Original Article

Evaluation Mannose - binding lectin gene and promoter polymorphism in renal transplant recipients

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Abstract

Objective: The aim of present study was to determine the distribution of the alleles of mannose-binding lectin gene and promoter variants in renal transplant recipients and seek correlation between these variants and diseases that cause renal dysfunctions.

Methods: One hundred and thirteen renal recipients' samples were compared with 120 normal controls from Azarbaijan population of Iran. Blood samples were obtained from renal transplant recipients who received a kidney between March 2004 and July 2005. Mannose-binding lectin genotypes were investigated by polymerase chain reaction and restriction fragment length polymorphism.

Results: Allelic and genotypic frequency of the polymorphism at position- 550, -221, +4 and at codon 52, 54 and 57 did not show statistical differences between recipients and controls (P>0.05) but significant frequency of allele B (codon 54) (P=0.02) and Lx haplotype (P=0.002) of promoter was observed in patients with Lupus Erythematosus and infection source of renal dysfunctions.

Conclusion: Our findings provide evidence that presence of different alleles and haplotypes that cause low concentration of mannose-binding lectin in serum is a risk factor for severity of systemic Lupus Erythematosus and susceptibility to renal infections that cause renal dysfunction (JPMA 58:294;2008).

Introduction

Mannose-binding lectin is a member of the collectin family of proteins found in serum[1] and binds to mannose and N-acetylglucosamine residues, while presented in the orientations and densities commonly found on microorganisms.[2-3] On binding, it activates the complement system independently of antibodies via two associated serin protease, Mannose-binding lectin- associated serin protease 1 and 2.[4]

Several studies have shown direct interaction of mannose-binding lectin with phagocyte cells, resulting in enhanced phagocytosis and modification of cellular activation.[5,6] Mannose-binding lectin therefore plays an important role in the innate immunity of the immune system.

Human mannose-binding lectin is derived from a single gene on chromosome 10 (mbl2),[7] The normal structural mannose-binding lectin allele is named A, while the common designation for the 3 variant structural alleles B (Mutation in codon 54, Gly to ASP), C (mutation in codon 57, Gly to Glu), and D (mutation in codon 52, Arg to Cys) are O.[8,9]

In general, individuals with a normal genotype (A/A) have 6-8 times higher mannose-binding lectin concentration in serum than those in individuals heterozygous for one of the variant alleles. (A/O: A/B, A/C or A/D), while individuals with a defective genotype (2 variant alleles B/B, C/C, D/D, B/C, B/D or C/D) have almost undetectable mannose-binding lectin serum levels.[10,11]

Moreover, MBL expression is influenced by polymorphic sites in the upstream part of the mannose-binding lectin gene nucleotides, substitutions at position -550, -221 and +4 give rise to H/L, Y/X and P/Q respectively[12,13]; while LX haplotypes are associated with low mannose-binding lectin plasma levels.[14-16]

Renal transplant recipients' have lost their kidneys as a result of infections, Lupus Erythematosus, high blood pressure, polycystic, diabetes and other unknown reasons. The aim of the present study was to determine the frequency of the mannose-binding lectin gene and promoter variants and to correlate different allele variants and disease which lead to the loss of the kidney and resulted in transplantation. It can reveal genetic relationship of diseases and renal disorders. In this study we investigated genetic polymorphism of mannose-binding lectin in all patients with renal failure caused by different causative agents and tried to find relations between them.

Patients and Methods

Blood samples were obtained from 113 renal transplant recipients who received renal grafts from March 2004 to July 2005. Blood samples of 120 normal controls from Healthy Medical University staff and students was randomly collected. Age range of both patients and controls were from 21 to 55 year. The association between mannose-binding lectin deficiency and renal failure can not be

Table 1. Oligonucleotides used for genotyping Mannose-binding lectin by Polymerase Chain Reaction (PCR).

