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Mannan-Binding Lectin Serum Levels in 593 Healthy Iranian Children and Adults

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ABSTRACT

Mannan-binding lectin (MBL) is a vital protein of innate immune system and has two critical functions: complement activation through the lectin pathway and opsonization. MBL deficiency has been classified as the most common inherited immunodeficiency known in humans (about 30% of the population), and is associated with predisposition to infections and high risk of some autoimmune diseases. The purpose of this study was to determine the profile of MBL serum level in Iranian healthy population in association with sex and age groups for the first time.

We studied the serum concentration of MBL in 593 Iranian healthy cases: 340 males and 235 females in 4 different age groups by using enzyme-linked immunosorbent assay.

The mean serum levels of MBL were 3.854± 2.77μg/ml at the age of less than 6 months, 4.147±3.54μg/ml at 6 months to 2 years of age, 4.410±3.09μg/ml at 2-6 years and 2.207±1.73μg/ml in adults. There was significant differences in the mean concentration of MBL among different age groups of children and also between children and adults (p<0.05). No association was observed between sex and MBL concentrations.

MBL serum levels of Iranian population seem to be different from some of other populations which may be explained by genetic variations. The MBL values in this study can be used as a normal reference range for future studies in Iranian population.

Keywords: Iran; Mannan-Binding Lectin; Reference value

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INTRODUCTION

Mannan-binding lectin (MBL) is a calcium-dependent collectin protein, which contains collagen

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and lectin domains, synthesized in the liver and is an acute phase reactant.[1,2] The MBL gene is located on chromosome 10q11.2-q21 [3]. MBL has a major role in innate immune system. MBL binds to N-acetylglucosamine and mannan structures on the surface of yeasts, bacteria, fungi, viruses and protozoa, leading to opsonization, phagocytosis and activation of complement system through lectin pathway, independent of an antibody.[1,4,5] MBL deficiency is considered as the most common inherited immunodeficiency in humans,[6] with a frequency of 5% (homozygote) and 30% (heterozygote). Most MBL-deficient people appear to be clinically healthy. However, low serum MBL levels have been associated with a range of bacterial infections in children and adults.[4,9-13] Especially, a heightened risk of infection occurs when passively acquired maternal immunity has waned (at 6 months) but adaptive antibody responses are still immature.[9] MBL deals with a wide range of pathogens, especially gram positive and negative bacteria, so MBL deficiency is associated with susceptibility to recurrent infections often in the form of upper respiratory infections, abscess, meningococcal infections and sepsis.[3,10] In addition to increasing the risk of different infections, MBL deficiency is associated with non-infectious diseases including systemic lupus erythematosus, rheumatoid arthritis, cystic fibrosis and common variable immunodeficiency.[8]

Like most of the immune human factors, MBL serum levels vary in different ages; MBL increases significantly after birth, and is at its highest at the age of 1 month. Thereafter, it declines to the initial level until the age of 5 months. Its concentration continues to decrease during childhood, and after the age of 12 years, the MBL values reach the adult level.[11] MBL deficiency is defined as an undetectable MBL serum level. Individuals with homozygous variation have plasma concentrations less than 1% normal levels, heterozygote subjects have about 10% of normal concentration.[8]

MBL replacement therapy and it's possible beneficial effect has not been established yet, but there are hopes that MBL replacement therapy is associated with decrease of burden of disease in susceptible individuals to infections including cystic fibrosis patients and after chemotherapy, especially in patients for whom common treatment was not effective.[13]

According to genetic variations and different MBL serum levels in different populations, this study was conducted in order to establish the reference value of MBL serum levels for the first time in Iranian healthy population.

MATERIALS AND METHODS

Study Subjects

The study subjects consisted of 593 Iranian healthy individuals. The subjects, based on sample size and the study of Chen, *et al.*[12] were divided into 4 age groups: <5 months (126 subjects), 5 months to 2 years (166 subjects), 2-6 years (180 subjects) and 18-55 years old (adults) (121 subjects).

Children (<6 years old) were the subjects referred to Children Medical Center, Tehran University of Medical Sciences, Tehran, Iran, for out-patient surgery such as circumcision, hernia repair, and adenoidectomy or screening tests to rule out of some diseases like thalassemia, iron deficiency anemia, hypothyroidism, and etc. They did not have any history of infections, hospitalizations or growth disorders, and their health were approved by the visiting physician. Sampling was done with informed consent. Adult blood samples were taken from healthy volunteer blood donors, Iranian Blood Transfusion Center.

Since MBL is an acute phase reactant protein, its serum level is unreliable in infections and according to ELISA kit guide, jaundice leads to disturbance measurement, so concurrent infection or jaundice were excluded from the subjects in this study. MBL serum level has an intense relation with thyroid hormones, and increasing and decreasing of MBL concentrations are seen in hyperthyroidism and hypothyroidism, respectively,[14] therefore these were ruled out for all of the samples.

MBL Assay

The serum concentration of MBL was determined by an enzyme-linked immunoassay according to the protocol in the kit (Mannose Binding Lectin ELISA kit, Sanquin, Amsterdam, the Netherlands).

Statistical Methods

The differences of MBL levels among various age/gender groups were evaluated using T-Test and ANOVA. Statistical significance was accepted at $p<0.05$. All statistical calculations were performed with SPSS (version 18).

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This study was approved by ethical committee of IAARI. Informed consent was taken from children's parents and adult blood donors.

