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Association between interlukin1B gene polymorphisms and keratoconus disease in a sample of Iraqi patients

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Abstract: The objective of this study was to investigate the association of polymorphisms in *IL1B* gene with the keratoconus disease in samples of Iraqi patients. Sample collection was carried out in the duration of two months from November 2016 to January 2017. The genomic DNA was extracted using Promega kits, with subsequent amplification of the fragments via PCR with specific primers followed by molecular detection of single nucleotide polymorphisms (SNPs) in specific regions along IL1B gene by DNA sequence analysis. The -511 (C>T) rs16944 is considered to be a risk factor for KC disease as the C allele frequency being higher in patients than controls (76% versus 40% respectively, OR 4.7500, 95% CI 2.1741-10.377, P 0.0001) also CC genotype (64% in patients versus 30% in controls, OR 4.1481, 95% CI 1.3573-12.677, P 0.0126). In addition -31 TATA box (T>C) rs1143627 was also considered as a risk factor to KC disease due to the results of allele and genotype frequency (T allele frequency 72% in patients to 33% in controls, OR 5.3407, 95% CI 2.4173-11.799, P 0.0001) and (TT genotype frequency 60% in patients to 25% in controls, OR 4.5000. 95% CI 1.4113-14.348, P 0.0110). Furthermore the two promoter SNPs (-31 and -511) are proved to be in complete linkage disequilibrium (LD) and the frequency of (T>C) haplotype (84% in KC patients versus 35% in controls, OR 9.7500, 95% CI 2.9663-32.047 and P 0.0002) which make it clear that the presence of those two SNPs together considered as a risk factor of KC disease for Iraqi patients. It was expected that the expression of *IL1B* may be enhanced due to the presence of T>C haplotype in the promoter region. Thus promote apoptosis consideres as a fundamental cause for keratoconus disease.

Keywords: Keratoconus; IL1B gene; Haplotype; Cornea.

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Introduction:

Keratoconus (KC) is a degenerative disease of the eye in which the peripheral or central cornea suffering gradual bulging and steepening, thus the shape of cornea looks like a cone. The advancement of the disease gives rise to reducing in the biomechanical rigidity of the cornea so that the major characteristics of keratoconus are distortion in the curveting of the cornea, anterior bulge and apical scarring leading to the progression of irregular short-sightedness astigmatism (1,2,3). One of the most important histopathological defects in keratoconic cornea is the presence of morphological change in keratocytes in addition to alteration in its density due to apoptosis. As a result, the disease advancement gives rise to reducing in the biomechanical rigidity of the cornea (3,4).

Apoptosis can cause due to aberration of epithelium following by increased production of Interleukin 1 (IL1) that cause apoptosis of keratocyte. This is in agreement with the finding in keratoconic cornea the IL1-recptor is four fold increased as well as the production of apoptotic cytokine can be

triggered by eye rubbing, eye lenses and atopy can cause keratocyte apoptosis (5) so that IL1 has been proposed as a candidate gene for KC. Interleukin 1 is a proinflammatory cytokine that stimulate the production of chemokines and cytokines, the interleukin 1 family is a group of pleiotropic cytokines that are encoded by IL1 superfamily genes which are located as a cluster on chromosome 2q14. IL1 family genes occupy nearly 400Kb including three well-defined genes IL1B, IL1A and IL1RN and their products IL1α and IL1β are agonists, while IL1RN is antagonist (6,7,8).

This gene cluster includes several polymorphisms which may be situated in the regulatory regions of the genes which in turn affect transcription activity leading to defect in the production of IL1. There are two SNPs in the *IL1β* gene -31 TATA box rs1143627 and -511 rs16944 were reported to be associated with various multifactorial diseases including KC. Those SNPs are involved in increased expression of *IL1β* mRNA (9,10,11) so the goal of this study is to investigate SNPs associated with keratoconus disease in Iraqi patients.

Materials and Methods:

Samples:

In the present study the ages of the samples chosen were within the range of (11- 41) years old. Fifty patients (24 male and 26 female) diagnosed with keratoconus were collected from LASIK Unit in Ibn al-Haytham Hospital (Baghdad) and from Al-Baseer eye specialist center (Baghdad / Palestine Street). All diagnosed keratoconus patients were informed about the study and gave written consent for their involvement in this study. They also filled a questionnaire form about their infection with keratoconus. Twenty intact people (10 female and 10 male) were chosen as controls for this study. All of them ensured that they had no symptoms of keratoconus.

