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Original Article

Interleukin-8-251 A/T and CXCR2 +1208 C/T Genes Polymorphisms in Chronic Rhinosinusitis

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Abstract

Introduction:
IL-8 is one of the pro-inflammatory cytokines which can play an essential role in the pathogenesis of chronic rhinosinusitis (CRS) as well as nasal polyposis (NP). The ability of individuals in producing IL-8 is partially determined by IL-8-251 A/T polymorphism. Hence, the aim of the present study was to investigate the association between IL-8-251 A/T and CXCR2 +1208 C/T genes polymorphisms and susceptibility to CRS and NP.

Materials and Methods:
Two hundred and forty fiveCRS patients and 204 healthy controls were included in this study. CRS patients were categorized by the existence or absence of NP. IL-8 promoter-251 A/T and CXCR2 +1208 C/T gene polymorphisms were genotyped via the allele specific PCR (AS-PCR) method.

Results:
While no remarkable difference was demonstrated between patients and controls for both CXCR2 +1208 C/T and IL-8 -251 A/T polymorphisms, a significant increase in IL-8-251 AA genotype was detected in CRS patients with NP compared to those without it (29.3% and 16.2%, respectively; *P*=0.03). Interestingly, this association got far stronger when only non-asthmatic CRS patients were taken into consideration (*P*=0.001).

Conclusion:
The results of the present study indicate that the inheritance of IL-8-251 Aallele is associated significantly with NP development in CRS patients. Therefore, NP formation might be a result of the exposure to an intense inflammatory environment, which is more likely in genetically susceptible CRS patients.

Keywords:
Chronic rhinosinusitis, CXCR2, InterleukinL-8, Nasal polyposis, Polymorphism

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Introduction

Chronic rhinosinusitis (CRS) is one of the most common chronic inflammatory diseases affecting up to 15% of the adult population in the western world, Because of the magnitude of this problem, significant resources have been allocated to understand the molecular basis and pathophysiology of the disease. However, our knowledge regarding the role of inflammation and various inflammatory mediators in the pathogenesis of CRS is still rudimentary. CRS is defined as an inflammatory condition involving the paranasal sinuses and the lining of the nasal passages which persists longer than 12 weeks. Approximately 20% of patients with chronic rhinosinusitis have nasal polyposis (NP) (1,2). NP is also a chronic inflammatory disease of the paranasal sinus mucosa leading to a protrusion of benign edematous polyps from the middle meatus into the nasal cavities. It is assumed that production of chemokines by the epithelium could play a critical role in recruiting inflammatory cells to the sinus mucosa during the disease process. In this respect, several studies have shown that IL-8 levels are raised in NP in comparison to normal nasal mucosa (3,4,5). This chemokine was first characterized for its ability in recruitment and activation of neutrophils at inflammatory sites (6,7). IL-8 also promotes inflammatory processes by attracting some subsets of T lymphocytes (8) to the site of inflammation, inducing cytokine production (9) as well as releasing tissue damaging mediators by neutrophils (10). IL-8 exerts its biological functions through high affinity binding to a promiscuous receptor, CXCR2. CXCR2 is expressed on different cells including endothelial cells, eosinophils, neutrophils, macrophages and monocytes (11). Thus, IL-8 and its receptor may be involved in the pathogenesis of inflammatory diseases like CRS with NP or CRS without NP.

IL-8 gene is mapped on chromosome 4q12-q13 (12). Interestingly, the host ability in production of IL-8 can be controlled by the-251 A/T polymorphism in the promoter region of this chemokine gene (13). The A-allele in this single nucleotide polymorphism was found to be related to higher in vitro levels of IL-8 production after stimulation with lipopolysaccharide or cytokines such as IL-1 β and TNF-α (13). Several studies have shown the association of IL-8 -251 A/T polymorphism with different infectious, malignant and neurological diseases (13-16). Moreover; for IL-8 receptor (CXCR2) gene three single nucleotide polymorphisms at positions +785 (C/T), +1208 (T/C) and +1440 (G/A) were reported 17. The CXCR2 T +1208C is located in the non-coding region of CXCR2 gene and there are several reports indicating that T +1208C variant might provide valuable information forboth pathogenesis and susceptibility to chronic inflammatory diseases(17). Due to the critical role of IL-8 in inflammation and its probable role in the pathogenesis of CRS and NP, polymorphisms in either IL-8 or its receptor (CXCR2) might be linked to disease induction or progression. However, there has been no report on a possible association between the IL-8 -251A/Tand CXCR2 +1208 C/T gene polymorphism and different clinical forms of CRS, including CRS with and without NP. Therefore, the objective of this study was to investigate the effects of thetwo above mentioned polymorphisms on susceptibility toCRS and NP in Iranian patients.

