High Frequency of Clinically Significant Infections and Cytomegalovirus Disease in Kidney Transplant Recipients With Serum Mannose-Binding Lectin Deficiency

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Introduction. Mannose-binding lectin (MBL) constitutes defense against infections when adaptive immune response is compromised. Elevation in serum MBL levels has been shown in patients with kidney failure. We compared serum MBL levels before and after kidney transplant and evaluated association of MBL deficiency with infectious complications in kidney transplant recipients.
Materials and Methods. This study was performed in 71 kidney transplant recipients and 48 healthy controls. In 36 recipients (group 1), serum MBL levels were tested before and on days 7 and 14 after transplantation. They were followed up for 6 months. In 35 recipients (group 2), serum MBL was measured during their posttransplant follow-up visits. In both groups, frequencies of clinically significant infections and acute rejection were compared between those with low MBL (< 500 ng/mL) and normal/high MBL (≥ 500 ng/mL).
Results. Serum MBL levels (1744 ± 905 ng/mL) were not higher in group 1 before transplantation than in controls. One and 2 weeks after transplantation, MBL levels decreased to 1699 ± 1030 ng/mL and 1562 ± 1020 ng/mL, respectively. Five patients who had low serum MBL levels experienced more frequent episodes of infections (P = .008) and CMV disease (P < .001). Ten patients in group 2 with low MBL levels had more frequent episodes of CMV disease (P = .01).
Conclusions. These findings suggest a potential role for MBL in defense against developing posttransplant CMV disease and that low serum MBL levels in kidney transplant recipients be considered an indicator of the need for CMV prophylaxis.

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INTRODUCTION

Kidney transplantation is now considered the treatment of choice for most patients with end-stage renal disease, because it prolongs patient survival and improves the quality of life compared with dialysis therapy.[1] A successful kidney transplantation demands that immunosuppressive medications be taken by the transplant recipient in order to prevent allograft rejection. These drugs decrease the number of rejection episodes; however, by compromising the adaptive immune system, they significantly increase the risk of infections and malignancies. Currently, infection is one of the main causes of patient death and graft failure

after kidney transplantation.

Mannose-binding lectin (MBL), the key component of innate immune system, is the first element to recognize microorganisms and control infections when adaptive immune response is immature during childhood or when it is compromised by immunosuppressive drugs. Mannose-binding lectin is a multimeric C-type lectin produced in the liver and presented in human serum. It is made up of 96-kDa structural units, which in turn has 3 identical 32-kDa primary subunits. Each subunit consists of an N-terminal, a collagen-like domain, and a C-terminal called carbohydrate-recognition domain. In the circulation, MBL is present in oligomeric form (mainly tetramers and hexamers) bound to MBL-associated serine proteases (MASPs), of which MASP2 plays the most important role in immune response. Mannose-binding lectin binds to carbohydrates present on a wide variety of bacterial, fungal, viral, and parasitic surfaces. Upon binding to the pathogen, the MBL-MASP2 complex mediates either direct opsonophagocytosis or complement activation. Activation of compliment via the lectin pathway, involving MBL as recognition molecule, leads to cleavage of complement 4 (C4) and C2 and formation of the complement 3 convertase C4b2a. Cleavage of C3 by C3 convertase initiates the terminal complement pathway, finally resulting in formation of the membrane attack complex C5b-9.

Exon 1 of the *MBL2* gene, which is located on chromosome 10, contains 3 functional single nucleotide polymorphism at codons 52-54 and 57 referred to variant alleles of D, B and C respectively.[2] This impairment of polymerization causes low serum levels of high-molecular-weight MBL or impaired MBL function. Mannose-binding lectin deficiency can be detected by MBL genotyping, serum MBL measurement, and MBL-MASP complex assay. Most serum MBL measurements by enzyme-linked immunosorbent assay have a strong preference for measurement of high-molecular-weight (wild-type) MBL, and therefore, they provide an excellent correlation with the MBL function. Most MBL-deficient individuals are apparently healthy, because they have alternative mechanisms for antimicrobial protection. However, MBL deficiency can become a strong risk factor of developing infectious disease in immunocompromised individuals such as organ transplant recipients.[3]