Codon 57 (wild type)	forward	5'-GAGGGCTAGACCTATGGGGCTAG-3'
	reverse	5'-TACCTGGTTCCCCCTTTTCTC-3'
Codon 57 (mutant)	forward	5'-GAGGCTTAGACCTATGGGGCTAC-3'
	reverse	5'-TACCTGGTTCCCCCTTTTCTT-3'
Codon 54 (wild type)	forward	5'-GAGGCTTAGACCTATGGGGCTAG-3'
	reverse	5'-CCCCTTTTCTCCCTTGGTGC-3'
Codon 54 (mutant)	forward	5'-GAGGCTTAGACCTATGGGGCTAG-3'
	reverse	5'-CCCCTTTTCTCCCTTGGTGT-3'
Codon 52 (wild type)	forward	5'-CTTCCCAGGCAAAGATGGGC-3'
	reverse	5'-GAGGCAGTTTCCTCTGGAAGG-3'
Codon 52 (mutant)	forward	5'-CTTCCCAGGCAAAGATGGGT-3'
	reverse	5'-CAGGCAGTTTCCTCTGGAAGG-3'
MBL allele H	forward	5'-GCTTACCCAGGCAAGCCTGTG-3'
	reverse	5'-CAGGCAGTTTCCTCTGGAAGG-3'
MBL allele L	forward	5'-GCTTACCCAGGCAAGCCTGTC-3'
	reverse	5'-CAGGCAGTTTCCTCTGGAAGG-3'
MBL allele P	forward	5'-GTAGGACAGAGGGCATGCTC-3'
	reverse	5'-CAGGCAGTTTCCTCTGGAAGG-3'
MBL allele Q	forward	5'-GTAGGACAGAGGGCATGCTT-3'
	reverse	5'-CAGGCAGTTTCCTCTGGAAGG-3'
haplotypes Hy	forward	5'-GCTTACCCAGGCAAGCCTGTG-3'
	reverse	5'-GGAACACTATAAACATGCTTTCC-3'
haplotypes Ly	forward	5'-GCTTACCCAGGCAAGCCTGTC-3'
	reverse	5'-GGAAGACTATAAACATGCTTTCC-3'
haplotypes Lx	forward	5'-GCTTACCCAGGCAAGCCTGTC-3'
	reverse	5'-GGAAGACTATAAACATGCTTTCG-3'
haplotypes Hx	forward	5'-GCTTACCCAGGCAAGCTTGTG-3'
	reverse	5'-GGAAGACTATAAACATGCTTTCG-3'

explain by confounding factors such as differences in age at disease onset or disease duration and sex.[11]

DNA was Isolated from either granulocytes or mononuclear cells by the modified proteinase K, sodium dodecyl sulfate (SDS), N-acetyl-N, N-trimethyl ammonium bromide (CTAB).[17]

Polymerase Chain Reaction (PCR) was performed in 20 to 100 µL volumes that contained 50 ng to 500 ng of genomic DNA, 0.5 µm of specific primers (Table 1) in the presence of 1.5 mM MgCl2 , 100 µM of each dNtp, 50 mM KCl ,20 mM tris-HCl, pH 8.4, and 1 to 2.5 unit recombinant DNA polymerase (fermentas). DNA was amplified by general PCR and sequence-specific primed polymerase chain reaction (SSP-PCR).[15,16,18]

All PCRs were initiated by a 4-min denaturizing step at 94°C and completed by a 7-min extension step at 72°C. The temperature cycles for different types of PCRs were as follows: 32 cycles of 40 second at 94°C, annealing temperature for 40s and 72°C for 55s.

Annealing temperatures used were: 60, 63, 63, 62, 66, 63, 66, 67, 64, 67, 67,65, 65 and 66°C for codon 57 (wild type), 57 (mutant), codon 54 (wild type), 54 (mutant), codon 52 (wild type), 52 (mutant), allele H, L, P, Q, haplotypes Hy, Ly, Lx and Hx amplification, respectively.

In addition to SSP-PCR, B and C alleles were detected by Ban I and Mbo ? restriction enzyme digestions (Cinnagen, Iran) (Figure 1), of the 328-bp product amplified by the allele P and Q primers respectively (table 1), followed by a 2.5% agarose gel electrophoresis. Ban I cleaves the A allele into two fragments allele (245 and 83 bp) and leaves the B allele undigested, while Mbo Π specifically cleaves the C allele into two fragments (266 and 62 bP).[18,19]

Statistical analyses were performed by $\chi 2$ test (chi-square test). P values below 0.05 were considered statistically significant.

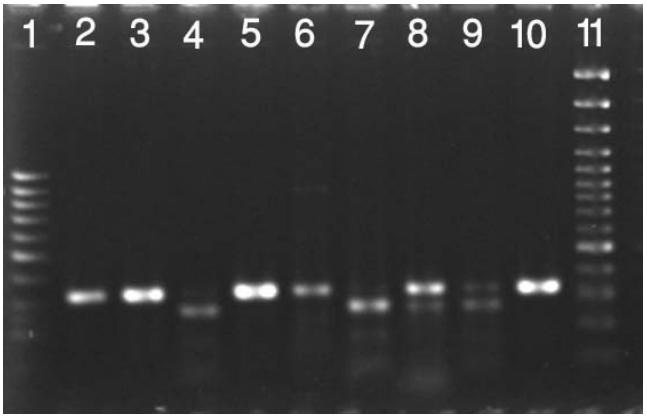

Figure 1. Gel electrophoresis of PCR in products digested with mbo II enzyme. 1: size marker (50 bp) (fermentas). 2, 3, 5, 6, 10: mbo II enzyme had not effect (natural allele A, B, D); 4, 7, 8, 9: mbo II digested products (allele C); 11: size marker plus (100 bp).

Results

From the 113 Renal transplant recipients 51.3% had lost their kidneys to infections, 16.81% were polycystic, 15.93% due to high blood pressure, 8.85% Lupus Erythematosus, 3.53% diabetes, 2.65% reflux and 0.88% Acute thrombotic necrosis. The distribution of haplotypes in recipients population were: 30.05%HYPA, 20.35% LXPA , 19.45% LYPB, 16.8% LYQA, 5.3% HYPD, 3.95% LYPA , 2.65% LYQC, 0.85% LYPD and 0.4% HXPA. And in normal population were 34.5% HYPA, 16% LYPB, 15.5% LYQA, 15% LXPA, 7.6% HYPD, 6.05% LXQC, 4.5% LYPA and 0.85% LYPD. There were not any statistical differences between populations.

