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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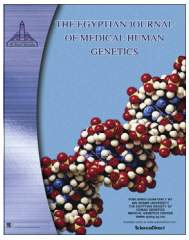

ORIGINAL ARTICLE

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

Rabah M. Shawky [a,*], Sherine M. Abd El-Fattah [a], Tarek M. Kamal [b], Mohammed A. Esa [a], Ghada H. El Nady [c]

[a] *Pediatrics Department, Faculty of Medicine, Ain Shams University, Egypt*
[b] *Medical Genetics Research Centre and Genetics Unit Children's Hospital, Ain Shams University, Egypt*
[c] *Ain Shams University Center for Genetic Engineering and Biotechnology and Medical Research Center, Egypt*

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

* Corresponding author. Tel.: +20 2 22585577.
E-mail address: shawkyrabah@yahoo.com (R.M. Shawky).
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.

Thermocycling for 30 cycles each consists of 94 °C/15 s – 58 °C/30 s – 72 °C/30 s, then final extension at 72 °C/3 min.

MBL alleles were analyzed as previously described by Madsen et al. [11] and Garred et al. [13] to detect the structural variant allele (*B*), and the normal allele (designated as *A*). All structural variant alleles including the variant allele (*B*) having a considerable effect on MBL concentrations can be grouped into one category (called allele *O*) (as sometimes referred to later in the discussion) [13]. The following MBL genotypes are to be expected: the *A*/*A* group, i.e. two normal structural alleles with high-expression promoter activity in position −221 (*YA*/*YA*), one high expression promoter and one low-expression promoter (*YA*/*XA*), or two low expression promoters (*XA*/*XA*); the *A*/*B* group, i.e. one variant structural allele (i.e. defective B allele) and one normal structural allele (A allele) combined with a high expression promoter (*YA*/*B*) or a low-expression promoter (*XA*/*B*); and the *B*/*B* group (=*O*/*O*), i.e. two defective structural alleles. The *X* allele is not carried on a haplotype containing structural *B* allele. The *A*/*B* individuals carrying the low-expression *X* promoter allele on the functional *A* chromosome have very low MBL levels in their serum, therefore, these patients were added to those homozygous for two defective structural alleles (*B*/*B*) into one group. The HY, LY and LX haplotypes are associated with high, medium and low serum MBL concentrations, respectively.

2.1. Statistical methodology

Data were expressed as means ± SD. Values for the measured parameters among studied and control groups were compared using ANOVA (with the application of Welch statistics). Analysis of data was done using SPSS VERSION 14.5. Unpaired *t*-test was used to compare two independent groups as regards a quantitative variable. Chi-square (χ^2) test was used to compare qualitative variables. Correlation (*r*-value) between variables was determined using Sperman's correlation and Karl Pearson's correlation co-efficient test.

3. Results

The ICU neonates group was associated with higher grades of respiratory distress (grades III and VI appeared in 92%) compared to infants group (60%) while grade II was evident in 40% of infants group compared to only 4% in ICU neonates ($p < 0.001$). The hemoglobin level and PNL were lower among infants group while lymphocytes level was higher than among the ICU neonates group. The difference was significant statistically. The C-reactive protein was positive in only 17 patients (34%) of our patients' sample ($p > 0.05$). All of the infants group showed abnormal X-ray findings especially exaggerated broncho-vascular markings (92%). Pneumonic patches were more evident among ICU neonates (24%) compared to (20%) of the infants group while 15 of the ICU neonates showed normal X-ray findings (Table 1).

There is a significantly increased incidence of LX promoter haplotype among the patients (58%) compared to control group (28%) ($p < 0.05$; $r = 0.912$).The most prevalent codon 54 genotype among both patients and control was wild/wild type with high expression activity (allele *A*) (72% and 76%, respectively; $p > 0.05$). The heterozygote W/M genotype appeared more in patients group (18%) ($p < 0.05$). On the other hand, the mutant genotype with too low expression activity appeared in (10%) of the patients compared to (16%) in control group but the difference was not significant statistically. Collectively, the mutant allele (glycine to aspartic

Table 1 Comparison between ICU neonates and infants group as regards respiratory distress grading, laboratory and radiological findings.

