The Egyptian Journal of Hospital Medicine (October 2020) Vol. 81 (3), Page 1690-1698

# Serum Microrna155 Expression Level in Systemic Lupus Erythematosus Related Peripheral Neuropathy

**Nearmeen M. Rashad[1], Hanan S. Ahmed[*2], Mona M.Amer [3], Rehab S. Abdul-Maksoud[4], Amany M. Ebaid[5], Magda M. Sherif[1]**

Departments of [1] Internal Medicine, [2]Clinical Pathology, 3Neurology, [4]Medical Biochemistry and [5]Rheumatology and Rehabilitation, Faculty of Medicine, Zagazig University, Zagazig, Egypt.

*** Corresponding author:** Hanan S. Ahmed**, Mobile:(+20):** 01128885180**, Email:** Hananclpath@yahoo.com

## ABSTRACT

**Background:** miRNA-155 (miR-155) became a focus in several studies because of its essential functions in autoimmune diseases and inflammatory responses such as multiple sclerosis (MS) and rheumatoid arthritis (RA).

**Objective:** We aimed in the current study to explore the expression profile of micro RNA-155 in SLE and evaluate its association with the clinical, immunological, and electrophysiological tests of patients with peripheral neuropathy (PN). **Patients and methods:** Ninety-five recently diagnosed systemic lupus erythematosus (SLE) patients according to 2012 Systemic Lupus International Collaborating Clinics classification criteria were enrolled in addition to 100 controls. Peripheral nerve conduction was evaluated by performing nerve conduction studies (NCSs). The serum miRNA-155 expression profiles were measured using a quantitative real time-polymerase chain reaction (qRT-PCR).

**Results:** Of the 95 SLE patients, PN was present in 35 patients (36.8%). The serum miR-155 expression levels were upregulated the in serum of SLE patients (1.17±0.21) compared to controls (0.826±0.169), $P < 0.001$. Among SLE groups, patients with PN had a higher level of miR-155 expression (1.24±0.1781) than patients without PN (0.913±0.046), $P < 0.001$. There was a significantly positive correlation of miR-155 expression levels with The Toronto Clinical Scoring System (TCSS), immunological markers, and electrophysiological tests of median and ulnar nerve.

**Conclusion:** This is the first Egyptian study report that miR-155 expression profile is upregulated in SLE patients especially patients with PN indicating miRNA-155 might be a potential biomarker for the diagnosis and treatment of SLE related PN.

**Keywords:** Polyneuropathy, Nerve conduction studies, miR-155, SLE, Expression.

## INTRODUCTION

Systemic lupus erythematosus (SLE) is a multisystem autoimmune disease. Genetic and environmental hypothesis are closely linked with the pathogenesis of the SLE [(1)]. Accumulating studies suggest that SLE affects the whole brain. Interestingly, peripheral neuropathy (PN) is an apparent manifestation of SLE [(2)]. The most common forms of PN in SLE, including symmetrical polyneuropathy, mononeuropathy, and cranial neuropathy [(3,4)]. A preponderance of evidence suggests that the pathogenesis of SLE-related neuropathy is obscure, and the few pathological studies of the peripheral nerves in SLE have revealed axonal degeneration, inflammatory changes, and vacuities [(5)].

Increasing evidence supports the hypothesis of immune system dysregulation in pathogenesis of SLE. Epigenetic modulation by DNA methylation, histone post-translational modifications and microRNAs (miRNAs) allows regulation of B cell mechanisms and plasma cell differentiation [(6,7)]. Dysregulation of epigenetic elements or mediators, including miRNAs, can result in aberrant immune responses, including dysregulated antibody production, and compound genetic susceptibility to mediate autoimmunity [(8)].

MicroRNAs (miRNAs) are small non-coding, single-stranded RNA molecules that regulate gene expression at the post-transcriptional level by degrading or blocking translation of messenger RNA (mRNA) [(8)]. MicroRNA-155 (miR-155) is located at 21q21.3, the B cell integration cluster, originally considered to be a proto-oncogene associated with lymphoma. Moreover, miR-155 was implicated in the innate immune function, and silencing miR-155 could ameliorate the disease severity and delay the onset of experimental autoimmune encephalomyelitis [(9)].

MiRNAs-based diagnosis and therapy are highly likely to be the future of treatment and prevention, especially in multifactorial disease processes. The pathogenesis of SLE-related neuropathy is obscure, therefore, the aim in the current study is to explore the expression profile of micro RNA-155 in SLE and evaluate its association with the clinical, immunological and electrophysiological tests of PN patients.

## SUBJECTS AND METHODS

This cross-sectional controlled study comprised 95 SLE patients recently diagnosed according to 2012 Systemic Lupus International Collaborating Clinics classification criteria [(10)] and recruited from the Rheumatology, Neurology and Internal Medicine outpatient clinics, Zagazig University Hospitals. 100 healthy age and sex-matched participants were included as a control group.