The genotype frequency of three mannose-binding lectin structural alleles was compared in transplant recipients and normal controls. No statistical difference was noted (Table 2). Also no difference was observed in the frequency of promoter gene variant. Haplotypes frequency was also similar.

Table 2. Geotype frequency of mannose-binding lectin structural alleles, promoter variants and position +4 in renal recipients and Healthy controls.

Alleles And Variants	Frequency%* Renal recipients (n =113) †	Controls (n =120)	P-value
Codon54mutation (Allele B)	19.5	14.2	.2785
Codon 57 mutation (Allele C)	3.1	2.1	0.6252
Codon 52 mutation (Allele D)	5.8	5.8	0.9788
Wild type (Allele A)	71.6	77.9	0.2726
H	41	38.33	0.6604
L	59	61.67	0.6604
P	81	72.5	0.1267
Hy	40.27	38.33	0.7627
Ly	38.94	42.9	0.5381
LX	20.35	18.75	0.7575

* Mannose-binding lectin Variants frequency in patients and controls †Each Patient has two variants on its genotype

Table 3. Frequency of defective allele in patients (Renal recipients).

Cause of renal failure	Percent of defective allele in renal recipients			
	B	C	D	LX
Lupus Erythematosus	40.0% *	0.0% ‡	10.0% ‡	40.0% ‡
Polycystic	41.0% *	5.9% ‡	17.6% ‡	32.3% ‡
Infection	35.0% †	7.0% ‡	8.7% ‡	49.1% *
Blood pressure	15.8% ‡	15.8% ‡	0.0% ‡	5.2% ‡

* P=0.002 in comparing with Controls via Chi-square test. † P=0.02 in comparing with controls via
Chi-square test. ‡ P>0.05 in comparing with controls via Chi-square test

Most patients with Lupus Erythematosus had at least one allele with dysfunction as B or Lx compared to controls (80 % vs. 32.95%) (P=0.002) and 89.47% patients with polycystic had at least one dysfunctive allele of B or LX (89.47% vs 32.95%) (p=0.002) compared to controls. In patients with infections causing renal dysfunction, 34.4% had allele B and in controls 14.2% (P=0.02) and 48.27% had Lx haplotype, It was statistically significant compared to controls (18.75%) (p=0.002) (Table 3).

Discussion

In population of East Azarbaijan of Iran, there is an increased frequency of LXPA haplotype, which has previously demonstrated an association with significantly lower serum mannose-binding lectin levels than other haplotypes.[20] This shows a low concentration of mannose-binding lectin in the Irani population. This low level can be associated with an increased risk of different types of infections and a poor prognosis.

Comparing the polymorphism of alleles between transplant recipients and normal controls, no significant differences were found in polymorphism in promoter region in renal recipients and normal controls. In our study patients with Lupus Erythematosus (SLE) with renal dysfunctions, had a high frequency of dysfunctional allele of B or Lx and these evidences confirm the results of previous studies on the relation of mannose-binding lectin defunctive alleles B and Lx and outcome of SLE.[7,11,21,22]

A low serum mannose-binding lectin level could cause defective activation of the complement system, leading to poor complement fixation onto the lattice of Immune complexes. Inadequately complement-coated Immune complexes might not be sequestrated in the liver, but circulate to other sites, such as the kidney, giving rise to inflammation.[4] Therefore, low serum mannose-binding lectin level can be considered as a risk factor for development of renal dysfunction.

In patients who lost their kidneys due to infections, a high frequency of allele B (35.08%) was encountered, which causes low concentration of mannose-binding lectin. This group had a high frequency of promoter Lx haplotype which was associated with low serum mannose-binding lectin concentration.[14] This low concentration of mannose-binding lectin in infectious patients can explain the reason of renal dysfunction in these patients. According to different Antimicrobial activities such as its role in the first line of defense at the time of primary contact that called ante-antibody[23] and its role in complement activation, phagocytosis and opsonic activities, Mannose-binding lectin deficiency leads to a defect of complement activation or opsonization of pathogens.[24]

In glomerulonephritis mannose-binding lectin has a different role, Mannose-binding lectin is present in the glomeruli of patients and can activate the lectin pathway of complements, but it has a marginal role in glomerular deposition.[25]

In polycystic kidney, prevalence of mannose-binding lectin defective alleles, can be a risk factor as seen in our study results.

In conclusion, the present study failed to demonstrate a clear association of renal dysfunction and polymorphic regions of the promoter and the first exon of mannose-binding lectin in general. It clearly revealed the higher frequency of allele B and Lx haplotype in patients with SLE as a susceptibility factor for renal failure. Similarly in cases of renal infections and polycystic kidneys developing renal failure, there was a high frequency of B allele and LX haplotype.

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