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Association of miR-146a Gene Polymorphism with Systemic Lupus Erthymatous Disease

Methak Jasim AL-Jboory

University Musstansiryah, College of Sciences, Iraq.

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The present study was carried out to detect the association of microRNA-146a haplotypes polymorphisms with Systemic lupus erythematous (SLE) in Iraqi patients, PCR-SSCP technique used in present study, blood was used to DNA extraction, the results show that there was strong association between microRNA-146a and SLE in one haplotype from two .The present study concluded that there was association between microRNA-146a polymorphisms with SLE, our finding need more investigation to use this polymorphism as early indication of SLE incidence.

Keywords: Lupus ,MicroRNA, miR-146a, PCR-SSCP technique, Haplotypes, Polymorphisms.

Systemic lupus erythematous is autoimmune disease lead to tissue damage due to inflammation (Lourenço. and La Cava ,2009). This disease begin with minimal or no activity and gradually increased its activity and its symptoms include skin rashes, arthralgia, and fatigue (Davies *et al.,*2012). It is more prevalent in female than in male with estimated ratio 9:1 (Petri *et al.,*2012). The factors that contribute in the etiology of the disease are environmental factors and genetic susceptibility. Viral infection or administration of certain drugs together with genetic factors can lead to promote SLE disease development (Ceccarelli *et al.,*2015) .

MicroRNAs are regulatory genes which have crucial role in regulation of the response of the innate and adaptive immunity by the post-transcriptional regulation of most genes regulatory pathways and this may lead to development of systemic autoimmunity including lupus , one of these microRNAs is miR-146a .(Liang and Shen, 2012).

* To whom all correspondence should be addressed.
E-mail: dr.methak@uomustansiriyah.edu.iq

MATERIALS AND METHODS

1. Sample and data collection; about 2 ml of whole blood was collected from patients of Systemic lupus erythematous in Marjan hospital .All subjects in this study were taken written consent before participation in this study according to ethical approval of Iraq ministry of health, while control collected from healthy, the clinical features of disease were diagnostic by specialist prof Dr Ali al- kazaz also all data were collected for other genetic detection in this disease .
2. DNA extraction; DNA was extracted from whole blood using Favor gene extraction kit and concentration and purity were detected using nanodrope (Al-Terehi *et al*.,2016a).
3. MiR-146a primer was (5'-GGGTCTTTGCACCATCTCTG-3' for the upstream primer and 5'-TCCAGTCTTCCAAGCTCTTCA -3' for downstream. (Vinci *et al.,* 2013).
4. PCR conditions and size products MiR-146a denaturation for 5 min at 94°C, then 30 cycles (30 s at 94°C, 20 s at 57.8°C, 50 s at 72°C, and finally 10 min at 72°C). PCR products were determined by electrophoresis pattern in agarose

gel (1.5% agarose, 70 V, 20 mA for 45 min) with ethidium bromide staining, the PCR size product were (195) bp for MiR-146a .Statics, the results were statically analysis using odd ratio at CI 95% nd p value <0.05).

5. SSCP technique, PCR products were denaturation using SSCP dye (EDTA, formamid and bromophynol blue) 1/1 V:V in water bath for 5 min at 95°C then its child in ice for 2 min.

6. SSCP electrophoresis, the products were electrophoresis as a following About 10 µl of the samples (sample+ dye) were loaded into wells of 8% acrylamide/bis gel containing 7% glycerol, and 1X TBE buffer. In more details; for recipe a 20 × 20 0.1 cm gel format. 8 ml of 40% acrylamide/bis (stoke solution 37.5:1) mixed with 8 ml of 5X TBE, 2.8 ml,100% glycerol, then 40 µl TEMED and 400 µl of 10% ammonium per sulfate were added with 20.8 ml of δH_2O After gel was casting sample were loaded and Run under the following conditions. Buffer 5.5 X TBE, Buffer temperature 10°C, Run time 1.5 h and 100V. Then gel was staining using ethedium bromide for 15 min.

7. Haplotype frequency were determination by variety of bands pattern between patients and control (Al-Terehi *et al*., 2016).