Received: 3/7 /2020
Accepted: 2/9 /2020

**Ethical approval:**

**An approval of the study was obtained from Zagazig University academic and ethical committee.** Every patient signed an informed written consent for acceptance of the operation.

The enrolled patients were naïve patients did not receive any immune-modulating medication divided into 60 patients without peripheral neuropathy (PN) and 35 patients with PN. The diagnosis of PN according to the American College of Rheumatology proposed case definitions and classification criteria for 19 CNS and PNS syndromes observed in SLE [(2)].

All participants underwent complete history taking, thorough clinical examination. Patients with a history of diabetes mellitus, myocardial infarction, angina, stroke, drug-induced lupus, pregnancy, hepatitis or other connective tissue disease were excluded. Disease activity was measured using the SLE disease activity index (SLEDAI) [(11)]. Neurological examination was performed in the Outpatient Clinic of Neurology Department using the 10-grams Semmes-Weinstein monofilament, applying the test on 9 different sites on the plantar surface of the foot and diagnosing sensory neuropathy when less than seven sites were felt by the patient. Vibration Perception Threshold (VPT) was also measured, using a biothesiometer, to define the presence of diabetic neuropathy with a cutoff VPT of more than 25 volts for the diagnosis of loss of protective sensation.

**Assessment of peripheral nerve conduction**

**The severity of neuropathy** was graded according to Toronto Clinical Scoring System (TCSS): 1–5 points for no neuropathy; 6–8 points for mild neuropathy; 9–11 points for moderate neuropathy; and 12–19 points for severe neuropathy. Symptoms, reflex, and sensory tests, including pinprick, temperature, light touch, vibration, and position sensation, were performed as part of the TCSS [(12)].

**Nerve Conduction Study**

NCS in the median, ulnar, peroneal, tibial, and sural nerves were carried out for all participants with the Micromed machine in the neurology outpatient clinic [(13)].

**Laboratory assessments**

Laboratory assessments included high-sensitivity C-reactive protein (hsCRP) by Cobas 8000 (Roche, Germany) and complement C3, C4 and 24 hr urine protein by Cobas 6000 (Roche, Germany). Antinuclear antibodies (ANA), Anti-double-stranded DNA antibody (anti-dsDNA), and anticardiolipin (ACL) were estimated. The ANA was measured by indirect immunofluorescence technique using the Indirect Immunofluorescent Kit NOVA Lite® HEp-2 ANA kit (INOVA Diagnostics, Inc, San Diego, USA). For anti-dsDNA, we used the anti-dsDNA indirect immunofluorescence Kit NOVA Lite® dsDNA Crithidia Iuciliae kit (INOVA Diagnostics, Inc, San Diego, USA). Anticardiolipin was performed by ELISA anticardiolipin IgG/IgM ORG515 (ORGENTEC Diagnostika Gmbh, Mainz, Germany).

**Measurement of miRNA -155 gene expression**

The expression of serum miRNA-155 was measured via quantitative real-time-polymerase chain reaction (qRT-PCR). Three milliliters of venous blood was collected and placed in a serum separator tube gel. The blood was centrifuged at 1600 rpm for 5 min and serum was transferred into 1.7 ml Eppendorf tubes, then another centrifugation step was done at high speed 12,000 rpm for 15 min to remove cell debris completely, leaving only circulating RNA.

RNA isolation was done according to the manufacturer's instructions by using the miRNeasy Mini Kit (QIAGEN GmbH, Hilden, Germany) that combines phenol/guanidine-based lysis of samples and silica-membrane-based purification of total RNA. Purified RNA was then used for one-step reverse transcription using miScript II RT kit (Qiagen, Hilden, Germany, Cat. No. 218161, Lot no. 163012117) following the manufacturer's instruction. qRT-PCR was carried out by Stratagene Mx3005P" platform (Agilent Technologies, USA). Small RNA (SNORD-68) was used as internal control (catalog no. MS00033712, Qiagen). The miRNA specific primer (miRNA155-5 p, Cat. No. MS00031486, Qiagen); was used. SYBR Green Master Mix (Qiagen/ SABiosciences Corporation, USA) was used in the (RT-PCR) reaction according to the manufacturer's suggested protocol, along with the manufacturer-provided miScript Universal primer and miRNA-specific forward primer. The relative gene expression (fold change) of serum microRNAs expression levels were analyzed using the comparative threshold cycles method [(14)].

***Statistical analysis***

Statistical analyses were performed using the Statistical Package for the Social Sciences for Windows (version 21.0; SPSS Inc, Chicago, IL, USA). Quantitative data were expressed using descriptive statistics (mean ± standard deviation (SD)) and were analyzed using the "t" test. A comparison of several means was done by one-way analysis of variance (ANOVA), followed by the least significant difference (LSD) test for multiple comparisons between groups. Qualitative data were expressed as frequency and percentage and were compared using $Chi^2$ test. Pearson correlation coefficient was used to assess the association between miR-155 expression, with anthropometric measures as well as electrophysiological parameters in patients. Receiver operating characteristic (ROC) analysis was performed to assess the potential accuracy of miR-155 expression, the area under the curve (AUC). We considered *P* to be significant at <0.05 with a 95% confidence interval (CI).