Blood Collection:

Three milliliters of blood were collected using 5ml syringe from the veins of all patients and controls and preserved in EDTA tubes followed by gentle mixing in order to prevent blood clotting and stored at 4º C then transported to lab. for future DNA extraction and purification.

IL1B Analysis:

DNA extraction from blood samples were carried out using the genomic purification kit of Wizard® (Promega, USA) followed by using 2µl of each sample to evaluate DNA concentration and purity by using NAS99 Nanodrop Spectrophotometer with easy computerized software control and data storage is preceded by using TE buffer as a blank solution accompanied with DNA integrity detection 0.8% agarose gel was used (0.8 gm of agarose in 100 ml of 1X TBE).

The *IL1B* gene was chose based on a study by (3). in which it was considered as a candidate gene in which there were SNPs associated with keratoconus. Two primers were used its sequences and information are listed in table (1).

Following optimization experiments, the selection of concentration that yield a single sharp band for sequencing reaction (shown in table 2) with a total volume of reactions 50µl.

The PCR reaction mixture was subjected to an initial denaturing step of 5 min at 95 °C, then 35 cycles of denaturing for 1 min at 95 °C, annealing for 1 min at 57 °C for -511 region and 61 °C for -31 region, extension for 1min at 72 °C, and a final extension step of 10 min at 72 °C followed by the examination of PCR product 1.5% agarose gel. After amplification of the targeted regions with specific molecular sizes (305, 499 bp), 45µl of PCR product of several samples (control and patient) with primers were sent abroad the country to Macrogen Company (South Korea) for direct sequencing. Alignment was established by tools that available online at the National Center Biotechnology Information (NCBI) (https://www.ncbi.nlm.nih.gov) by using BLASTn for nucleotide sequence and BLASTp for peptide sequence alignments by the translation of exons to their amino acids sequences (http://web.expasy.org/translate). Haploview 4.2 software program was used for LD and Haplotype analysis.

Table (1): Sequences and information of used primers.

Gene	Target site for amplification	Primer sequences (F: Forward R: Reverse)	PCR product (bp)
***IL1B* (12)**	Promoter-511	F:5`- TGGCATTGATCT GGT TCATC-3` R:5`- GTTTAGGAATCT TCCCACTT-3`	305
	Promoter -31	F:5`- AGTCCCCTCCCCTAAGAAGC-3` R:5`-TGTATTGGCTAGGAGAGCTGG-3`	499

Table (2): Components of optimized amplification reactions of IL1B regions.

Components	Concentration	Components of one sample (µl)	Components of 10 samples (µ)
D.W.	---	13	130
Go Taq® green master mix	1X	25	250
Forward primer	10 picomols/µl	1	10
Revers primer	10 picomols/µl	1	10
DNA Sample	100 ng/µl	10	*
Total volume	---	50	400

Results and Discussions:

Sampling step demonstrated that the keratoconus incidence is roughly equal in males and females as from the 50 patient there are 24 (48.6%) males and 26 (51.4%) female with higher frequencies at the ages (16 – 29 years). Two promoter regions (-511 and -31) in *IL1B* gene were amplified by PCR as shown in figures 1 and 2. Which were targeted for sequencing, the frequency of the genotypes and alleles in two patient and control groups were estimated according to Hardy-Weinberg equilibrium (HWE) that listed in Table (3 and 4).

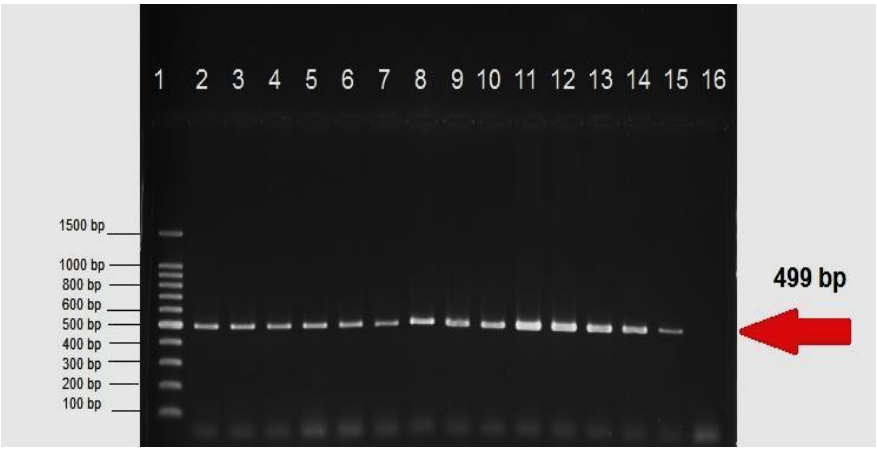

Figure (1): IL1B gene PCR product (-511 promoter) of 503 bp molecular size which is referred by the red arrow. Electrophoresis was applied as 2% agarose at 90 voltages (5 cm/min) for two hours followed by 30 minutes ethidium bromide staining and visualization with the aid of gel documentation system

