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The role of cytokine gene single nucleotide polymorphism in the development of recurrent acute otitis media

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Abstract

The study aimed at examining the role of single nucleotide polymorphism (SNP) of cytokine genes in the development of recurrent acute otitis media (AOM) among children. Single nucleotide polymorphism of IFN-γ, IL-6, IL-10, TNF-α, and TGF-β1, were analyzed by the polymerase chain reaction with sequence-specific primers (PCR-SSP) in 82 children with recurrent AOM and compared with a similar control group. There was a significant higher incidence of IL-10 polymorphisms (loci -592, -819 and -1082) in children with recurrent AOM (*P*=0.0137, 0.0137 and 0.0072, respectively). However, there was no significant difference in the distribution of other cytokine genotypes between the two study groups. Among the 5 studied cytokine genes, only IL-10 loci showed significant correlation to the development of recurrent AOM.

Keywords: Cytokines; Gene polymorphism; Otitis media

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Introduction

Acute otitis media (AOM) is an acute inflammatory disease of the middle ear (ME) that results from the interaction between the host and other environmental and microbial factors. Recurrent AOM is defined as having at least three attacks of AOM in 6 months or having at least four attacks of AOM in 12 months.[1]

Children are more prone to recurrent episodes of acute otitis media. The prevalence of frequent ear infection in children was 6.6% according to a national survey, during 1997-2006, in the United States.[2]

There are many risk factors for recurrent AOM which may be either host related factors or environmental related factors such as tobacco smoke exposure, overcrowding and socioeconomic status. The role of genetic

factors in single episode of AOM may be less prevalent than recurrent AOM.[3]

There are many genes and gene variants, including genes encoding different inflammatory cytokines, that can predispose to recurrent AOM that have been determined by many animal and genetic studies and reviewed in literature.[4-8] Additionally, the heritability of otitis media and recurrent ear infection was evidenced by twin and triplet studies.[9]

Single nucleotide gene polymorphisms (SNPs) in genes encoding inflammatory cytokines was found to cause modulation of the production rate and the distribution pattern of inflammatory cytokines resulting in modulation of the innate immune response in case of AOM.[6]

Polymorphisms of IFN-γ, IL-6, IL-10, TNF-α, and TGF-β1 was found to be associated with recurrent AOM.[5,7,8,10-12] However, there was inconsistency in the results of the significance of SNP of these cytokine genes. The goal of the current study was to evaluate the significance of SNP of some cytokine genes in the development of recurrent AOM among Saudi population.

Materials and Methods

Settings

The study was conducted in Al-Lith Hospital, Saudi Arabia. The study protocol was reviewed and approved by the research ethics committee of Al-Lith Health Sciences College (REC 190003, 10/2019). A written informed consent was obtained from the parents of all involved children.

Study design

This is a retrospective study that included 142 children whose ages ranged from 1 to 7 years and have received their routine vaccination regularly. Exclusion criteria included presence of any serious disease or genetic syndromes, presence of chronic suppurative otitis media with or without cholesteatoma, and absence of well documented medical records. The involved children were divided into two groups: The first groups, recurrent AOM group, included 82 children who have a previous documented diagnosis of at least 3 episodes of AOM in 6 months period or at least 4 episodes of AOM in 12 months. The second group, a control group, included 60 children who have no previous history or documented diagnosis of recurrent episodes of AOM.

Sample collection and DNA extraction

Blood samples were withdrawn and stored at -70 until DNA extraction. DNA was extracted from blood samples using QIAamp DNA Blood Mini Kit (Qiagen NV, Venlo, Netherlands), according to the manufacturer's instructions.

Genotyping of Cytokine Genes

Polymorphisms of IFN-γ, IL-6, IL-10, TNF-α, and TGF-β1, were analyzed by the polymerase chain reaction with sequence-specific primers (PCR-SSP) method using Cytokine Genotyping Tray (One Lambda, Inc., Los Angeles, CA, USA).

For TNF-α, one polymorphism −308 G/A (rs1800629) at the promoter region was analyzed. For TGF-β1, two polymorphisms, codon 10 +869 T/C (rs1982073) and codon 25 +915 C/G (rs1800471), in the coding region were analyzed. For IL-10, three polymorphisms −1082 G/A (rs1800896), −819 C/T (rs1800871), and −592 C/A (rs1800872) in the promoter region were analyzed. For IL-6, one polymorphism, -174 G/C (rs1800795) in the promoter region was analyzed. Finally, for IFN-γ, one polymorphism, +874 T/A (rs2430561), in the coding region was analyzed. DNA fragments corresponding to each cytokine were amplified in accordance with the manufacturer's instructions.