Materials and Methods

Patients

Two hundred and forty five patients (102 females, 143 males) who underwent a functional endoscopic sinus surgery in Khalili hospital (Shiraz, Iran) entered the study. The diagnosis of CRS was based on nasal symptoms (e.g. nasal obstruction - congestion, facial pain-pressure-fullness,

nasal drainageand hyposmia-anosmia) not responding to the optimal medical treatment and lasting longer than 12 weeks. The diagnosis of CRS was also confirmed by sinus mucosal thickening on computed tomography scan while other suspicious sinusal diseaseswere ruled out. The presence or absence of protruding nasal polypsfrom the middle meatus was recorded on the basis of rigid nasal endoscopy. The patients with immunodeficiency, systemic diseases and neoplasia were excluded from the study. The extent of sinus disease was assessed for each patient by using the sinus CT staging system developed by Lund and Mackay (18). Moreover, any previous history of documented asthma was recordedby a single internist.

The control group consisted of 204 healthy volunteers (90 females and 114 males) who were recruited from Shiraz Blood Transfusion Organization after being screened by filling a designed questionnaire to ensure the absence of CRS.Patients and controls were residents of the same area (Fars province). The present study was approved by the local ethics committee of Shiraz University of Medical Sciences.

Determination of IL-8 and CXCR2 genotypes

Genomic DNA was extracted from peripheral blood leukocytes by a salting out method. An allele-specific oligonucleotide polymerase chain reaction (ASO-PCR) was usedto distinguish polymorphisms at location -251 of IL-8 and +1208 of CXCR2 genes. As an internal control, the β-globin specific primers were added in the ASO-PCR. For IL-8 genotyping, 10 µl of PCR reaction mixture consisting of 250 ng ofgenomic DNA, 200 µmol/L dNTPs, 2.25 mM $MgCl_2$, 1X Taq DNA polymerase buffer, 2units of Taq DNA polymerase (Bohringer Manheim, Germany), 10 pmol of each testprimer and 5 pmol of internal control primers were used. Then, it was followed by a touch-downprocedure which included 25 seconds at 95°C, annealing for 45 s at temperatures decreasing from 68°C (four cycles) to 61°C (20 cycles), and an extension step at 72°C for 40 s. The annealing temperature for the lasting 5 cycles was 58°C lasting for 40 s.

CXCR2 gene polymorphism was determined in the identical PCR reactionmixture except for the concentration of $MgCl_2$ which was 1.7 mM. In addition, the touch-downmethod was parallel to IL-8 genotyping with the exception of the annealing temperatures in the three consecutive steps that were 70°C, 65°C, and 55°C, respectively. The reaction productsof IL-8 and CXCR2 gene amplification were separated on 2.5% agarose gel and stainedwith ethidium bromide.

Statistical analysis

Data were analyzed by either the Chi-square or Fisher's exact test in the appropriate condition. All tests wereperformed two tailed with a confidence interval (CI) of 95%. In the same way, genotypefrequencies were compared within the patient series, stratified according to the presence or absence of NP or asthma,using the Chi-square analysis or Fisher's exact test.Statistical calculations were carriedout using SPSS version 11.5 and Epi Info 2000 statistical software packages.

Results

IL-8 -251 A/T and CXCR2 +1208 C/T polymorphisms were genotyped in CRS patients and controls. The genotype distributions of these polymorphisms are demonstrated in (Table 1). As shown in this table, no significant difference was detected for CXCR2 +1208

C/T polymorphism and IL-8 -251 A/T polymorphism in both patients and controls. However, when CRS patients were classified to patients with and without NP, a significant association was seen between the presence of high

producer IL-8 -251 AA genotype and the occurrence of NP in CRS patients (Table 1; 29.3% and 16.2%, respectively; *P*=0.03).

Table 1: Comparison of genotypes of IL-8 -251 A/T and CXCR2 +1208C/T gene polymorphisms between CRS patients and controls and also comparing CRS Patients with and without NP.