Parameter	ICU neonates	Infants	*p*-Value
(A) Respiratory distress			
1. Grade 0	1 (4%)	0	
2- Grade II	1 (4%)	10 (40%)	<0.001[a] (HS)
3. Grade III	14 (56%)	11 (44%)	
4. Grade IV	9 (36%)	4 (16%)	
(B) Laboratory findings	(means ± SD)	(means ± SD)	
1. Hb (gm/dl)	13.98 ± 2.53 (range: 9–17.9)	9.89 ± 2.52 (range: 2.6–17)	<0.001[a]
2. WBCs (×10^3/Ul)	11.53 ± 6.25 (range: 1.31–25.1)	8.82 ± 2.9 (range: 2.8–20)	>0.05[c]
3. PNL (%)	52.01 ± 13.86 (range: 66–77)	42.54 ± 15.48 (range 0–70)	<0.05[b]
4. Lymphocytes (%)	39.72 ± 13.68 (range: 16–67.8)	49.03 ± 12.7 (range: 22–69.9)	<0.05[b]
5. CRP			
(positive)	11 (44%)	6 (24%)	>0.05[c]
(negative)	14 (56%)	19 (76%)	
(C) X-ray findings			
1. Exaggerated bronchovascular markings	2 (8%)	23 (92%)	<0.001[a] (HS)
2. Pneumonic patches	6 (24%)	5 (20%)	
3. White lung	2 (8%)	0	
4. Collapsed lung	1 (4%)	0	
5. Normal findings	15 (60%)	0	

[a] Highly significant difference.
[b] Significant difference.
[c] Insignificant difference.

Table 2 *MBL2* gene promoters haplotyping and codon 54 polymorphisms among patients compared to control group.

Group	MBL2 promoters			MBL2 codon 54-RFLP		
	LX	HY	LY	W/W	M/M	W/M
Patients (n = 50)	29 (58%)	24 (48%)	41 (82%)	36 (72%)	5 (10%)	9 (18%)
Control (n = 25)	7 (28%)	9 (36%)	21 (84%)	19 (76%)	4 (16%)	1 (4%)
p-Value	<0.05[a]	>0.05[b]	>0.05[b]	>0.05[b]	>0.05[b]	<0.05[a]

W: wild type. M: mutant type.
[a] Significant.
[b] Insignificant difference.

Table 3 Genotyping of *MBL2* gene promoters among patients and control group.

Genotype	Patients			Control (*no* = 25)%	p-Value
	ICU neonates (*no* = 25)%	Infants (*no* = 25)%	Total (*no* = 50)%		
YA/YA	4 (16%)	9 (36%)	13 (26%)	17 (68%)	>0.05[b]
YA/XA	12 (48%)	10 (40%)	22 (44%)	2 (8%)	<0.05[a]
YA/B	1 (4%)	3 (12%)	4 (8%)	2 (8%)	>0.05[b]
XA/XA	1 (4%)	0 (0%)	1 (2%)	1 (4%)	>0.05[b]
B/B (or) XA/B	7 (28%)	3 (12%)	10 (20%)	3 (12%)	>0.05[b]

[a] Significant difference.
[b] Insignificant difference.

Table 4 Relationship between *MBL2* gene promoters and grade of respiratory distress.

Respiratory distress	MBL promoters				p-Value
	LX	HY	LY	Total alleles	
(1) ICU neonates					
Grade II	1 (2.17%)	1 (2.17%)	1 (2.17%)	3 (6.52%)	>0.05[a]
Grade III	9 (19.56%)	7 (15.21%)	9 (19.56%)	25 (54.34%)	
Grade IV	8 (17.39%)	4 (8.69%)	6 (13.04%)	18 (39.13%)	
Total alleles	18 (39.13%)	12 (20.08%)	16 (34.78%)	46 (100%)	
(2) Infants					
Grade II	4 (8.69%)	3 (6.52%)	10 (21.73%)	17 (36.95%)	>0.05[a]
Grade III	5 (10.86%)	5 (10.86%)	10 (21.73%)	20 (43.47%)	
Grade IV	2 (4.34%)	3 (6.52%)	4 (8.69%)	9 (19.56%)	
Total alleles	11 (23.91%)	11 (23.91%)	24 (52.17%)	46 (100%)	

[a] Insignificant difference.

acid, allele *B*) (=allele *O*) appeared in (28%) of patients compared to (20%) in control ($p > 0.05$; $r = 0.962$) (Table 2).

