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Original Research Article

Study of miRNA-146a as a biomarker in Lupus Nephritis and its relationship to treatment

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Abstract: ***Background:*** Lupus nephritis is the most common complication among patients suffering from SLE and is associated with high morbidity and mortality rates among patients with SLE (Systemic lupus erythematosus). ***Objective:*** This research aimed to evaluate the diagnostic value of miRNA-146a as a biomarker in lupus nephritis and its role in response to treatment with azathioprine. ***Patients and methods***: This research comprised 34 patients with biopsy-proven active lupus nephritis, diagnosed according to the 2012 SLICC criteria. All Caseshave been recruited from the nephrology and dialysis unit at Mansoura University. Seventeen sex - and age - matched healthy individuals acted as control group. ***Results:*** miRNA-146a fold change medians were 0.24 (range 0.05–0.47) in control +ve group, 0.49 (0.02–1.89) in azathioprine-treated group, and 1.15 (0.93–2.80) in control -ve. Highly significant differences in miRNA-146a expression were found between control +ve group and azathioprine-treated group. ***Conclusion***: miRNA-146a demonstrated an important association with systemic lupus erythematosus activity, and served as a prognostic marker for cases with lupus nephritis (LN). Moreover, miRNA-146a may act as a valuable particular biomarker for the identification of LN in lupus cases in the future.

Keywords: Lupus nephritis, Azathioprine.

INTRODUCTION

Lupus nephritis is a significant and prevalent complication for people with systemic lupus erythematosus. About 74% of patients with lupus will develop lupus nephritis [1]. The goals of management of LN are to achieve rapid remission of active disease, avoid renal flares, avoid progression of CKD, decrease morbidity and mortality, reduce treatment-associated toxicity and preserve fertility. After clinical remission, treatment of patients with proliferative LN should change to maintenance therapy with low-dose steroids and immunosuppressants [2]. Azathioprine, 6-(1-methyl-4-nitroimidazole-5-yl) thiopurine, is an immunosuppressant drug, that is widely used in clinical management of autoimmune disorders as well as in prevention of graft rejection in organ and tissue transplantation [3]. In SLE, oral azathioprine is generally prescribed for active non-renal disease, using weight-based dosing with varying suggested adjustments for renal impairment [4]

The micro-ribonucleic acids (miRNAs) are small non-coding RNAs of 18–25 nucleotides that regulate the expression of multiple protein-encoding genes [5]. They play an essential role in regulation of gene expression, development of the immune system and also regulation of both innate and adaptive immunity [6]. Several studies have investigated differential miRNA profiles in the plasma of SLE with or without lupus nephritis by high throughput sequencing and reported a group of new and promising biomarker candidates as for diagnosing lupus nephritis [7].

This research aimed to evaluate the diagnostic value of miRNA-146a in lupus nephritis and its role in response to azathioprine therapy.

2. Patients and methods This study is a cohort research included 34 cases with active lupus nephritis (LN) diagnosed with SLE with regard to the 2012 SLE International Collaboration Clinics (SLICC) classification criteria [8] with biopsy-proven nephritis. Seventeen patients with active LN before taking any immunosuppressant and 17 LN patients taking azathioprine for 6 months for maintenance. All caseshave been recruited from the nephrology and dialysis unit at Mansoura University. Seventeen sex - and age -matched healthy persons acted as control group.

2.1. Grouping

Group 1: 17 patients received oral azathioprine two mg/kg/day for six months.

Group 2 (control +ve):17 newly diagnosed patients who had not yet received immunosuppressive treatment.

Control -ve group: 17 healthy individuals.

2.2. Laboratory assessment: included complete blood count, serum creatinine and miRNA-146a expression via quantitative real-time reverse transcription-polymerase chain reaction (qRT-PCR).

MATERIAL AND METHODS

2.3. Molecular Methods

2.3.1. Blood Collection and RNA Extraction

2 milliliters of peripheral blood samples have been obtained, red blood cells were lysed with a 1X red blood cell lysis buffer, produced via diluting a 10X stock solution containing ammonium chloride, potassium bicarbonate, and EDTA. The leukocyte pellet has been obtained through centrifugation and subsequently cleaned. Total RNA has been extracted from the leukocyte pellet utilizing the TRIzol reagent. The RNA pellet underwent two washes with seventy-five percent ethanol, was air-dried, and subsequently dissolved in RNase-free water.

2.3.2. Reverse Transcription

Complementary DNA (cDNA) has been produced with the ABT High-Capacity cDNA Reverse Transcription Kit. The thermal protocol comprised a thirty-minute period at fortytwo degrees Celsius, succeeded by five minutes at 70°C. Synthesized cDNA was preserved at –20°C for subsequent utilization.

