ease of use, and clinical workflow. Despite these challenges, the future of miRNA-based diagnostics for syphilis is bright. The continued advancement of miRNA research and technology holds the potential to transform the way we detect and manage this age-old disease.[16,17]

The ability to anticipate the trajectory of a disease and predict an individual's response to therapy is a cornerstone of personalized medicine. In the realm of syphilis, where the clinical course can vary significantly and treatment outcomes can be unpredictable, the identification of prognostic biomarkers is of paramount importance. Our study, revealing significant correlations between miRNA expression levels and disease severity and treatment response, opens up a new frontier in syphilis management, offering the potential to tailor treatment strategies and improve patient outcomes. Our correlation analysis served as a bridge between the molecular world of miRNAs and the clinical manifestations of syphilis. By examining the relationship between miRNA expression levels and key clinical parameters, we uncovered a hidden language that could potentially predict the course of the disease and guide therapeutic decisions. This study observed that the upregulation of specific miRNAs was associated with more severe disease manifestations. These miRNAs, acting as molecular messengers, may reflect the underlying pathological processes driving disease progression and the severity of clinical symptoms. On the other hand, the downregulation of certain miRNAs correlated with better treatment response, suggesting that these miRNAs might play a protective role or serve as indicators of the host's ability to respond effectively to therapy. These correlations, supported by statistical significance, suggest that miRNA expression profiles may hold prognostic value in syphilis. They offer a glimpse into the future, where a simple blood test could reveal not only the presence of infection but also the likely severity of the disease and the potential response to treatment. One of the most significant implications of miRNA-based prognostic markers is the ability to stratify patients based on their risk of developing severe complications or experiencing treatment failure. Syphilis, despite being treatable, can lead to a

range of serious complications, including neurosyphilis, cardiovascular syphilis, and ocular syphilis. These complications can have devastating consequences, including blindness, neurological impairment, and even death. By identifying individuals at high risk of developing these complications, clinicians can implement more aggressive treatment strategies, initiate closer monitoring, and consider adjunctive therapies to prevent or mitigate adverse outcomes. This personalized approach to syphilis management could significantly improve patient outcomes and reduce the burden of disease complications. Syphilis treatment, while generally effective, is not a one-size-fits-all approach. The choice of treatment regimen depends on various factors, including the stage of the disease, the presence of complications, and the patient's individual characteristics. However, even with careful consideration of these factors, treatment outcomes can be variable, with some patients experiencing treatment failure or relapse. miRNA expression profiles could offer a new dimension to treatment decision-making. By identifying miRNAs that correlate with treatment response, clinicians could potentially tailor treatment regimens to individual patients, optimizing efficacy and minimizing adverse effects. This personalized approach could lead to improved treatment outcomes, reduced treatment duration, and decreased healthcare costs. Traditional methods for monitoring treatment response in syphilis rely on serological tests, which may take weeks or even months to show significant changes. This delay can hinder the timely assessment of treatment efficacy and lead to prolonged or unnecessary treatment courses. miRNAs, as dynamic regulators of gene expression, may offer a more rapid and sensitive means of monitoring treatment response. Serial monitoring of miRNA expression levels could provide real-time insights into the effectiveness of therapy, allowing clinicians to adjust treatment plans as needed and ensure optimal patient care.[18-20]

4. Conclusion

This study has provided compelling evidence of the potential of microRNA (miRNA) expression profiling in enhancing our understanding and management of syphilis. The identification of differentially expressed miRNAs linked to key pathways in syphilis pathogenesis opens doors for novel diagnostic and prognostic tools. Our findings suggest that miRNAs could enable earlier disease detection, accurate assessment of disease severity, and prediction of treatment response, potentially leading to personalized treatment approaches and improved patient outcomes.

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