In clinical organ transplantation, serum MBL reveals a double-edged sword function, meaning that low serum MBL levels are indicative of increased risk of infections,[4] whereas high serum MBL levels is associated with transplant rejection.[5] In some studies, significant elevation of serum MBL levels has also been associated with advanced kidney failure predicting all-cause mortality.[6] Focusing on the role of MBL measurements in kidney transplant recipients, the first aim of our study was to see whether MBL levels are elevated in patients with advanced kidney failure, and if so, whether MBL levels will decrease after successful kidney transplantation and elimination of uremia. The second aim of the study was to see whether MBL deficiency in kidney transplant recipients is associated with increased infectious complications.

MATERIALS AND METHODS

This study was carried out on 71 living donor kidney transplant recipients and 48 healthy controls at Shahid Hasheminejad Medical Center in Tehran, Iran. The kidney transplant recipients consisted of 2 groups of patients. Group 1 included 36 consecutive kidney transplant recipients who provided consent to be enrolled in this study and their blood samples were collected for serum MBL levels on a day before transplantation and on days 7 and 14 after transplantation. Patients in this group were followed for 6 months since the date of transplantation for development of clinically significant infections and acute rejection episodes (prospective part of the study). Group 2 consisted of 35 stable kidney transplant recipients who were seen in the transplant clinic and provided consent for participation in the study. A blood sample was collected for testing MBL level, and their medical records were reviewed for the development of clinically significant infections and acute rejection episodes since the date of kidney transplantation (retrospective part of the study). Serum MBL levels of 48 healthy volunteers were also used for comparison and as control. This group consisted of couples referred for laboratory tests required before marriage.

In all of the 71 kidney transplant recipients, data on the following variables were obtained from their medical records: demographic data, laboratory data, immunosuppressive protocols, serologic status for

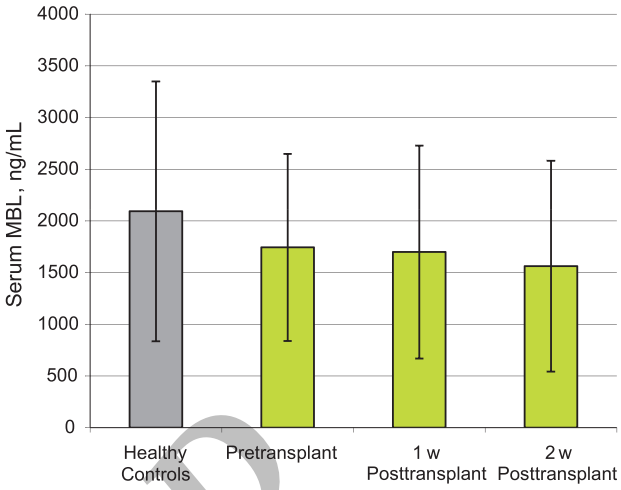
cytomegalovirus (CMV), the number of clinically significant infections (such as CMV disease), and the number of acute rejection episodes since transplantation. Clinically significant infection was defined when appropriate clinical findings were present. Diagnosis of CMV disease was made by documentation of CMV replication (positive pp65) in the presence of clinical expression of active infection followed by antiviral therapy. Acute rejection episode was defined as deterioration of allograft function requiring antirejection treatment.

Serum MBL concentration was measured by MBL oligomer enzyme-linked immunosorbent assay kit (BioPorto Diagnostics, Gentofte, Denmark). The MBL concentrations lower than 500 ng/mL were classified as low MBL, and MBL levels equal to or higher than 500 ng/mL, as normal/high MBL. Categorical characteristics among low- and high-MBL patients in both groups were compared using cross tables with calculation of *P* values. Continuous variables were compared between the groups using the Mann-Whitney test. Changes in MBL levels after transplantation was tested by the paired *t* test. A *P* value less than .05 was considered significant.