**RESULTS**

Among studied subjects, in the SLE group, 89.4 % were females and 10.5% were males, their mean age was 45.95±7.63 year. In the control group, 74.3% were females and 25.7% were males, their mean age was

46.98±7.98 years. SLE and control groups were matched for age, sex, and smoking.

**Clinical and biochemical characteristics of SLE groups**

As shown in table 1, SLE patients with PN had significantly higher values of SLEDAI, TCSS, vasculitis, CRP, WBC, ESR as well as ANA compared to SLE patients without PN. On the other hand, SLE patients with PN had significantly lower values of hemoglobin, platelet, C3 and C4 compared to SLE patients without PN.

**Comparison of miR-155 expression in the studied groups.**

Our results show that SLE patients had statistically significant higher values of miR-155 expression (1.17±0.21) compared to controls (0.826±0.169); P < 0.001 as shown in (Figure 1). Among SLE patients, patients with PN had statistically significant higher values of miR-155 expression compared to SLE patients without PN as shown in table 1.

**Table (1): Clinical characteristics and laboratory parameters of SLE patients**

| Variable | SLE without PN (n=60) Mean±SD or number (%) | SLE with PN *(n=35)* Mean±SD or number (%) | *P* value |
|---|---|---|---|
| Male/female, number | 6/54 | 4/31 | 0.541 |
| Duration of disease (years) | 5.44±5.96 | 6.29±7.012 | 0.526 |
| Fever | 16 (26.6%) | 8 (22.8%) | 0.438 |
| SLEDAI | 9.14±3.85 | 15.6±8.47 | <0.001* |
| TCSS | 0.57±0.21 | 9.98±2.96 | <0.001* |
| Discoid rash | 42 (70 %) | 23 (65.7%) | 0.416 |
| Photosensitivity | 38 (63.3%) | 18 (51.4%) | 0.178 |
| Oral ulcers | 39 (65%) | 18 (51.4%) | 0.139 |
| Alopecia | 38 (63.3%) | 19 (54.2%) | 0.257 |
| Pleurisy | 42 (70 %) | 22 (62.8%) | 0.311 |
| Pericarditis | 37 (61.7%) | 25 (71.4%) | 0.231 |
| Arthritis | 42 (70%) | 25 (71.4%) | 0.538 |
| Vasculitis | 16 (26.6%) | 0 (0%) | <0.001* |
| Myositis | 2 (3.3%) | 1(2.8%) | 0.695 |
| Cataract | 3 (5 %) | 2 (5.7%) | 0.611 |
| Retinal change/optic atrophy | 1 (1.6%) | 1 (2.8%) | 0.604 |
| Seizures | 5 (8.5 %) | 4 (11.4%) | 0.437 |
| Psychosis | 2 (3.3%) | 1 (2.8%) | 0.695 |
| Headache | 7 (11.6%) | 2 (5.7%) | 0.284 |
| WBC count (cell×103/µl) | 7.09±2.88 | 9.06±5.21 | <0.001* |
| Hemoglobin (g/dl) | 10.3±2.16 | 10.42±2.09 | <0.001* |
| Platelet (cell×103/µl) | 108.4±64.5 | 94.65±44.02 | <0.001* |
| Creatinine (mg/dl) | 1.28±0.69 | 1.87±1.55 | <0.001* |
| hs CRP (mg/dL) | 7.69±1.61 | 11.92±4.68 | <0.001* |
| ESR (mm/h) | 36.47±10.31 | 84.87±34.94 | <0.001* |
| ANA | 55 (91.6%) | 30 (85.7%) | <0.05* |
| Anti-dsDNA | 40 (66.6%) | 19 (54.2) | 0.065 |
| ACL | 22 (36.6) | 9 (25.7%) | 0.121 |
| C3 (mg/dl) | 75.06±29.34 | 42.87±24.02 | <0.001* |
| C4 (mg/dl) | 21.93±17.09 | 13.2±4.95 | <0.001* |
| MiRNA-155 expression | 0.913±0.046 | 1.24±0.1781 | <0.001* |
| Medications | | | |
| Corticosteroids | 55 (91.6%) | 27(77.1%) | <0.05* |
| Azathioprine | 10 (16.6%) | 16 (45.7%) | 0.875 |
| Cyclophosphamide | 27 (45%) | 12 (34.2%) | <0.001* |
| Mycophenolate | 23 (65.7%) | 7 (20%) | <0.001* |

SLE; Systemic lupus erythematosus, PN; peripheral neuropathy, SLEDAI; systemic lupus erythematosus disease activity index, TCSS; Toronto Clinical Scoring System C3; complement 3, C4; complement 4 ACL; anticardiolipin, ESR; erythrocyte sedimentation rate, ANA; antinuclear antibodies; CRP: C-reactive protein; dsDNA: double-stranded DNA. *: Significant P value.

