The Egyptian Journal of Medical Human Genetics (2014) 15, 31–38

Ain Shams University

The Egyptian Journal of Medical Human Genetics

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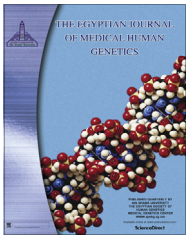

ORIGINAL ARTICLE

Genotyping of mannose-binding lectin (MBL2) codon 54 and promoter alleles in Egyptian infants with acute respiratory tract infections

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Received 10 September 2013; accepted 27 October 2013
Available online 17 November 2013

KEYWORDS

Mannose binding lectin;
Respiratory distress;
MBL2 gene;
Polymorphism

Abstract *Background:* MBL2 gene polymorphisms affect serum concentration of mannose-binding lectin and are associated with infectious conditions. Acute respiratory tract infections are among the most prevalent infections in childhood with the highest incidence among children younger than 2 years. This study aimed at correlation between the occurrence of acute respiratory tract infections and the prevalence of MBL2 gene codon (54) and promoter variants among the Egyptian infants in the study.

Subjects and methods: This case-control study included 25 neonates (0.21 ± 0.19 months), 25 infants (9.65 ± 8.5 months) with acute respiratory tract infection and normal control group. CBC, CRP and chest X-ray were done. DNA was extracted from peripheral blood. Genotypes of MBL gene codon 54-exon 1(G54D) were identified by PCR-RFLP analysis. MBL2 promoter genotyping was performed by allele-specific polymorphisms at −550 (H/L) and −221(X/Y).

Results: Incidence of LX promoter haplotype among the patients was (58%) ($p < 0.05$). Homozygosity for codon (54) allele A (high expression activity) among patients was (72%) ($p > 0.05$). Heterozygote codon 54 A/B genotype appeared more in patients (18%) ($p < 0.05$). Mutant genotype (too low expression activity) was more in patients but the difference was insignificant. Collectively the mutant allele (glycine to aspartic acid, allele B) appeared in 28% of patients compared to 20% in control ($p > 0.05$). YA/XA heterozygote promoter genotype was more prevalent among patients group (44%) ($p < 0.05$). Low-expression promoters (XA/B) and (B/B) appeared more in the patients (20%) compared to (12%) among control group ($p > 0.05$). Among ICU

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Peer review under responsibility of Ain Shams University.

Production and hosting by Elsevier

http://dx.doi.org/10.1016/j.ejmhg.2013.10.002

neonates, LX promoter was the most prevalent among all grades of respiratory distress (39.13%) followed by LY allele (34.78%). In the infants group, LY allele was (52.1%) with equal distribution of LY and HY (23.91% each).

Conclusion: Although there is a significantly increased incidence of LX promoter coding for low serum MBL concentrations among the ARTI patients; the YA/XA heterozygote promoter genotype was more prevalent over the homozygote mutant genotype. Also, the heterozygote codon 54 A/B genotype was more prevalent in the group of patients compared to the control. This may be an example of heterosis (heterozygote advantage) which may support the concept of balanced polymorphism.

1. Introduction

Many risk factors have been identified to contribute to the occurrence of respiratory tract infections; however, it is also possible that innate characteristics of the individuals such as genetic factors could play a role, and various attempts have been made to analyze the human genetic composition in relation to both infection susceptibility and development of clinical manifestations [1]. Mannose-Binding Lectin (MBL) is a serum protein [2] and believed to be particularly important in the early stages of primary infections in infants during the decay of maternal antibodies [3] The MBL is known to be an important component of innate immunity toward microbes by activating complement and augmenting opsonization and phagocytosis [4]. MBL is also known to play a role in enhancing attachment, ingestion and killing of opsonized pathogens by phagocytes [5] and activation of complement system through the MBL-associates serine protease [6]. There is evidence that the risk of developing bacteremia might be genetically modulated [7].