Statistical analysis

All data were analyzed using SPSS version 14.0 for Windows (SPSS Inc., Chicago, IL; USA). The genotype distributions for the studied polymorphisms between study groups were statistically compared using Pearson's chi-squared test. Results were considered statistically significant when the probability of findings occurring by chance was less than 5% (P<0.05). Odds ratios (ORs) with 95% confidence intervals (CIs) were calculated for disease susceptibility.

Results

This study included 142 children, 82 in the OM prone group and 60 in the control group. The OM prone group contained 46 male (56.1%) and 36 female (43.9%) while the control group contained 25 males (41.7%) and 35 females (58.3%).

Samples from both groups were genotyped for cytokine genes polymorphism, the frequencies of genotypes were expressed as wild vs polymorphic. Polymorphic is defined as the genotype that contain at least one polymorphic allele.

The frequencies of genotypes for different analyzed cytokines are shown in Table 1. The distribution frequencies were not significantly different among study groups except for IL-10 -592, IL-10 -819, and IL-10 -1082 loci.

The odds ratios (OR) of being recurrent AOM, when carrying polymorphic genes, are shown in Figure 1. The highest OR was 2.556 (95% CI 1.28-5.103) when carrying IL10 (-1082) polymorphic allele and the lowest was 0.91 (95% CI 0.465-1.78) when carrying IFN-γ (+874) polymorphic allele.

Table 1. Frequencies of wild vs polymorphic genotypes for the OM prone group vs control group.

Cytokine	Otitis Media Prone Group n (%)	Control Group n (%)	Total n (%)	*P* Value
IL-6 (–174) Polymorphic	18 (22.0%)	10 (16.7%)	28 (19.7%)	
Wild (GG)	64 (78.0%)	50 (83.3%)	114 (80.3%)	NS
IL10 (-592) Polymorphic	43 (52.4%)	19 (31.7%)	62 (43.7%)	
Wild (CC)	39 (47.6%)	41 (68.3%)	80 (56.3%)	0.0137
IL10 (-819) Polymorphic	43 (52.4%)	19 (31.7%)	62 (43.7%)	
Wild (CC)	39 (47.6%)	41 (68.3%)	80 (56.3%)	0.0137
IL10 (-1082) Polymorphic	46 (56.1%)	20 (33.3%)	66 (46.5%)	
Wild (GG)	36 (43.9%)	40 (66.7%)	76 (53.5%)	0.0072
TNF-α (-308) Polymorphic	26 (31.7%)	16 (26.7%)	42 (29.6%)	
Wild (GG)	56 (68.3%)	44 (73.3%)	100 (70.4%)	NS
IFN-γ (+874) Polymorphic	35 (42.7%)	27 (45.0%)	62 (43.7%)	
Wild (AA)	47 (57.3%)	33 (55.0%)	80 (56.3%)	NS
TGF-β1 (+868) Polymorphic	42 (51.2%)	24 (40.0%)	66 (46.5%)	
Wild (CC)	40 (48.8%)	36 (60.0%)	76 (53.5%)	NS
TGF-β1 (+915) Polymorphic	11 (13.4%)	4 (6.7%)	15 (10.6%)	
Wild (GG)	71 (86.6%)	56 (93.3%)	127 (89.4%)	NS

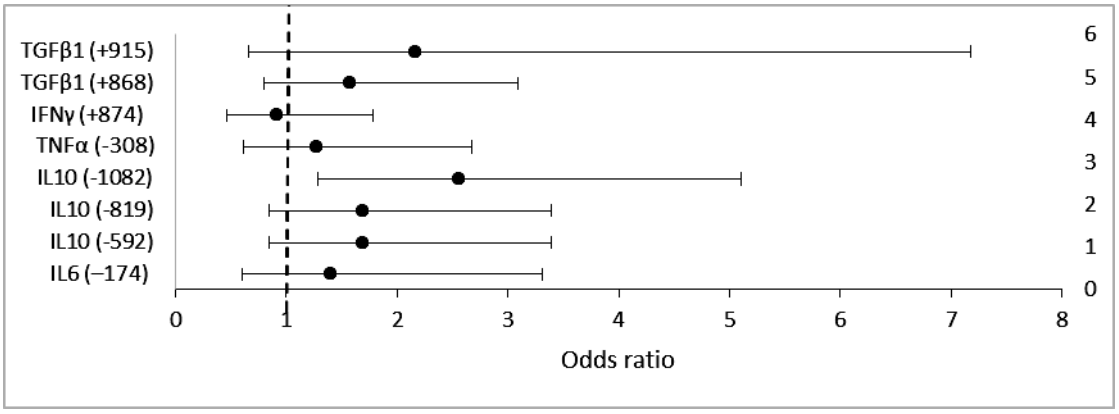

Figure 1. Odds ratio for being OM prone when carrying polymorphic genes.

