Original Article

Association between Serum Levels of MASP-2 and Neutropenic Febrile Attacks in Children with Leukemia

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Abstract

Background: Infectious complications are a major etiology of morbidity and mortality in febrile neutropenic patients. Low serum mannose-binding lectin (MBL)-associated serine protease-2 (MASP-2) concentration may represent a risk factor for infection in leukemia patients receiving chemotherapy. This study evaluates the relationship between serum levels of MASP-2 with neutropenic febrile attacks in children with leukemia.

Method: This prospective cohort study conducted between 2009–2010, we measured baseline serum MASP-2 levels by enzyme-linked immunosorbent assay (ELISA) prior to chemotherapy in leukemia patients less than 14 years of age. The relationship of febrile neutropenia (FN) episodes and duration of hospitalization with MASP-2 concentration was analyzed.

Results: We evaluated 75 children [38 girls (51%), 37 boys (49%); mean age, 61.6 ± 43.7 months]. There were 8 (10.7%) children with MASP-2 deficiency (< 200 ng/mL). Mean MASP-2 was 673.2 ± 288.7 ng/mL (range: 116–1112). Eight patients had no FN episodes. Of the 129 FN episodes recorded, 19 (average 2.4 times) were from the MASP-2deficient group and 110 (average 1.6 times) were in the normal group. There was a significant difference between the mean MASP-2 concentration and FN episodes ($P = 0.043$).

There was an inverse relationship between FN episodes (r = -0.332, $P = 0.004$) and the duration of hospitalization (r = -0.334, $P = 0.005$) with MASP-2 concentration. MASP-2 deficient patients were hospitalized longer than the normal group, which was strongly significant ($P < 0.001$).

Conclusion: Our study confirmed the results of several previous studies. MASP-2 deficiency in leukemic children treated with chemotherapy was associated with an increased risk of FN episodes, prolonged cumulative duration of hospitalization, and intravenous antimicrobial therapy.

Keywords: Associated Serine Protease, Fever, Innate Immunity, Leukemia, Mannose-Binding Protein, Neutropenia

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Introduction

In febrile neutropenic patients, infectious complications are one of the major reasons for morbidity and mortality.[1,2] Mannose-binding lectin (MBL) is a serum lectin that takes part in the hereditary immune response. Low levels of MBL in serum may be a risk factor for infection in leukemia patients receiving chemotherapy. The immune system has the ability to identify invading microorganisms or tumor cells and attempt to remove them.[3,4] MBL, an acute phase protein, adheres to carbohydrate structures on a wide range of bacteria and viruses, consequently activating the complement system through autoactivation of MBL-associated serine protease (MASPs); therefore, playing a fundamental role in eradication of external life-threatening factors.[5–7]

In order to activate the complement system, both MBL and ficolins depend on MASP-2. MBL/MASP-2 complexes bind to microbial surfaces. MASP-2 then generates the C3 convertase C4bC2b 4 and C2, finally leading to opsonization and direct destruction of pathogens and an increase in inflammatory cells. MASP-2 represents the common pathway of complement activation for MBL and ficolins; therefore, MASP-2 is more involved compared to MBL.[8] Recent surveys have shown a relationship between MBL deficiency and increased risk for bacterial and viral infections[9] in many diseases similar to leukemia,[10] rheumatoid arthritis,[11] and in those patients who receive treatment for hematological cancers.[12] In this study we evaluate the relationship between serum levels of MASP-2 with neutropenic febrile attacks in children with leukemia.

Patients and Methods

In this prospective cohort study, we measured baseline serum MASP-2 levels in 75 children under 14 years of age diagnosed with leukemia who were candidates for chemotherapy at Mofid Children's Hospital. We intended to determine their predictive value for the development of febrile neutropenia (FN). All patients with acute lymphoblastic leukemia (ALL) and acute myeloblastic leukemia (AML) underwent its own treatment protocol. FN was defined as an axillary temperature of 38.5°C that persisted for 2 hours or a single axillary temperature of 39°C during severe chemotherapy-induced neutropenia (absolute neutrophil count: 500 cells/L).[13] Patients were observed for one year and a ques-

