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# The Expression of Mirnas in Sudanese Patients with Systemic Lupus Erythematosus in Khartoum State

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## ABSTRACT

**Background:** MicroRNAs (miRs) are noncoding gene regulators that may have a role as diagnostic or prognostic biomarkers in systemic lupus erythematosus (SLE).

**Aim:** To measure the blood levels of miR-146a, miR-126 and miR-30a in Sudanese SLE patients and to investigate their potential role in disease pathogenesis and utility as biomarkers for SLE.

**Material and Methods:** A total of 48 SLE patients and 20 matched healthy individuals were enrolled in this study. SLE disease activity index (SLEDAI) was assessed. The blood levels of miR-146a, miR-126 and miR-30a were determined by Real-time polymerase chain reaction (PCR) in all participants. For γ-INF and IL-2 were analyzed by ELISA, and CD markers were used flowcytometery.

**Results:** The mean age of the patients was 31.5 ± 8.5 years with disease duration >5 years. In SLE patients, the mean blood level fold changes of miR-146a (0.33±0.277; P<0.001), miR-126 (2.44±1.771; P=0.007) and miR-30a (1.56±1.40; P >0.305) compared to controls. Down regulation of miR-146a increase expression of γ-INF (p < 0.002), whereas the up regulation of miR-126 increase expression of CD markers (p<0.000) in SLE patients. MiR-126 at a cut-off value 1.209 and miR-146a at cut-off value 0.9233 which can discriminate between SLE patients with significantly associated to SLE disease. Conversely, miR-30a was insignificantly associated with SLE disease (p value >0.05) as no differences between the SLE patients and healthy control.

**Conclusion:** Circulating miR-146a and miR-126 could be a potential noninvasive biomarker in SLE. This study providing an overview of the current state of research on the role of miRNAs in the immune pathogenesis and regulation of SLE. Further studies are needed in miRNAs profiling expressions of SLE diseases.

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## Introduction

Systemic Lupus Erythematosus (SLE) is a clinically heterogeneous autoimmune disease which affects multiple organ systems and causes significant morbidity and mortality [1]. One of the hallmarks of SLE is the production of antinuclear autoantibodies by uncontrolled over-activated B cells [2]. The auto-antibody auto-antigen immune complexes deposit in different tissues and organs, leading to chronic inflammation and tissue damage in many parts of the body [2]. Complicated interactions between genes, environment, hormones, smoking, infections, drugs, and abnormalities in the adaptive immune system all contribute to the onset and progression of SLE [3,4]. In recent years, studies have shown that aberrant epigenetic mechanisms also play an important role in the pathogenesis of SLE [5,6]. Although the specific cause of SLE is not known, multiple factors are associated with the development of the disease. These include: genetic, racial, hormonal and environmental factors. In patients who are predisposed genetically, exposure to natural ultraviolet radiation viruses and drugs have been implicated in precipitating or exacerbating SLE [7,8].

MicroRNAs (miRNAs) are small non-coding RNAs of approximately 19 to 25 nucleotides that post-transcriptionally regulate gene expression. They bind to the 3'-untranslated

region (UTR) of their target messenger RNAs (mRNAs) through complementary recognition, which then leads to mRNA degradation or repression of protein expression [9]. To date, over 2000 mature miRNA products have been identified in the human genome and the number registered on the miRNA database is still growing. More important than the expanding numbers is the complex regulatory network mediated by miRNAs, which controls a spectrum of biological events ranging from cell differentiation, proliferation and homeostasis, cell-cell interactions to intracellular signaling responses [10,11]. Furthermore, miRNAs play important roles in the development and function of innate and adaptive immune cells [12]. Many immunoregulatory genes, including transcription factors, cofactors and chromatin modifiers, are miRNA targets and some even harbor binding sites for eight or more different miRNAs [13]. Each miRNA could potentially recognize many, or even up to hundreds of, target genes, then dysfunction of miRNAs or dysregulation of their expression in the immune cells would lead to immunodeficiency or autoimmunity [14].