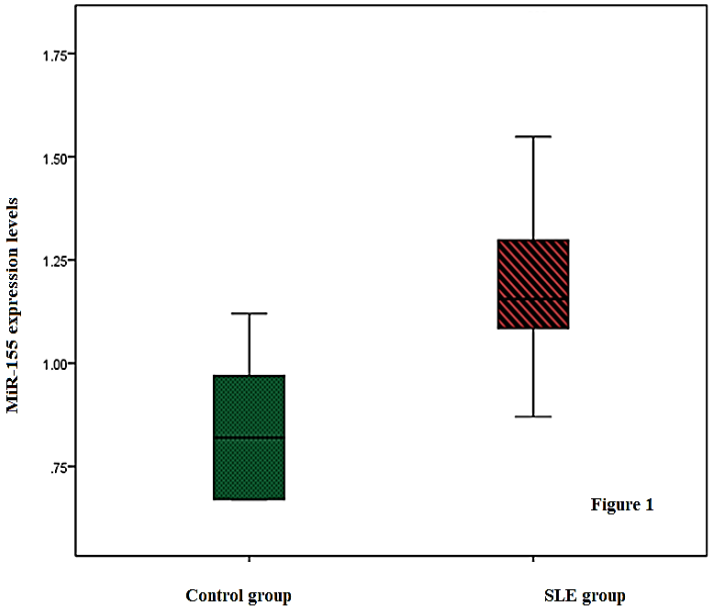


**Figure (1):** Comparison of MiR-155 expression levels in studied groups.

**Electrophysiological tests of the studied groups.**

Nerve conduction velocities in the studied group showed that motor nerve conduction velocities (MNCV) in the median and ulnar nerves were significantly decreased in SLE patients with PN compared to SLE patients without PN and control group. Moreover, sensory nerve conduction velocities (SNCV) in the median and ulnar nerves were significantly decreased in SLE patients with PN compared to SLE patients without PN and control group (Table 2).

Regarding amplitudes, compound motor action potential (CMAP) amplitude in median and ulnar was significantly decreased in SLE patients with PN compared to SLE patients without PN and control group. Sensory nerve action potential (SNAP) amplitude in median and ulnar was significantly decreased in SLE patients with PN compared to SLE patients without PN and control group while all other nerve amplitudes differences were not significant (Table 2).

**Table (2): Comparison of electrophysiological tests of the studied groups**

| Electrophysiological parameters | Control group (n=100) Mean±SD | SLE patients without PN (n=60) Mean±SD | SLE patients with PN (*n* =35) Mean±SD | P1 | P2 | P3 |
|---|---|---|---|---|---|---|
| MNCV (m/s) | | | | | | |
| Median | 54.27± 9.89 | 53.48± 11.35 | 46.2±4.3 | >0.05 | <0.001* | <0.001* |
| Ulnar | 54.09± 10.3 | 55.01±11.27 | 46.6±6.32 | >0.05 | <0.001* | <0.001* |
| CPN | 51.17± 9.8 | 51.95± 11.62 | 55.6±8.97 | >0.05 | >0.05 | 0.294 |
| SNCV(m/s) | | | | | | |
| Median | 52.29± 9.79 | 51.3±11.77 | 43.36± 4.65 | >0.05 | <0.001* | <0.001* |
| Ulnar | 51.50± 10.42 | 51.65±8.15 | 45.48± 5.26 | >0.05 | <0.001* | <0.001* |
| PTN | 52.20± 9.83 | 52.45±5.23 | 52.04± 8.26 | >0.05 | >0.05 | 0.554 |
| Sural | 52.03± 9.87 | 51.33± 4.93 | 50.78± 5.56 | >0.05 | >0.05 | 0.345 |
| CMAP amplitude(mV) | | | | | | |
| Median | 7.92±0.48 | 7.59±1.31 | 4.6±0.5 | >0.05 | <0.001* | <0.001* |
| Ulnar | 8.14±1.48 | 8.35±1.31 | 5.6±0.5 | >0.05 | <0.001* | <0.001* |
| CPN | 6.65±1.48 | 6.66±1.67 | 5.89±1.39 | >0.05 | >0.05 | 0.342 |
| SNAP amplitude(μV) | | | | | | |
| Median | 9.88±1.97 | 9.14±1.87 | 6.81±1.38 | >0.05 | <0.001* | <0.001* |
| Sural | 10.25± 1.85 | 10.61±2.34 | 9.91±2.75 | >0.05 | >0.05 | 0.234 |
| Ulnar | 7.42±1.93 | 7.64±1.85 | 4.31±1.39 | >0.05 | <0.001* | <0.001* |
| PTN | 8.83±1.94 | 8.44±1.68 | 8.64±1.89 | >0.05 | >0.05 | 0.611 |

P1: Control group versus SLE without PN P2: Control group versus SLE with PN
P3: SLE without PN versus SLE with PN *: Significant P value.