2.3.3. Quantitative Real-Time PCR (qRT-PCR)

Quantitative real-time PCR has been performed applying the HERA SYBR Green qPCR Kit and a real-time PCR detection system. Thermal cycling conditions were as follows:Initial polymerase activation at 95°C for 2 minutes and forty amplification cycles: 95°C for ten seconds (denaturation), and 60°C for 20–30 seconds (annealing/extension).A melting curve analysis was performed post-PCR by gradually increasing the temperature from 60°C to 95°C to confirm product specificity. The expression of miRNA-146a was normalized to the expression of the housekeeping small nuclear RNA U6.

2.3.4. Data Analysis and Fold Change Calculation

The cycle threshold (Ct) values have been applied for relative quantification applying the ΔΔCt method [16]:ΔCt (sample) = Ct (miRNA-146a) – Ct (U6), ΔCt (control mean) was calculated from healthy controls and ΔΔCt = ΔCt (sample) – ΔCt (control mean). Fold change (Relative Quantitation, RQ) = 2^-ΔΔCt: This method allowed for assessment of relative expression changes in miRNA-146a between patient groups and control group.

RESULTS

Significant variances have been found between control +ve group and control -ve in RBCs, hemoglobin, and platelets and between azathioprine-treated group and control -ve in RBCs and hemoglobin. Serum creatinine differed significantly between control +ve group and azathioprine-treated group, with medians for creatinine of 2.1and 1.6 mg/dL, respectively. (Table 1)

miRNA-146a fold change medians were 0.24 (range 0.05–0.47) in control +ve group, 0.49 (0.02–1.89) in azathioprine-treated group, and 1.15 (0.93–2.80) in control -ve. Highly significant differences in miRNA-146a expression were found between control +ve group and azathioprine-treated group. (Table 2)

F: ANOVA test.KW: Kruskil Wallis test, *significant p≤0.05., **P1**: Azathioprine-treated Vs. Control +ve, , **P2:** control -ve Vs. control +ve, **P3:** Azathioprine-treated Vs. control -ve.

Table 1. laboratory findings among different groups

	Control -ve (n=17)	control +ve (n=17)	Azathioprine-treated (n=17)	Test of significance	Post hoc LSD test
HB g/dl Mean ± SD	10.73± 0.55	8.96± 1.95	9.44± 1.47	F=3.95 P=**0.012***	P1=0.398 P2=**0.007*** P3=**0.042***
RBC*10^12/L Mean ± SD	4.56±0.22	3.75± 0.53	3.87±0.62	F=6.40 P=**0.001***	P1=0.482 P2≤ **.001*** P3=**0.001***
WBCs*10^9/L Median (Min-max)	9.50 (6-12)	7.5 (2.5-21.7)	7.0 (2.9-17.9)	KW= 6.67 P= 0.083	P1=0.276 P2=0.912 P3=0.086
PLT*10^9/L Median (Min-max)	226.5 (121-380)	215 (29-386)	251.5 (127-400)	KW= 7.124 P=0.068	P1=**0.025*** P2=**0.035*** P3=0.799
Serum creatinine mg/dl Median (Min-max)	1.1 (1-1.3)	2.1 (1.2-8)	1.6 (0.84-6.1)	KW= 21.34 P≤ **0.001***	P1=**0.042*** P2≤ **0.001*** P3=0.064

Table (2): miRNA-146a expression among different groups

	Control -ve (n=17)	control +ve (n=17)	Azathioprine-treated (n=17)	Test of significance	Post hoc LSD test
miRNA – 146a fold change Median (Min-max)	1.15 (0.93-2.80)	0.24 (0.05-0.47)	0.49 (0.02-1.89)	F= 42.76 P≤ **0.001***	P1=**0.049*** P2≤**0.001*** P3=**0.002***

F: ANOVA test. *significant p≤0.05., **P1**: Azathioprine-treated Vs. Control +ve, **P2:** control -ve Vs. control +ve, **P3:** Azathioprine-treated Vs. control -ve.

DISCUSSION

miRNAs (microRNAs) are a sort of non-coding nucleotide sequences with 20-25 bp in length. Numerous miRNAs have an essential role in negatively regulating innate immune response [9]. More specific, great evidence from both animal and human studies reported that the expression of miRNAs is altered in SLE (systemic lupus erythematosus) [10]. In cases with diagnosed lupus nephritis, blood levels of miRNA-146a were elevated compared to controls in certain investigations [11,12]. However other studies showed lower expression of miRNA-146a than controls [13,14,15] which agrees with our results. Our results showed a significant decrease of miRNA-146a expression in patients with LN who did not receive any immunosuppressants (control +ve) than control -ve ($p<0.001$). Regarding azathioprine-treated LN patients, our results showed that there was a statistically significant increase in miRNA-146a expression in azathioprine-treated patients as compared to control +ve which is in consistent with other studies that showed increase in miRNA-146a expression in LN patients after treatment [16,17]. However, this increase did not reach the miRNA-146a level in control -ve group.

