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Serum Levels of Mannose-Binding Lectin in Atopic and Healthy Mongolian Adults

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Objectives: Mannose-binding lectin (MBL) is a vital protein of an innate immune system synthesized by the liver. The serum level of MBL is associated with an increased susceptibility to infection with a high risk of allergic and autoimmune diseases. The aim of this study was to determine the profile of serum levels of MBL in atopic and healthy Mongolian subjects. **Methods:** Serum samples were collected from 219 healthy Mongolian adult blood donors and 216 atopic subjects. We analyzed the MBL level in each serum by using the double-antibody sandwich ELISA method. **Results:** The mean serum level of MBL was 3088.28 ± 669.8 ng/ml in atopic subjects and 2165.07 ± 708.5 ng/ml in healthy groups. In males, the MBL mean serum level of atopic subjects was 3012.8 ± 783.16 ng/ml and 2073.33 ± 678.26 ng/ml in the healthy group of males. The MBL mean serum level of female atopic subjects was 3138.22 ± 580.6 ng/ml and 2263.61 ± 733.1 ng/ml in the healthy group of females. Low association and significant differences were observed between MBL levels of atopic and healthy subjects. **Conclusion:** The serum level of MBL in atopic subjects was comparatively higher than in subjects of the healthy group. Significant differences were observed between mean levels of MBL between the different age groups.

Keywords: Mannose-Binding Lectin, Atopic Hypersensitivity, Complement Activation, MBL-Associated Serine Proteases

Introduction

The prevalence of allergic diseases including; asthma, allergic rhinitis, anaphylaxis, drug allergy, food allergy and atopic eczema is increasing in developed and developing countries of the world. One of the main extrinsic risk factors of asthma and allergies is urbanization and changing lifestyles related to economic development. Increasing rates of morbidity and mortality caused by allergic diseases are interdependent with economic development [1]. The World Health Organization and World Allergy Organization have emphasized that 300 million people have asthma and 400 million individuals are suffering from rhinitis--worldwide [1, 2]. In a population-based epidemiological study published [3] in 1999, it was determined that the prevalence of asthma and allergic rhinoconjunctivitis was 1.1% and 9.3% in villages, 2.4% and12.9% in rural towns

and 2.1% and 18.4% in Ulaanbaatar, respectively. This shows the occurrence of allergic diseases is lower in rural areas and significantly higher within city populations of Mongolia [3]. In 2009, the prevalence of asthma and allergic rhinitis increased among adults 4.7% (95% CI: 4.3 – 5.6) and 23.6% (95% CI: 22.4 - 24.9) respectively, in Ulaanbaatar city [4].

The complement system is activated via three pathways: the alternative, the classical and the Mannose-binding lectin (MBL). MBL is a calcium dependent collecting protein, which contains collagen and lectin domains. MBL is synthesized in the liver as a vital component of innate immunity [5, 6]. The gene for human MBL lies on the long arm of chromosome 10q11.2-q21 [7]. MBL has a major role within the pattern recognition molecule of the innate immune system. MBL binds to N acetylglucosamine sugar motifs and mannose structures on the surface of bacteria, fungi, and viruses to opsonization and it helps phagocytosis of macrophages [5, 8].

Recently, Hawlisch H et al. explained an alternative mechanism of allergic diseases in 2004 Components such as C3a, C5a (anaphylatoxins) of the activated complement system play a role in the pathogenesis of asthma and allergic diseases. The strongest anaphylatoxin is C5a [9]. One of the important complement pathways is the lectin complement cascade, which is initiated by the multifunctional C-type serum lectin, MBL. The activation of the MBL-mediated lectin complement pathway is marked by the activation of C4 to C4a and C2 to C2a, which represent the first product of this pathway. The complex of C2a, C4a activate C3 and C5 components [10]. In a study by Varga, it was discovered that an allergen can bind to MBL and activate the lectin complement pathway. This verifies the lectin pathway in allergen-induced activation. [11].

In our study, we defined the serum levels of MBL in atopic and healthy subjects in Mongolia. It was important to define the reference value of serum levels of MBL and determine the different serum levels of MBL in different age groups with allergies and within the healthy population of Mongolia.