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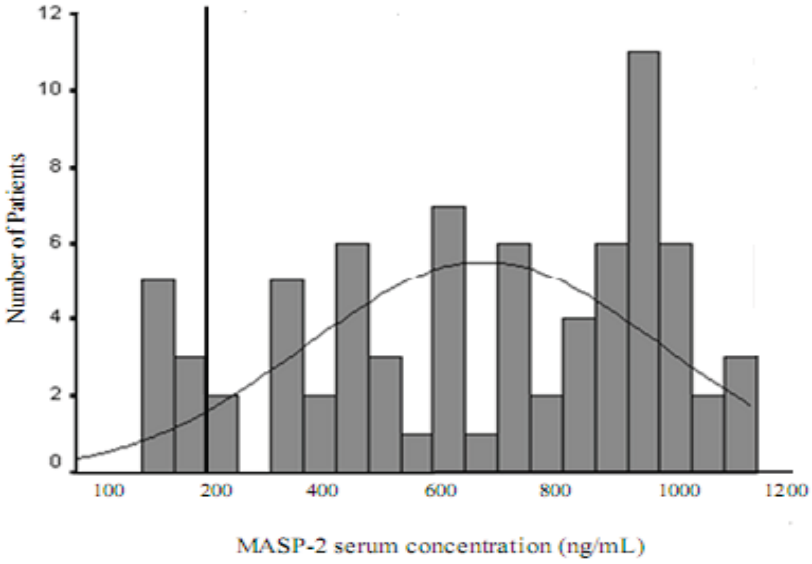

Figure 1. Distribution of MASP-2 serum concentration. The vertical line is set at 200ng/mL to distinguish MASP-2 deficiency from normal concentrations.

Table 1. Patients' characteristics and MASP-2 concentrations.

Characteristics		Patients	No. of patients based on MASP-2 levels	
			< 200 ng/mL	≥ 200 ng/mL
Gender				
	Female	38 (51%)	3(7.9%)	35(92.1%)
	Male	37 (49%)	5(13.5%)	32(86.5%)
Age at diagnosis (years)				
	<4	37 (49%)	4(10.8%)	33(89.2%)
	4–8	20 (27%)	2(10%)	18(90%)
	8–12	12 (16%)	1(8.3%)	11(91.7%)
	≥12	6 (8%)	1(16.7%)	5(83.3%)
Diagnosis				
	Acute Lymphoblastic Leukemia	65 (87%)	7(10.8%)	58(89.2%)
	Acute Myeloblastic Leukemia	10 (13%)	1(10%)	9(90%)

tionnaire was completed. Before chemotherapy, blood samples (3 cc) were obtained from all subjects. Samples were mixed with 75 µL anticoagulant (EDTA) and centrifuged at 2000 rpm for 10 minutes. Purified plasma was stored at -20ºC. MASP-2 was measured by enzyme-linked immunosorbent assay (ELISA). MASP-2 concentrations were subsequently categorized into MASP-2 deficient (< 200 ng/mL) and normal (≥ 200 ng/mL).

Statistical analysis

Descriptive analysis was performed using frequency tabulations for categorical variables and summary parameters for continuous variables. The Shapiro-Wilks test was used to assess normality. The association between MASP-2 serum level and other categorical variables was assessed by Fisher's exact test. Comparison of the mean MASP-2 in different groups of FN patients determined by the number of febrile attacks was performed by the Kruskal-Wallis test. The relationship between FN episodes and the duration of hospitalization with MASP-2 concentration was analyzed using Pearson's correlation. Significance level for all tests was $P < 0.05$.

Results

Totally, we evaluated 75 children, of which 38 were girls (51%) and 37 were boys (49%). The mean age of the participants was 61.6 ± 43.7 months (range: 5–168 months). All patients were observed for one year unless they died during this period (10 patients; 13.3%). Average follow up was 323.4 ± 107.8 days with a median of 365 days and range from 16–365 days.

