Mannan - binding Lectine Serum Level Dosing Method

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Mannan-binding lectine (MBL) is a protein implicated in immune response in children. Children are more prone to develop pathologies like infection, autoimmune disease, cystic fibrosis, liver disease. The aim of the study was to evaluate the MBL serum levels in children with cystic fibrosis, liver disease, compared to controls. Subjects with cystic fibrosis and liver disease were aged matched with controls, blood samples were collected. MBL was determined by solid-phase enzime-linked immunoabsorbent assay. In the group of subjects with liver disease associated with cystic fibrosis, the average was 2061.99 ng/mL, almost 50% lower than average level in the controls group (3986.827 ng.ml), being significantly low among children with cystic fibrosis and liver disease. In our study MBL values ranged from 2.3 ng/ml to 5432 ng/mL. Determining of MBL levels in children is suggestive for certain diseases, like cystic fibrosis liver disease and could be used as a predictive factor for early diagnosis and subsequent therapy.

Keywords: mannan-binding lectine, children, cystic fibrosis, inate immunity

Mannan-binding lectine(MBL) is a protein from lectins family, able to recognize carbohydrate patterns formed on the surface of pathogens and host cells, having a role in complement activation and activation of immune defense [1,2]. MBL serum deficiency has the ability to decrease complement activation, an action that is extremely important for the humoral immunity functionality [3]. Epidemiological studies show that MBL deficiency influences susceptibility and evolution of very common diseases like infections, neoplasm, metabolic and cardiovascular diseases [4-6]. MBL 2 gene has an oligomer structure, with three subunits containing identical peptides [7], the homozygous genotype for structural mutations are associated with low levels of MBL [1,8]. MBL deficiency may be associated with autoimmune diseases and immunodeficiency [8], being a risk factor for severe infections. It is well known that low levels of MBL are found in patients with immunodeficiency and susceptible to serious infections as well as in premature infants or in patients with autoimmune pathology [9, 10]. Published studies suggest that the severity of liver disease in patients with cystic fibrosis(CF) is influenced by low MBL levels, these patients having an increased risk of developing cirrhosis[1]. It is possible that associated immunodeficiency with MBL variants, to facilitate the development of hepatotoxic lesions, thereby facilitating degradation of liver status in patients with cystic fibrosis [11].

Gabolde and collaborators have started from the finding that low serum levels of MBL status, associated with homozygous or heterozygous allele-specific MM is associated with more severe evolution of patients with chronic viral hepatitis due to a lack of opsonization and induced immunodeficiency [1]. There are studies that suggest that MBL deficit can be a predictor for the development of cystic fibrosis associated liver disease(CFLD) [12] , but to produce clear evidence, longitudinal studies are needed in larger groups of patients. In this context we consider interesting to perform the dosage of serum MBL levels in our patients liver disease associated with cystic fibrosis, in order to interpret the mentioned hypothesis.

Experimental part

Material and methods

Blood samples were collected from 35 subjects (21 with liver disease associated) with cystic fibrosis monitored for annual evaluation and in parallel were collected blood samples from a control group formed by children present for annual biological assessment(without diseases which could influence the MBL levels) . Patients and their parents signed their informed consent and approval of Clinics Hospital Committee was obtained. Patients with severe lung disease, autoimmune diseases, immunodeficiency, liver failure, were excluded from the group of patients with cystic fibrosis liver disease. Samples were obtained during clinical care of patients and were separated according to the standard operating procedures. After separation, serum aliquots were stored at – 80°C. Serum was diluted to 1/5-1/150 and results were corrected with dilution factor. Serum MBL concentration were determined by solid-phase enzime-linked immunoabsorbent assay using MBL Oligomer Elisa Kit 029 (AntybodyShop, Copenhagen, Denmark) in Chemistry and Biochemistry Laboratory in the Faculty of Agriculture of Banat's University of Agricultural Science and Veterinary Medicine from

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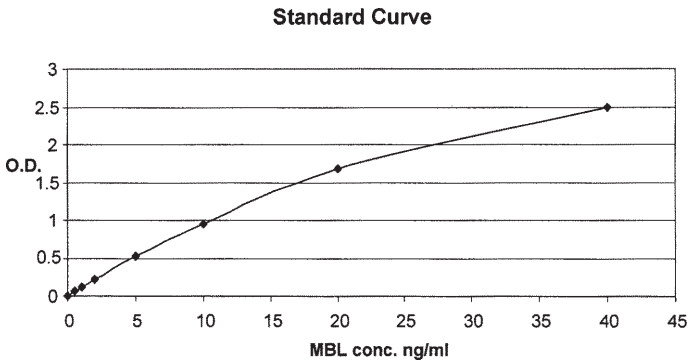

