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Original article

The role of Mannose-binding lectin 2 (MBL2) gene polymorphisms in adenoid hypertrophy among young children

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ABSTRACT

Objective: Is to determine the role of Mannose binding lectin (MBL) 2 (SNP 49 C/T rs#5030737) gene polymorphism among patients with adenoidal hypertrophy in Iraqi population.
Methods: From July through December 2015, a total of 60 adenoid hypertrophy (study group) young child patients (35 males and 25 females) with an age between (4 and 12) years old, were enrolled in this study according to selection criteria. A second group of otherwise healthy young child who did not have any symptoms or signs of adenoid hypertrophy were considered to be a control group. Confirmation of adenoid hypertrophy was achieved by: clinical examination, radiological assessment of postnasal space and an endoscopic nasopharyngoscopy. Blood samples were collected from both groups and genotyping of MBL-2 gene polymorphism was performed using traditional PCR and allele-specific technique.
Results: MBL2 gene polymorphism and allele frequencies among adenoid hypertrophy patients and their control were studied and the results showed that CC = 40 (66.7%), TT = 13(21.7%), and CT = 7 (11.6) in study group, while in control group CC = 5(8.3), TT = 9 (15%).CT = 46 (67.7%). The P-value of genotypes (CC, TT and CT) in study groups versus the control group were $P < 0.001$ (highly significant), $P = 0.435$ (non-significant) and $P < 0.001$ (highly significant) respectively.
Conclusion: The difference between the MBL2 (SNP 49 C/T rs#5030737) gene polymorphism among adenoid hypertrophy patients and healthy people may indicate it could be used as an early predictive factor for children whom will be prone to adenoid hypertrophy. The genotype CC could be considered as a risk factor while CT genotype could be considered a protective factor against adenoid hypertrophy in the current study. A further study needed to evaluate the use of the above mentioned polymorphism as a prognostic value for adenoid hypertrophy.

1. Introduction

The Adenoids, a group of lymphoid tissues in nasopharynx becomes apparent clinically when they undergo hyperplasia.[1] Adenoidal hypertrophy producing nasal airway obstruction remains one of the most frequent indications for surgery in children ,[2] otherwise may cause mouth breathing during sleep, snoring, anterior and posterior nasal discharge, cough, speech disturbances, behavioral disturbances, lethargy and oro-facial development disorders.[3] Chronic sinusitis, recurrent otitis media with effusion, and chronic serous otitis media associated with pediatric adenoidal hypertrophy are common indications for surgical removal of adenoid.[4–6] Adenoidal hypertrophy and its measurement by clinical examination, imaging techniques, and endoscopic evaluation has been reported.[7] Lateral airway radiographs have long been used as a diagnostic tool in the assessment of adenoid size.[8] Although each of these approaches; (patient symptoms, lateral airway neck radiography, and endoscopy) have their merits, currently none of them has been accepted by the majority of clinicians as standard practice.[9] Hence using the 3 methods together for confirming Adenoid hyperatrophy could be a wise manner for a perfect diagnosis.

As adjunctive treatments of chronic sinusitis and chronic otitis media, nonsurgical alternatives for reduction of adenoid size are limited. Several days of oral steroids will produce a prompt, but temporary, reduction in adenoid size. More commonly, medical management is indirect, treating concurrent infections and the complications of adenoidal enlargement.[5] Mannose-binding lectin

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Table 1
Primers sequences, PCR conditions, length of PCR products.

SNPs	Primers sequences	PCR Conditions	Size of PCR Products digestion products
MBL2gene (SNP 49 C/T rs#5030737)	**C-allele specific primer:** **F1:** 5-TTCCCAGGCAAAGATGGG**C**-3 **T-allele specific primer:** **F2:** 5-TTCCCAGGCAAAGATGGG**T**-3 **Common reverse primer:** 5-CTGGGCTGGCAAGACAACTA-3	**An initial denaturation** at 95oC for 5 min – Then, **30 cycles** each cycle consisted of denaturation at 94 °C for 60 s, annealing at 60 °C for 30 s and extension at 72 °C for 30 s. – **A final extension** at 72 °C for 10 min.	**Allele C:** 251 bp **Allele T:** 251 bp

(MBL) is the first component of the complement lectin pathway and an acute-phase reactant secreted by the liver.[9] It is encoded in humans by a single functional gene (MBL2) at chromosome 10q11.2–q21.[10] Low concentration and insufficiency of MBL are caused by polymorphisms in codons 52 (CGT → TGT; designated D allele), 54 (GGC → GAC; B) and 57 (GGA → GAA; C) in exon 1 of the structural MBL2 gene, which result in amino acid substitutions Arg → Cys, Gly → Asp and Gly → Glu in the peptide, respectively.[11,12]

The aim of this study was to examine the role of Mannose-binding lectin 2 (MBL2) gene polymorphism rs5030737 which is a C > T transition at codons 52 (CGT > TGT) in genetic susceptibility for adenoid hypertrophy in a sample of Iraqis patients.