Based on the normal range of MASP-2 in this study (≥ 200 ng/mL), 8 (10.7%) children were MASP-2 deficient and 67 (89.3%) were normal. The mean serum level of MASP-2 was 673.2 ± 288.7 ng/mL (range: 116–1112 ng/mL).Since the distribution of MASP-2 concentration was not normal (Shapiro-Wilks: 0.929; $P < 0.001$), we used nonparametric analysis (Figure 1). There were no significant differences between the patients with and without MASP-2 deficiency regarding patients' characteristics (Table 1).

Although 8 patients had no FN episodes, there were 129 FN episodes recorded in 67 patients (median: 2; range: 0–5), of which 19 (average 2.4 times) episodes were in the MASP-2deficientgroup and 110 (average 1.6 times) were in the normal group. The mean MASP-2 concentrations based on FN episodes are summarized in Table 2. The mean neutrophil granulocyte count was 4708.6 ± 42185.8 10^9/µL (range: 100–45700 10^9/µL) in the normal group and 796.7 ± 316.8 10^9/µL (range: 100–1200 10^9/µL) in patients with MASP-2 deficiency. There were no significant differences between patients with and without MASP-2 deficiency ($P = 0.451$). Severity of the neutropenia in patients and MASP-2 levels are summarized in Table 2. There were no significant differences between neutropenia severity in patients with and without MASP-

Table 2. Association between severity of neutropenia in patients and MASP-2 levels.

Neutropenia	MASP-2 levels		Total
	< 200 ng/mL	≥ 200 ng/mL	
Mild	44 (37.6%)	1 (11.1%)	45 (35.7%)
Moderate	61 (52.1%)	7 (77.8%)	68 (53.9%)
Severe	12 (10.3%)	1 (11.1%)	13 (10.3%)
Total	**117 (92.9%)**	**9 (7.1%)**	**126 (100%)**

Table 3. Comparison of mean MASP-2 in different groups of FN patients determined by the number of febrile attacks.

No. of FN	Patients	Mean ± SD*	Range	95% Confidence interval for mean	
				Lower bound	Upper bound
0	8	845.6 ± 269.8	348–1112	620.1	1071.2
1	30	728.7 ± 276.1	145–1060	625.6	831.8
2	20	640.9 ± 317.7	132–1002	492.3	789.6
More than 3	17	532.2 ± 229.46	116–964	414.2	650.1
Total	**75**	**673.2 ± 288.7**	**116–1112**	**606.8**	**739. 7**

*P-value was 0.043 according to Kruskal-Wallis test.

2 deficiency (P = 0.264). The mean MASP-2 concentration in 8 patients who had no FN episodes was 854 ± 269.8 ng/mL, it was 728.7 ± 276.1 ng/mL in 30 patients with one episode of FN, 640.9 ± 317.7 ng/mL in 20 patients with two episodes of FN and 532.2 ± 229.43 ng/mL in 17 patients with more than three episodes of FN, of which the difference was significant (P = 0.043; Table 3).

The duration of hospitalization was 20.79 ± 16.74 days in the normal group and 56.25 ± 32.45 days in patients with MASP-2 deficiency, which was strongly significant (P < 0.001). There was an inverse relationship between MASP-2 concentration and FN episodes (r = -0.332, P = 0.004) and the duration of hospitalization (r = -0.334, P = 0.005) using Pearson's correlation.

Discussion

This study was carried out to evaluate the relationship between serum levels of MASP-2 with neutropenic febrile attacks in children with leukemia. The results showed that FN episodes in children were inversely related to MASP-2 concentrations.

The duration of hospitalization in patients with MASP-2 deficiency was longer than the normal group, which was significant. Similar results have been reported by Schlapbach et al., who have noted that MASP-2 deficient children had a significantly increased risk of developing FN, experienced prolonged hospitalization and had more intravenous antimicrobial treatment. They concluded that MASP-2 deficiency was a novel risk factor for chemotherapy-related infections.[13]The FN episodes did not have a microbiological etiology confirmed by blood culture, as the sensitivity of blood cultures in children is low partly because of the inoculation of small blood volumes into blood culture bottles.[14]

In another study performed by Vekemans, it was noted that MBL-deficient patients had greater numbers of severe infections and their first severe infection occurred earlier compared to patients without this deficiency.[15] Several other studies worldwide have shown an association between MBL deficiency and increased risk of infection. For example, Lambourne demonstrated an association between MBL deficiency and invasive aspergillosis.[16] On the contrary, other studies[17,18] have shown different results. For instance, Kilpatrick[19] in a retrospective study observed 128 adult patients who received treatment for hematological cancer and found no relationship between MBL levels and chemotherapy-related infections. Possibly, different studies have considered different ranges for the normal level of MASP-2.

