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SOME MICR-RNA GENES POLYMORPHISMS IN ASTHMA PATIENTS

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ABSTRACT : Asthma defined as a chronic inflammatory disease of the airways, which associated with airway hyper responsiveness. The present study carried out to investigated the relation between two MIR types (146a and 149) with asthma disease. Study enrolled 30 patients and 30 healthy control, blood samples used to DNA extraction and PCR, PCR-SSCP used to detection genotyping of MIR genes. The results show that there was 3.33% of patients have deletion mutation in MIR-149 and 57% in control with significant differences. The genotyping of MIR-146a shows four haplotypes three of them had non-significant differences with odd ratio (1) and CI (0.0196 - 50.8937), only one haplotype had significant differences between patients and control it was appeared in 80% of patient and disappeared in control at odd ratio 229.9231 and CI (12.3359 to 4285.4233). From this results can be concluded that the two types of MIR 146a and 149 associations with asthma disease in Iraqi patients.

Key words : MIR-149, asthma, MIR-146a, PCR-SSCP, genotyping.

INTRODUCTION

Asthma is one of the most common chronic factors that affected in different age categories. The risk factors for each types of asthma genetic, environmental and host factors. Family history also common, but didn't sufficient necessary for the development disease(Burke *et al*, 2003).

microRNAs (miRNAs) is one of the important discovering in lase decades, which have major roles in some biological processes in cells of different organism, it is short single-stranded of RNA molecules, its contributed with degradation target mRNAs and inhibit their translation. A miRNA have hundreds targets; thus it is influence large proportion of genes expression (Rebaneand Akdis, 2012). miR-146a is an anti-inflammatory agents, which suppressed activation of NF-êB in some studies, its directly downregulates IL-1 receptor-associated kinase 1 (IRAK1), TNF receptor-associated factor 6 (TRAF6) (Boldin *et al*, 2011).

Asthma is a chronic inflammatory airway disease characterized by airway hyper responsiveness and reversible airway obstruction, it can be categorized classified intonumerous phenotypes according to the inflammatory profile (eosinophilic, neutrophilic, mixed granulocytic and paucigranulocytic), presence of allergy (atopic vsnonatopic) and age of onset (childhood vs adult onset) (Wenzel *et al*, 2012).

MATERIALS AND METHODS

Study design and subject

Case-control study was in Babylon province, Iraq. The study subjects comprised from 30 patients suffer from asthma these patient under biological therapy randomly selected from mrjan teaching hospital (16 male and 14 female) with age average (7-60 year), the control group study included 40 people apparently healthy that included (12 male and 17 female) with age average (7-60 year). This control group matched with patient group. All subjects in this study were taken written consent before participation in this study according to ethical approval of Ministry of Health in Iraq.

Exclusion criteria

The excluded include patients DM, hypertension, hepatitis, heart failure, renal failure, liver disease, malignant disease, patients on chemotherapy, etc. and excluded patients, who suffer from complication AS. Data collected from patients according to ethical approval of Ministry of Health of Iraq.

Materials and methods

1. DNA was extracted from whole blood according to kit leaflet (genaid, genomic DNA extraction kit).
2. After DNA extraction**;** consternation and purity of DNA were estimated using nanodrpe. MiR-146a

primer was (5'-GGGTCTTTGCACCATCTCTG-3' for the upstream primer and 5'-TCCAGTCTTCCAAGCTCTTCA -3' for downstream and F:5'-GTGTCTTCACTCCCGTGCTT-3'; R: 5'-ACCTCTCACACCCCCTCAC-3" was amplified by PCR with annealing temperature 60.5 for MIR-149. (Vinci *et al*, 2013; Al-Terehi *et al*, 2018).

3. PCR conditions and size products MiR-146a denaturation for 5 min at 94^{0}C, then 30 cycles (30 s at 94^{0}C, 20 s at 57.8^{0}C, 50 s at 72^{0}C and finally 10 min at 72^{0}C) for MiR-146a and 30 cycles (30 s at 94^{0}C, 20 s at 60.5^{0}C, 50 s at 72^{0}C and finally 10 min at 72^{0}C) for MiR-149. 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 PCRsize product were (196) bp for MiR-146a and (178 bp) for MiR-149. Statics, the results were statically analysis using odd ratio at CI95% nd p value <0.05.
4. 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^{0}C then its child in ice for 2 min.
5. SSCP electrophoresis, the products were electrophoresis as a following about 10 ìl of the samples into wells of an 8% acrylamide/bis gel (37.5:1), containing 7% glycerol and 1x TBE buffer. And for recipe a 20 × 20 × 0.1 cm gel format. 8 ml of 40% acrylamide/bis (37.5:1) mixed with 8 ml of 5 × TBE, 2, 8 ml 100% glycerol, then 40 ìl TEMED and 400 ìl of 10% ammonium per sulfate were added with 20.8 ml of dH_2O. After gel was casting sample were loaded and Run under the following conditions. Buffer 0.5X TBE, Buffer temperature 10°C, Run time 3.5 hours and 100V. Then gel was staining using ethedium bromide for 15 min (Al-Terehi *et al*, 2016).

Table 1 : The association of study groups by study variables.

Categories	p	c
Age	33.83±19.73	33.8±12.08
BMI	26.819±5.7844	23.45±3.28
Male	53.33%	80.00%
Female	46.66%	20.00%
Smoker	20%	26.66%
Non-smoker	80%	73.33%
Urban	63.33%	86.66%
Rural	36.66%	13.33%

RESULTS

The present study was carried out to investigate association micro RNA -146a and 149 with asthma patients, the results show that the mean of age were 33.83±19.73 and 33.8±12.08 for patients and control respectively, males have high percentage than females in patients, it was 53.33% also in control it was 80%. Smoker habitat was 20% in patients and 26.66% in control. According to residence study groups ware 63.33% of patients were urban while 36.66 of them was rural (Table 1).

