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Polymorphisms and Serum Level of Mannose-Binding Lectin: An Iranian Survey

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ABSTRACT

Mannose-binding lectin (MBL) is a Ca^{+2} -dependent collagenous lectin, that is produced by liver and mediates innate immune responses by opsonization of pathogens. The serum level of MBL varies widely among healthy individuals, ranging from 0.05μg/ml (or lower) to over 5μg/ml, mainly depending on genetic variations.

This study has examined promoter and exon 1 of *mbl2* genotype among 117 Iranian healthy blood donors. MBL Single Nucleotide Polymorphisms (SNPs) were genotyped using polymerase chain reaction (PCR), and serum levels of MBL were quantified using a double-antibody enzyme linked immunosorbent assay (ELISA).

Results of this study showed that there are two promoter polymorphisms at -550 (H/L variants) and -221 (Y/X variants) positions, and three polymorphisms in exon 1 at codon 52 (D Allele), 54 (B Allele), and 57 (C Allele) in this population. B allele was significantly correlated with the lowest serum MBL level. Our results also showed that the most frequent genotype was HYA/LXA, and the genotype that associated with the highest serum level of MBL was HYA/HYA. The genotype that causes lowest MBL production in Iranian population was LYB/LXA.

These results showed some differences compared to that of the other populations. To verfiy the originality of these differences we may need to extend the study to a larger samples of respective populations; meanwhile the importance of a new mutation, nucleotide 101 of MBL2 exon1, reported in the current study should be taken in considerations in terms of its possible pathobiological effects in following studies.

Keywords: Complement System; Haplotype; Innate immunity; Mannose-binding lectin; Polymorphisms; Single Nucleotide Polymorphism (SNP)

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INTRODUCTION

Mannose Binding Lectin (MBL) is a vital factor in the triggering of the innate immunity.[1,2]

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MBL belongs to the C-type lectin superfamily; its function appears to be a pattern recognition receptor at the first line of innate immune defense.[3,4] MBL recognizes carbohydrate patterns, found on the surface of a wide number of pathogenic microorganisms. Binding of MBL to a microorganism leads to the activation of the Lectin Pathway (LP) of the complement system.[2,3,5] MBL has an oligomer structure (400-700kDa), consisting of subunits which contain three identical peptide chains. MBL can acquire several oligomer structures; however, for the activation of the complement, a tetramer form is required. Following the pathogen binding, MBL takes a conformational change and associates with some molecules such as MBL-Associated Serine Proteases and then takes part in initiation of complement activation.[6-8]

MBL is an acute phase protein synthesized by hepatocytes.[3] After parturition, the MBL concentration will rise from 1000ng/ml to 2500ng/ml within few weeks, and there after falls to a level of 1700ng/ml in adults. The incidence of MBL deficiency is more common compared to deficiency in other components of complement system.[9] MBL deficiency is associated with increased susceptibility to infections, such as otitis media, pneumonia and sepsis; due to these clinical implications, its analysis and study, especially in recurrent infections, is very important.[9,10]

The gene encoding MBL, *mbl2,* is located on chromosome 10 at q11.2-q21.[8] The serum level of MBL varies highly among individuals, ranging from 50µg/L to over 5000µg/L, mainly due to genetic variations in the respective gene.[1] Low serum level of MBL has been reported to be associated with the polymorphisms found in both coding and promoter regions. Three mutations have been found along the exon 1 of MBL molecule at codons 52, 54, 57, leading to three allelic variant called D, B and C, respectively, and the wild type allele is called A.[8] D, B and C mutations occur at nucleotides 223 (C to T), 230 (G to A) and 239 (G to A) of exon 1, respectively. These mutations bring about the substitution of arginine by cysteine at codon 52, the substitution of glycine by aspartic acid at codon 54, and the substitutions of glycine by glutamic acid at codon 57, respectively.[8,11]