Genotypes	CRS patients		Controls	p1	p2	p3
	With NP *N* (%)	Without NP *N* (%)	*N* (%)			
IL-8 -251A/T						
AA	39(29.3)	16(16.2)	58(28.4)	0.2	0.3	
AT	57(42.9)	43(43.4)	70(34.3)			
TT	37(27.8)	40(40.4)	76(37.3)			
CXCR2 +1208 C/T						
CC	45(39.13)	31(37.8)	61(42.4)	0.33	0.5	
CT	58(50.43)	46(56.1)	64(44.4)			
TT	12(10.43)	5(6.1)	19(13.2)			

p1: Comparison between total CRS patients and controls.
p2: Comparison between CRS patients with NP and without NP.
P3: Comparison between CRS patients with NP and normal controls.

Moreover, when the CRS patients were categorized into asthmatic and non asthmatic groups (Table 2); the association between the existence of IL-8 -251 AA genotype and the occurrence of NP remained significant only in the non asthmatic group (*P*=0.001).

Table 2: Comparison of IL-8 -251 A/T genotype frequencies between NP^+ and NP^- patients after classification of CRS patients according to their asthma status.

Genotypes	CRS patients with asthma		CRS patients without asthma		p1	p2
	with NP *N* (%)	Without NP *N* (%)	With NP *N* (%)	Without NP *N* (%)		
IL-8 -251A/T						
AA	13(21.66)	6(31.6)	26(36.1)	10(12.7)	0.63	0.001
AT	28(46.66)	7(36.8)	29(40.3)	35(44.3)		
TT	19(31.66)	6(31.6)	17(23.6)	34(43)		

p1: Comparison of IL-8 polymorphism between NP^+ and NP^- groups in CRS patients with asthma.

p2: Comparison of IL-8 polymorphism between NP^+ and NP^- groups in CRS patients without asthma.

Interestingly, after stratification of patients according to the age of NP onset (Table 3), the frequency of IL-8 -251 AA genotype was significantly higher in CRS patients who developed NP under the age of 20 compared to those who presented NP after the age of 20 (58.9% versus 25%, *P*=0.017).

In marked contrastto IL-8 -251 A/T polymorphism, there existed no significant association between the CXCR2 +1208 C/T polymorphisms and the occurrence of NP in the whole CRS patients (*P*=0.5), CRS patients without asthma (p= 0.19) and also the development of NP before and after the age of 20, (*P*=0.45). In addition, like IL-8 -251 A/T (*P*=0.18), CXCR2 +1208 C/T polymorphisms did not show any significant association with the radiological Mackay score, (*P*=0.42).

Discussion

CRS is defined as a spectrum of disorders characterized by inflammation of the nasal mucosa and paranasal sinuses lasting longer than 12 weeks.CRS could be clinically classified as CRS with NP and CRS without NP. CRS with nasal polyposis which consists 20% of patients with CRS is characterized by massive tissue edema and is infiltrated by a variety of cells including eosinophils, neutrophils, lymphocytes, plasma cells and mast cells (14). While noticeable efforts have been made to understand the molecular basis and pathophysiology of CRS and NP, yet significant gaps in our knowledgeconcerning the role of inflammation and various inflammatory mediators exist.

IL-8 is the prototype of ELR^+ CXC chemokine which has a key role in inflammation via recruitment and activation of neutrophils and lymphocytes (6-8). It is also documented that inflammatory processes are promoted by IL-8 through inducing cytokine production (9) and release of mediators with tissue damaging activity by neutrophils (10). In this regard, the direct role of IL-8 in the

pathogenesis of NP is documented by several studies, whichclearly showsthat IL-8 levels are conspicuously higher in patients with NPas opposed to healthy volunteers (3-5). Among them, Kostamo et al. reported significant elevation of IL-8 and MMP-8 (collagenase-2) in NP patients unlike controls; proposing that IL-8 and MMP-8 form an inductive cytokine-proteinase cascade in NP pathogenesis (3). Therefore, it is not surprising that aside from their function in mediating the recruitment of leukocytes, IL-8 and CXCR2 system contribute to the pathogenesis of CRS with NP. As a matter of interest, the IL-8 -251 A/T polymorphism affects the host ability in IL-8 production and it has been shown that the A allele is associated with enhanced promoter activity (13). As a result, it could be concluded that individuals with higher ability in IL-8 production (carriers of A alleles) have a greater susceptibility for developing NP. For this reason, in the present study we investigated the association of IL-8 -251 A/T polymorphism and IL-8 receptor (CXCR2) +1208 C/T gene polymorphism with the occurrence of NP in a population of Iranian CRS patients.