8. The statics analysis implemented using Qi square and odd ration at p value <0.05.

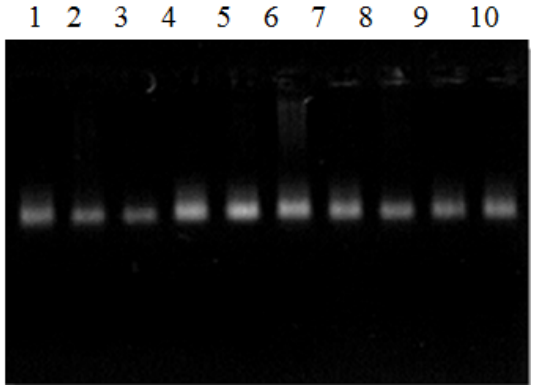

Fig. 1. Electrophoresis pattern of gnomic DNA in study groups, lane 1-5 DNA from patients lane 6-10 DNA from control

RESULTS AND DISCUSSION

The results of study show DNA extraction from whole blood (figure 1)

The results of miR-146a of gene polymorphism which show in Table 1 and Figure 2 clarified the variation of haplotypes in patients and control, there were 2 pattern (A and B,), polymorphisms show significant differences(p>0.05) between patients and control where haplotype A was appeared in control with (75%) while in patients it was absent, and haplotype B was appeared in control with (25%

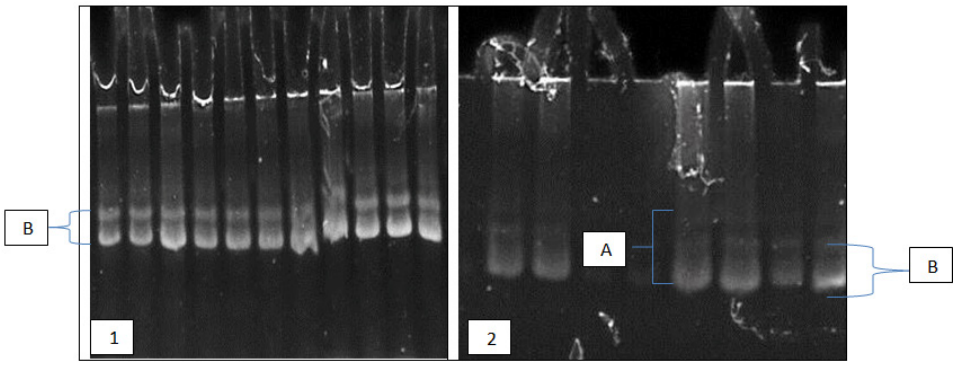

Fig. 2. Electrophoresis pattern of PCR-SSCP of miR-146a gene polymorphism for patients 1, and control 2

Table 1. Haplotypes frequency of miR-146a gene in patients and control

P value	95 % CI	Odd ratio	patient	Control	pattern
< 0.0001	0.0001 to 0.0280	0.0017	0%	75%	3 bandsA
< 0.0001	35.6584 to 9932.1679	595.1176	100%	25%	2 bandsB

) while in patients it was (100%). Systemic lupus erythematous is autoimmune disease which cause inflammation throughout the body and this lead to trigger the innate and adaptive immune response. The study of (Boldin *et al.,*2011) found that mice suffering from severe lupus-like autoimmune disorder characterized by multi-organ inflammation ,lymphadenopathy, splenomegaly, , and greatly increased autoantibody production when they have deficient of miR-146a .MicroRNAs genes are regulatory genes which have important role in regulation and maintenance of the immune system response.MiR-146a participate in the regulation of T-cell lineage. Several studies indicated that miR-146a act on the decrease the function of interferon and toll like receptor(TLR) signaling by repressing the target genes of TLR and interferon such as (*Traf6/Irak1*, *Stat1* and *Tlr7* or *Tlr9*) and thus miR-146a play important role in the pathogenesis of lupus (Chan *et al.,*2013 ; So *et al.,*2013) .

The study of (Luo *et al.,*2011) investigated that the genetic variant(rs57095329) in the promoter region of *miR-146a* in SLE patients are reduced where the risk allele G in this variant had contributing in the decrease binding to transcription factor Ets-1, and lead to reduce the levels of miR-146a in SLE patients. The meta-analysis in other study found that the SNPs (rs57095329 and rs2431697) of miR-146a are associated strongly with SLE susceptibility (Ji *et al.,*2014). Our finding need more investigation to detect the mutations in microRNAs that may be related with the etiology of SLE.

CONCLUSION

The present study concluded that there were one haplotype in miR-146a is associated strongly with SLE in Iraqi patients.

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