Measurement of Serum Levels of Mannose-binding Lectin in Hemodialysis Patients

A Comparison With Healthy Individuals

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Keywords. mannose-binding lectin, hemodialysis, immune system

Introduction. Mannose-binding lectin (MBL) is a part of the innate immune system. Many studies showed an association of low serum MBL levels with decreased host defense against various infectious agents. Considering paradoxical reports about the serum level of MBL in hemodialysis patients, this study aimed to measure and compare serum MBL levels in hemodialysis patients and healthy individuals.

Materials and Methods. In a cross-sectional study, 70 hemodialysis patients and 70 volunteers with normal routine laboratory tests and physical examination were assessed for serum MBL level (measured by an enzyme-linked immunosorbent assay). In addition, serum C-reactive protein levels in hemodialysis patients were measured to rule out correlation of increased serum MBL level with inflammation.

Results. In hemodialysis patients, 32 (45.7%) were men and 38 (54.3%) were women. In the control group, 34 (48.6%) were men and 36 (51.4%) were women ($P = .87$). The mean age showed no significant difference in hemodialysis (44.5 ± 13.5 year) and control (46.4 ± 12.4 years) groups. Serum level of MBL was significantly higher in hemodialysis patients (2.12 ± 1.49 µg/mL) than that in the controls (1.49 ± 2.12 µg/mL; $P < .001$). No significant correlation was found between serum MBL and C-reactive protein levels ($r = 0.002$, $P = .98$) among the hemodialysis patients.

Conclusions. Serum MBL level in hemodialysis patients was significantly higher than that in the control group of healthy individuals. This may have some implications in management of patients and prediction of kidney allograft survival.

IJKD 2011;5:255-9
www.ijkd.org

Original Paper

INTRODUCTION

Complement system not only is a part of innate immunity system, but also has an important regulatory function in initiation and control of adapting immunity. Complement is activated by 3 different pathways. These three pathways reach to a common pathway at complement 3 (C3) level, and ultimately, membrane attack complex is made. Activation pathways include classic, alternative, and mannose-binding lectin (MBL). The process of complement activation in MBL pathway is through activation of MBL-associated serin protease 2 (MASP-2) and then cleavage of C2 and C4 and formation of C4b2q.[1] Mannose-binding lectin is composed of 6 trimeric parts and has structural similarity with C1q. Plasma MBL level can be

highly variable, sometimes up to 1000 fold, even in healthy individuals.[2,3] This difference in serum levels is related to polymorphism in *MBL2* gene exon and promotor areas.[1,4-6] In addition, low level of MBL is a predisposing factor for infections, particularly when other deficiencies complicated the situation. Deficiency of MBL is a risk factor for infection in infants, patients with chemotherapy treatments, and recipients of transplant organs. Mannose-binding lectin neutralizes bacteria after attachment and opsonize them by complement activation.[1]

One study by Lam and colleagues revealed the significantly lower levels of MBL in hemodialysis patients compared with the general population.[7] Another study performed by Satomura and coworkers showed a completely different result. In that study, the average serum level of MBL in patients with kidney failure before and after hemodialysis was significantly higher than that in the normal (control) group.[8] He suggested that since MBL, like C-reactive protein (CRP), is an acute-phase reactant, and considering that most hemodialysis patients are susceptible to many inflammatory reactions, increased serum levels of MBL in this group of patients is in concordance with serum CRP levels and thus explainable. Recently, Satomura and coworkers reported the effect of low serum levels of MBL in mortality of hemodialysis patients.[9] They measured serum MBL level in a group of patients who were under maintenance hemodialysis for about 2 years. Then, they followed patients for 36 months. Eighteen patients died during this period primarily due to cardiovascular complications and infections. Serum level of MBL was significantly lower in patients who died compared with the survived patients. Berger showed that serum MBL level in hemodialysis patients before transplantation is similar to healthy individuals.[10] The different result of previously published studies may be partly attributed to background genetic variability of hemodialysis patients. The aim of this study was to investigate the serum MBL in Iranian hemodialysis patients compared to healthy individuals.

MATERIALS AND METHODS

During a 6-month period between January 2008 and July 2008, a cross-sectional study was carried out in Hasheminejad Kidney Center, Tehran, Iran. A group of 70 hemodialysis patients were enrolled in this study by simple random sampling. A group of 70 healthy individuals (controls) with no abnormal finding in physical examination or laboratory findings were randomly selected and their serum MBL level was measured. The control group was selected from those referred to the laboratory for routine checkups and had no abnormality in routine serum biochemical assessments and urine tests. On all healthy individuals, physical examination including blood pressure measurement was performed to rule out underlying diseases. Exclusion criteria included systemic infectious diseases in the past 2 months and history of malignancy.

