Research Article
Volume 12:7, 2021

Journal of Molecular Biomarkers & Diagnosis

ISSN: 2155-9929 Open Access

Role of Micro-Rna132 and its Long Non Coding Sox2 in Diagnosis of Lupus Nephritis

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ABSTRACT

Skin and kidney are commonly affected in systemic lupus erythematosus (SLE) with similar molecular mechanisms. Although clinical indicators of renal injury in SLE are fairly uncontroversial, few biomarkers are reliable. The role of Micro-RNAs (micro-RNAs) in lupus nephritis (LN) pathogenesis has been investigated to help in early diagnosis. The aim of work is to evaluate micro-RNA132 expression in SLE Egyptian patients with and without nephritis and the relation between micro-RNA132 and its long non-coding gene SOX2 in both patients groups. This is a case-control study involving 100 SLE patients with and without LN (LN and non-LN groups), and 50 age-and sex-matched healthy controls were carried out to detect micro-RNA132 and SOX2 expression with quantitative Real-Time Polymerase chain reaction methods. The SLE disease activity index (SLEDAI) was assessed. SLEDAI was (2-7) and (4-14) in non-LN and LN respectively.micro-RNA132 expression was significantly increased in patient groups compared to controls (1-1) (P<0.001*) but increased in LN (1.3-7.8) compared to non-LN group (0.5-2.3)(P<0.001*).SOX2 was significantly decreased in patient groups compared to controls (1-1) (P<0.001*) more in LN (0.01-0.14) compared to non-LN group (0.05-0.45) (P<0.001*) There was negative correlation between miRNA132 and SOX2 expression in both patients groups r=-0.677 in non LN, r=-–0.756 in LN, (P<0.001*).In conclusion,micro-RNA132 and SOX2 may play a role in SLE activity and helping in the early diagnosis of LN.

Key words: Systemic lupus erythematosus • Real-time PCR• Micro-RNA132 • SLEDAI score.

Introduction

Systemic lupus erythematosus (SLE) is a potentially fatal, chronic, multisystem autoimmune disorder that typically affects women from puberty till menopause. Defects can occur in many parts of the immune cascade resulting in different clinical presentations, and delay in diagnosis resulted in increased damage to vital organ systems [1].

Kidney disease in SLE is a major cause of morbidity and often leads to chronic kidney disease and ultimately renal failure if left untreated [2].

The particular reason for systemic lupus erythematosus(SLE) is obscured; however, hereditary, racial, hormonal, infection, and environmental variables are involved, Persistent auto antibodies directed against nuclear antigens and various organ systems affection is described in SLE [3].

The micro-RNAs play vital roles in controlling the cell growth cycle, differentiation, and survival by modulating mRNA stability and translation adequacy its role in the cause of proliferative lupus nephritis (LN) was the subject of intensive research [4].

Long non coding RNA (lncRNA) are characterized by a length of more than 200 nucleotides, and their effects on gene expression in the development of diseases are based on a variety of mechanisms of action, including recruitment of chromatin modifiers, controlled recruitment of TFs (both activators and repressors of gene transcription), regulation of RNA splicing, regulation of chromosome looping, control of mRNA translation and decay, and miRNA sponging [5].

Studies on lncRNAs on the patterns of expressed genes have revealed increasing richness and complexity of mechanisms through which lncRNAs control gene expression, at both transcriptional and post-transcriptional levels .

Micro-RNAs and lncRNAs regulate gene expression on all levels – transcriptional, posttranscriptional and post-translational. Through this multi-leveled influence on protein expression patterns, these vast families of non coding RNAs affect all aspects of cell metabolism, including cell division, senescence, differentiation, stress response, immune activation, and apoptosis.

We aimed in this work to study the expression of micro-RNA132 and its long non coding SOX2 in SLE patients with or without nephritis to help in early non invasive diagnosis of renal complication.

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Received: 02 August, 2021; **Accepted:** 16 August, 2021; **Published:** 23 August, 2021

Materials and methods

Patient and tissue samples

After obtaining informed consent, this case-control study comprised 150 participants: 51SLE with nephritis, 49 SLE without nephritis and 50 case controls.