Polymorphic positions are also reported at promoter and 5´-untranslated regions of the *MBL2* gene. Single nucleotide polymorphisms (SNPs) are present at positions -550 (G to C, alleles H/L), -221 (G to C, alleles X/Y) and +4 position of the 5´-untranslated region (C to T, P/Q alleles) (Figure 1). Strong linkage disequilibrium exists between polymorphic positions at the promoter and exon 1 of the *mbl2* gene, though only 7 of 64 possible haplotypes, HYPA, LXPA, HYPD, LYQA, LYPA, LYPB and LYQ have been reported so far.[2,8]

The aim of this study was to search for MBL polymorphisms in healthy Iranian population and their possible relations to MBL serum level.

MATERIALS AND METHODS

Population

The population under study consisted of 117 adult Iranian healthy volunteers as blood donors. All subjects gave informed consent for participating in scientific studies. The blood samples were obtained from Tehran Blood Transfusion Organization which is dedicated to collect and store blood samples for applied purposes throughout country. Each sample, 10 ml. of whole blood, was collected in two tubes. One part in Ethylene Diamine Tetra Acetic acid (EDTA) which was used for genomic DNA extraction, and the second part collected in the tube contained silicone gel and used for serum separation. The serum was separated by centrifugation at 3000 rpm for 5 minutes. Donors' sera were stored at -80°C and the MBL level was quantified using a double-antibody Enzyme Linked Immunosorbent Assay (ELISA).

DNA Extraction and Genotyping of *MBL2*

DNA samples were extracted by salting-out method, and amplified by Polymerase Chain Reaction (PCR). The sequences of PCR primers used in *MBL* genotyping are shown in table 1. PCR reactions were performed in a volume of 20 µl, containing 1 µl (2.1 µg/µl) of the genomic DNA, 0.7µl of each specific primer in the presence of 0.8 µl $MgCl_2$, 0.5 µl dNTP mixture, 2.5 µl of 10X reaction buffer, 0.2 µl of Taq polymerase (2U/µl) and 13.6 µl dH_2O.

All PCR sets were initiated by a 2-min denaturizing step at 95°C and completed by a 5-min extension step at 72°C. The temperature for different sets of PCR cycles were accomplished as follows: 35 cycles of 30-second denaturation at 95°C, 30-second annealing at 60°C followed by 30-second extension at 72°C. Annealing temperatures are described in table 1 The PCR products were sequenced (MBL exon 1 and promoter) by Macrogen Inc. Soul, South Korea.

Table 1. PCR primers used in *MBL* genotyping

PCR Primers	Polymorphism	SNP	Left Primer	Right Primer	Product Size	Annealing Temperature
Set 1	Promoter	-550	TTCCTGCCAGAA AGTAGAGAGG	TGGTGATCCAAAGA GGAAGG	193	61°C
Set 2			CCTTCCTCTTTG GATCACCA	GCTGGCTTATGCCT GTTAGC	213	60°C
Set 3		-221	GGAATCAGCTG CCCAGATAC	GAGCATGCCCTCTG TCCTAG	399	62°C
Set 4	Exon 1		CCAGGGATGGG TCATCTATTT	ACAGAACAGCCCA ACACGTA	336	58°C

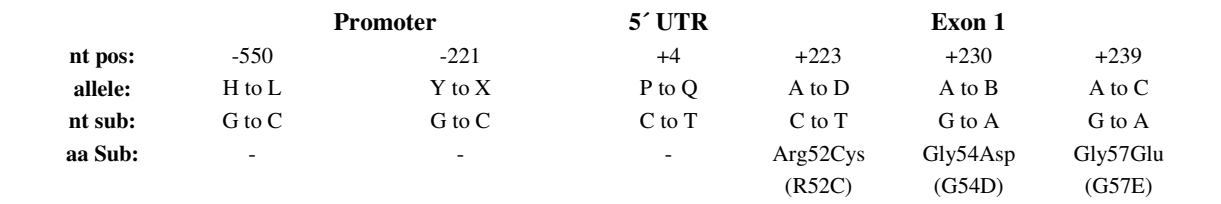

Figure 1. Organization of the promoter and exon 1 of *MBI2* gene, "aa sub": amino acid substitution, "nt sub": nucleotide substitution.[8]