Discussion

The pathogenesis of otitis media is multifactorial involving host, pathogen, and environmental factors. A genetic role is among well-established factors in susceptibility to recurrent AOM.[4]

Single nucleotide polymorphisms (SNPs) of different cytokine genes affect the liability for otitis media.[4,5,7,8,10,11,13] Additionally, it may influence the clinical outcome of respiratory tract inflammation in response to viral infection, such as respiratory syncytial virus and rhinovirus.[5,13] This effect may be caused by affection of the balance between pro-inflammatory and anti-inflammatory cytokines.[4]

Different SNPs were identified to be related to higher incidence of AOM. Moreover, there are racial and geographical variations among different population in the distribution of genetic polymorphisms.[14] In this study we evaluated the significance of eight different SNPs on the recurrence of AOM on Saudi population.

Otitis media is a broad term that includes many clinical conditions which differ in pathogenesis and risk factors. AOM is characterized by rapid onset of signs and symptoms of inflammation in the middle ear. Otitis media with effusion (OME) is defined as presence of middle ear effusion without signs and symptoms of acute ear infection. Chronic OME is considered if the middle ear effusion persisted for more than three months.[15]

The pathogenesis of early onset AOM is usually started by exposure to an infective agent[16] while Eustachian tube dysfunction plays an important role in the pathogenesis of OME.[15] Genetic factors have a higher significant influence on recurrent AOM rather than single episode of AOM.[3]

Some of the previous studies,[5,7,8,17] which were conducted on the influence of cytokines on otitis media, involved different clinical categories under one group which was named as otitis media prone group. Therefore, in our study, we focused on recurrent AOM as the main inclusion criteria for the case group.

Our results showed that polymorphism of IL-10 was linked to recurrent AOM, in all the three studied loci (-592, -892, and -1082), polymorphic alleles were found more frequently in recurrent AOM group 52.4%, 52.4% and 56.1% (*P*=0.0137, 0.0137 and 0.0072) respectively. Illia et al., 2014, found that polymorphic IL-10 (–592, –819 and –1082) genotypes were linked to the frequency of AOM episodes in addition to the rate of tympanostomy tube insertion and the age of onset of AOM.[10] However, Miljanović et al., 2016, found that only polymorphic genotypes IL10 -1082 GA and GG were more frequent in OM-prone children than in the control group.[8]

In our study, IL-6 (-174) polymorphic genotypes were not significantly frequent in recurrent AOM (*P* =0.43). However, Revai et al., 2009,[5] and Alper et al., 2009,[13] found that IL-6 (-174) polymorphic genotypes was linked to the risk of AOM[5] and the risk of OM coincidence with viral upper respiratory infections.[13]

Regarding IFN-γ (+874) polymorphism, our study found no significant difference in its

distribution between the two study groups (*P*=0.78). Insignificant role was also noticed in other studies.[10,13]

Our study found an insignificant role for SNPs in TGF-β1 codon 10 and codon 25 (*P*=0.186 and 0.196, respectively), although Ilia et al. (2014), found that only SNP in TGF-β1 codon 10 was significantly linked to the frequency of AOM episodes.[10]

TNFα is considered a strong inflammatory inducer that can induce otitis media with effusion regardless the infectious state of the middle ear. However, TNFα deficiency in mice was found associated with prolonged duration and persistence of the bacterial infection in middle ear.[18] Our study, as well as other previous studies.[7,8,10,11,13] found no significant correlation of SNP of TNFα gene and recurrence of AOM (*P*=0.52). Although, Ravi et al. (2009), documented that polymorphic TNFα (-308) was associated with increased risk for acute otitis media following URI.[5]

Our study, in comparison to other studies, was focused on one clinical condition, which is recurrent AOM, and raised the concern of the importance of IL10 polymorphism against other cytokines polymorphism in the pathogenesis of recurrent AOM especially in Saudi population. Further studies are needed to evaluate the relation of SNPs of different cytokine genes with the pattern of the immune response and the distribution of the inflammatory mediators in different types of viral and bacterial infection.