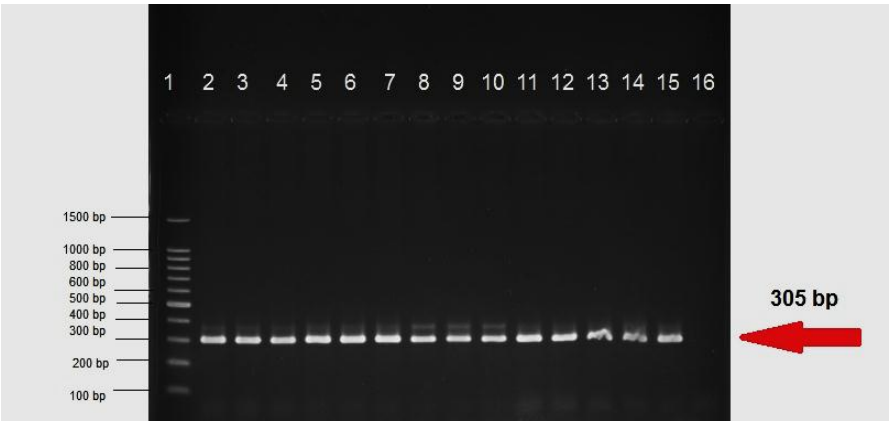

Figure (2): *IL1B* gene PCR product (-31 promoter) of 499 bp molecular size which is referred by the red arrow. Electrophoresis was applied as 2% agarose at 90 voltages (5 cm/min) for two hours followed by 30 minutes ethidium bromide staining and visualization with the aid of gel documentation system.

Table (3): Observed numbers and percentage frequencies in -511 *IL1B* alleles in keratoconus patients and controls.

Genotype	Patients No=50		Controls No=20		OR	95%CI	P value
	No.	%	No.	%			
CC	32	64	6	30	4.1481	1.3573-12.677	0.0126 *
CT	12	24	4	20	1.7895	0.4464-7.1732	NS
TT	6	12	10	50	0.1364	0.0401-0.4633	0.0014 **
Allele freq.							
C	76	76	16	40	4.7500	2.1741-10.377	0.0001 **
T	24	24	24	60	0.2105	0.0964-0.4600	0.0001 **
*** (P<0.05), **(P<0.0001) NS: Non-Significant.**							

P: Fishers exact propapility; CI: Confidence Interval; OR: Odds ratio

Table (4): Observed numbers and percentage frequencies in -31 *IL1B* alleles in keratoconus patients and controls.

Genotype	Patients No=50		Controls No=20		OR	95%CI	P value
	No.	%	No.	%			
TT	30	60	5	25	4.5000	1.4113-14.348	0.0110 *
TC	12	24	3	15	1.7895	0.4464-7.1732	NS
CC	8	16	12	60	0.1270	0.0394-0.4097	0.0006 **
Allele freq.							
T	72	72	13	32.5	5.3407	2.4173-11.799	0.0001 **
C	28	28	27	67.5	0.1872	0.0848-0.4137	0.0001 **
*** (P<0.05),**(P<0.0001) NS: Non-Significant.**							

P: Fishers exact propapility; CI: Confidence Interval; OR: Odds ratio

The C>T -511 promoter rs16944 was significantly different between patients and control group as the frequency of CC genotype was higher in patients than controls (64% versus 30%).As well as the C allele frequency (76% in patients, 40% in control group). The odds ratio was (4.1481and 4.7500) of CC genotype and C allele respectively approved its consideration as a risk factor to KC disease in Iraqi population. The CT genotype do not consider as a risk factor because it is not statistically significant to be associated with KC disease. Finally the TT genotype and T allele are recommended to be a protective factor against the disease. This is consistent with the results of other population studies suggested that the C>T -511 SNP was significantly associated with increased risk to KC disease such as: Korean population (11), Japanese population (13) and Chinese population (8).