Fig. 1 Standard Curve for MBL

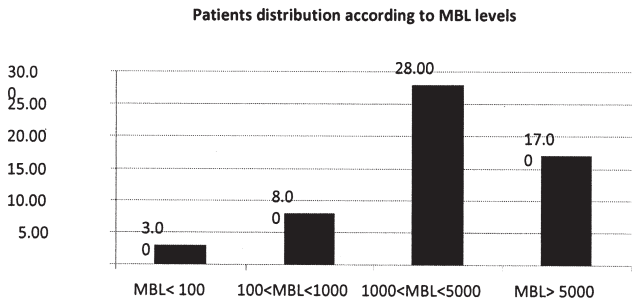

Fig. 2.Distribution of Group 1(CFLD) patients according to the MBL values

Timisoara. The testing was performed in microwells coated with a monoclonal antibody against the MBL carbohydrate-binding domain. Bound MBL was detected with the same antibody that has been labeled with biotin, followed by development with horseradish peroxidase (HRP)-conjugated streptavidin and incubation with a chromogenic substrate. Method required four steps:

1. 100 µL volumes of each standard solution (0.5, 1.0, 2.0, 5.0, 10.0, 20.0, 40.0 ng/mL) and diluted serum samples were incubated in microwells precoated with monoclonal antibody against MBL for 60 min at room temperature on a shaking platform set at 200/min. Unbound material was removed by washing tree time with 300 µL diluted Wash Solution.

2. 100 µL of Biotinylated MBL Antibody into each microwell was added and incubated for 60 min at room temperature on a shaking platform (200/minute). Unbound detection antibody was removed by washing.

3. 100 µL HRP-conjugated streptavidin was added to each test well and allowed to form a complex with the bound biotinylated antibody. Unbound conjugate was removed by washing.

4. 100 µL of tetramethylbenzidine (TMB) Substrate was added into each microwell. The bound HRP-streptavidin reacts with the substrate to generate a colored product. The enzymatic reaction was stopped with Stop Solution, and the color intensity was read at 450 nm in MPT READER GVD 990BV6. The data for the standards were used to construct a standard curve (fig. 1) from which the concentrations of MBL in the test specimens were read using RIDAWIN software program.

After evaluating the average value of MBL to study groups we have made comparative analysis between the three groups. ANOVA was used for comparison between values.

The results were interpreted according to the following scale:

- values < 100 ng/mL = severe deficiency;
- values > 100 ng/mL and < 1000 ng/mL = moderate deficiency;
- normal levels between 1000-5000 ng/mL;
- elevated > 5000 ng/mL.

Results and discussions

MBL range of values found in the general population are ranging from 0-7000 ng/mL [1, 3].

In our study MBL values ranged from 2.3 ng/mL to 5432 ng/mL. The lowest level in our patients was 2.1 ng/mL, in a 3 year old child with liver disease that associated hepatomegaly, cytolysis and hepatic steatosis, specific for a mild form of liver disease. Clinical studies to date have used as a lower limit values 50 ng /mL or 100 ng / mL to define severe MBL deficiency [1, 14, 15].

Low levels of MBL < 1000 ng/ml were measured in 38% of patients with liver disease, recorded as moderate deficiency, only in 3 cases were recorded serum levels specific for severe MBL deficit (fig. 2). Children majority serum levels were between 1000 - 5000 ng/mL or higher. The explanation could be the influence of growth hormone

Table 1

MBL VALUES IN PATIENTS WITH LIVER DISEASE AND CYSTIC FIBROSIS COMPARED WITH CONTROLS

Patients with CFLD	MBL in patients with CFLD	Controls	MBL Controls (ng/ml)
P1	1589	M1	5169
P2	5023.1	M2	5003
P3	732	M3	2700
P4	1657	M4	4948.5
P5	2.1	M5	2700
P6	5512	M6	4948.5
P7	4432.4	M7	5003
P8	1400	M8	5186.3
P9	765	M9	4978
P10	800	M10	5432
P11	58	M11	5186.3
P12	340	M12	4978
P13	4823.1	M13	2100
P14	783	M14	5186.3
P15	1900	M15	4978
P16	1340	M16	4948.5
P17	5023.1	M17	4978
P18	2630	M18	4948.5
P19	1780	M19	4978
P20	512	M20	5186.,3
P21	1200	M21	1750

Table 2
MBL MEDIAN VALUES AMONG THE STUDIED GROUPS

Liver disease		N	Average	Standard deviation
MBL	YES	21	2061.991	1495.589
	NO	14	3432.800	1828.672
	CONTROL	21	3986.827	2050.152

during childhood which might raise the MBL levels [14, 16]. Another reliable hypothesis could be a "wild" heterozygous genotype or homozygous genotype for MBL mutations in our study group.