MBL Serum Level Measurement

This assay was performed using ELISA kit (Sanquin Reagents, The Netherlands) according to manufacturer's instructions. The enzymatic reaction was stopped chemically and the color intensity was read at 450nm in ELISA reader.

Statistical Method

All calculations performed on the data were analyzed by SPSS 17. Confidence Interval (CI) was calculated with 95% confidence and *p* value less than 0.05 was considered to be meaningful.

RESULTS

MBL Serum Levels

Mean age in entire cohort (N=117) was 38 years. Quantified data obtained from the MBL measurement illustrated wide range of MBL level from 0.06µg/ml to 8.05µg/ml. The mean MBL level in our population was 2.34µg/ml (CI 2.00 – 2.67 µg /ml).

Alleles and Genotypes

As shown in table 2, there are two promoter polymorphisms at -550 (H/L variants) and -221 (Y/X variants) positions, as well as three exon polymorphisms at codons 52 (D Allele), 54 (B Allele), 57 (C Allele) of exon 1. Among 117 samples, 17 samples had no polymorphisms, the genotype of this group was HYA, and also had maximum range of MBL level. Twenty samples were shown to be of D allele, and B allele was demonstrated in 27 individuals. In addition, in promoter region, the L and X alleles were found among 84 and 67 individuals, respectively.

From five SNPs, the polymorphisms at -550 position and the codon 54 were found to be correlated with low MBL levels ($p<0.0001$). The HH, HL and LL genotypes at -550 polymorphism found to be correlated with high (mean 2.96 µg/ml), medium (mean 2.27 µg/ml), and low (mean 0.867 µg/ml) MBL serum levels, respectively. The effect of the X allele at -221 does not seem to show statistically significant effect on MBL level ($P=0.414$). The AA genotype polymorphism at codon 54 (B allele) were correlated with high MBL levels (mean 2.82 µg/ml) and AB and BB genotypes seemed to be associated with low MBL levels (with the means of 0.42 µg/ml and 0.01 µg/ml, respectively). The effects of codon 52 (D allele) on MBL level was demonstrated to be less significant compared to that of the B allele. Codon 57 (Allele C) variant was found only in one sample and therefore it is not meaningful to infer any conclusion based on

Table 2. The mean MBL concentrations of various genotypes

Loci	Common Nomenclature	Genotype	Number	Mean (µg/mL)	95% CI (µg/mL)[a]	*P*[b]
-550	H/H	GG	33	2.966	2.351 - 3.582	0.001
	L/H	GC	62	2.279	1.730 - 2.828	
	L/L	CC	22	0.867	0.294 - 1.440	
-221	Y/Y	GG	50	2.345	1.809 - 2.882	0.414
	Y/X	GC	65	2.144	1.611 - 2.677	
	X/X	CC	2	0.824	-4.970 - 6.618	
52	A/A	CC	97	2.53	2.15 - 2.9	0.021
	A/D	TC	18	1.5	0.9 - 2.1	
	D/D	TT	2	0.01	-0.12 - 0.15	
54	A/A	GG	90	2.82	2.45 - 3.19	0.001
	A/B	GA	25	0.42	0.25 - 0.59	
	B/B	AA	2	0.01	-0.08 - 0.1	
57	A/A	GG	116	2.35	2.02 - 2.69	-
	A/C	GA	1	0.01	-	
	C/C	AA	-	-	-	