Materials and Methods

1. Study Subjects.

We collected 216 serums samples of atopic subjects from 4 different age groups (21 - 30, 31 - 40, 41 - 50 and 51–60 years old). Atopic subjects were diagnosed based on clinical symptoms, laboratory findings and positive results from a skin prick test for different kinds of aeroallergens by "Effect" Allergy and Asthma Clinic. We also collected 219 serums from healthy adult blood donors from the National Center for Transfusion Medicine, Mongolia. All donors signed a donor health questionnaire, to determine donors' allergy histories, and other respiratory symptoms and issues.

2. Sample collection

After collection, we allowed each sample to clot in a serum separator tube (SST) for 30 minutes. After sufficient clotting was achieved, the sample was centrifuged for 15 minutes at 1000 rpm at room temperature then separated in a Eppindorff tube for the future assay. Serum samples required at least a 1:50 dilution using Assay Diluent. Serums to be tested within 8 hours were stored at 40C or frozen at -800C for future testing.

3. MBL Assay

Serum levels of MBL were determined by an enzyme-linked immunosorbent assay according to the protocol in the kit (Human Mannose Binding Lectin ELISA kit, Gen Way Biotech Inc ™, San Diego CA, USA). The kit contains components necessary for quantitative determination of natural or recombinant Human MBL concentrations of any experimental sample including; cell lysates, serum and plasma. This particular immunoassay utilizes the quantitative technique of a "Sandwich" Enzyme-Linked Immunosorbent Assay (ELISA) where the target protein (antigen) is bound in a "sandwich" format by the primary capture antibodies coated to each well-bottom. Secondary detection antibodies were added subsequently by the investigator. The capture antibodies [coated to the bottom of each well] are specific for a particular epitope of Human MBL while the user-added detection antibodies bind to epitopes on the captured target protein. After incubation and "sandwiching" of the target antigen, a peroxidase enzyme is conjugated to the constant heavy chain of the secondary antibody (either covalently or via Avidin/Streptavidin-Biotin interactions), allowing for a colorimetric reaction to ensue upon substrate addition. When the substrate TMB (3, 3', 5, 5'-Tetramethylbenzidine) is added, the reaction catalyzed by peroxidase yields a blue color that represents the antigen concentration. Upon sufficient color development, the reaction can be terminated by adding a Stop Solution (2 N Sulfuric Acid) causing the color of the solution to

turn yellow. The absorbance of each well was read at 450 nm using a microplate reader.

4. Statistical analysis

All analyses were performed with SPSS Statistics version 23.0. Intergroup comparisons in demographic characteristics of atopic and healthy subjects. Age groups of atopic and healthy subjects were analyzed using independent t-test or the Chi – square test. The ANOVA test was used in the atopic and healthy groups to compare MBL levels between age groups in males and females. A correlation analysis was performed between MBL levels according to age and gender. Statistical significance was accepted at $p<0.05$.

5. Ethical statement

The study was conducted after obtaining the approval of the Biomedical Research and Ethical subcommittee of the Mongolian National University of Medical Sciences. Each patient signed a consent form before participating in the study.

Results

1. Study subjects

Two hundred nineteen healthy adult subjects (113 males and 106 females) and two hundred sixteen atopic subjects (86 males and 130 females) from different age groups (21 - 30, 31 - 40, 41 - 50 and 51 – 60 years old) were chosen for this study. General characteristics of all subjects of this study are shown in Table 1.

2. Serum level of MBL in Healthy groups

In the healthy group, the mean age was 37.5 years and the mean serum level of MBL was 2165.1 ± 708.5 ng/ml. The mean levels of all four age groups are reported in Figure 1. We found statistically significant differences in MBL serum levels between the different age groups ($p = 0.0001$). Comparisons of mean MBL serum levels of healthy male and female subjects within the age groups are illustrated in Table 2. There was a significant difference in MBL levels among healthy age groups of females ($p = 0.0001$). Especially, in the 21-30 age group which was significantly higher than in the remaining groups: 30-40 age group ($p = 0.001$); 41 - 50 age group ($p = 0.0001$); and 51-60 age group ($p = 0.041$). However, we did not find statistically significant differences between the MBL serum levels of different age groups in males ($p = 0.07$). The highest serum levels of MBL were defined in 21 year old subjects. Serum levels of MBL then continuously decreased within the remaining age groups. In this study, we found a statistically significant difference of serum levels of MBL between both gender groups ($p = 0.045$).