In conclusion, many cytokines may contribute to the pathogenesis of OM, however, among studied cytokines, our results showed significant contribution of IL-10 loci to risk for being prone to OM.

Author Contributions

Authors contributed in sample collection and processing, performing PCR, data collection and interpretation, writing the manuscript and revision.

Declaration of Conflicting Interests

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Ethical approval

The study protocol was reviewed and approved by the research ethics committee of Al-Lith Health Sciences College (REC 190003, 10/2019).

Informed consent

A written informed consent was obtained from the parents of all involved children.

References

1. McDonald S., Langton Hewer C.D., Nunez D.A. (2008). Grommets (ventilation tubes) for recurrent acute otitis media in children. *The Cochrane database of systematic reviews*(4):Cd004741.

2. Vakharia K.T., Shapiro N.L., Bhattacharyya N. (2010). Demographic disparities among children with frequent ear infections in the United States. *The Laryngoscope*;120(8):1667-70.

3. Casselbrant M.L., Mandel E.M. (2005). Genetic susceptibility to otitis media. *Current opinion in allergy and clinical immunology*;5(1):1-4.

4. Geng R., Wang Q., Chen E., et al. (2019). Current Understanding of Host Genetics of Otitis Media. *Front Genet*;10:1395.

5. Revai K., Patel J.A., Grady J.J., et al. (2009). Association between Cytokine Gene Polymorphisms and Risk for Upper Respiratory Tract Infection and Acute Otitis Media. *Clinical Infectious Diseases*;49(2):257-61.

6. Patel J.A., Nair S., Revai K., et al. (2009). Nasopharyngeal acute phase cytokines in viral upper respiratory infection: impact on acute otitis media in children. *Pediatr Infect Dis J*;28(11):1002-7.

7. Nokso-Koivisto J., Chonmaitree T., Jennings K., et al. (2014). Polymorphisms of immunity genes and susceptibility to otitis media in children. *PloS one*;9(4):e93930.

8. Miljanović O., Cikota-Aleksić B., Likić D., et al. (2016). Association of cytokine gene polymorphisms and risk factors with otitis media proneness in children. *European journal of pediatrics*;175(6):809-15.

9. Casselbrant M.L., Mandel E.M., Fall P.A., et al. (1999). The heritability of otitis media: a twin and triplet study. *Jama*;282(22):2125-30.

10. Ilia S., Goulielmos G.N., Samonis G., et al. (2014). Polymorphisms in IL-6, IL-10, TNF-α, IFN-γ and TGF-β1 Genes and Susceptibility to Acute Otitis Media in Early Infancy. *The Pediatric Infectious Disease Journal*;33(5).

11. McCormick D.P., Grady J.J., Diego A., et al. (2011). Acute otitis media severity: association with cytokine gene polymorphisms and other risk factors. *International journal of pediatric otorhinolaryngology*;75(5):708-12.

12. Tsujimoto S., Ozaki Y., Ito T., et al. (2021). Usefulness of Cytokine Gene Polymorphisms for the Therapeutic Choice in Japanese Patients with Rheumatoid Arthritis. *International journal of general medicine*;14:131-39.

13. Alper C.M., Winther B., Hendley J.O., et al. (2009). Cytokine polymorphisms predict the frequency of otitis media as a complication of rhinovirus and RSV infections in children. *European archives of oto-rhino-laryngology : official journal of the European Federation of Oto-Rhino-Laryngological Societies (EUFOS) : affiliated with the German Society for Oto-Rhino-Laryngology - Head and Neck Surgery*;266(2):199-205.

14. Huang T., Shu Y., Cai Y.D. (2015). Genetic differences among ethnic groups. *BMC Genomics*;16:1093.

15. Rosenfeld R.M., Shin J.J., Schwartz S.R., et al. (2016). Clinical Practice Guideline: Otitis Media with Effusion (Update). *Otolaryngology--head and neck surgery : official journal of American Academy of Otolaryngology-Head and Neck Surgery*;154(1 Suppl):S1-s41.

16. Schilder A.G., Chonmaitree T., Cripps A.W., et al. (2016). Otitis media. *Nature reviews Disease primers*;2(1):16063.

17. Morris M.C., Almudevar A.L., Casey J.R., et al. (2015). Familial and microbiological contribution to the otitis-prone condition. *International journal of pediatric otorhinolaryngology*;79(12):2174-77.

18. Wine T.M., Alper C.M. (2012). Cytokine Responses in the Common Cold and Otitis Media. *Current Allergy and Asthma Reports*;12(6):574-81.