An aliquot of serum sample of the control group was kept in a temperature of -20°C. Serum aliquots of hemodialysis patients were collected and stored in a similar situation during monthly routine clinical laboratory assessments. Serum MBL levels were measured in both groups using enzyme-linked immunosorbent assay kits (Sanquin, Amsterdam, Netherland), according to the manufacturers' guidelines. In addition, serum levels of CRP were measured in the hemodialysis group using enzyme-linked immunosorbent assay kits (BioVendor, Helsinki, Finland). Results of laboratory tests, age, and gender of patients were recorded and compared in between the two groups.

Data were analyzed using the SPSS software (Statistical Package for the Social Sciences, version 11.0, SPSS Inc, Chicago, Ill, USA). The Fisher exact test, the *t* test, and the Mann-Whitney test were used for comparison of qualitative variables, quantitative variables with a normal distribution, and qualitative variables without a normal distribution, respectively. To assess the presence of normal distribution of variables the 1-sample Kolmogorov-Smirnov test was used. The Kendal test was used to assess the relationship of serum MBL with quantitative variables in the two groups. P values less than .05 were considered significant.

RESULTS

In the hemodialysis group, 32 patients (45.7%) were men and 38 (54.3%) were women. In the control group, 34 patients (48.6%) were men and

36 (51.4%) were women (P = .87). The mean of the patients' age in the hemodialysis group was 44.5 ± 13.5 years, and in the control group was 46.4 ± 12.4 years (P = .37).

Serum level of MBL in hemodialysis patients varied between zero and 7.35 µg/mL with median and mean levels of 0.52 µg/mL and 2.12 µg/mL, respectively. In the control group, serum MBL levels varied between zero and 7.02 µg/mL. In this group, the median and mean levels of MBL were 1.61 µg/mL and 1.49 µg/mL, respectively. Serum MBL level in hemodialysis patients was significantly higher than that in the control group (P < .001; Figure 1). No significant difference was detected in serum MBL level between men and women in either patients or control groups (Table). No significant correlation was detected between age and serum MBL level in either hemodialysis (r = 0.072, P = .55) or control (r = 0.158, P = .19) groups.

Serum measurements for CRP in hemodialysis patients showed no correlation with MBL level (r = 0.002, P = .98; Figure 2). The mean serum albumin level in this group was 4.0 ± 0.4 mg/dL. There was no correlation between serum albumin and MBL levels in this group (r = 0.009, P = .94).

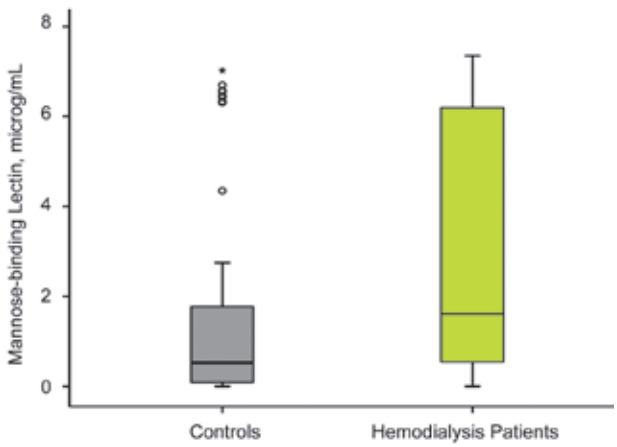

Figure 1. The distribution of serum mannose-binding lectin level in hemodialysis and control groups.

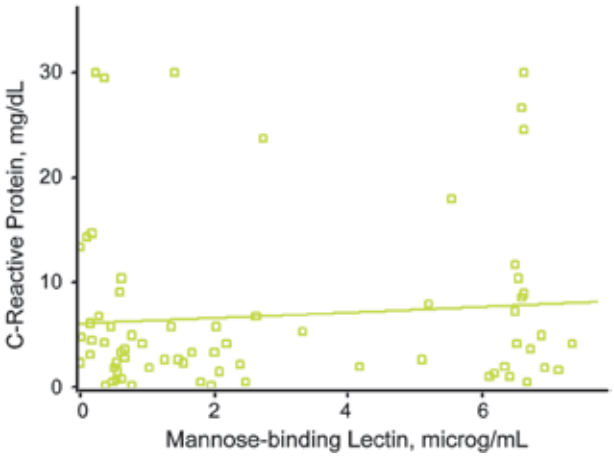

Figure 2. The correlation between serum mannose-binding lectin and C-reactive protein in hemodialysis patients.