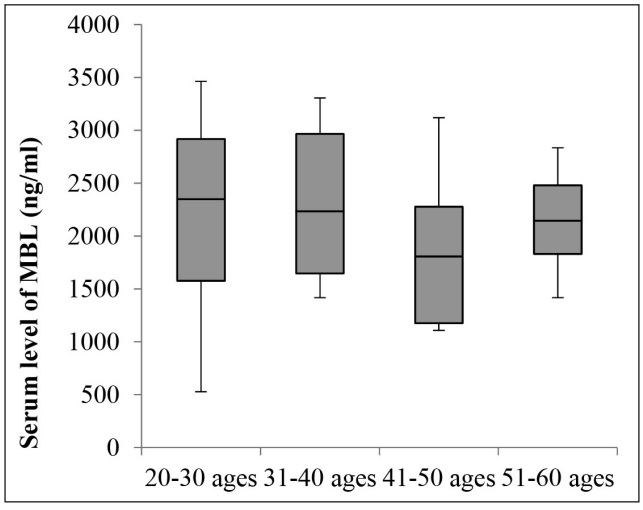

Figure 1. Serum level of MBL in healthy groups ($p = 0.0001$)

3. Serum level of MBL in Atopic subjects

All patients with atopic diseases had positive results [having at least one common allergen] from the skin prick test. In atopic subjects, the mean age was 32.5 years and the mean serum level of MBL was 3088.3 ± 669.8 ng/ml. Figure 2, shows mean serum

Table 1. Patient characteristics in atopic and healthy groups

		Healthy (n = 219) n	Atopic (n = 216) n	p-value
Gender	Male	113	86	0.024[b]
	Female	106	130	
Age		37.5 ± 10.7[c]	32.5 ± 10.1[c]	0.15[a]
MBL		2165.1 ± 708.5[c]	3088.3 ± 669.8[c]	0.0001[a]

[a]t-test. [b]Chi-square test. [c]values are mean ± SD.

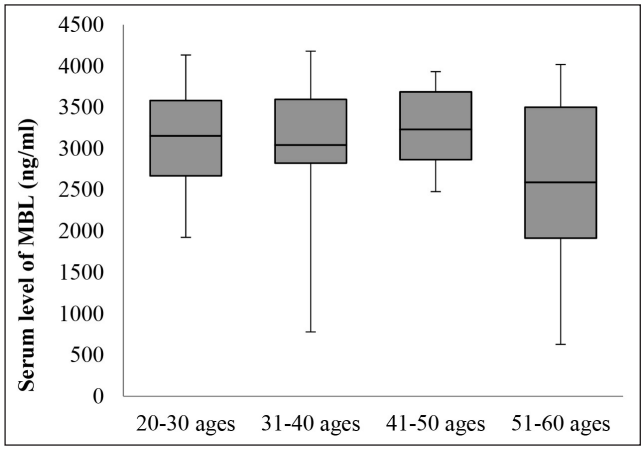

Figure 2. Serum level of MBL in atopic subjects (p = 0.01)

years old age group than in the 51-60 age group (p = 0.0001). However, , there were not significant differences of serum levels of MBL between four age groups in females (p = 0.303).

4. Comparing serum level of MBL both groups

Table 4, shows serum levels of MBL in healthy and atopic subjects of both genders within different age groups. Levels of MBL in the 21 – 30 age group of healthy females was comparatively higher (p = 0.0001) than in other age groups. In males, levels of MBL of the 41 – 50 age group with atopic males was statistically significantly higher than within the same age group of females (p = 0.002). Levels of MBL in 21 - 30, 31 - 40, and 41 - 50 age groups were statistically significantly different between atopic

Table 2. Serum level of MBL between age groups [male and female] in healthy groups

	Age groups				p-value
	21-30 years	31-40 years	41-50 years	51-60 years	
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	
Gender					
male	2066.3 ± 728.8	2336.8 ± 533.7	1737.6 ± 843.5	2095.4 ± 440.4	0.07
female	2788.6 ± 642.7	2132.4 ± 731.9	1834.3 ± 492.7	2241.2 ± 613.1	0.0001

levels of MBL in atopic subjects and the statistically significant difference of serum levels of MBL between 4 different age groups (p = 0.01). Table 3, shows serum mean levels of MBL of atopic subjects in males and females of the different age groups.

subjects and healthy subjects (Table 5). There were no differences between 51 - 60 age groups in both atopic and healthy subjects.