The T>C -31 promoter rs1143627 was obviously different between control and patient groups as the frequency of TT genotype was presented in 60% of patients and 25% of controls and the frequency of CC genotype were 16% in patients versus 60% in controls, while the TC genotype was not significantly associated with KC disease. The P-value was lower than 0.01in other genotypes and allels which indicated that it's statically significance and the odds ratio were greater than 1 proposed that the odds of exposure among case-patients were greater than the odds of exposure among controls as a result the TT genotype and Tallele may be a risk factor for KC disease with the exception of CC genotype and C allele odd ratio was lower than 1, therefore; it might be a protective factor against KC disease in Iraqi population. This agreed with the result in Korean population (11), Japanese population (13) and in Chinese population (8). Another promoter A>T rs940780825 was found in some Keratoconus patients and controls thus it maybe correlated with the disease.

The Haplotype analysis of the block 1 SNPs (-31 and -511) were proved to be in complete linkage disequilibrium (LD). As the (D`=0.929, LOD=19.85 and r^2=0.696) indicated strong index of LD as in (Figure 3) (T>C) haplotype (84% in KC patients versus 35% in controls, OR 9.7500, 95% CI 2.9663-32.047 and P 0.0002). The presence of these two SNPs together were considered as a risk factor for KC disease as demonstrated in Table 5.

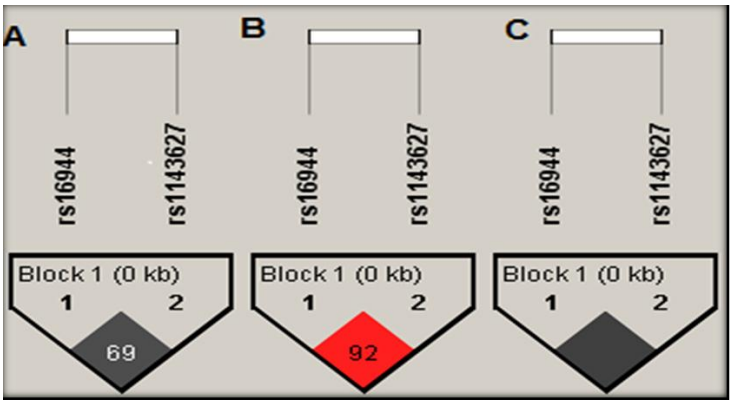

Figure (3): Haploview linkage disequilibrium (LD) plots for two promoter *IL1B* SNPs using three parameters: A.r^2 gray color B.D`and LOD red color C.CI dark gray color.

Table (5): *IL1B* rs1143627 (-31) and rs16944 (-511) Haplotype frequencies.

-31/-511 Haplotype	Patients No=50		Controls No=20		OR	95%CI	P value
	No.	%	No.	%			
TC	42	84	7	35	9.7500	2.9663-32.047	0.0002 *
CT	6	12	9	45	0.1667	0.0489-0.5681	0.0042*
TT	0	0	1	5	0.1287	0.0050-3.2965	NS
CC	2	4	3	15	0.236	0.0363-1.5362	NS
*** (P<0.05),**(P<0.0001) NS: Non-Significant.**							

P: Fishers exact propapility; CI: Confidence Interval; OR: Odds ratio.

SNPs -31 and -511 within the regulatory region (promoter) of the *IL1B* gene may influence its function. Thus protein expression was altered and it is reported to be associated with many diseases including keratoconus disease. Previous single SNP analysis is reported that either the -31 or -511 SNPs should affect the expression of the gene. This confirms the hypothesis that those two SNPs in complete linkage disequilibrium due to the fact of cis-interaction (14). The TC haplotype of -31 TATA box and -511 have been involved to increase *IL1B* gene expression and decrease *IL1A* expression. This may result in trigger extracellular matrix (ECM) apoptosis and degradation. TC haplotype was significantly increased in patients 84% compared to controls 35% thus it was proved to be a risk factor for keratoconus disease in Iraqi population while the CT haplotype was found in(12% patients and 45% in controls). In addition OR 0.1667 that confirmed its being a protective factor for keratoconus disease in Iraqi population those results consistent with the results of previous studies in Korean population (11), Japanese population (13) and Chinese population (8).