Evaluation of results in three groups of children

We performed a comparative study of serum MBL (mannan-binding lectin) between three study groups: group 1 patients with cystic fibrosis and associated liver disease (21 subjects), group 2 which included 14 patients with cystic fibrosis and without liver disease and group 3, the controls group, included 21 healthy children, age matched. The groups were homogeneous regarding the age. Dosage serum levels of MBL in the 3 groups are shown in table 2.

In the group of patients with liver disease associated with cystic fibrosis, the average was 2061.99 ng/mL ± 1495.589, compared to the average in the second group 3432.80 ± 1828.672 and the control group in which the average value was 3986.827 ng/mL ± 2050.152 (table 2). Comparison of serum levels of MBL in patients with cystic fibrosis liver disease revealed a median value of MBL in CFLD lower by almost 50%, compared to the median value of the control group, with a statistically significant difference ($p = 0.001$). Among group 2 (patients with cystic fibrosis without liver disease), the average value was 3423.800ng/mL, reduced by 13% compared with controls, without statistical significance ($p = 0.394$). In group 1, patients with CFLD, the mean was 40% lower than in group 2, with a statistical significance of $\alpha=0.05$.

Figure 3 illustrates the conglomeration of patients with liver disease and cystic fibrosis in the area corresponding values below 1000 ng/mL, while the higher values MBL > 5000 ng/mL were found in the control group. An interesting distribution have MBL values of patients with cystic fibrosis without liver disease in which there is a bipolar distribution, 2 subjects are with medium deficiency and one with severe deficiency, range 2000-4000 ng / mL remains "free" for the rest of the patients to be concentrated to values above 4000 ng / mL. A possible explanation of the predominance of medium MBL deficiency and intermediate values in group 1 study, belongs to growth hormone intervention [15] , our group with mean age of 10.5 years, belongs to prepubertal period, characterized by hormone storm and association with significant growth hormone increase.

Conclusions

The results obtained for MBL concentration by ELISA Method are accurate and could be correlated with clinical expressions. MBL levels varies in our patients, from lowest to higher levels, signifying a heterogeneity of MBL levels and the influence of other unknown factors. Serum levels of MBL varied in our study population, lowest levels being present in children with cystic fibrosis and associated liver

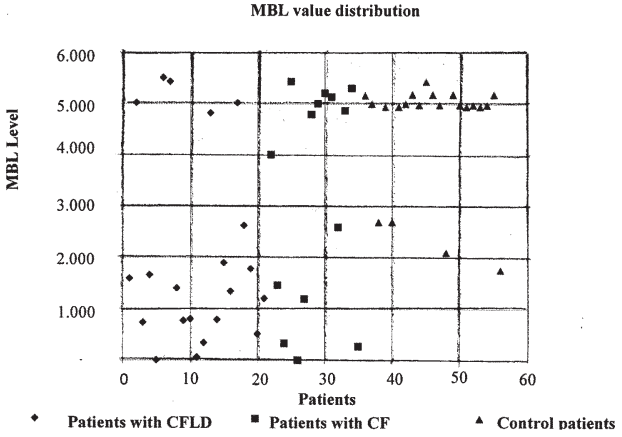

Fig. 3. Patient's distribution according MBL levels

disease. Cystic fibrosis is the most frequent monogenic disease and its evolution is marked by complication like liver disease, the second cause of death in cystic fibrosis. It is very important to try to find a predictive factor for liver disease occurrence. MBL deficiency was frequent in patients with CFLD, suggesting that low levels of MBL could be considered a risk factor for liver disease development in patients with cystic fibrosis. Longitudinal assessment of MBL levels would be useful for early diagnosis of children with CFLD and disease monitoring. Levels of children with cystic fibrosis, without liver disease, are similar with values detected in healthy children, fact that strengthen the idea that MBL deficit associates liver disease in cystic fibrosis children. Early detection of MBL deficiency could identify children with risk for this complication in a time when a correct therapy could influence the disease evolution and increase life expectancy.

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Manuscript received: 28.01.2013