Full history was taken and complete general and dermatological examinations were done. SLE was diagnosed according to the American College of Rheumatology (ACR) revised classification criteria for SLE (10), The global disease activity was assessed by the SLE Disease Activity Index (SELDAI score).

Laboratory investigations were done including complete blood picture (CBC), erythrocyte sedimentation rate (ESR)(normal value for>14 years; for males :0-15ml/h and female:0-20ml/h) , and for serum complements 3 (C3) (normal values for >14 years; for male: 82–185 mg/dl and female: 83–193 mg/dl) and complements 4 (C4) (normal values >14 years; for male: 15–53 mg/dl and for female: 15–57 mg/dl). Antinuclear antibodies (ANA) were assessed by immunofluorescence technique, the antibody kit ANAFAST™, Wilmington, USA U/ml as per kit instructions (indirect fluorescentg assay was done in dilutions of 1:40, 1:80, 1:160, 1:320 as per kit instructions . Anti-double-stranded DNA antibody (anti-dsDNA) was done by enzyme-linked immuno-sorbent assay (ELISA) (using anti-dsDNA ELISA, Abnova™, Philadelphia, USA) according to manufacturer's instruction, urinary creatnine and total 24h urinary proteins were measured.

Detection of miRNA and its long non coding SOX2OT gene expression by quantitative real-time polymerase chain reaction (PCR)

Sampling: A venous blood sample was withdrawn from all subjects,blood was delivered into centrifuge tubes containing EDTA for RNA extraction and for make CBC analysis and stored at −80°C until analysis.

Another serum blood sample were taken for measure to ESR (erythrocyte sedimentation rate) and for measure to C3, C4(complement 3,complement 4)immune complex.

RNA extraction

Total RNA was extracted from 100 µL of serum using the mineareasy kit (Ambion, Warrington, UK according to the manufacturer's instructions). The concentration and purity of RNA were determined using the Nanophotometer.

CDNA synthesis

Chain reaction (RT-PCR) RNA (5 µg) was used per 20-µLreaction to generate cDNA using Gene-specific primers,microRNA Assays and reagents from Quiagen. Micro-RNA Reverse Transcription kit (miscript RT kit ID: 218160), real-timePCR was studied using an miscript syper green pcr kit (ID: 218073) Primers for microR-132 obtained from Quiagen (ID: M rd68S00031409), and for SOX2 from Quiagen(ID: 330701LPH15037A).micro-RNA expressions were normalized to SNORD68 obtained from Quiagen(ID: MS00033712) with a similar efficiency of miRNAs.

Statistical analysis

Data were presented statistically by means ± standard deviation (SD), range, frequency, and percentage, if any. Mann–Whitney U for independent samples was used to compare the numerical variables between the three studied groups, while for categorical data Chi-square test was used, Correlation between two continuous variables using Pearson's correlation coefficient. Receiver Operator characteristics (ROC) curve, was used to assess optimal cutoff point, AUC, Sensitivity, Specificity, PPV, NPV and accuracy of different variables Statistical analysis was made using SPSS software whereas P value was considered significant<0.05.

Results

Clinical diagnosis of systemic lupus

There are different clinical manifestation for diagnosis of systemic lupus, in our results there was no significant difference between both diseased groups(LN and non-LN)with highly significance between the diseased groups and control group in different clinical manifestations as skin ,joint ,cardiovascular ,neurological manifestations(p value<0.001*)(table 1).