CONCLUSION

miRNA-146a served as a prognostic marker for cases with LN. Furthermore, miRNA-146a could act as a valuable particular biomarker for the identification of LN in lupus cases in the future. Our findings indicate that miRNA-146a differentially expressed in patients with lupus nephritis, both pre- and post-treatment with azathioprine.

REFERENCES

1. Li Y, Fang X, Li QZ. Biomarker profiling for lupus nephritis. Genomics Proteomics Bioinformatics. 2013;11(3):158–65.
2. Anders H.-J., Saxena R., Zhao M.-h., Parodis I., Salmon J. E. & Mohan C. 2020. Lupus

nephritis. Nature reviews Disease primers, 6: (1), 7.
3. Lewis J. D., Schwartz J. S. & Lichtenstein G. R. 2000. Azathioprine for maintenance of remission in Crohn's disease: Benefits outweigh the risk of lymphoma. Gastroenterology, 118: (6), 1018-1024.
4. Croyle L., Hoi A. & Morand E. F. 2015. Characteristics of azathioprine use and cessation in a longitudinal lupus cohort. Lupus Science & Medicine, 2: (1), e000105.
5. Wu L., Fan J. & Belasco J. G. 2006. MicroRNAs direct rapid deadenylation of mRNA. Proceedings of the National Academy of Sciences, 103: (11), 4034-4039.
6. Dai R. & Ahmed S. A. 2011. MicroRNA, a new paradigm for understanding immunoregulation, inflammation, and autoimmune diseases. Translational Research, 157: (4), 163-179.
7. Navarro-Quiroz E., Pacheco-Lugo L., Lorenzi H., Díaz-Olmos Y., Almendrales L., Rico E., et al. 2016. High-Throughput Sequencing Reveals Circulating miRNAs as Potential Biomarkers of Kidney Damage in Patients with Systemic Lupus Erythematosus. PLOS ONE, 11: (11), e0166202.
8. Petri M., Orbai A. M., Alarcón G. S., Gordon C., Merrill J. T., Fortin P. R., et al. 2012. Derivation and validation of the Systemic Lupus International Collaborating Clinics classification criteria for systemic lupus erythematosus. Arthritis Rheum, 64: (8), 2677-86.
9. Taganov K. D., Boldin M. P., Chang K.-J. & Baltimore D. 2006. NF-κB-dependent induction of microRNA miR-146, an inhibitor targeted to signaling proteins of innate immune responses. Proceedings of the National Academy of Sciences, 103: (33), 12481-12486.
10. So B. Y. F., Yap D. Y. H. & Chan T. M. 2021. MicroRNAs in Lupus Nephritis–Role in Disease Pathogenesis and Clinical Applications. International Journal of Molecular Sciences, 22: (19), 10737.
11. Zununi Vahed S, Nakhjavani M, Etemadi J. Altered levels of immuneregulatory microRNAs in plasma samples of patients with lupus nephritis. BioImpacts. 2018; 8:177–83.
12. Tawfik NA, El-Dydamoni OA, Abozaid SY, Ebrahem EE, Abd El Rahim M. Serum miRNA-146a and miRNA-155 as novel biomarkers in lupus nephritis activity with systemic lupus erythematosus. Am J Biochem. 2019; 9:21–34.
13. Su YJ, Lin IC, Wang L. Next generation sequencing identifies miRNA-based biomarker panel for lupus nephritis. Oncotarget. 2018; 9:27911–9.
14. Zhu Y, Xue Z, Di L. Regulation of MiR-146a and TRAF6 in the diagnosis of lupus nephritis. Med Sci Monit. 2017; 23:2550–7.
15. Hashad DI, Abdelmagid MH, Elsherif SH. microRNA-146a expression in lupus patients with and without renal complications. J Clin Lab Anal. 2012; 26:35–40.
16. Ayeldeen G., Nassar Y., Ahmed H., Shaker O. & Gheita T. 2018. Possible use of miRNAs-146a and -499 expression and their polymorphisms as diagnostic markers for rheumatoid arthritis. Molecular and Cellular Biochemistry, 449: (1-2), 145-156.
17. Labib D. A., Koptan D., Ghoniem S., Salah S. H., El Shazly R. & El Refai R. M. 2020. Dysregulation of microRNA146a-5p expression in systemic lupus erythematosus females: Diagnostic potential and association with ocular manifestations. The Egyptian Rheumatologist, 42: (2), 117-121.