Conclusion:

Samples of the current study revealed that keratoconus incidence in Iraqi population were in the range of 11-41 years with the highest frequencies at the ages 16-29 years. -511 promoter C>T rs16944 and TATA box -31 T>C rs1143627 in *IL1B* gene are demonstrate to be a risk factor for keratoconus disease in Iraqi population. The T>C haplotype for the two *IL1B* -31 and -511 promoter SNPs are confirmed to be a risk factor for keratoconus disease in Iraqi population.

It was expected that the expression of *IL1B* may be enhanced due to the presence of T>C haplotype in the promoter region. Thus promote apoptosis consideres as a fundamental cause for keratoconus disease.

References:

1. Mohsin, T.M. and Hassan, Z.M. (2011). Results of corneal collagen cross-linking with riboflavin and ultraviolet-A light for patients with keratoconus in Ramadi City. Tikrit Journal of Pharmaceutical Sciences, 7(1): 83-89.

2. Partida, G.C. (2016). Complex trait genetics: mapping, correlation and causation. Phd Thesis. School of Medicine, University of Queensland, Australia

3. Bykhovskaya, Y.; Margines, B. and Rabinowitz, Y. S. (2016). Genetics in Keratoconus: where are we?. Eye and Vision Journal, 3:16.

4. Hodge, C. (2014). Keratoconus in the Australian Population: Characteristics, Diagnosis and Treatment. Phd Thesis. Department of Ophthalmology and Eye Health, Sydney Medical School, University of Sydney, Australia.

5. Koppen, C. (2012). Corneal Cross-Linking and Keratoconus: The Change in Treatment Paradigm of Keratoconus. Phd. Thesis. Medical Science, University of Antwerp, The Netherlands.

6. Kornman, K.S. (2006). Interleukin 1 genetics, inflammatory mechanisms, and nutrigenetic opportunities to modulate diseases of aging. The American Journal of Clinical Nutrition, 83(2): 475-483.

7. Nicklin, M.J.H.; Barton, J.L.; Nguyen, M.; FitzGerald, M.G.; Duff, G.W. and Kornman, K. (2002). A Sequence-Based Map of the Nine Genes of the Human Interleukin-1 Cluster. Genomics Journal, 79(5): 718-725.

8. Wang, Y.; Wei, W.; Zhang, C.; Zhang, X.; Liu, M.; Zhu, X. and Xu, K. (2015). Association of Interleukin-1 Gene Single Nucleotide Polymorphisms with Keratoconus in Chinese Han Population. Current Eye Research, 1–6.

9. September, A.V.; Nell, E.; O'Connell, K.; Cook, J.; Handley, C.J.; Merwe, L.V.; Schwellnus, M. and Collins, M. (2012). A pathway-based approach investigating the genes encoding interleukin-1β, interleukin-6 and the interleukin-1 receptor antagonist provides new insight into the genetic susceptibility of Achilles tendinopathy. Advance of the Print Journal.

10. Palamar, M.; Onay, H.; Ozdemir, T.R.; Arslan, E.; Egrilmez, S.; Ozkinay, F. and Yagci, A. (2014). Relationship Between IL1b-511C.T and ILRN VNTR Polymorphisms and Keratoconus. Cornea Journal, 33(2): 145-147.

11. Kim, S.; Mok, J.; Kim, H. and Joo, C.K. (2008). Association of −31T>C and −511 C>T polymorphisms in the interleukin 1 beta (IL1B) promoter in Korean keratoconus patients. Mol. Vis. J., 14: 2109–2116.

12. Bhata, I.A.; Naykoob, N. A.; Qasima, I.; Ganiec, F.A.; Yousufd, Q.; Bhate, B.A.; *et al.* (2014). Association of interleukin 1 beta (IL-1β) polymorphism with mRNA expression and risk of non-small cell lung cancer. Meta Gene Journal, 2: 123-133.

13. Mikami, T.; Meguro, A.; Teshigawara, T.; Takeuchi, M.; Uemoto, R.; Kawagoe, T.; *et al.* (2013). Interleukin 1 beta promoter polymorphism is associated with keratoconus in a Japanese population. Molecular Vision Journal, 19: 845-851.

14. Chen, H.; Wilkins, L.M.; Aziz, N.; Cannings, C.; Wyllie, D. H.; Bingle, C.; *et al.* (2006). Single nucleotide polymorphisms in the human interleukin-1B gene affect transcription according to haplotype context. Human Molecular Genetics, 15(4): 519-529.