In this study, we identified a weak, negative correlation (r =

Table 3. Serum level of MBL between age groups [male and female] in atopic subjects

	Age groups				p-value
	21-30 years	31-40 years	41-50 years	51-60 years	
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	
Gender					
male	3100.8±556.6	2706.4±1058	3442.8±453.8	2048.9±1286	0.0001
female	3199.9±525.4	3108.5±639.1	2741.1±203.7	3134.8±741.8	0.303

There was a statistically significant difference in serum levels of MBL in male atopic subjects (p = 0.0001). Serum levesl of MBL was comparatively higher in the 21- 30 age group than in the 51 - 60 age group (p = 0.001) and and higher in the 41 - 50

-0.219) of levels of MBL within the age groups of atopic subjects. There did not appear a correlation between gender groups (Table 6). In healthy groups, we observed a weak, negative correlation (r = -0.237) between levels of MBL and the ages of subjects.

Table 4. Serum levels of MBL of healthy and atopic subjects [male and female] in different age groups

	MBL level				p-value
	Male		Female		
	Mean	SD	Mean	SD	
Healthy group by age groups					
21-30 years	2066.28	728.88	2788.63	642.68	0.0001
31-40 years	2336.83	533.73	2132.37	731.89	0.319
41-50 years	1737.59	843.48	1834.28	492.66	0.595
51-60 years	2095.43	440.40	2241.22	613.00	0.418
Atopic subjects by age groups					
21-30 years	3100.84	556.58	3199.97	525.45	0.322
31-40 years	2706.41	1058.01	3108.56	639.15	0.110
41-50 years	3442.84	453.79	2741.11	203.75	0.002
51-60 years	2048.99	1286.57	3134.80	741.81	0.058

Table 5. The mean serum level of MBL by age group

	Groups						p-value
	Atopic subject			Healthy group			
	n	Mean	SD	n	Mean	SD	
Age groups							
21-30 years	118	3154.61	539.87	87	2348.58	778.06	0.0001
31-40 years	62	3043.70	727.28	40	2234.60	640.67	0.0001
41-50 years	20	3232.32	510.56	56	1806.66	606.67	0.0001
51-60 years	16	2591.89	1159.16	36	2144.03	500.41	0.056

Table 6. Correlation coefficients of the serum level of MBL by age and gender in atopic subjects

		MBL	Age	Gender
MBL	r	1		
	p			
Age	r	-0.219	1	
	p	0.001		
Gender	r	0.092	0.002	1
	p	0.179	0.981	

r - correlation coefficient; p- p-value

Table 7. Correlation coefficients of the serum level of MBL by age and gender in healthy groups

		MBL	Age	Gender
MBL	r	1		
	p			
Age	r	-0.237	1	
	p	0.0001		
Gender	r	0.121	0.030	1
	p	0.075	0.659	

