

ORIGINAL PAPER

Analysis of serum levels of interleukin-18 in the acute and past infection of mononucleosis in children

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ABSTRACT

Introduction: The objective of the current study was to assess the serum level of interleukin-18 (IL-18) in children with mononucleosis and to compare the expression of this cytokine in the acute and past phase of the infection.

Material and method: The level of IL-18 was determined using commercial enzyme-linked immunosorbent assay kits (Cloud-Clone Corp China). Other laboratory findings (white blood cells – WBC, C-reactive protein – CRP, alanine aminotransferase – ALT, aspartate transaminase – AST, lactate dehydrogenase – LDH, γ -glutamyl transferase – GGT) were determined using routine laboratory methods using a Cobas c501 and Sysmex 4000i.

Results: The level of IL-18 was significantly higher in the acute phase compared to the past stage of the disease (45.3 pg/ml vs. 21.6 pg/ml; $p = 0.001$) and in the acute phase compared to the control group (45.3 pg/ml vs. 23.8 pg/ml; $p = 0.045$).

Conclusions: In conclusion, this study shows that serum concentration of IL-18 in children with mononucleosis in the acute phase is increased, thus confirming the ongoing inflammatory process. We also suggest that IL-18 can be a useful new marker to differentiate between the acute and past phase of the disease. The determination of IL-18 with the activities of AST, ALT, LDH, GGT, leukocytosis (WBC) and the level of acute phase protein (CRP) may help assess the phase of the disease.

KEY WORDS:

mononucleosis, interleukin-18, acute and past phase.

INTRODUCTION

Infectious mononucleosis (IM) is a disease caused by the Epstein-Barr virus. It is most frequently characterized by swollen lymph nodes, fever and sore throat [1].

Interleukin-18 (IL-18) is an immunoregulatory cytokine that acts mainly on T-cells and natural killer (NK) cells, by inducing IFN- γ and IL-2 secretion [2, 3]. Interleukin-18 affects cytotoxicity of CD8⁺, CD4⁺ T-cells and NK cells. It can also trigger the secretion of IL-4 and IL-13 through the effect exerted on CD4⁺ T-cells, basophils,

mast cells or NK cells, which seems to indicate its involvement in cellular and humoral responses [4].

The study objective was to assess the serum level of IL-18 in children with mononucleosis and to compare the expression of this cytokine in the acute and past phase of the infection.

STUDY AND CONTROL GROUP

The tested group consisted of 58 ambulatory children successively reporting to physicians in the hospital emergency

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department with symptoms suggestive of infectious mononucleosis, including fever, cervical or generalized lymphadenopathy, tonsillitis, coryza and hepatosplenomegaly. The children were divided into two subgroups according to the disease phase: acute and past. Acute infectious mononucleosis was defined as a febrile illness with signs of infection in the upper respiratory tract and presence of heterophile and/or viral capsid antigen (VCA) IgM antibodies. There were 31 children (14 boys and 17 girls) aged from 16 months to 17 years (mean: 10.35 ± 5.43) determined to be in the acute phase of the disease. Past Epstein-Barr virus (EBV) infection was diagnosed in children with serologic traits: presence of VCA IgG and/or Epstein-Barr nuclear antigen (EBNA) IgG antibodies. In this subgroup, all children were also diagnosed with a nonspecific viral upper respiratory tract infection. There were 27 children (9 boys and 18 girls) aged 21 months to 17 years (mean: 10.99 ± 5.42) in the subgroup with past EBV infection.

The control group comprised 30 healthy children (13 boys and 17 girls) at the age of 3 months to 16 years (mean: 8.64 ± 4.12). None of these children showed signs of infection or had received drugs within four weeks prior to the study.

The children were assigned to groups in the Department of Pediatric Infectious Diseases, Medical University of Białystok.

The study was approved by the local Bioethics Committee at the Medical University of Białystok (Approval No. APK.002.149.2020).

MATERIAL AND METHODS

Blood (1.2 ml) for hematological investigations (WBC) was collected into tubes containing anticoagulant ethylene-diamine tetraacetic acid (EDTA-3K). Blood samples (2.7 ml) for biochemical assays (C-reactive protein – CRP, alanine aminotransferase – ALT, aspartate transaminase – AST, lactate dehydrogenase – LDH, γ -glutamyl transferase – GGT) and IL-18 were obtained from a peripheral vein once in the emergency unit. The sera were separated by centrifugation at 3500 g for 5 minutes.