**Correlations between miR-155 expression levels with laboratory, TCSS as well as electrophysiological parameters in SLE patients.**

The current results demonstrated a significantly positive correlation of *miR-155 expression levels* with TCSS, ESR, ANA and ACL. On the contrary, there was a significant negative correlation with C3, C4, and electrophysiological tests; MNCV (median and ulnar nerves), SNCV (median and ulnar nerves), CMAP amplitude (median and ulnar nerves) and SNAP amplitude median and ulnar nerve (Table 3).

**Table (3): Pearson correlation between MiR-155 expression levels with laboratory, TCSS as well as electrophysiological parameters in SLE patients**

| | MiR-155 | |
|---|---|---|
| | **r** | **p** |
| hsCRP | 0.608 | <0.001* |
| ESR | 0.364 | <0.001* |
| ANA | 0.329 | <0.001* |
| Anti-phospholipid antibody | 0.259 | <0.001* |
| C3 | -0.357 | <0.001* |
| C4 | -0.506 | <0.001* |
| **TCSS** | 0.192 | <0.05* |
| **MNCV** | | |
| Median | -0.258 | <0.001* |
| Ulnar | -0.263 | <0.001* |
| CPN | -0.063 | >0.05 |
| **SNCV** | | |
| Median | -0.268 | <0.001* |
| Ulnar | -0.264 | <0.001* |
| PTN | -0.021 | >0.05 |
| Sural | -0.016 | >0.05 |
| **CMAP amplitude** | | |
| Median | -0.274 | <0.001* |
| Ulnar | -0.274 | <0.001* |
| CPN | -0.053 | >0.05 |
| **SNAP amplitude** | | |
| Median | -0.194 | <0.001* |
| Ulnar | -0.371 | <0.001* |
| PTN | -0.059 | >0.05 |

**Linear regression analyses in SLE patients to assess the main independent parameters associated with miR-155 expression levels**

As summarized in table 4, a linear regression analysis test revealed that miR-155 expression levels were independently correlated with ANA, TCSS and ACL.

**Table (4): Linear regression analyses to test the influence of the main independent variables against MiR-155 expression levels (dependent variable) in SLE**

| Model | Unstandardized Coefficients | | Standardized Coefficients | t | P | 95% CI | |
|---|---|---|---|---|---|---|---|
| | B | SE | Beta | | | Lower Bound | Upper Bound |
| (Constant) | 0.144 | 0.485 | | 0.29- | >0.05 | 1.109 | 0.821 |
| hsCRP | 0.099 | 0.064 | 0.113 | 1.542 | >0.05 | 0.029 | 0.227 |
| ESR | 0.005 | 0.003 | 0.425 | 1.785 | >0.05 | 0.012 | 0.001 |
| ANA | 0.430 | 0.153 | 1.286 | 2.804 | <0.001* | 0.734 | 0.125 |
| TCSS | 0.015 | 0.007 | 1.253 | 2.274 | <0.05* | 0.002 | 0.028 |
| APL | 0.047 | 0.016 | 0.541 | 2.959 | <0.001* | 0.015 | 0.078 |
| | 0.286 | 0.392 | 0.341 | 0.730 | >0.05 | 0.494 | 1.066 |
| | 0.404 | 00.299 | 0.502 | 1.349 | >0.05 | 0.999 | 0.192 |

ESR: erythrocyte sedimentation rate; hsCRP: high sensitive C-reactive protein; C.I.: Confidence interval; *: Significant P value (P<0.05).

**The accuracy of miR-155 expression levels for discriminating SLE patients from the control group by ROC analysis**

We investigated the potential diagnostic value of miR-155 expression by ROC tests (**Figure 2)**, the cutoff values of was (0.8879) and the AUC was 0.906 (95% CI =0.874-0.937), additionally, the sensitivities and the specificities were (88.7 %) and (617%).

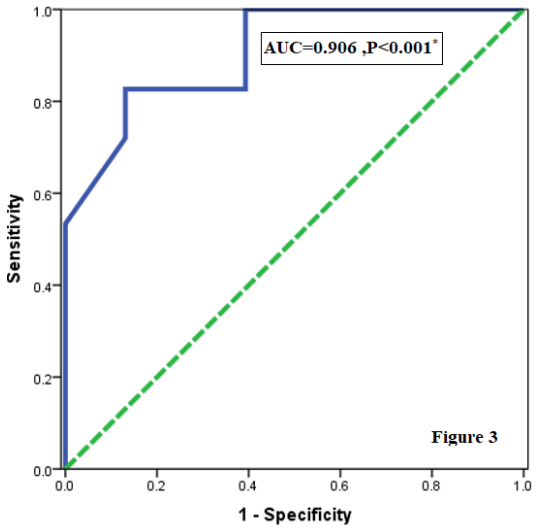


**Figure (2):** ROC curve of MiR-155 expression levels for discriminating SLE from controls

**The accuracy of miR-155 expression levels for discriminating PN among SLE patients by ROC analysis**

We further investigated the potential diagnostic value of miR-155 expression by ROC tests (**Figure 3).** In SLE patients, when we discriminated patients with PN from patients without PN, we found the cutoff values of 1.012 and the AUC was 0.894 (95% CI =0.796-0.992), additionally, the sensitivities and the specificities were (98.3 %) and (97.7%), (p< 0.001).