RESULTS

Results of the study in group 1 showed that serum MBL levels were not elevated in advanced kidney failure. Serum MBL levels was 1744 ± 905 ng/mL in the 36 patients prior to kidney transplantation and it was 2093 ± 1257 ng/mL in the 48 healthy controls (*P* = .14). After successful kidney transplantation, serum MBL levels decreased from 1744 ± 905 ng/mL to 1699 ± 1030 ng/mL on posttransplant day 7 and to 1562 ± 1020 ng/mL in on 14 (*P* = .16, compared to pretransplant level). These changes were not statistically significant (Figure).

Five of 36 patients (13.9%) in group 1 had low serum MBLs and the remaining 31 had normal/high MBL levels. Table 1 demonstrates the demographic and clinical characteristics as well as the number of clinically significant infection episodes and acute rejection episodes during 6 posttransplant months in the patients with low and normal/high MBL levels. The immunosuppressive protocol was the same in all of the patients including cyclosporine A, mycophenolate mofetil, and prednisolone. Also, none of the patients had received prophylaxis for CMV disease. There were no significant differences between the patients with low and with normal/high serum MBL levels regarding sex, age, allograft



The Effect of successful kidney transplantation on serum Mannose-Binding Lectin (MBL) levels in comparison with that in healthy controls. The difference between the MBL levels of the healthy controls and the pretransplant levels of the transplant recipients was not significant (*P* = .14). Also, the MBL levels did not decrease significantly on day 14 compared to those before transplantation (*P* = .16).

Table 1. Kidney Transplant Recipients' Characteristics in Group 1 According to Pretransplant Serum Mannose-Binding Lectin Levels*

Characteristic	MBL, ng/mL		*P*
	< 500	> 500	
Number of patients	5	31	…
Sex			
Mal	2	17	
Female	3	14	.65
Mean age, y	42.2 ± 13.7	39.2 ± 13.4	.64
Serum creatinine, mg/dL†	1.8 ± 1.5	1.4 ± 1.2	.51
Hemoglobin, g/L†	9.5 ± 2.1	10.9 ± 1.8	.12
CMV IgG status			
Donor+/recipient+	4	24	
Donor+/recipient-	1	4	
Donor-/recipient+	0	2	
Donor-/recipient-	0	1	.88
Infection episodes	10	12	.008
UTI	4	7	< .001
URI	1	1	…
Wound infection	0	1	…
Herpes zoster	1	0	…
Tuberculosis	1	0	…
Herpes simplex	0	2	…
CMV disease	3	1	< .001
Acute rejection episodes	1	6	.97

*MBL indicates mannose-binding lectin; CMV, cytomegalovirus; IgG, immunoglobulin G; UTI, urinary tract infection; and URI, upper respiratory infection. Ellipses indicate not applicable or not calculated.
†These were measured 2 weeks after transplantation.

function, hemoglobin levels, and CMV serostatus. However, patients with low serum MBL levels developed more episodes of clinically significant infections ($P = .008$), such as urinary tract infection ($P < .001$) and CMV disease ($P < .001$), compared to those with normal/high MBL levels. The frequency of acute rejection episodes was the same in patients with low and with normal/high MBL levels.

In group 2, there were 10 patients (28.6%) who had low serum MBL levels. Table 2 outlines the demographic and clinical characteristics as well as the number of clinically significant infection episodes and acute rejection episodes during 6 posttransplant months in the patients of group 2 with low and normal/high MBL levels. There were no significant differences between the patients with low and normal/high serum MBL levels regarding sex, follow-up months, immunosuppressive protocols, allograft function, hemoglobin levels, clinically significant infection episodes, and acute rejection episodes since transplantation. However, the 10 patients with low serum MBL levels had developed significantly more episodes of CMV disease compared with 25 patients who had normal/high MBL levels ($P = .01$).