		Control	Non-nephritis	Nephritis	P value
		N=50	N=49	N=51	
Skin lesion	No	50(100%)[b]	17(34,7%)[a]	33(64.7%)[a]	<0.001*
	Yes	0(0%)	32(65.3%)	18(35.3%)	
Arthritis	No	50(100%)[b]	6(15%)[a]	6(16.2%)[a]	<0.001*
	Yes	0(0%)	34(85%)	31(83.8%)	
Neurological Symptoms	No	50(100%)[b]	16(32, 7%)[a]	11(26,2%)[a]	<0.001*
	Yes	0(0%)	33(67.3%)	31(73.8%)	
C.V.S Symptoms	No	50(100%)[b]	39(79.6%)[a]	40(78.4%)[a]	<0.001*
	Yes	0(0%)	10(20.4%)	11(21.6%)	

Table 1: Measures of different clinical data in control, complicated LN and non LN:

Laboratory diagnosis of SLE

The mean age was 33.5 ± 9.4 for the control, 33.6 ± 9.4 for non-LN ,30.4 ± 7.7 for LN with no significant difference between groups (P value=0.121). There was significant difference between control and both LN non LN in serum level of Anti DNA, LAC, ANA (P value<0.001) but no significant difference between LN and non LN groups (table 1). Both C3 and C4 decreased in patient groups than control C4 significantly decreased in patient groups than control (P value<0.001) but C3 significantly decreased in LN than non-LN and control groups (P value <0.001).

Discussion

Systemic lupus erythematosus (SLE) is a potentially fatal, chronic, multisystem autoimmune disorder that typically affects women between puberty and menopause. Defects can occur in many parts of

the immune cascade resulting in a striking heterogeneity of clinical presentations. Delay in diagnosis is associated with increased damage to vital organ systems. .

The criteria of the American College of Rheumatology (ACR) was diagnosed SLE as Four of the 11 criteria have to be fulfilled., different clinical symptoms as constitutional symptoms, malar rash ,arthritis, cardiovascular, and neurological more evident in systemic lupus patients, application of the ACR criteria without analysis of auto antibodies may result in an overestimation of SLE.

In the current study, ANA and anti-dsDNA were highly positive in SLE patients. Because of the high frequency, sensitivity, and specificity of anti-dsDNA, it represents the hallmark of SLE patients (16). However, in our study, the results of ANA and anti-ds-DNA showed no statistical difference between LN and non-LN patients.

One of the main laboratory diagnosis of systemic lupus is Lupus anti-coagulant, which elevated in SLE patients than control that may be explained by the presence of auto-antibodies targeting platelet membrane glycoproteins leading to peripheral platelet destruction and deposition of them in the blood vessels.

In our results we reported that, C3 and C4 levels were significantly lower in SLE patients than control, and C3 was lower in LN than non-LN. This may be explained by the consumption of C3 and C4 in immune complex formation and by reduced synthesis. In LN, the initial event is likely caused by immune complex deposition in the interstitium of glomeruli.

Lupus nephritis is the most common complication in systemic lupus patients, almost in all cases deposits of immunoglobulin are found in the glomeruli.

Urine analysis of asymptomatic patients often shows hematuria and proteinuria,in our results we detected that there was significant increase in both urinary proteins and creatnine in lupus nephritis patients than nonlupus nephritis (P value =0,0001*) which indicated renal involvement.

SELDIA score was assested in our study, the severity of disease increased in diseased groups than control more in lupus nephritis than non-nephritis this may be explained by increasing serositis and the inflammatory changes in LN group that increase the severity of the disease .

Although kidney biopsy is considered to be the basis for LN diagnosis, it is an invasive process, and therefore, the presence of biomarkers as microRNA detection in the serum is vital to predict and diagnose this condition.

Different micro-RNAs have been dysregulated in the serum of lupus nephritis patients as (micro-RNA155, micro-RNA 423, and micro-RNA 663).

In this study, we selected patients with SLE, who suffered from lupus related kidney involvement, so that we can test a marker that helps in non invasive prediction and diagnosis of kidney morbidity, which easy the early intervention.

Circulating micro-RNAs play a significant role in the immune response, they are essential in both adaptive and innate immunity, including controlling the differentiation of various immune cell subsets as well as their immunological functions.

The dysregulation of micro-RNAs can lead to aberrant immune response and antibody production to exogenous antigens or self-antigens, which may contribute to the development of LN.