Consequently, our study confirmed the results of several previous studies and showed that MASP-2 deficiency in children treated with chemotherapy for leukemia was associated with the increased risk of FN episodes, prolonged cumulative duration of hospitalization and intravenous antimicrobial therapy.

Acknowledgments

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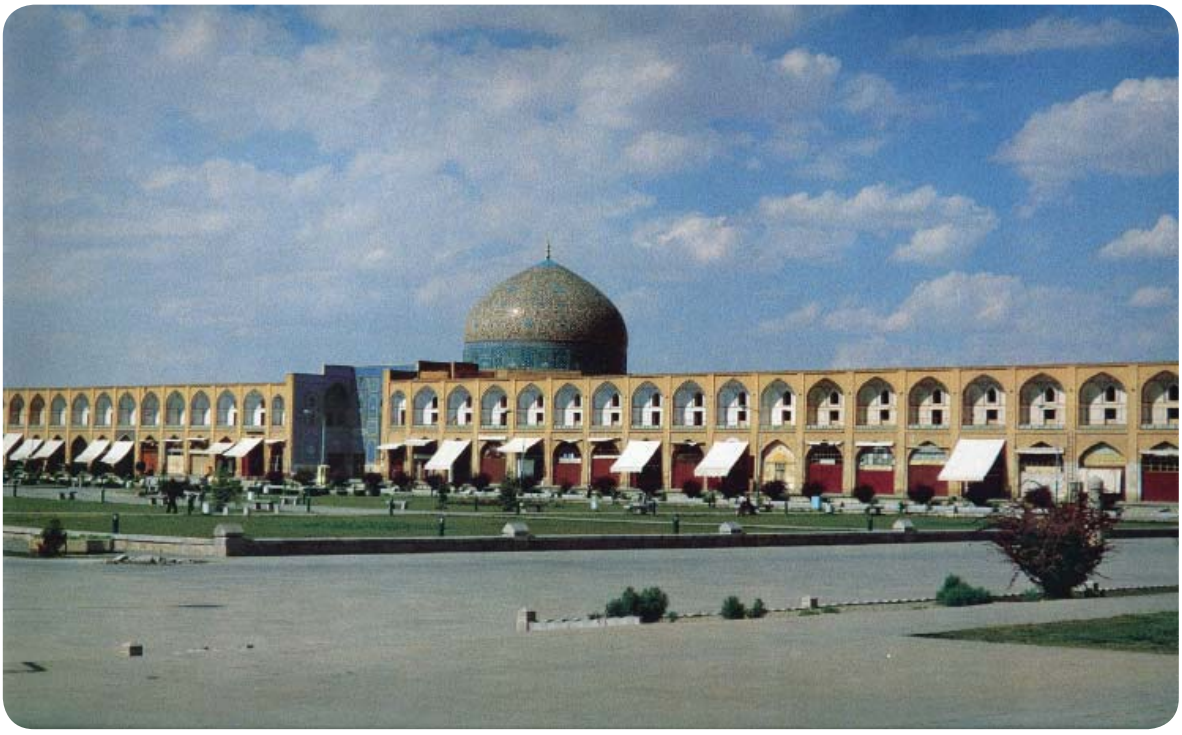

Sheikh Lotfollah Mosque located on the eastern side of the Naqsh-e Jahan Square in Isfahan, Iran, constructed between 1598 – 1629 AD, Safavids Era, one of the UNESCO's world heritage sites. (*Source:* 'A Glance at Esfahan, City of Art' by Parviz Dabiri MD, Mehr Afrouz Publication, Isfahan, Iran, 2005; p: 16)