The YA/XA heterozygote genotype with one high expression promoter and one low-expression promoter was more prevalent among patients group (44%) ($p < 0.05$) while the YA/YA homozygote genotype (high-expression promoter activity) was the most prevalent among control group (68%) ($p > 0.05$). The low-expression promoter (*XA*/*B*) and the *B*/*B* group (i.e., two defective structural alleles) appeared more in the patients (20%) compared to (12%) control group, however, the difference was not statistically significant (Table 3). In the ICU neonates, the LX promoter was the most prevalent allele among all grades of respiratory distress (39.13%; $r = 0.894$) followed by LY allele (34.78%; $p > 0.05$). In the infants group, the LY allele was detected in a percentage of (52.1%; $r = -0.142$) with equal distribution of LX and HY (23.91% each; $p > 0.05$; $r = 0.866$) (Table 4). Respiratory distress grade III was the most prevalent among the ICU neonates (56%) followed by grade IV (36%) while in the infants group , grades II and III respiratory distress were more evident (44% and 40%, respectively). The wild type genotype for codon 54 was the highest among the ICU neonates and the infants group (64% and 76%, respectively; $p > 0.05$) followed by the heterozygote genotype (W/M) (24%) among ICU neonates ($r = -0.5$). In the infants group the mutant genotype was equally detected in a percentage of (12%). However, no statistically significant correlation was detected with genotypes of codon 54 ($r = 0.052$) (Table 5 and Fig. 1).

4. Discussion

Mannose-binding lectin (MBL) is one of the genetically determined factors that have been suggested to be involved in systemic inflammation and sepsis [14]. MBL concentration is highly dependent upon several promoter region

Table 5 Relationship between *MBL2* codon 54 genotypes and grade of respiratory distress.

Respiratory distress	RFLP genotypes of MBL2 codon 54				*p*-Value
	Wild (W)	Mutant (M)	W/M	Total genotypes	
(1) ICU neonates					
Grade II	0	0	1 (4%)	1 (4%)	>0.05[a]
Grade III	11 (44%)	1 (4%)	2 (8%)	14 (56%)	
Grade IV	5 (20%)	1 (4%)	3 (12%)	9 (36%)	
Total	16 (64%)	2 (8%)	6 (24%)	25 (100%)	
(2) Infants					
Grade II	8 (32%)	1 (4%)	1 (4%)	10 (40%)	>0.05[a]
Grade III	8 (32 %)	1 (4%)	2 (8%)	11 (44%)	
Grade IV	3 (12%)	1 (4%)	0	4 (16%)	
Total	19 (76%)	3 (12%)	3 (12%)	25 (100%)	

[a] Insignificant difference.

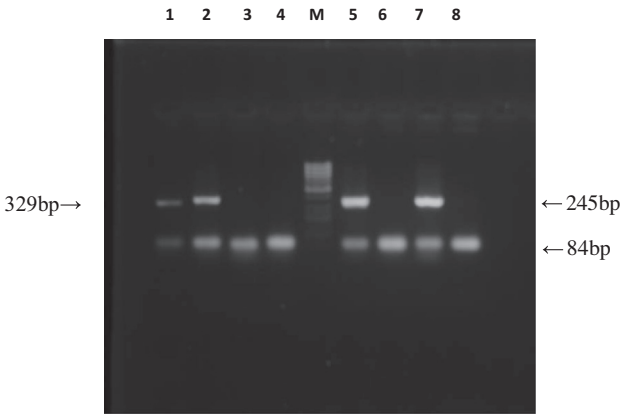

Figure 1 Lane no. 1: mutant allele CD 54 (329 bp). Lane no. 2: positive variant allele LX in the promoter of *MBL2* gene. Lanes no. 3 and 4: negative HY and LY variant alleles in the promoter of *MBL2* gene. M: molecular weight marker (50 bp). Lane no. 5: positive for HY variant allele in the promoter of *MBL2* gene. Lane no. 6: negative LX variant allele in the promoter of *MBL2* gene. Lane no. 7: wild type 54 allele (245 and 84 bp). Lane no. 8: negative LY variant allele in the promoter of *MBL2* gene.

polymorphisms of the MBL2 gene alleles: Y and X which are clinically the most important as well as polymorphisms in codon 54 in exon 1 [15].