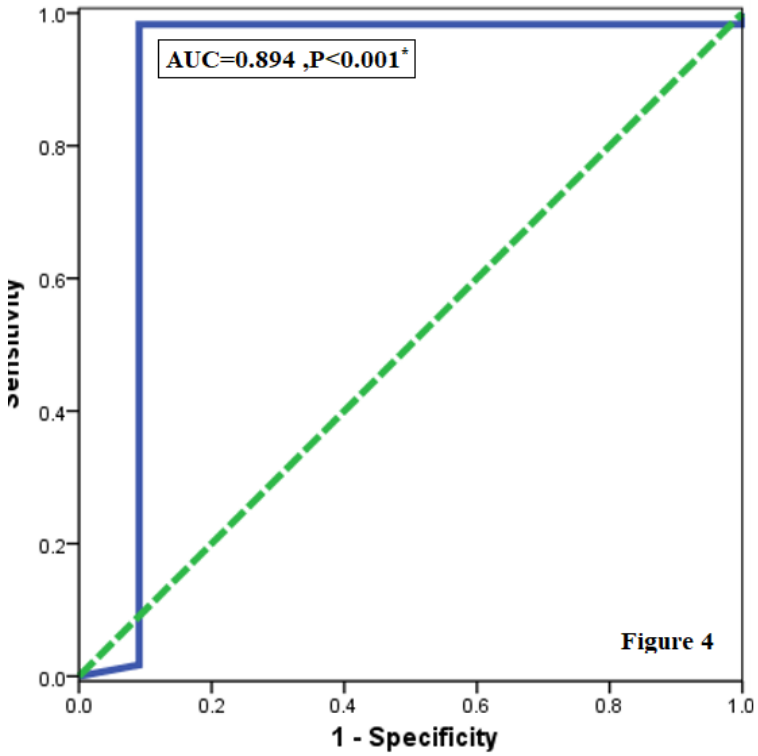


**Figure (3):** ROC curve of MiR-155 expression levels for discriminating SLE with PN from SLE without PN.

## DISCUSSION

It has been postulated that different mechanisms are associated with polyneuropathy in SLE and most of these mechanisms can be influenced by microRNAs. As a consequence, dysregulation of these miRNAs might participate in the pathogenesis of autoimmune disease. This hypothesis was further supported by previous observations that discovered increased miR-155 expression in patients with rheumatoid arthritis in comparison with general population [(15)].

Despite the growing evidence that the symptoms of PN are not a reliable indicator for the presence of neuropathy in the disease course, as about 50% of patients with neuropathy are asymptomatic; therefore, they are prone to insensate foot complications[(16)]. Thereby, early recognition of the high-risk population is enormously important.

According to the current study, among 95 studied patients with SLE patients, the prevalence of PN was 36.8%. Another Egyptian study conducted on SLE patients to evaluate the pattern of neuropathy among SLE observed that the prevalence of neuropathy was 39.4% [(17)]. A noted feature of the pathological process of PN was the finding of inflammatory infiltrates around epineurial and perineural blood vessels from a biopsy specimen, suggesting the inflammatory process was responsible for the occurrence of PN in addition to nerve ischemia [(18)].

Moreover, SLE patients with PN had significantly higher values of SLEDAI, TCSS, vasculitis, CRP, ESR as well as ANA compared to SLE patients without PN. On the other hand, SLE patients with PN had significantly lower values of C3 and C4 compared to SLE patients without PN.

Regarding the activity of SLE, similar results detected by **Imam *et al.*** [(17)] as they found the disease activity SLAM index was significantly higher in SLE patients with PN compared to SLE patients without PN. Similar results observed by **Saigal *et al.*** [(18)] proposed a significant association of PN in SLE patients with ESR level**.** In a conflicting report by **Imam *et al.*** [(17)], they observed no statistically significant difference between the two studied groups with or without PN regarding ANA, ACLs, C3, C4, and anti-dsDNA. These variations can be explained by the theory of chronicity and effect of medication as our patients were recently diagnosed as having SLE; however in Imam study, median duration disease in both SLE groups with or without neuropathy was 36 months and patients received immune modulating medication.

The results presented herein are innovative; as this study performed a robust estimation of nerve conduction studies. Our study revealed that there was 35 patients (36.8%) of studied SLE patients had sensorimotor polyneuropathy of median and ulnar nerve, which was a significant result.