Table 2. Kidney Transplant Recipients' Characteristics in Group 2 According to Pretransplant Serum Mannose-Binding Lectin Levels*

Characteristic	MBL, ng/mL < 500	MBL, ng/mL > 500	*P*
Number of patients	10	25	…
Sex			
Mal	2	9	
Female	8	16	.60
Mean age, y	32.5 ± 8.6	40.3 ± 13.4	.05
Immunosuppression			
CA + MM + P	10	20	
Others	0	5	.32
Follow-up, mo	66.2 ± 55.7	81.3 ± 65.4	.49
Serum creatinine, mg/dL	1.3 ± 0.6	1.3 ± 0.5	.97
Hemoglobin, g/L	12.7 ± 2.2	12.7 ± 2.1	.93
Infection episodes	37	92	.98
URI	15	50	
UTI	9	25	
Gastroentritis	6	11	
CMV disease	6	3	.01
Sepsis	0	1	
Herpes zoster	1	2	
Acute rejection episodes	2	8	.94

*MBL indicates mannose-binding lectin; CA, cyclosporine A; MM, mycophenolate mofetil; P, prednisolone; URI, upper respiratory infection; UTI, urinary tract infection; and CMV, cytomegalovirus.

DISCUSSION

Satomura and coworkers measured MBL levels in the sera of 178 patients with end-stage renal disease who were receiving maintenance hemodialysis, in 23 patients with chronic kidney failure before they began dialysis therapy (serum creatinine 3.2 ± 1.4 mg/dL), and in 22 healthy controls.[6] The mean levels of serum MBL were significantly higher in patients with chronic kidney failure (4343 ± 2533 ng/mL) and in patients who were on dialysis (8897 ± 4920 ng/mL), than in healthy controls (1452 ± 692 ng/mL). Also the MBL levels were significantly higher among patients on dialysis than in patients with chronic kidney failure. The authors hypothesized that some factors of chronic kidney failure increase serum MBL concentrations. They found no relationship between serum MBL levels and the glomerular filtration rate. Because MBL has a large molecular size and it cannot be excreted by the kidneys, kidney failure cannot explain per se the elevation of serum MBL levels. This elevation of serum MBL levels in patients with advanced kidney failure has not been found by other investigators. Berger and colleagues measured serum MBL levels in the pretransplant sera of 266 deceased donor kidney transplant recipients and sera of 70 MBL-genotyped healthy controls.[5] The mean serum MBL concentration in the patients with advanced kidney failure (before transplant) was 1112 ng/mL, which was very similar to the levels in the healthy controls who had a mean MBL level of 1054 ng/mL.

In our study, we measured MBL levels in sera of 36 patients with advanced kidney failure on a day before kidney transplantation and on days 7 and 14 after transplantation. If MBL levels had been increased because of kidney failure, it was expected that the levels decrease by successful kidney transplantation and elimination of uremia. After transplantation, serum MBL levels decreased in these patients from 1744 ± 905 ng/mL to 1699 ± 1030 ng/mL and to 1562 ± 1020 ng/mL, 1 and 2 weeks after transplantation, respectively. However, these changes were not significant. Again serum MBL levels in 36 patients with advanced kidney failure before receiving kidney transplantation (1744 ± 905 ng/mL) was not higher than the mean serum MBL levels of our 48 healthy volunteers (2093 ± 1252 ng/mL). The results of our study is similar to the findings of Berger and colleagues and does

not confirm the results presented by Satomura and colleagues.[5,6] We also showed that elimination of uremia by successful kidney transplantation did not significantly change serum MBL levels.