r - correlation coefficient; p- p-value

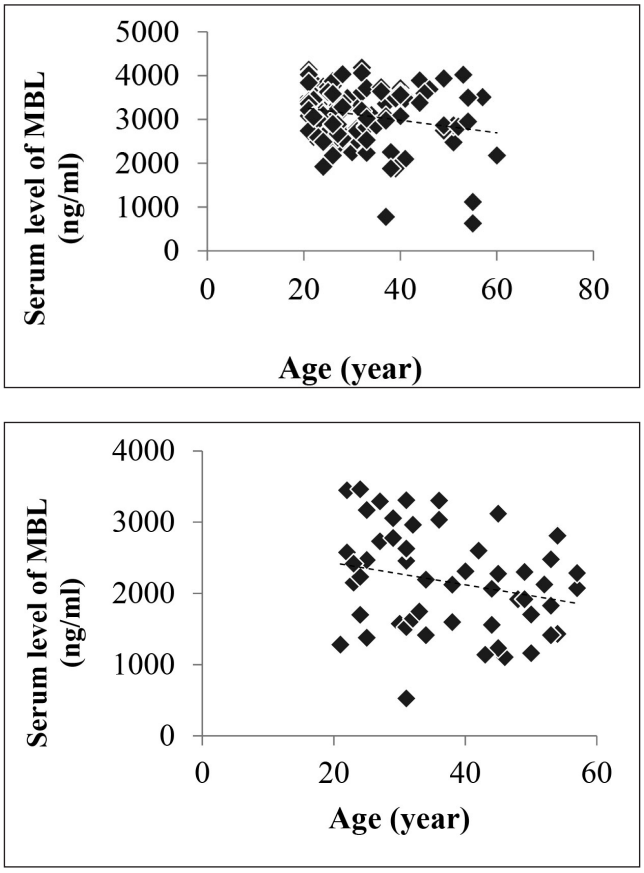

Figure 3. Correlation of the serum level of MBL and ages in atopic (a) and healthy (b) subjects (Pearson correlation coefficient (a) r = -0.219, p = 0.001 and (b) r = -0.237, p = 0.0001).

However, there was no correlation between genders (Table 7). In Figure 3, we defined a weak, negative correlation between atopic and healthy groups (atopic subjects r = -0.219, p = 0.001, healthy group r = -0.237, p = 0.0001, and also between levels of MBL and age groups .

Discussion

In atopic individuals, most allergic diseases are dominated by the preferential development of specific Th2 type of adaptive immune responses against innocuous antigen. Recently, several studies have mentioned the important role of MBL on pathophysiological mechanisms of allergies [9-11]. Some researchers have defined that MBL concentration of serum was significantly different in allergic patients and healthy people [11-13].

In our study of atopic subjects, the mean age was 32.5 and the mean serum level of MBL was 3088.3 ± 669.8 ng/ml. The mean level of MBL of all atopic subjects was statistically significantly higher than of healthy groups (p = 0.0001). The results of this study are comparable with Japan and Switzerland studies [15-17].

The mean MBL levels of allergic patients (3.28 ± 0.86 µg/ml) were significantly elevated in comparison with the controls (2.08 ± 0.50 µg/ml) in Indian individuals [12]. Another study by Uguz et al. on pediatric mild asthma patients in an African population reported the presence of significantly higher serum MBL levels in asthmatic children with allergy (65.7%), (3.6 mg/l) than in healthy patients (2.8 mg/l) [13].

In healthy Mongolian subjects, the mean age was 37.5 and the mean serum level of MBL concentration was 2165.1 ± 708.5 ng/ml. Similar observations were reported in the study among Korean population. In Korea, subjects below 30 years in age, the mean MBL level was 2139 µg/l. In the age group 30-39 the mean MBL level was 1466 µg/l [14]. In Switzerland, the mean MBL level was 1.960 µg/ml in healthy children <16 and 1.130 µg/ml in adults ages 18-64. In Iran, the mean and median of MBL were 2.207 ± 1.73 µg/ml and 1.858 µg/ml, In Iranian adults, the mean and median of MBL were 1.56 ± 1.04 µg/ml. In Japan, the median MBL was 1.28 µg/ml within age group of 20-39. It seems mean concentration of MBL in healthy Mongolian adults in the present study was close to other studies [15-17].

Salazar et al., suggest lectin recognizes glycoallergens from diverse sources and that this engagement elicits different intra and extracellular responses, which in some cases can lead to opposing effects. Some of these interactions could form the basis for new method for immunotherapy of allergies [18].

Conclusion

The results of this study showed a significant difference in the mean MBL concentrations among different age groups. In atopic males, ages 21 -30, MBL levels were higher than other age groups. A weak and negative correlation between MBL levels occurs with age as a decline in MBL level is associated with aging. Increasing the MBL level in plasma might be a sensitive marker of allergic diseases. It shows that Mongolian young people may be more sensitive to allergies. Mean MBL levels in atopic subjects of 21 - 30, 31 – 40, 41 - 50 age groups were higher than of in healthy groups.

Levels of functional MBL in plasma which significantly

increase in atopic groups suggest a possible role of MBL in the pathophysiology of allergy..

Furthermore, these data of MBL level of healthy subjects can be used as reference values for other disease association studies.

Currently, it is thought that non-adequate interactions between adaptive and innate immune systems may cause atopic diseases such as allergic rhinitis, allergic dermatitis and asthma. Recent data indicate genetic polymorphisms in genes are involved in innate immune responses showing a tight association with increased incidence of allergic diseases. [19]. Therefore, in the future, we will study MBL2 genotypes in atopic Mongolian subjects by including those with autoimmune diseases and common prevalent infectious diseases.

Conflict of Interest

The authors state no conflict of interest.

Acknowledgements

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