Our findings are in agreement with **Imam *et al.*** [(17)] as they observed that 60 % of patients with PN had sensorimotor polyneuropathy. Similar results were described in previous studies, which detected that the most common PN among SLE patients was sensorimotor polyneuropathy [(19, 20)].

Our study revealed clear evidence that SLE patients had statistically significant higher values of miR-115 expression compared to controls. Among SLE

patients, patients with PN had statistically significant higher values of miR-155 expression compared to SLE patients without PN. MiRNAs are vital regulatory molecules involved in the pathogenesis of immune and inflammatory diseases, miR-155 might be a proinflammatory factor in immune-related disease, and its deprivation might prevent autoimmunity [(21)].

Growing evidence highlights the link of type I IFN and miR-155 as IFN is one of the key cytokines promoting the development of SLE [(22)]. There is some controversy regarding the levels of miR-155 in patients with lupus nephritis as **Wang *et al.*** [(23)] found miR-155 expression level was overexpressed in the urine of SLE patients but was lower in the serum. However, **Zhou *et al.*** [(24)] found higher levels of miR-155 in a mouse model of lupus alveolar hemorrhage.

**Tan *et al.*** [(25)] found that miR-155 expression levels were markedly increased in the spinal cord. Inhibition of miR-155 significantly attenuated mechanical allodynia, thermal hyperalgesia, and proinflammatory cytokine expression.

**Heyn *et al.*** [(26)] observed that neuropathic pain patients had overexpression of miR-155 compared to control. They explained their findings as in neuropathic pain; enhanced targeting of SIRT1 by miR-124a and miR-155 induces a bias of CD4+ T cell differentiation towards Tregs, thereby limiting pain-evoking inflammation.

**Chen *et al.*** [(27)] findings suggested that miR-155 mimics leads to suppression of NF-κB mediated inflammation by targeting Notch2 and TRAF2, causing improvement in peripheral tissue perfusion.

To the best of our knowledge, this study is the first Egyptian study that had explored the correlation of miR-155 expression with clinical scoring; CSS and electrophysiological tests among patients with SLE. Noteworthy, our results confirmed that miR-155 expression levels were significantly positively correlated with TCSS, ESR, ANA and ACL. On the contrary, there was a significant negative correlation with C3, C4, and electrophysiological tests of median and ulnar nerves. Linear regression analysis test revealed that miR-155 expression levels were independently correlated with ANA, TCSS, and ACL. Interestingly, we further investigated the potential diagnostic power of miR-155 expression by ROC tests in differentiating SLE from controls as well as in differentiating SLE patients with PN from ones without PN.

## CONCLUSION

The prevalence of PN in our study was 36.8% as 35 patients had sensorimotor polyneuropathy of the median and ulnar nerve. The serum miRNA-155 expression levels were upregulated in SLE groups especially patients with PN. The diagnostic power of circulating miR-155 was highly significant and it could be a useful diagnostic biomarker of SLE related PN. Further future multicenter studies with a bigger sample size are needed to validate our findings.

## DECLARATIONS

- **Availability of data and material:** data available on demand
- **Competing interests:** The authors declare that they have no competing interests.

**Funding:** No fund
**Data availability:** Not applicable.

**Authors' contributions:**

**Nearmeen M. Rashad, Mona M.Amer , Rehab S. Abdul-Maksoud , Amany M.Ebaid , Shady E. Shaker** , conceived of the presented idea, collected patients' samples and clinical data developed the theory.

**Hanan S. Ahmed**, prepared sample for laboratory investigations and performed laboratory analysis. **Hanan S. Ahmed and Nearmeen M**. **Rashad** wrote the paper, statistical analysis, interpretation of data and preparation the paper for international submission. Critical revision of the manuscript was performed by all of the authors. All authors have read and approved the final manuscript.

## ACKNOWLEDGEMENTS

The authors thank the patients whose participation made this study possible.

## REFERENCES

1. **Cooper G, Dooley M, Treadwell E *et al.* (1998):** Hormonal, environmental, and infectious risk factors for developing systemic lupus erythematosus. Arthritis Rheum., 41: 1714-1724.
2. **Liang M, Corzillius M, Bae S *et al.* (1999):** The American College of Rheumatology nomenclature and case definitions for neuropsychiatric lupus syndromes. Arthritis Rheum., 42: 599–608.
3. **Bertsias G, Ioannidis J, Aringer M *et al.* (2010):** EULAR recommendations for the management of systemic lupus erythematosus with neuropsychiatric manifestations: report of a task force of the EULAR standing committee for clinical affairs. Ann Rheum Dis., 69: 2074–2082.
4. **Florica B, Aghdassi E, Jiandong S *et al.* (2011):** Peripheral neuropathy in patients with systemic lupus erythematosus. Semin Arthritis Rheum., 41: 203–211.
5. **Yoshio T, Okamoto H (2015):** Pathogenesis of neuropsychiatric syndromes of systemic lupus erythematosus. Open J Rheumatol Autoimmune Dis., 5: 46–56.
6. **Xu Z, Zan H, Pone E *et al.* (2012):** Immunoglobulin class-switch DNA recombination: induction, targeting and beyond. Nat Rev Immunol., 12:517–531.
7. **Li G, Zan H, Xu Z *et al.* (2013):** Epigenetics of the antibody response. Trends Immunol., 34:460–470.
8. **Krol J, Loedige I, Filipowicz W (2010):** The widespread regulation of microRNA biogenesis, function and decay. Nat Rev Genet., 11 (9) 597–610.
9. **Murugaiyan G, Beynon V, Mittal A *et al.* (2011):** Silencing microRNA-155 ameliorates experimental autoimmune encephalomyelitis. J Immunol., 187: 2213–2221.

