Mannose-Binding Lectin and Diabetic Nephropathy in Type 1 Diabetes

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Objective: The aim of this study was to investigate serum levels of mannose-binding lectin (MBL) in type 1 diabetes with diabetic nephropathy (DN) and persistent normoalbuminuria (PN). **Method:** Serum MBL levels were determined in 224 type 1 diabetes with overt nephropathy and 224 type 1 diabetes with PN matched for sex, age, and duration of diabetes. The prediction value of MBL was compared with hemoglobin A1c (HbA1c), high-sensitivity C-reactive protein (Hs-CRP) and other known predictors. Multivariate analyses were performed using logistic regression models. **Results:** The serum MBL levels were significantly higher in diabetes with DN as compared to with PN ($P < 0.0001$). Based on the receiver operating

characteristic (ROC) curve, the optimal cut-off value of serum MBL levels as an indicator for diagnosis of DN was projected to be 1,680 $\mu\text{g/l}$, which yielded a sensitivity of 75.4% and a specificity of 78.8%, with the area under the curve at 0.768 (95% confidence interval [CI], 0.724–0.815). Multivariate logistic regression analysis adjusted for common factors showed that serum MBL level $\geq 1,680 \mu\text{g/l}$ was an independent indicator of DN (odds ratio [OR] = 6.99; 95% CI: 2.83–17.15). **Conclusion:** In type 1 diabetic patient, evaluated serum levels of MBL can be seen as an independent marker of DN even after correcting for possible confounding factors. *J. Clin. Lab. Anal.* **30**:345–350, 2016. © 2015 Wiley Periodicals, Inc.

Key words: mannose-binding lectin; diabetic nephropathy; type 1 diabetes; risk; Chinese

INTRODUCTION

Diabetes has become a major public health problem in China. In 2009, the age-standardized prevalence of total diabetes and prediabetes was 9.7% and 15.5%, respectively, accounting for 92.4 million adults with diabetes and 148.2 million adults with prediabetes (1). Diabetic nephropathy (DN) is one of the major complications of type 1 diabetes (T1DM) and it is associated with end-stage renal failure, cardiovascular disease, and increases mortality of diabetic patients (2). An emerging amount of data suggested that the complement system plays an important role in the pathogenesis of diabetic vascular complications (3). A connection between the complement system and vascular dysfunction had been suggested, with a special focus on the relation to diabetic kidney disease.

Mannose-binding lectin (MBL) is synthesized by hepatocytes and belongs to the family of C-type lectins [4]. Functional MBL deficiency occurs in as many as 10% of the normal population, and these individuals may be at increased risk of infections (5). MBL may aggravate local

and systemic inflammation through complement activation (6). Hansen et al. (7) reported that concentrations of both MBL and high-sensitivity C-reactive protein (Hs-CRP) were associated with the progression of renal disease in type 1 diabetes.

Inflammation and complement activation initiated by MBL may be implicated in the pathogenesis of diabetes and diabetic vascular complications. Hansen et al. (4) suggested that MBL may be involved in the pathogenesis of micro- and macrovascular complications in T1DM. Bouwman et al. (8) reported that MBL serum concentration was significantly higher in new-onset patients with diabetes compared with their siblings matched for

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high-producing MBL genotypes, while Megia et al. (9) found that MBL gene polymorphisms are associated with gestational diabetes mellitus.

Previous studies found that in patients with T1DM, high levels of circulating MBL have been associated with the development of DN and the presence of cardiovascular disease (4, 10). Interestingly, Hansen et al. (5) reported that in patients with T2DM, measurements of MBL alone or in combination with C-reactive protein (CRP) can provide prognostic information on mortality and the development of albuminuria. The relationship between MBL levels and DN in Chinese patients with T1DM remains unknown. Thus, the aim of this study was to evaluate serum MBL levels in T1DM with DN and with persistent normoalbuminuria (PN).

METHOD

From the outpatient clinic at Linyi people's hospital and Qilu Hospital of Shandong University, China, all patients with long-standing T1DM and DN were recruited for this study. A total of 224 patients with DN and 224 patients with PN (urinary albumin excretion [UAE] < 30 mg/24 h), matched for sex, age, and duration of diabetes, were included and recruited for this study. All patients and control subjects were of Chinese origin. DN was diagnosed clinically based on the following criteria: persistent albuminuria > 300 mg/24 h in at least two of three consecutive 24-h urine collections, presence of retinopathy, and no evidence of other kidney or renal tract disease (4). Diabetes was defined as self-report of a previous diagnosis of the disease by a clinician (excluding gestational diabetes mellitus) or hemoglobin A1c (HbA1c) of 6.5% or greater (American Diabetes Association's new diagnostic criterion for undiagnosed diabetes) [11].