Tables 2 and 3 show genotyping of MIR-146a and MIR-149 for patients and control, the PCR products of MIR-146a were 196 bp and it show four haplotypes (A, B, C and D) three of it was appeared in patients and control without any variations, haplotype D show significant differences between patients and control it was appeared in 80% in patients while it disappeared in control (Fig. 1 A and B).

The results of MIR- 149 shows DNA sequence deletion in 3.33% in patients and 53% in control (Table 2 and Fig. 1C), statics shows significant differences between patient and control as in Table 3.

DISCUSSION

Asthma is auto immune disease has been recorded in all age categories in Iraqi population. The present study show there was no differences between patents and control in age, also in BMI there was less differences between study groups, the present study show that males were

Table 2 : The genotype distribution and odd ratio of MIR-146a gene polymorphism for patients and control.

Haplotypes name	Patients%	Contro l%	OD ratio	CI 95%	P-value
A	100	100	1	0.0196 - 50.8937	1
B	100	100	1.0000	0.0196 - 50.8937	1
C	100	100	1.0000	0.0196 - 50.8937	1
D	80	0	229.9231	12.3359 - 4285.4233	0.0003

Table 3 : The genotype distribution of MIR-149 gene polymorphism in patients and control.

Pattern name	Patients %	Contro l%	OD ratio	CI 95%	P-value
+	96.66%	43	0.0425	0.0056 to 0.3248	0.0023
–	3.33%	57			

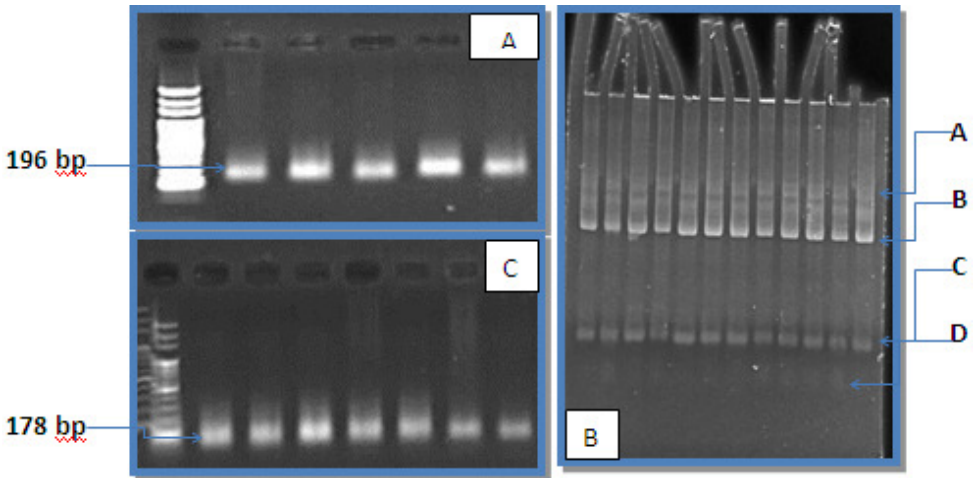

Fig. 1 : The electrophoresis pattern of PCR product for MIR-149 and MIR-146a gene for both patients and control A, amplification product one band 196 bp of MIR-149; B electrophoresis pattern of SSCP technique of MIR-149, CPCR products 178 bp of MIR-146a gene, 1% agarose,75V, 20Am for 1 h.

more than females in the study groups, the frequency of sample according to sex didn't depended on clinical evidence of disease although of higher percentage of males in present study m other study found that females have increment in Clinical evidence of asthma symptoms that starting at puberty compared to males. Because of sex hormones role in asthma symptoms (Fuseini *et al*, 2017); thus present study need more investigation about role of sex hormones in regulation of these symptoms in Iraqi patients.

High percentage of patients was urban in present study than rural, this because high level of pollution and high level of heavy metals in air, water and soil which may effect in airway and allergic response (Taha *et al*, 2005; Hasan *et al*, 2010). This performed by Guo and Chen (2018) when they improved effect asthma disease by air pollutions in Shanghai city.

The relationship between MIR types and asthma have been studied, the MIR-149 and MIR-146a gene polymorphisms were studied in present study, the results show association between asthma and MIR-146a in one haplotype its appeared in 80% of patients, other studies concluded that other types of MIR have role in asthma inflammatory phenotype; the expression of miR-223-3p, miR-142-3p and miR629-3p were increased in patients sputum of severe asthma and is related to neutrophilic airway inflammation (Maes *et al*, 2016). Haj-Salem *et al* (2015) found that MIR-19a was upregulated in epithelia of severe asthmatic compared with mild asthmatics and controls.

In the other hand, Comer *et al* (2015) investigated of miR-146a and miR-146b expression is inducible in human airway smooth muscle cells, they found that miR-146a expression was high in asthmatic cells, which suggested that miR-146 can be used in asthma treatment.

In study deal with some MIR genotyping, Hu *et al* (2017) found that miR-149 rs2292832 variant is strongly related with allergic rhinitis and asthma, it lead to change in miR149 expression when allergies happened, which following the stimulation with an allergen. Also, the CC genotypes were more susceptible to asthma patients.

CONCLUSION

The present study concluded that there was association between MIR-146a and 149 and asthma disease.

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