Our results showed no differences in allele or genotype frequencies of IL-8 -251 A/T and CXCR2 +1208 C/T genes polymorphisms between CRS patients and normal controls (*P*=0.2 and P=0.33), indicating that IL-8 or CXCR2 genes polymorphisms do not predispose patients to CRS. However, a significant higher frequency of AA genotype (high IL-8 producing genotype) was detected in CRS patients with NP compared to non polyposis CRS patients (29.3% and 16.2%, respectively; *P*=0.03).

In other words, in the CRS patients with an AA genotype, 70.9% developed NP while only 29.1% did not develop this problem.

This finding is compatible with other studies that reported higher levels of IL-8 in patients with NP as opposed to controls (3-5). Accordingly, we hypothesize that the inheritance of high IL-8 producer allele (-251 A allele) causes higher inflammatory cells infiltration in the epithelium of paranasal sinuses of CRS patients which gradually leads to NP development.

On the other hand, there is a controversy in our results; although there is no significant difference between CRS patients with NP and normal controls but there is a significant difference between CRS patients with NP and non polyposis CRS patients (*P*=0.03). Therefore, we have explained this controversy as follows: IL-8 -251 A/T polymorphism per se does not precipitate in the development of NP in healthy sinuses; whereas if IL-8 -251 A/T polymorphism is activated by chronic inflammation in CRS patients, then the high IL-8 producing genotype predisposes the patient to NP. In simple words, CRS is mandatory for beginning the effect of IL-8 -251 A/T polymorphism in NP formation. Therefore, we modify our hypothesis in the way that, after CRS initiation the high IL-8 producer allele (-251 A allele) causes a bigger number of inflammatory cells infiltration in the epithelium of paranasal sinuses of CRS patients which gradually leads to NP development.

Moreover, the results of the present study showed a strong association between IL-8 -251 A/T polymorphism and formation of NP in non-asthmatic CRS patients (*P*=0.001), while this association did not remain significant for asthmatic CRS patients (*P*=0.63). Therefore, it might be concluded that other Th2-dependent pro-inflammatory cytokines (IL-4 and IL-5) play a role in the formation of NP in asthmatic CRS patients. In accordance to our findings, microarray analysis revealed over expression of IL-8 mRNA in non-eosinophilic CRS patients with NP(20).

In addition, when the CRS patients with NP were categorized according to their age, a significant association was observed between the inheritance of IL-8 -251 AA genotype and disease manifestation under the age of 20 (Table3; *P*=0.017). In addition, after classifying the patients with

NP into high (AA) and intermediate + low (AT + TT) IL-8 producer genotypes, a greater statistically significant association was obtained between the heritage of AA genotype and occurrence of the disease under the age of 20 (OR=4.29, 95% CI=1.34-13.95; *P*=0.008). This result could be explained by the higher tendency of CRS patients with IL-8-251 AA genotype to produce IL-8 and inflammatory responses that eventually lead to the development of NP in younger ages.

Table 3: Comparison of IL-8-251 A/T genotype frequencies between those over and under the age of 20 in CRS patients both with and without NP.

Genotypes	CRS patients with NP		CRS patients without NP		p1	p2
	Age<20 N (%)	Age>20 N (%)	Age<20 N (%)	Age>20*N* (%)		
IL-8 - 251A/T						
AA	10 (58.9)	29(25.0)	2(13.33)	13(15.8)	0.017	0.5
AT	4 (23.5)	53(45.7)	8(53.33)	35(42.7)		
TT	3(17.6)	34(29.3)	5(33.33)	34(41.5)		

p1: Comparison of IL-8 polymorphism in over and under 20-year-old CRS patients with NP.
p2: Comparison of IL-8 polymorphism in over and under 20-year-old CRS patients without NP.

Conclusion

The results of the present study show that after initiation of CRS, the host's ability in producing higher levels of IL-8 seems to be an important factor in the development of NP. Regarding the rarity of similar studies, further examination of this hypothesis in other populations is highly recommended.

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