At admission, we requested individual participant data regarding presence and severity of DN, age, sex, ethnicity, diabetes duration, HbA1c, systolic and diastolic blood pressure, cigarette smoking status, body mass index (BMI), and current use of diabetes, antihypertensive, and lipid-lowering medications. The study followed the tenets of the Declaration of Helsinki and was approved respectively by the Institute ethics committee of Linyi people's hospital, Qilu Hospital of Shandong University, with written informed consent obtained from each participant.

All investigations were performed in the morning after an overnight fast. Venous blood was drawn with minimal stasis from an antecubital vein. Clotted blood was centrifuged within 1 h and serum was stored at -80°C . HbA1c was measured by high-performance liquid chromatography (HLC-723 G7; TOSHO, Japan) with a normal range of 4–6%. The UAE was determined in 24-h urine collections by enzyme-linked immunosorbent assay thereafter (sensitivity, 0.001 mg/l). MBL was measured by

time-resolved immune-fluorometric assay on serum samples. Microwells coated with anti-MBL antibody were incubated with dilutions of patient serum, were developed with europium-labeled anti-MBL antibody, and europium was quantified with time-resolved fluorometric assay (Baoman Biological Technology Co., Ltd, Shanghai, China). The detection limit was 1.5 $\mu\text{g/l}$. The coefficients of variation (CV) for the intra- and inter-assay reproducibility were 8.5% and 10% for a sample of 3,500 $\mu\text{g/l}$, 6.05% and 7.5% for a sample of 1,800 $\mu\text{g/l}$, 4.2% and 4.9% for a sample of 750 $\mu\text{g/l}$. Other biomarkers, such as Hs-CRP and creatinine were tested by standard laboratory methods.

Results are expressed as percentages for categorical variables and as medians (interquartile ranges, IQRs) for the continuous variables. Univariate data on demographic and clinical features were compared by Mann-Whitney *U*-test or Chi-square test as appropriate. Correlations among continuous variables were assessed by the Spearman rank correlation coefficient. To investigate whether MBL allows predicting of DN in diabetes, different statistical methods were used. First, the relation of MBL with the DN was investigated with the use of logistic regression models. For multivariate analysis, we included confounders, known risk factors, and other predictors as assessed in univariate analysis. Results were expressed as adjusted odds ratios (OR) with the corresponding 95% confidence interval (CI). Second, receiver operating characteristic (ROC) curve was used to test the overall predict accuracy of MBL, and results were reported as area under the curve (AUC). All statistical analysis was performed with SPSS for Windows, version 20.0 (SPSS Inc., Chicago, IL). Statistical significance was defined as $P < 0.05$.

RESULTS

Patient Characteristics

There were 224 patients with DN and 224 patients with PN were eligible for the study. The median age of patients included in this study was 66 (IQR, 55–79) years and 58.9% were men. The median time of diabetes duration was 12.8 (IQR, 8.3–18.8) years. Basal characteristics of those patients are provided in Table 1.

Main Results

The results indicated that the serum MBL levels were significantly higher in diabetes with DN as compared to with PN (2,231 [IQR, 1,542–3,110] $\mu\text{g/l}$ vs. 1,100 [IQR, 856–1,550] $\mu\text{g/l}$; $P < 0.0001$; Fig. 1. Serum MBL levels increased with worse of diabetes control as defined by the HbA1c level. There was a significant positive

TABLE 1. Basal Characteristic of Diabetes Patients With DN or Normoalbuminuria

Characteristics	T2DM		P
	DN (n = 224)	Normoalbuminuria (n = 224)	
Age at baseline (IQR, years)	66 (55–79)	66 (55–79)	NS
Male (%)	58.9	58.9	NS
Diabetes duration (IQR, years)	12.8 (8.3–18.8)	12.7 (8.2–18.7)	NS
BMI (IQR, kg/m ²)	27.2 (25.9–30.4)	26.9 (25.3–29.9)	NS
Systolic blood pressure (IQR, mmHg)	148 (127–156)	124 (118–142)	0.011
Smoking status (%)	49.6	44.2	NS
Intensive glucose treatment (%)	49.1	33.5	0.016
Blood pressure treatment (%)	58.9	31.3	0.008
Use of lipid-lowering medication (%)	39.3	26.8	0.011
Laboratory findings (IQR)			
HbA1c (%)	8.6 (7.8–9.9)	7.1 (6.2–8.1)	<0.001
UAE (mg/24 h)	890 (330–2,230)	11 (5–18)	<0.0001
Serum creatinine (μmol/l)	112 (72–144)	70 (55–86)	<0.001
Total cholesterol (mmol/l)	5.3 (4.2–6.0)	4.4 (3.7–5.2)	0.032
Hs-CRP (mg/dl)	1.68 (0.76–3.22)	0.61 (0.36–1.46)	<0.001
MBL (μg/l)	2,231 (1,542–3,110)	1,100 (856–1,550)	<0.0001

Results are expressed as percentages or as medians (IQR); DN, diabetic nephropathy; BMI, body mass index; Hs-CRP, high-sensitivity C-reactive protein; UAE, urinary albumin excretion; HbA1c, hemoglobin A1c.