The present study tried to make a correlation between the prevalence of *MBL2* allele genotypes and occurrence of acute respiratory tract infections in Egyptian infants and to find out the most prevalent *MBL2* variant alleles among the Egyptian samples in the study. We chose *MBL2* genotypes over MBL levels in serum, because serum MBL level can be influenced by infection, drugs, and hormones and that MBL serum levels strongly correlate with the *MBL2* polymorphism genotypes [16]. Yokota et al. [17] stated that genetic analyses are important since neonates with wild-type *MBL2* genotypes but low MBL levels at birth were able to obtain normal levels within time, in contrast to neonates with variant *MBL2* genotypes. The apparent serum concentration and complement-activating activity of MBL markedly depend on *MBL-2* gene point mutations in codons 52, 54, and 57 of exon 1 [18]. The present study concerned the G54D allele to be studied in Egyptian infant patients. This allele is the most prevalent among Caucasian populations [9]. The functionality of the *MBL2* exon 1 and promoter polymorphisms termed *Y/X*, has been well documented in Caucasian patients. Low serum levels of MBL due to polymorphisms in the MBL gene are found in 10% to 15% of white populations. [19,11,20]. In the present study, the mean age ranged from 0.21 ± 0.19 months in ICU neonates to 9.65 ± 8.5 months in infants. MBL is believed to be particularly important during the 'window of vulnerability' experienced by infants during the decay of maternal antibodies [9]. The presence of *MBL2* polymorphisms is associated with the increased risk of respiratory infections during early childhood, especially during the first 6–18 months of life when the adaptive immune system is immature [21,22]. The *MBL2* gene mutations occur about twice as frequently in pediatric patients with recurrent infections of the respiratory system [18]. Both heterozygosity and homozygosity for MBL mutations are important risk factors for acute respiratory infections [23,24] and seem to predispose to respiratory infections, bronchiolitis and more severe post bronchiolitis wheezing in full term infants before the age of 6 months [24,25]. There is a proved association between variant *MBL2* genotypes causing MBL deficiency at birth both with an increased risk of developing pneumonia and culture-proven sepsis during the first month of life [26].

In the present study, only the full term neonates and infants were selected. In neonates, low MBL levels are not only associated with variant *MBL2* genotype, but also with low gestational age [26–28]. The combination of prematurity and low MBL levels increases the risk of sepsis to 70% [29]. However, in a study on Egyptian preterm neonates [30], there was no statistical significant difference between preterm and full term groups regarding MBL levels.

In the present study, C-reactive protein was positive in 34% of the studied population (44% in ICU neonates and 24% in infants group). Abnormal chest X-ray findings were evident in the entire infants group (100%) while it was evident in only 40% of the ICU neonates group ($p < 0.001$). In agreement with our results, Castellano et al. [31] stated that no correlation was observed between the levels of MBL and CRP in either of the acute or recovery pneumonia phases. MBL does not act uniformly in all patients as an acute-phase reactant. There was an absence of parallelism between both of MBL protein and C-reactive protein. Interestingly, the patients with the

wild-type MBL genotype had a greater risk of developing bacteremia. Like an acute-phase protein, MBL serum levels may rise under stress [32–35].

In this study, *MBL2* promoter genotyping revealed a statistically significant increase in LX promoter among patients followed by LY and HY promoters compared to control group. The respiratory distress grade III was the most prevalent (54.4%) among all MBL promoter variants in ICU neonates followed by grade IV (39.1%). In the infants group, the LY promoter variant was more evident in patients with grade II and III equally, while HY and LX alleles were more evident in the patients with grade III respiratory distress. Promoter polymorphisms influence transcription activity and synthesis of MBL [35]. The HY, LY and LX haplotypes are associated with high, medium and low serum MBL concentrations, respectively. Koch et al. [22] suggested that approximately 70% of LX homozygotes might be considered MBL insufficient. MBL levels were reported in persons of the same genotype [11,20]. In contrast, the Y variant is associated with high MBL plasma levels. The presence of an increased LX promoter among patients of the present study is in agreement with Hellemann et al. [21] who stated that in particular, a base substitution at codon-221 (G to C; promoter allele X) is associated with a lower MBL serum concentration. MBL deficiency arising from mutations and promoter polymorphisms in the *MBL2* has been associated with increased risk, severity, and frequency of infections [13,36]. The strongest and most significant inverse correlations between serum MBL and respiratory disease were found in patients with grade III [18]. Each of the three variants reduces the amount of functional MBL subunits in heterozygous individuals 5- to 10-fold [37].