This study aimed to investigate of miRNAs expression in Sudanese patients with Systemic Lupus Erythematosus in sudan-Khartoum state

## Material and Methods

This is an analytical case-control study, conducted at Al-Ryan special immunology laboratory centre (ARSILC), Khartoum, Sudan, in the period from February 2019 to November 2021. In which, a total of 48 patients with a confirmed diagnosis of SLE and 20 age- and sex-matched, apparently healthy volunteers- as a control group- were enrolled. Venous blood samples were collected from all participants in Ethylene diaminetetraacetic acid (EDTA) then centrifuged at 2000 rpm for 5 min at room temperature. The supernatant was transferred to Ependorf tubes. These samples were re-centrifuged at 15,000 rpm for 10 min to precipitate cell debris and the supernatants, used to RNA extraction. RNA was isolated from supernatants using AQUA gene kits and stored at -30°C until PCR is carried out. Micro RNA was analysed by real time PCR system (Applied Biosystem). The qPCR reaction mixture was performed as 10µl qPCR mix (SYBR Green QIAGEN-Korea), 0.5 µl of gene-specific forward and reverse primers, as shown in (Table 1) ,5 µ of template, made up to a final volume of 20 with DEPC water. The cycling parameters were set as follows: The melting curve analysis was performed at temperatures from 58°C to 95°C, with stepwise fluorescence acquisition at every 1°C/second. The level of miRNA expression was measured using the cycle threshold (CT). The expression for each miRNA was calculated as the difference between its CT value and the average CT value of the endogenous reference gene (β-Actin). All samples were amplified in duplicate, and the data analysis was carried out using MxProqPCR system software (Strata gene). The ΔCt value was calculated by substracting the Ct value for β-Actin from the Ct value for the gene of interest. Relative expression (fold change) was calculated for each target miRNA within each group using the equation 2-ΔΔCT [14].

**Table 1: Primers specific for miRNA expression and reference gene used in qPCR with sequence:**

| Primers Name | Sequence (5´-3´) | Reference No |
|---|---|---|
| miR-126 | FTTACTAGTCTTCTTCAGCACAACCGTCA<br>RATAAGCTTGCCACAAACACCATGTACCA | (Luo et al.,2015)[16] |
| miR-146a | FGTGAGATCTGCATTGGATTTACC<br>RGACCTCGAGACTCTGCCTTCTGT | (Luo et al.,2015)[16] |
| miR-30a | FCTAGCCTGCAGGATAAACTTACTCATGTTCTA<br>RATCCGGCCGGCCTACTCTGAGATTTGATAAAT | (Zheng etal.,2015)[17] |
| β-Actin | FCTGTGGCATCCACGAAACTA<br>RAGTACTTGCGCTCAGGAGGA | (Dai et al.,2015)[18] |

## Results

A total of 68 subjects were enrolled in this study, 48 patients with SLE and 20 age- and sex-matched healthy volunteers as a control group. Majority of participants (73%) were from Khartoum state, other sociodemographic characteristics are shown in (Table 2). The average CT value of target miRNAs in blood of patients and control is shown in (Figure 1), the present study showed that there was statistically significant association in the relative expression of target miR-146a and miR-126 in SLE patients when compared to healthy controls (p. value=0.00). Moreover; there is no statistically significant association in the relative expression of target miR-30a in SLE patients when compared to healthy controls (p. value= 0.3) as shown in (Table 3). The mean value and standard deviation of fold changes of miR- 146a, miR-126 and miR-30a expression in SLE patients versus healthy control are shown in (Figures 2, 3 and 4).

**Table 2: Socio-demographic characteristics of SLE patients and Healthy control**

| Demographic features | | SLE patients(n=48) | H. control (n=20) |
|---|---|---|---|
| Male: Female ratio | | 1 (16.7%) / 5 (83.3%) | 1(50.0%) / 1(50.0%) |
| Ages (mean) | | 31.5 ± 8.5 years | 25.6 ± 4.4 years |
| Residence | Khartoum state | 35 (72.9%) | (100.0%) |
| | Outside Khartoum state | 13 (27.1%) | 0 (0.0%) |
| Disease duration/ years | | >5 years SLE onset | ----- |

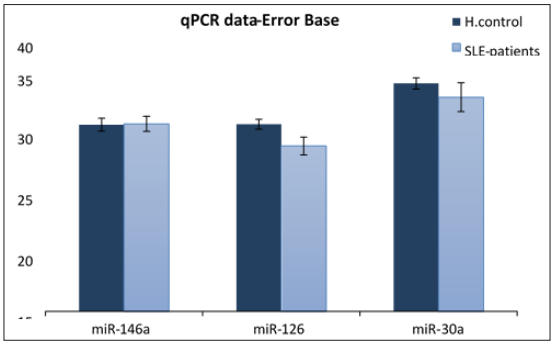


**Figure 1:** Shows the Average Ct Value of Target Mirnas in Blood From Sle Patients Compared to Healthy Controls

**Table 3: The Relative Expression of Target Mirnas in Sle Patients Compared to Healthy Controls**

| Fold Changes Median (range) | SLE patients (n = 48) | H. control (n = 20) | t-test | P-value |
|---|---|---|---|---|
| miR-146a | 0.33 (0.06-0.96) | 1.21 (0.34-2.57) | -5.093 | 0.000 |
| miR-126 | 2.44 (0.25-5.95) | 1.14 (0.51-2.47) | 4.297 | 0.000 |
| miR-30a | 1.56 (0.07-4.75) | 1.18 (0.43-2.62) | 1.054 | 0.305 |