DISCUSSION

This study was performed to determine serum MBL levels in hemodialysis patients and in comparison with that in healthy individuals. Taking the effects of MBL gene polymorphism in different serum levels of MBL even in the general population into consideration,[4-6,10] we measured the serum MBL levels in both hemodialysis patients and healthy volunteers. No genetic study was done in either the patients or the controls. The results showed that the serum MBL level in hemodialysis patients is elevated as compared to the control group. Previous similar studies have shown conflicting results. A study performed by Satomura and colleagues[8] revealed that serum MBL level is higher in hemodialysis patients compared with that in the general population. On the other hand, a study conducted by Lam and colleagues[7] revealed that serum MBL level in hemodialysis patients was significantly lower than that in healthy adults. Satomura and colleagues believed that such a difference was related to the method of measurement. In Lam and colleagues' study, like the present study, serum MBL captured by mannan was measured. In another study in Japan, the median serum functional MBL levels were

Serum Mannose-binding Lectin (MBL) Levels in Hemodialysis and Control Groups by Gender

	Hemodialysis Group			Control Group		
Parameter	Men	Women	*P*	Men	Women	*P*
Number of patients	34	36	…	32	38	…
Median MBL, µg/mL	1.98	1.04	…	0.71	0.43	…
Mean MBL, µg/mL	2.99	2.55	.34	2.06	1.01	.11

significantly reduced in both prehemodialysis and hemodialysis patients compared with healthy subjects.[11]

Most hemodialysis patients are candidates of transplantation in future. It was shown that MBL had a role in survival of a transplant organ. In a 12-year study performed on a group of patients with pancreas and kidney transplants, those with serum MBL level less than 400 ng/mL showed significantly longer transplant survival in comparison with those with levels higher than 400 ng/mL.[12] This study has shown that in the future, serum MBL level may be used as a predictor factor in evaluation of kidney transplant survival.

Hemodialysis patients are susceptible to different infections which may be due to uremic state and therapeutic interventions like catheterization. Considering the proved role of MBL deficiency in predisposition to various infections, particularly after organ transplantation,[13-16] there might be other potential indications for measuring serum MBL before and after kidney transplantation, including adjustment of methods and dosage of immunosuppression, to achieve the optimum balance between increased transplant survival and susceptibility to infections.

The relationship between serum MBL levels with survival of hemodialysis patients was noted by Satomura and colleagues who reported that hemodialysis patients who died during a 36-month follow-up period had significantly lower serum MBL levels in comparison with survived patients.[9] The authors described that serum MBL level in hemodialysis patients may be a marker of transplant survival and detection of high-risk patients. We have begun to follow the transplant survival in patients enrolled in this study and the result will be available in the future.

In present study, we noticed no correlation between serum MBL level and age or gender in patients and control groups. Although a study conducted by Hovind and coworkers on diabetic patients followed for 18 months revealed that in early stages of type I diabetes mellitus, high serum levels of MBL were related to higher risk of microalbuminuria and permanent macroalbuminuria,[17] in the present study, there was no correlation between serum albumin and serum MBL level in hemodialysis patients.

Knowing that MBL, like CRP, is an acute-phase protein, there is a question whether it is possible that the higher serum levels in hemodialysis patients are related to concomitant inflammatory processes or not. In this study, we did not find any significant correlation between serum CRP and MBL levels in hemodialysis patients. This finding is in concordance with previous similar studies.[9]

CONCLUSIONS

Results of this study showed that MBL level in hemodialysis patients was significantly higher than that in control healthy individuals. This may have some implications in management of patients and prediction of kidney allograft survival.

ACKNOWLEDGEMENTS

This study was accomplished as thesis of Dr Akbari for nephrology subspecialty and was supported financially in part by the research deputy of Iran University of Medical Sciences. Contributions of Mrs Shahghale in collection of samples and Mr Ghelman for performing laboratory measurements are gratefully acknowledged.

CONFLICT OF INTEREST

None declared.

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Received October 2009
Revised February 2011
Accepted March 2011