Fig. 1. Distribution of serum MBL levels in diabetic patients with diabetic nephropathy (DN) and with persistent normoalbuminuria (PN). All data are medians and interquartile ranges (IQRs). The horizontal lines indicate medians and interquartile ranges levels. *P* values refer to Mann–Whitney *U* tests for differences between groups.

correlation between levels of MBL and HbA1c ($r = 0.348$, $P < 0.0001$) or when the DN and PN groups were analyzed separately ($r = 0.392$, $P < 0.0001$ and $r = 0.212$, $P = 0.001$, respectively). Similarly, serum MBL levels increased with severity of DN as defined by the UAE level, and MBL concentrations were positively correlated with UAE ($r = 0.285$; $P < 0.0001$). In addition, there was a significant, albeit weak, positive correlation between MBL concentrations and Hs-CRP in the entire study group ($r = 0.224$, $P = 0.002$) or when the DN groups were analyzed separately ($r = 0.275$, $P < 0.0001$). There were no

significant sex differences, age, creatinine, duration of diabetes, or daily insulin dose.

MBL and DN

In univariate logistic regression analysis, we calculated the OR of MBL levels as compared with other factors as presented in Table 2. With an unadjusted OR of 1.002 (95% CI, 1.001–1.002; $P < 0.0001$), MBL had a strong association with DN. In multivariate analysis, after adjusting for all other significant predictors, MBL remained can be seen as an independent DR predictor with an adjusted OR of 1.001 (95% CI, 1.001–1.002; $P < 0.0001$).

Based on the ROC curve, the optimal cutoff value of serum MBL levels as an indicator for diagnosis of DN was projected to be 1,680 μg/l, which yielded a sensitivity of 75.4% and a specificity of 78.8%, with the AUC at 0.768 (95%CI, 0.724–0.815; Fig. 2). With an AUC of 0.768, MBL showed a significantly greater discriminatory ability as compared with Hs-CRP (AUC, 0.661; 95% CI, 0.583–0.772; $P = 0.006$), HbA1c (AUC, 0.701; 95% CI, 0.643–0.758; $P = 0.012$) and creatinine (AUC, 0.654; 95% CI, 0.556–0.748; $P = 0.005$). Further, in our study, we found that an increased diagnosis ability of DN was associated with MBL levels $\geq 1,680$ μg/l (unadjusted OR: 10.20, 95% CI: 4.06–30.12). This relationship was confirmed in the dose–response model. In multivariate analysis, there was an increased diagnosis ability of DN associated with MBL levels $\geq 1,680$ μg/l (OR: 6.99, 95% CI: 2.83–17.15; $P < 0.0001$) after adjusting for above possible confounders. In addition, male sex, HbA1c, Hs-CRP,

TABLE 2. Univariate and Multivariate Logistic Regression Analysis for DN

Parameter	Univariate analysis			Multivariate analysis		
	OR ^a	95% CI ^a	<i>P</i>	OR ^a	95% CI ^a	<i>P</i>
Indicator: DN						
MBL	1.002	1.001–1.002	< 0.0001	1.001	1.001–1.002	<0.0001
MBL ($\geq 1,680 \mu\text{g/l}$) ^b	10.20	4.06–30.12	< 0.0001	6.99	2.83–17.15	<0.0001
Male sex	1.24	1.12–1.36	0.005	1.14	1.05–1.26	0.009
HbA1c	1.55	1.30–1.76	< 0.001	1.31	1.10–1.48	0.003
Hs-CRP	1.13	1.06–1.21	< 0.001	1.07	1.03–1.18	< 0.001
Creatinine	1.09	1.03–1.17	< 0.001	1.05	1.02–1.163	< 0.001
Systolic BP	1.17	1.11–1.32	0.008	1.13	1.04–1.35	0.013

^aNote that the odds ratio corresponds to a unit increase in the explanatory variable.

^bMBL ($\geq 1,680 \mu\text{g/l}$) as a variable instead of MBL in the multivariate analysis model.

OR, odds ratio; CI, confidence interval; Hs-CRP, high-sensitivity C-reactive protein; DN, diabetic nephropathy.

Fig. 2. Receiver operating characteristic (ROC) curves were utilized to evaluate the accuracy of serum levels of MBL to diagnose diabetic nephropathy (DN).

creatinine, and systolic BP were also can be seen as DN indicators in multivariate analysis Table 2.