P[b] : P-values Kruskal-Wallis Test

Table 3. The relation between common genotype combinations and MBL serum level

Genotype	Number	Mean MBL serum level (µg/mL)	95% CI
HYA/HYA	17	3.770	2.998 - 4.541
HYA/HXA	3	3.893	-0.328 - 8.114
HYA/LYA	6	2.992	0.608 - 5.376
HYA/LXA	38	3.000	2.279 - 3.721
LYA/LXA	5	1.654	0.506 - 2.802
LYA/LYA	2	3.924	-6.977 - 14.825
LXA/LXA	2	0.824	-4.970 - 6.618
HYA/HYD	10	1.994	1.115 - 2.873
HYD/LXA	5	0.255	-0.059 - 0.570
HYD/HYD	1	-	-
HYD/LXD	1	-	-
HYA/LYB	10	0.738	0.464 - 1.011
HYB/LXA	1	-	-
LYB/LXA	11	0.116	0.000 - 0.233
LYB/LYB	1	-	-
LYB/LXB	1	-	-

only one sample and needs further studies.

Then combined effect of four polymorphisms (-550, -221, 52, 54) analyzed. Of 16 possible genotypes, only seven genotypes –HYA/HYA, HYA/LYA, HYA/LXA, LYA/LXA, HYA/HYD, HYA/LYB,LYB/LXA- found to be common, with frequencies greater than 5% (Table 3).

HYA/LXA genotype (mean 3.00µg/ml) was the most frequent in our population (N=38) of study. The highest MBL producing genotype was demonstrated to be HYA/HYA (mean 3.77µg/ml), and it seemed LYB/LXA to be associated with the lowest MBL level (mean 0.11µg/ml).

DISCUSSION

In this study, mean MBL concentration was 2.34µg/ml (in range of0.06-8.05), while the mean value for Korean (N=129) and Australian (N=236) populations were 1.70 and 1.94µg/ml, respectively,[1,2] The results of another report among Iranian population reported while current study was under revision. The mean value for MBL serum level (2.207±1.73 µg/ml) among adults in this study was comparable to the level found in that study[12].

Previous studies have shown two polymorphisms at promoter (-550, -221), one polymorphism (+4) at

5´UTR and 3 coding variations 52, 54, 57 for *mbl2* gene.[8] In Korean population, L and P alleles were significantly correlated with Low MBL level. In addition, the effect of L allele was more powerful than polymorphisms seen at 5´UTR region.[1] On the contrary, in Caucasian population, P allele did not seem to have any known association with low MBL level.[1] In Danish and Australian population studies, two promoter polymorphisms (-550,-221) were found to be correlated with low MBL level, but the effect of -221 polymorphism on MBL level demonstrated to be stronger than the effect of -550 polymorphism.[2] The effect of -221 promoter polymorphism on MBL level was demonstrated to be more significant in Australian population compared to that of Koreans.

In the United States Midwestern population, the most prevalent mutation in coding region of *mbl2* gene was found to be at codon 54 (B allele).[3] Also, a similar study in Dane population[8] showed similar result, that this result was in line with Iranian population. In our study, according to the result of Korean population, the effect of polymorphism at -221 (X allele) was rather less significant than that of L allele. Interestingly, in contrast to Stefenson, et al.[8] findings, among 117 samples studied, we did not find any Q allele, while in the Dane population (N=100)[8] the frequency of this allele was 25.5%. Our results demonstrated that the highest MBL producing haplotype is HYA/HYA, followed by HYA/LXA, and HYA/LYA. In addition, LYB/LXA had the lowest MBL level. The most frequent genotypes in our population were found to be HYA/LXA, HYA/HYA, and LYB/LXA, respectively. Furthermore, in 3 samples we found a different SNP in nucleotide 101 of *MBL2* exon 1. In this substitution, the G nucleotide was changed with A, though this finding needs to be tested in future studies. However, MBL polymorphisms and MBL deficiency that has various ranges between different populations, as an influential factor in health, especially in recurrent infections, needs to be further studied.

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