Different micro-RNAs have been detected in the serum of systemic lupus patients with nephritis as micro-RNA (miR- 155, miR-663, and miR-423), These results opened the way for the identification of vital indicators of the disease for the early diagnosis of LN .

In this context, our purpose of the study was to investigate the expression of micro-RNA132 and its long non coding SOX2 in patients with or without lupus nephritis and their sensitivity, specificity in early diagnosis.

We reported that micro-RNA 132 expression were significantly higher in SLE patients than control subjects and were lower in non-LN than LN (P value<0.001*). This may be explained by different micro-RNAs as (micro-RNA 132, micro-RNA 155, micro-RNA 663a,micro-RNA 423-5p, and Let-7)are well represented by both nuclear factor (NF)-κB and interferon (IFN- γ) causing lupus nephritis, and NF-κB can induce inflammation and fibrosis in LN by regulating the transcription of proinflammatory cytokines.

Studies on lncRNAs on the patterns of expressed genes have revealed increasing richness and complexity of mechanisms through which lncRNAs control gene expression, at both transcriptional and post-transcriptional levels.

SOX2 is along non coding gene of micro-RNA132, in this study we reported that the expression of SOX2 was significantly decreased in SLE patients and lower in LN than non-nephritis (P value<0.001*).

This may be explained as the regulation of lncRNA expression and function affected by micro-RNAs and vice versa, besides their direct actions upon target complementary mRNAs, pre-mRNAs, and DNA, indirect actions need to be carefully evaluated, For example, a micro-RNA that appears to repress transcriptionally the levels of an mRNA lacking a micro-RNA target site may in fact be lowering the abundance of a lncRNA required for the transcriptional activation of the mRNA in squestion .

As regard the correlation between micro RNA 132 expression and SOX2OT, there is a negative correlation between them in both groups(non lupus nephritis and lupus nephritis) this negative correlation may be explained as there is a reciprocal regulation of ncRNAs on the effects of micro-RNAs (for example, in reducing lncRNA stability), since lncRNAs resemble mRNAs in many respects .

In this study,ROC curve analysis was performed for predicting sensitivity and specificity of micro-RNA132 and SOX2 in the early diagnosis of lupus nephritis, this analysis showed that micro-RNA132 sensitivity was 49.02% and specificity was 97.96%,and SOX2 sensitivity was100% and specificity was 88% so both can help for early non invasive diagnosis of LN.

Both micro-RNA132 and SOX2 are sensitive and specific in prediction of lupus nephritis; however SOX2 is more sensitive than micro-RNA132 help in early diagnosis of renal involvement of systemic lupus patients.

Conclusion

Nevertheless, our results showed an increased expression of miRNA132 in SLE especially with LN ,in contrast to the expression of its long non coding gene (SOX2) , there was decreased the expression in lupus nephritis patients than non lupus nephritis . We support the hypothesis that LN is due to the effects of many micro-RNAs instead of single micro-RNA. In conclusion, the patterns of miRNA132 expression and its long non coding (SOX2) may represent a new non invasive diagnostic method for predicting renal involvement, but SOX2 is more sensitive than micro-RNA132 in diagnosis of LN, however, the contribution to other circulating miRNAs on larger samples should be studied.

Acknowledgments

Many thanks to DR,Rasha Foaud, Assistant Professor of MedicalBiochemistry and Molecular Biology, Faculty of Medicine,Minya University,DR Hnd Mohamed, Professor of Medical Biochemistry and Molecular Biology, Faculty of Medicine, Minya University,DR Olfat Gamil, Professor of Medical Biochemistry and Molecular Biology, Faculty of Medicine, Cairo University,DR Bothaina Ahmed, MD,of Medical Biochemistry and Molecular Biology, Faculty of Medicine,Minya University, Egypt for performing the laboratory works and great.

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How to cite this article: Ahmed Rasha F. "Role of Micro-Rna132 and its Long Non Coding Sox2 in Diagnosis of Lupus Nephritis." *J Mol Biomark Diagn* 12 (2021) : 1