*SLE: systemic lupus erythematosus. Bold values are significant at $p < 0.05$

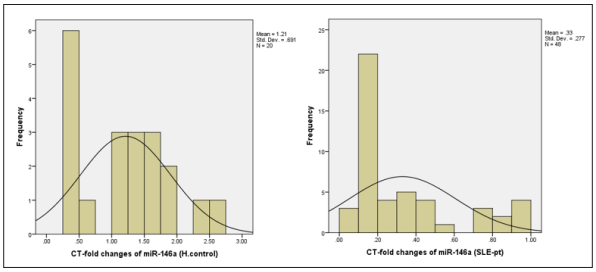


**Figure 2:** Shows the mean Value and Standard Deviation of Fold Changes of Mir- 146a Expression of in Sle Patients Versus Healthy Control

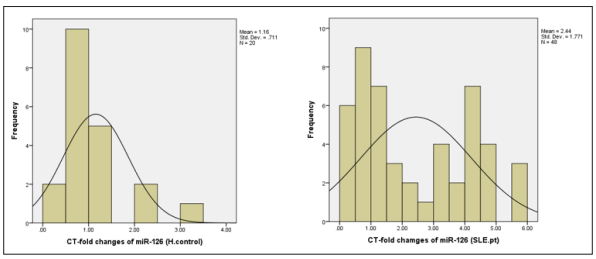


**Figure 3:** Shows the Mean Value and Standard Deviation of Fold Changes of Mir-126 Expression of in Sle Patients Versus Healthy Controls

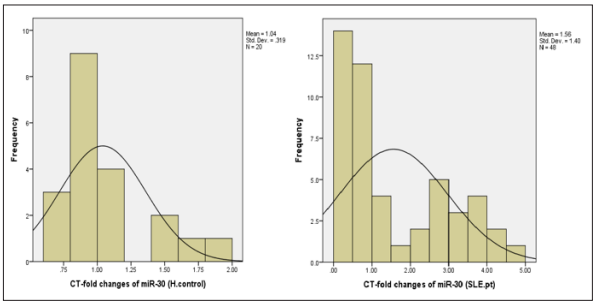


**Figure 4:** Shows the Mean Value and Standard Deviation of Fold Changes of Mir-30a Expression of in Sle Patients Versus Healthy Controls

## Discussion

In recent years, miRNA quantitation by RQ-PCR has developed substantially. The technique is now sensitive enough to reliably quantify miRNAs from a minute starting RNA volume, as was the case with SLE blood specimen, as the sample is easier to obtain in larger quantities and can be re sampled at various time-points without great difficulty or inappropriate distress to patients. The evidence indicates that micro RNAs (miRNAs) can regulate gene expression and play an important role in the pathogenesis of autoimmune diseases, particularly SLE which often associates with difficulties in diagnosis [19]. Although circulating antibodies in the blood help in patients' classification, obtaining a clear distinction between SLE and other autoimmune diseases is still a major challenge for clinicians. The recognition of the complexity of interactions between epigenetic mechanisms and immunity disorders in autoimmune diseases is essential for the research on fast and precise diagnosis as well as effective therapeutic strategies. The Epigenetic factors, including DNA methylation, post-translational histone alterations and miRNAs, interplay with genetic programs to control the immune functions [20]. In the present study, we investigated the expression and regulation of three target miRNA (miR-146a, miR-126 and miR-30a) from blood samples of Sudanese SLE patients. Reduced blood levels of miR-146a in SLE patients found in the present study is consistent with Löfgren et al supporting a possible role in the immune pathogenesis of the disease [21]. The finding that blood miR-126 was significantly higher in SLE patients compared to controls. This is harmonized with the findings of previous studies [22]. A study done by [23,24]. Found that; miR-126 is up regulated in T cells isolated from SLE patients and affect the DNA methylation by reducing the expression of DNA methyltransferase 1 (DNMT1) and thus suppressing DNMT1 transcription activity. This block was suggested to upregulate miR-126 expression and an overproduction of CD4+ cells that enhance IgG production with a consequent worsening of the disease [25]. Additionally, high levels of miR-126 was reported as positively correlate with DNA hypomethylation in lupus CD4+ Tcells, and suppression of the miR-126 is beneficial in diagnosis of Lupus disease [26]. Recently, Kim et al stated that the methylated status in a promoter region blocks the accessibility to transcriptional activators and thus inhibits the gene transcription, serving as oppressive ''lock,'' while an un methylated state at the promoter permits transcription [27]. No significant difference in the MiR-30a levels was observed in SLE patients compared to healthy controls in this study. This is in contrast to increase expression of IL-2 levels observed. On the contrary, further validations have shown that miR-30a is over expressed in SLE compared to healthy controls, and have been identified by computational means as targets for IL-2. These findings, in accordance with previous studies, demonstrate miR-30a as a dramatic increase of expression in IL-2 from SLE disease activity, autoantibodies production and renal involvement with increases T cell proliferation [28,29]. This data implies that the miR-30a is not significant biomarker for diagnosis of SLE disease, as studies by [30,31,32]. Plays an important role in cancer development and progression by modulating target genes, including inhibiting proliferation, invasion, and migration, inducing apoptosis, depending on the type of cancer; similarly.