10. **Petri M, Orbai A, Alarcon G *et al.* (2012):** Derivation and validation of the Systemic Lupus International Collaborating Clinics classification criteria for systemic lupus erythematosus. Arthritis Rheum., 64:2677–2686.
11. **Gladman D, Ginzler E, Goldsmith C *et al.* (1996):** The development and initial validation of the Systemic Lupus International Collaborating Clinics/American College of Rheumatology damage index for systemic lupus erythematosus. Arthritis Rheum., 39(3): 363–369.
12. **Sachedina S, Toth C (2013):** Association of comorbidities with increasing severity of peripheral neuropathy in diabetes mellitus. World Journal of Diabetes, 4(4): 135–144.
13. **Dyck P, Carter R, Litchy W (2011).** Modeling nerve conduction criteria for diagnosis of diabetic polyneuropathy. Muscle and Nerve, 44(3): 340–345.
14. **Livak K, Schmittgen T (2001).** Analysis of relative gene expression data using real-time quantitative PCR and the 2 (– delta delta C (T)) methods. Methods, 25:402–408.
15. **Stanczyk J, Pedrioli D, Brentano F *et al.* (2008):** Altered expression of microRNA in synovial fibroblasts and synovial tissue in rheumatoid arthritis. Arthritis Rheum., 58:1001–1009.
16. **Wu S, Driver V, Wrobel J *et al.* (2007):** Foot ulcers in the diabetic patient, prevention and treatment. Vasc Health Risk Manag., 3:65–76.
17. **Imam M, Koriem H, Hassan M *et al.* (2019):** Pattern of peripheral neuropathy in systemic lupus erythematosus: clinical, electrophysiological, and laboratory properties and their association with disease activity. Egypt Rheumatol Rehabil., 46: 285-98.
18. **Saigal R, Bhargav R, Goyal L *et al.* (2015):** Peripheral neuropathy in systemic lupus erythematosus: clinical and electrophysiological properties and their association with disease activity parameters. J Assoc Physicians India, 63: 15–19.
19. **Xianbin W, Mingyu W, Dong X *et al.* (2015):** Peripheral neuropathies due to systemic lupus erythematosus in China. Medicine (Baltimore), 94: 625-632.
20. **Toledano P, Orueta R, Rodríguez-Pintó I *et al.* (2017):** Peripheral nervous system involvement in systemic lupus erythematosus: prevalence, clinical and immunological characteristics, treatment and outcome of a large cohort from a single centre. Autoimmun Rev., 16: 750–755.
21. **Dai R, Zhang Y, Khan D *et al.* (2010):** Identification of a common lupus disease-associated microRNA expression pattern in three different murine models of lupus. PLoS One, 5(12): 14302-6.
22. **Zhou H, Huang X, Cui H *et al.* (2010):** MiR-155 and its star-form partner miR-155∗ cooperatively regulate type I interferon production by human plasmacytoid dendritic cells. Blood, 116(26):5885–5894.
23. **Wang G, Tam L, Kwan B *et al.* (2012):** Expression of miR-146a and miR-155 in the urinary sediment of systemic lupus erythematosus. Clin Rheumatol., 31: 435–440.
24. **Zhou S, Wang Y, Meng Y *et al.* (2016):** In vivo therapeutic success of microRNA-155 antagomir in a mouse model of lupus alveolar hemorrhage. Arthritis Rheumatol., 68(4): 953-64.
25. **Tan Y, Yang J, Xiang K *et al.* (2015):** Suppression of MicroRNA-155 Attenuates Neuropathic Pain by Regulating SOCS1 Signalling Pathway. Neurochem Res., 40: 550–560.
26. **Heyn J, Luchting B, Hinske L *et al.* (2016):** MiR-124a and miR-155 enhance differentiation of regulatory T cells in patients with neuropathic pain. Journal of Neuroinflammation, 13(1): 248-.
27. **Chen J, Liu W, Yi H *et al.* (2019):** MicroRNA-155 mimics ameliorates nerve conduction velocities and suppresses hyperglycemia-induced pro-inflammatory genes in diabetic peripheral neuropathic mice. Am J Transl Res., 11(6):3905-3918.