RESULTS

Our study subjects consisted of 340 males and 235 females. The mean of MBL concentrations in 4 different age groups is illustrated in Table 1. At the age of <5 months the mean±standard deviation and median of MBL were 3.854±2.77 µg/ml and 4.065 µg/ml, repectively. At the age of 5-24 months the mean and median were 4.147± 3.54 µg/ml and 3.761 µg/ml. The mean and median were 4.410±3.09 µg/ml and 4.211µg/ml at the age of 2-6 years. In adults the mean and median were 2.207±1.73 µg/ml and 1.858 µg/ml. There was a significant difference in the MBL concentrations among the 4 age groups (p<0.0001). The percentile of MBL serum level in different age groups are presented in Table. 2. Also according to age, the subjects were divided into 2 groups: children (≤ 6 years old) and adults. There was significant differences between the mean of MBL concentration in the 2 groups (p<0.05). There was no significant difference between the sex groups (p=0.074).

Table 1. The mean and Std. deviation of MBL Serum Levels in deferent age groups

Age	Sex groups	Number (N)	Mean (µg/ml) (%95 [1]CI)	[2]Std. Deviation (µg/ml)	Median	Percentile (%5,%95)
0-5 m	Male	85	4.280 (3.68,4.87)	2.768	4.378	0.583,8.458
	Female	41	2.970 (2.15,3.79)	2.595	1.682	0.002,7.380
	Total	126	3.854 (3.36,4.34)	2.772	4.065	0.068,8.219
6-24 m	Male	90	4.511 (3.77,5.24)	3.503	4.121	0.053,11.284
	Female	76	3.716 (2.90,4.53)	3.572	3.21	0.031,9,775
	Total	166	4.147 (3.60,4.69)	3.546	3.761	0.043,9.725
24-72 m	Male	104	4.300 (3.69,4.91)	3.138	3.758	0.054,10.929
	Female	76	4.560 (3.86,5.25)	3.051	4.791	0.061,9.086
	Total	180	4.410 (3.96,4.86)	3.096	4.211	0.062,9.255
Adult	Male	61	2.132 (1.73,2.52)	1.530	1.858	0.031,5.006
	Female	60	2.284 (1.78,2.78)	1.938	1.808	0.051,6.695
	Total	121	2.207 (1.89,2.52)	1.739	1.858	0.041,5.899

[1]CI: Confidence Interval

[2]Std. Deviation: Standard Deviation

Table 2. Percentile of MBL serum level in different age groups

Mean of MBL serum level (µg/ml)							
Percentile of population							
Age	5	10	25	50	75	90	95
0-5 m	0.068	0.485	1.181	4.065	5.945	7.088	8.219
6-24 m	0.043	0.099	1.087	3.761	6.112	8.772	9.725
24-72 m	0.062	0.637	1.440	4.211	6.641	8.491	9.255
Adults	0.041	0.263	0.787	1.858	3.146	4.772	5.899

Table 3. The mean of MBL levels among children and adults

Age Groups	N	Mean(µg/ml) (%95 CI)	Std. Deviation	Median	Percentile (%5,%95)
Children (<6yr)	472	4.196(3.88,4.45)	3.184	3.960	0.060,9.510
Adults (18-55)	121	2.207(1.89,2.52)	1.739	1.858	0.041,5.899

DISCUSSION

In the present study, MBL serum concentrations of 593 healthy Iranian population, the mean and median of MBL concentration in children, were 4.196±3.18 µg/ml and 3.960 µg/ml respectively And in adults, its mean and median were 2.207±1.73 µg/ml and 1.858 µg/ml respectively. The results in this study are comparable with other studies. The median of MBL concentration were reported 2.536 µg/ml in healthy Chinese children (<6 yr),[12] 1.960 µg/ml in healthy neonates and children <16 yr and 1.130 µg/ml in adults (18-64 yr) in Switzerland.[16] The mean and median of MBL were 2.30±1.30 and 2.33 µg/ml under the 20 yr, 1.56±1.04 µg/ml and 1.28 µg/ml in older cases (20-100 yr) in normal Japanese population.[15] In Finnish adults the mean and median concentration of MBL were 4.48 µg/ml and 4.02 µg/ml.[11] It seems the median of MBL concentration in adults in the present study was close to other studies in spite of Finnish, but in children there were differences in the median of MBL concentration in these studies. This can be explained by different age range in each study or other factors, like differences in the prevalence of infectious agents or genetic background.

The results of this study showed that there was a significant difference in the mean of MBL concentration between age groups, especially between children (6 yr) and adults which was lower in adults. The findings were consistent to previous studies. So it should be noted that the adult normal ranges can not be applied to pediatrics. In children, the higher level of MBL probably reflects childhood infections and the need of MBL and its important role in the first line of antimicrobial defense until it is replaced by a more specific immune component such as the antibody system.

We found that the MBL concentration varies with age, so it is important to take age into consideration in the evaluation of MBL levels among patients or normal individuals. We propose that the MBL reference values in Iran are 0.002-7.380 at the age of <5 months, 0.031-9.775 at the age of 5-24 months, 0.061-9.086 at the age of 24-72 months and 0.051-6.695 in adults (Table 2). Individuals with MBL concentration below 5th percentile in table 2 should be considered as MBL deficient. These data can be used as reference values for future studies of MBL in Iran.

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