The susceptibility to *Wuchereria bancrofti* infection also appears to be significantly affected by the MBL expression genotype of the host [8]. Trans-racial studies have looked at the association between the status of MBL protein production, the MBL genotype and the clinical phenotype [9]. A single gene, *MBL2* located at chromosome 10, codes for human MBL and exerts its action through binding to high mannose and N-acetyl glucosamine oligosaccharides present on various micro-organisms [10].

The present study aimed at characterization of the structural alleles of *MBL2* gene located on chromosome 10 and trying to make a correlation between genotyping of MBL2-codon 54 and promoter alleles with the occurrence of acute respiratory tract infections in Egyptian infants in addition; trying to find out the most prevalent *MBL2* variant promoter alleles among the Egyptian samples in study.

2. Subjects and methods

This case-control study included 25 neonates (mean age 0.21 ± 0.19 months) and 25 infants (mean age 9.65 ± 8.5 months) (males to females ratio 3:1) with acute respiratory tract infection. Inclusion criteria included: full term infants, infants with pneumonia, sepsis and recurrent acute respiratory tract infections. Normal control group of healthy infants of matching age and sex was also included ($n = 25$).

The work is carried out in accordance with "The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments in Human." Also the work was carried out after taking approval of the parents and approval of the ethics committee of Ain Shams University.

All infants were subjected to full history taking, thorough clinical examination in addition to complete blood picture (CBC), C-reactive protein (CRP) and chest X-ray.

Genomic DNA extraction: DNA was extracted from peripheral blood leucocytes by spin column method of GeneJET™ Genomic DNA purification kit #K0722, Fermentas Life Sciences, Finland .The eluted DNA was stored at −20 °C till application.

Genotypes of MBL gene codon 54-exon 1(G54D) point mutations were identified by PCR using the following primers listed: Forward 5′-GTAGGACAGAGGGCATGCTC-3′. Reverse 5′-CAGGCAGTTTCCTCTGGAAGG-3′ [11,12]. Template DNA (500 ng) was amplified in the presence of 25 µl Green Taq master mix containing 4 mM $MgCl_2$, 0.4 mM for each of the dNTP (dATP, dCTP, dGTP and dTTP) and 2.5 U/µl Ampli *Taq* DNA polymerase, in addition to 1 µM for each of the forward and reverse primers and nuclease free water to 50 µl total volume. Analysis of the amplified products was done on agarose gel electrophoresis 1.5% to detect the corresponding amplified fragments. The PCR products were subsequently digested with the restriction enzyme *Ban*I (BshNI) FastDigest® (Fermentas Life Sciences, Cat#FD1004) which permits identification of the mutation through its unique cleavage site. The genotypes were determined by electrophoresis on 2% agarose gels stained with ethidium bromide. A fragment with the wild type (A) allele is cleaved into two bands (245 bp and 84 bp), while that with the mutant allele (B) shows one band (329 bp). Three patterns were determined 54 W/W (wild/wild), 54 W/m (wild/mutant), and 54 m/m (mutant/mutant).

Genotyping of MBL promoter variants was performed by allele specific PCR Polymorphisms in the promoter region of the gene, at −550 (H/L variants) and −221 (X/Y variants). Each primer ends with an allele specific base. LY, LX and HY promoter regions were amplified using the appropriate pairs of primers in parallel reactions by PCR. The genotypes were determined by electrophoresis on 2% agarose gels stained with ethidium bromide. The primer sequences were as follows: (1) HY promoter: Forward 5′ GCTTACCCAGGCAAGCCT GTG-3′. Reverse 5′-GGAAGACTATAAACA TGC TTT CC-3′. (2) LY promoter: Forward 5′-GCTTACCCAGGCAA GCCTGTC-3′. Reverse 5'-GGAAGACTATAA ACATG CT TTCC-3′. (3) LX promoter: Forward 5′ GCT TAC CCAGGC AAGCCTGTC-3′. Reverse 5′-GGAAGACTATAAACATGC TTTCG-3′. The PCR protocol was performed on thermal cycler HVD™, Austria as follows: Pre-PCR 94 °C/2 min.