It has been shown that in individuals with MBL deficiency, when the adaptive immune response is immature or is compromised, the incidence of infectious diseases significantly increases. Hibberd and associates from Meningococcal Research Group studied on 266 children with meningococcal disease and showed an association between MBL deficiency and meningococcal infections.[7] Garred and coworkers also showed that the presence of MBL deficiency in patients with cystic fibrosis is associated with poor outcome. They demonstrated that the low survival of MBL-deficient patients was due to more aggressive course of lung disease caused by chronic *Pseudomonas aeruginosa* infection.[8]

Mannose-binding lectin has been studied in many viral infections, too. Persistent hepatitis B virus infection has been reported to be associated with low MBL serum levels.[9] The role of MBL in human immune deficiency virus (HIV) infection has been extensively studied. It has been suggested that MBL is involved in the recognition of HIV. The envelope protein gp120 of the HIV-1 virus is highly glycosylated, enabling MBL to bind, opsonize, and neutralize the HIV-1 virus.[2] Garred and colleagues showed that men with MBL deficiency had a shorter survival time following the onset of acquired immune deficiency syndrome than did patients with normal MBL.[10] It has also been shown that MBL is able to neutralize and inhibit the infection of influenza A virus independent from complement activation.[11] On the other hand, MBL has been shown to be an important antimicrobial defense in the immunocompromised patients such as transplant recipients. Manuel and associates measured plasma MBL levels in 16 kidney transplant recipients with high-risk CMV serostatus (donor positive/recipient negative).[4] All patients received CMV prophylaxis for 3 months after transplantation. With a follow-up of at least 12 months after transplantation, they found that 82% of patients who had late-onset CMV infection or disease had MBL deficiency, while none of the patients without CMV infection had MBL deficiency. They concluded that MBL deficiency is a significant risk factor of the development of CMV infection in kidney transplant recipients, demonstrating a role for innate immunity in the control of CMV infection after organ transplantation. Bouwman and colleagues showed that the transplantation of livers with variant MBL genotype resulted in low MBL levels in the recipients and a significantly increased rate of severe infections.[12] Verschuren and coworkers studied infectious complications after simultaneous pancreas-kidney transplantation. During the first year after transplant, 152 consecutive recipients of pancreas-kidney transplant developed 529 clinically significant infections. Pretransplantation serum MBL levels were low (< 400 ng/mL) in 44 patients and high (> 400 ng/mL) in 108 patients. In transplant recipients with low serum MBL, clinically significant infections such as urinary tract infection, cystitis, and urosepsis were significantly more frequent compared with patients who had high MBL levels.[13]

In this study, we investigated the frequency of clinically significant infectious complications prospectively in 36 consecutive kidney transplant recipients for a period of 6 months after transplantation, and we also performed a retrospective study on 35 kidney transplant recipients attending transplant clinic. We used a cutoff point of 500 ng/mL to differentiate low MBL level from its normal and high levels. This cutoff level has been adopted in most studies and strongly correlated with the presence of MBL polymorphisms. We documented that in both groups, patients with low serum MBL levels developed more frequent CMV disease episodes compared to patients who had normal or high serum MBL levels. In the first 6 months after transplantation, infections including urinary tract infection were also more frequent in patients with low MBL.

MBL deficiency has been reported to have a dual effect depending on the clinical situation. Its detrimental effect is an increased susceptibility to infection in immunosuppressed patients. Its beneficial effect is its association with lower mortality and less graft loss in organ transplant recipients. Berger and coworkers[5] studied graft survival in deceased donor kidney transplant recipients and found that at 10 years, graft survival rate was 89.9% in patients with MBL levels below 400 ng/mL compared to 78.8% in those with a high MBL level. In another study, this group of investigators has shown that low pretransplant MBL levels can predict superior patient and graft

survival rates after simultaneous pancreas-kidney transplantation.[14]

In this study, we did not find any relationship between serum MBL levels and number of acute rejection episodes. This is most likely because of small number of our patients and because our study was carried out on living donor transplants with no remarkable ischemia reperfusion injury.

CONCLUSIONS

The results of our study showed that serum MBL levels were not elevated in patients with advanced kidney failure. We also showed that MBL deficiency is a risk factor for developing infectious complications and CMV disease in kidney transplant recipients.

CONFLICT OF INTEREST

None declared.

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