2. Patient and methods

A prospective study was conducted in AL-Karama Teaching Hospital/College of Medicine/University of Wasit / Iraq. A total of 60 adenoid hypertrophy (group A) young child patients (35 males and 25 females) with an age between (4 and 12) years old, were enrolled in this study. A second group of otherwise healthy young child who did not have any symptoms or signs of adenoid hypertrophy were considered to be a control group (group B).

Inclusion criteria was: a patient with symptoms and signs of adenoid hypertrophy with a history of nasal obstruction symptoms for at least 3 months prior to presentation, snoring with or without sleep apnea. Confirmation of adenoid hypertrophy was achieved by: clinical examination, radiological assessment of postnasal space and an endoscopic nasopharyngoscopy. Exclusion criteria were: Patients with obstruction due to septal deviation, concomitant rhinosinusitis, uncontrolled allergic rhinitis, or palatine tonsilar hypertrophy.

Informed consent was obtained from the parents, and parents were present at the time of examination of their children.

A lateral nasopharyngeal X-ray was taken in all patients in an erect position, with the mouth closed and slightly extended neck. The films were then assessed by a radiologist who was blinded to the physical and endoscopic findings.

Final confirmation of adenoid hypertrophy was done by endoscopic nasopharyngoscopy through using 2.7 mm flexible video nasopharyngoscope, (Italian, Euro-clinic company 2012) Topical anesthesia in the form of a mixture of lidocaine/phenylephrine was applied prior to endoscopic examination. Patients were asked to sit in a semi-sitting position. After all the above assessments, patients with moderate - severe hypertrophy were included in our study.

3. Blood sampling

One ml of venous blood was collected in EDTA tubes from each individual (patient or healthy control) and was stored as whole blood at −20 °C for subsequent DNA isolation. Genomic DNA was isolated from whole blood according to Sambrook et al.[13]

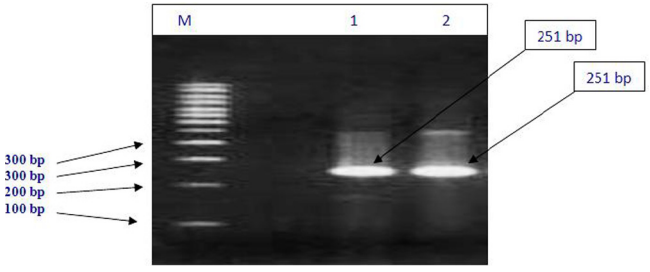

Fig. 1. 2% agarose gel electrophoresis for allele specific PCR for MBL2gene (SNP 49 C/T rs#5030737) M: 100 bp DNA ladder from GeneDireX®. Lanes 1 and 2: PCR products upon using allele specific C primer and allele specific T primer, respectively. Heterozygous genotype will give positive reaction upon using both allele specific primers. However, homozygous genotype will give positive reaction upon using only one of these allele specific primers.

4. Genotyping of MBL-2 gene polymorphism

One SNP (C/T rs#5030737 transition at codons 52 CGT > TGT) in MBL2gene was genotyped among the participants groups in this study. The MBL2 C/T polymorphic region (rs#5030737) was amplified by polymerase chain reaction (PCR) using allele specific PCR technique as shown in Table 1. Three primers (two allele specific primers and common reverse primer) were designed based on the nucleotide sequence of a partial fragment (retrieved from the online dbSNP) of the gene containing the target SNP. The polymorphism was visualized by separating the DNA fragments in a 2% agarose gel that was stained with ethidium bromide and illuminated by UV to validate the PCR- allele specific results as shown in the Fig. 1. All primers used in this study were newly designed using Primer Blast online programme (http://www.ncbi.nlm.nih.gov/tools/primer-blast/).

5. Statistical analysis of data

Statistical analysis of data was done to correlate genotype distribution and allele frequencies were performed by SPSS package version 17. The frequencies of alleles, genotypes in different groups were compared using the Chi-squared test (X2), *t*-test were used to test the significance of results of quantitative variables. Odds ratio and 95% confidence interval (95% CIs) were calculated for different studied parameters. The confidence interval (CI) at 95% was used to describe the amount of uncertainty associated with the samples.[14,15] The significance of the results was taken at the $P < 0.05$ level of significance.

6. Results

The mean ± SD age of cases were, (10.5000 ± 6.79606), also the mean ± SD age of healthy individuals were (31.0167 ± 11.30845).

MBL2 gene polymorphism and allele frequencies among adenoid hypertrophy patients and their control were studied and the