In the present study, MBL codon 54 wild type genotype (AA) was detected in 72% of the patients. The heterozygote genotype was more in the group of patients (18%) compared to 4% in the control ($p < 0.05$). The mutant genotype appeared equally in grades III and IV in both groups. There was no statistically significant relationship between MBL codon 54 genotype and the grades of respiratory distress in both of the ICU neonates and the infants group. This is in agreement with Kronborg et al. [38] who stated that in patients who have pneumococcal bacteremia, MBL genotypes do not differ from those of healthy control persons. It is possible that a putative effect of MBL is revealed only in patients with a concomitant disturbance in the immune system that exposes an MBL phenotype [39]. Also in agreement with our results, Frakking et al. [40] found that of the eleven studied neonates with a severe infection, five had variant, three had wild-type, and three had unknown *MBL2* genotypes. The homozygous B variant of the *MBL2* gene codon 54 and the down regulating promoter LX in combination with the heterozygous B variant were related to MBL deficiency [16]. There are positive findings between the MBL codon 54 variants and hospital admission in patients with chronic obstructive pulmonary disease (COPD) [41]. The XA/O (heterozygote) and O/O homozygote genotype-carrying individuals are considered MBL insufficient. Overall, MBL insufficiency (genotypes *XA/O* + O/O) was associated with a significant 2-fold increased respiratory rate in acute respiratory tract infections [22]. Summerfield et al. [42] found that the prevalence of variant alleles of the MBL gene in children with infections was twice that in children without infections. Mombo et al. [9] found that homozygosity for *MBL2* variant alleles (O/O) causing MBL structural defects was associated with the highest adjusted mortality rate followed by homozygosity for the normal *MBL2* allele (A/A) encoding high MBL levels, whereas heterozygous A/O patients had the most favorable outcome. The variant alleles in exon 1 and the *X* allele in the *MBL XY* polymorphism have been found to be associated with serum MBL deficiency especially in individuals homozygous for the variant alleles [32]. Individuals who are homozygous for the mutant MBL alleles display an increased susceptibility to infections while those who are heterozygous for MBL mutations are much less susceptible to infections than those who are homozygous for the wild-type MBL allele[9] .This protective effect contrasts with the increased susceptibility of homozygotes (for the mutant MBL alleles) to various infections. [43]. Helden et al. [44] stated that, subjects homozygous for the wild-type MBL allele display an increased risk of bacterial infections including tuberculosis. Patients homozygous for the variant *codon 54B* allele (*54BB*) had worse severity of illness on admission, greater likelihood of septic shock and increased odds of acute respiratory distress syndrome (ARDS) when compared with heterozygotes and homozygotes for the wild-type allele. On the other hand, no association was found between ARDS and the *MBL XY* allele [32].

In the present study, the appearance of heterozygosity in 18% of the studied patients group may support the concept of a balanced polymorphism (the deleterious effects of the mutant homozygotes in terms of high susceptibility to infections is outweighed by the advantageous effects of heterozygotes) which is probably applicable to the MBL gene, with an interesting extension toward homozygotes for the wild type alleles [9]. There are some discrepancies in the literature regarding the relative importance of homozygosity and heterozygosity for variant alleles. Only homozygosity for MBL mutant alleles predisposes to recurrent infections. However, heterozygosity as well as homozygosity increased susceptibility to meningococcal disease [45]. Heterogeneity for exon 1 mutations was significantly more prevalent in children (age 0–18 years) hospitalized for various infective diseases than in patients admitted with other diagnoses, but homozygotes presented as well [42]. Heterozygosity for *MBL2* alleles confers a protective effect whereas homozygosity is associated with the worst outcome soon after discharge from ICU. This may be an example of heterosis. Similarly, heterozygosity for the *MBL2* structural genotypes (A/O) was significantly associated with improved survival compared with the normal A/A genotype and the homozygous variant O/O genotype after follow-up period following admittance to the ICU [46]. Due to selective pressure promoting heterozygosity, a heterozygous advantage (heterosis) of the *MBL2*-variant alleles has been proposed [47]. Thus, the normal A allele may confer disadvantages to the host under some circumstances, such as sepsis. Although heterozygosity associated with a low MBL level showed an advantage for severity in the sepsis, there was no demonstrable influence on outcome [21]. Summerfield et al. [42] stated that increased susceptibility to infections was found in both heterozygotic and homozygotic children, but homozygotic children had more severe infections (including recurrent URTI). In the present study, the appearance of the wild type alleles in 72% while the heterozygote genotypes in an incidence of 18% is comparable with other studies [23,42] where children aged less than 2 years who were heterozygous or homozygous for variant alleles had an increased risk for acute respiratory infections.

5. Conclusion

In this study, we detected an increased incidence of LX promoter which codes for low serum MBL concentrations among the patients with acute respiratory tract infections. However, the prevalence of both YA/XA heterozygote promoter genotype together with the heterozygosity for codon 54 A/B genotype among patients over the homozygosity for the mutant genotypes may be an example of heterosis and heterozygote advantage which may support the concept of a balanced polymorphism.

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