Sensitivity and specificity of systemic miRNAs for SLE patients in our region; as ROC analysis determined the optimal cut-off value for miR-146a, miR-126 and miR-30a to differentiate SLE cases from controls were 0.9233-, 1.2097- and 1.1024-fold changes respectively. The combination of circulating levels of miR-146a and miR-126 increased the sensitivity for differentiating SLE

cases from controls to 77.1% and 83.3% respectively (logistic regression analysis; $p<0.05$). Circulating miR-30a levels did not correlate with the existing SLE marker lupus antigen with a determined sensitivity of 37.5% (logistic regression analysis; $p=0.737$). Meanwhile, many investigations had been carried out to assess whether miRNAs have sufficient capability to be used as biomarkers for SLE [33,34,35]. Which suggested that miR-146a and miR-126 had a moderate diagnostic accuracy in detecting SLE whereas miR-30a had no sensitivity or specificity in diagnosis of SLE as may effect on the abnormalities in T cell populations.

To the best of our knowledge, this is the first analysis that focused on the accuracy of chosen three miRNAs (miR-146a, miR-126 and miR-30a) in detecting SLE and confirmed the diagnostic accuracy of miRNAs as potential biomarkers for SLE. There are number of advantages favoring the use of miRNAs as disease indicators. Their expression in serum is stable, reproducible, and consistent. Moreover, compared with other biomarkers, detection of miRNAs seems to be more available with low complexity. A wide study has identified specific genetic variants in the promoter or intergenic region of miR-146a, miR-126 that account for regulation and could be responsible for the genetic susceptibility of SLE disease [36]. By contrast, miRNAs play key roles in the post-transcriptional regulation of most gene-regulatory pathways and regulate both the innate and the adaptive immune responses. Overall, although it is difficult to identify a stable, specific and sensitive "miRNA signature" for SLE at the current stage, it may be possible to pinpoint distinct groups of either up regulated or dysregulated miRNAs according to their shared functional consequences in SLE. Although these findings demonstrate only three types of miRNAs as SLE-specific biomarkers, it is important to acknowledge that the sample size of SLE patients evaluated in this study is relatively small, particularly for the panel of miRNAs selected for evaluation was biased towards our search for SLE specific markers. Additionally, the control group was small in order to determine the normal reference range for circulating miRNAs, before one could define what levels were abnormal. Nonetheless, these data suggest sustained effort toward developing circulating miRNAs as SLE specific biomarkers is warranted. Regarding SLE disease; different patterns of miRNA sex pression may serves a better diagnostic and therapeutic use in SLE disease.

## Conclusion

This study concluded that blood miR-146a was significantly lower, miR-126 significantly higher in Sudanese SLE patients whereas no significant alteration was observed in miR-30a level compared with healthy controls.

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## Ethical Approval and Consent

The study was approved by the research committee at the faculty of Medical laboratory sciences, Gezira university. Ethical approval was achieved from the university, Informed consents were taken from each subject before enrollment in the study.

## Competing Interests

Authors have declared that no competing interests exist.

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