DISCUSSIONS

DN affects approximately one-third of people with type 1 or type 2 diabetes mellitus (12). This will produce significant social and economic ramifications, particularly in the developing world, such as China and India. Mounting evidence suggests that there may be a link between complement activation and the development of diabetic renal complications (13,14). In this study, we first assessed

the serum MBL levels with regard to their accuracy to predict DN in patients with T1DM in Chinese sample. There have been several papers in the literature linking MBL and DN complications in T1DM, as far as I could find, none in an ethnic Chinese sample. As such the manuscript adds significantly to the literature, especially as Asian patients with diabetes account for more than 60% of the world's diabetes population (15).

In our study, we reported that serum MBL levels were significantly higher in patients with DN as compared to patients with PN ($P < 0.0001$). Importantly, for the entire group, when adjusting for other possible risk factors, an elevated MBL level was an independent DN protection factor, and serum MBL levels $\geq 1,680 \mu\text{g/l}$ was associated with a 6.99-fold increase in DN, suggesting a possible role of MBL in the pathogenesis of DN complications in T1DM. It could thus be hypothesized that in patients with T1DM, high levels of MBL may contribute to the development of nephropathy through aggravated complement activation. Similarly, Hansen et al. (7) reported that concentrations of both MBL and Hs-CRP were associated with the progression of renal disease in T1DM. Further, we found that the serum MBL levels increased with decreasing severity of DN as defined by the UAE.

In previous study, Hansen et al. (4) reported that there were no correlations between Hs-CRP and MBL levels ($P = 0.12$), whereas there was a significant, albeit weak, positive correlation between MBL concentrations and HbA1c ($P = 0.001$), UAE ($P = 0.013$) in another study (16). In our study, we found that serum MBL levels were correlation with HbA1c and UAE, and the relationship between Hs-CRP and MBL was also found. Different information was reported, as there was no correlation between the two proteins in previous studies of patients with T1DM (4,5,17). Several studies have shown that deficiency of MBL increases the overall susceptibility of an individual to infectious disease (18). The most

striking example of this is the association of acute respiratory tract infections with MBL deficiency in early childhood (19). Clinical studies have shown that MBL insufficiency is associated with bacterial infection in patients with neutropenia and meningococcal sepsis. Numerous other potential infectious disease associations have been described (20). In contrast, there is evidence that for some intracellular parasites MBL deficiency may be protective and this might explain the high frequency of *MBL* mutations in sub-Saharan Africa and South America (18). MBL is an example of a pattern recognition molecule that plays a dual role in modifying inflammatory responses to sterile and infectious injury (21).

The biological mechanism linking MBL with DN is still unclear. Only 30–40% of patients with diabetes mellitus develop DN, which suggests that other contributing factors besides the diabetic state are required for the progression of DN (22). First, the distinct difference in MBL levels between diabetic patients with nephropathy and patients with normoalbuminuria is in part attributable to differences in the MBL genotype distribution, indicating that inherited high concentrations of circulating MBL may be a risk factor for DN (4). Second, many lines of evidence show that inflammation is a cardinal pathogenetic mechanism in DN (23). Inflammatory cells have all been implicated in the pathogenesis of DN via increased vascular inflammation and fibrosis (2). In addition, MBL may aggravate local and systemic inflammation through complement activation and modulation of pro-inflammatory cytokine production (24). Third, MBL could play a role in the progression of DN through oxidative stress (25). Diabetic mice with severe endothelial dysfunction owing to deficiency of endothelial nitric oxide synthase develop progressive nephropathy and retinopathy similar to the advanced lesions observed in humans with diabetes mellitus (18). Fourth, circulating MBL has the ability to effectively initiate inflammation through the enzymatic activation cascades of complement. Complement activation from any cause may thus have more widespread consequences in diabetic patients and contribute to the ongoing inflammation and microvascular and macrovascular complications of diabetes (5).

A number of issues have to be taken into account when interpreting the results of the present study. One limitation of our analyses is the relatively small study size and the modest size of the observed effects as well as the unavailability of DNA samples for the analysis of MBL genotypes. Second, without serial measurement of the circulating MBL, this study yielded no data regarding when and how long of MBL was elevated in these patients. Additionally, it should be investigated whether serial MBL testing further improves the risk stratification of these patients. Third, further studies should investigate whether MBL can help physicians tailor the therapy in view of

the relative risk and allocate resources accordingly and whether this strategy might affect DN outcome.

CONCLUSIONS

In type 1 diabetic patient, evaluated serum levels of MBL can be seen as an independent marker of DN even after correcting for possible confounding factors. We suggested that further studies should be carried out with respect to what was the cause of the increased MBL levels and the role in the pathology of the DN. If it is possible to elucidate this, more intensive efforts could be directed toward the cause, thus hopefully improve the prognosis of these patients.

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CONFLICT OF INTEREST

On behalf of all authors, the corresponding author states that there is no conflict of interest.

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