The level of IL-18 was determined using commercial enzyme-linked immunosorbent assay (ELISA) kits (Cloud-Clone Corp China). Readings were performed using an ELISA microplate reader (ANTHOS, Australia) at an absorbance value of 450 and 630. The sensitivity of the test was 5.6 pg/ml. This test allows recognition of both natural and recombinant human IL-18.

Antibodies were determined using the immunochromatographic cassette test VIRAPID MONO M&G for the qualitative determination of EBV antibodies (heterophile antibodies, VCA-IgM, VCA-IgG, EBNA).

White blood cells (WBC) were measured using a Sysmex XT-4000i hematology analyzer (Sysmex Corporation, Kobe, Japan). Alanine aminotransferase (normal range: 5–41 U/l), AST (normal range: 5–40 U/l), LDH (normal range: 240–480 U/l), GGT (normal range:

5–40 U/l) and CRP (normal range: 0–5.0 mg/l) were determined by using routine laboratory methods with Roche reagents (Roche, Germany) using a Cobas c501 analyzer (Roche, Germany).

STATISTICAL ANALYSIS

All analyses were performed in TIBCO Software Inc. (2017) Statistica, version 13 (Palo Alto, CA, USA). Results were expressed as median and interquartile range.

Differences between the groups were compared using the Kruskal-Wallis one-way analysis of variance with multiple comparisons using the rank sums post hoc test.

The results were considered to be statistically significant when p -values were less than 0.05. Correlations between variables were analyzed using the Spearman rank correlation test.

ETHICAL APPROVAL

This clinical investigation (APK.002.149.2020) was approved by the Bioethical Committee of Medical University of Białystok, Poland.

RESULTS

The level of IL-18 was significantly higher in the acute phase compared to the past stage of the disease (45.3 pg/ml vs. 21.6 pg/ml; $p = 0.001$) and between the acute phase and control group (45.3 pg/ml vs. 23.8 pg/ml; $p = 0.045$) (Figure 1, Table 1). The data concerning other laboratory parameters for the acute and past phase of mononucleosis are presented in Table 1. The serum ALT, AST, GGT and LDH activities, CRP concentration and WBC count

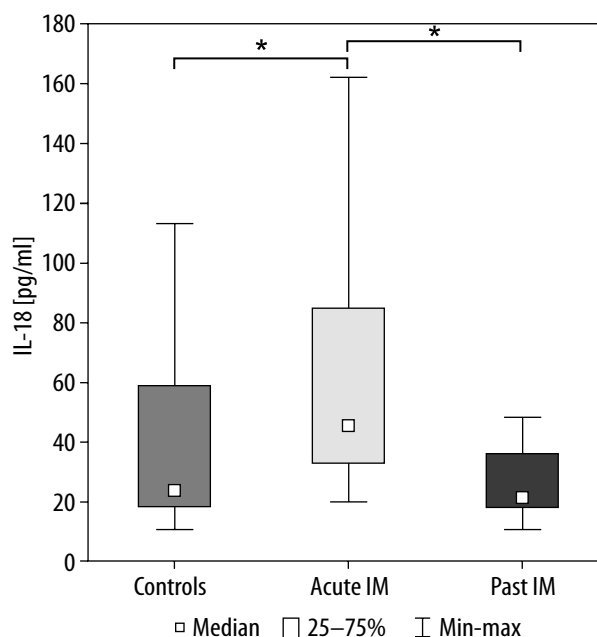


FIGURE 1. Comparison of interleukin-18 level for the acute and past phase of mononucleosis

*Indicates $p < 0.05$

TABLE 1. Results of interleukin-18 levels and other laboratory parameters in the acute and chronic phase subgroups of mononucleosis and control group

Group/subgroups	IL-18 [pg/ml]	ALT [IU/l]	AST [IU/l]	GGT [IU/l]	LDH [IU/l]	CRP [mg/l]	WBC [$\times 10^3/\mu\text{l}$]
Control (n = 30)	23.8 18.3–58.4	10.5 8.0–17.0	18.0 17.0–22.0	37 9–37	388 364.5–407	1.9 0.2–3.9	9.1 6.2–12.8
Acute phase (n = 31)	45.3 32.9–84.9 $p = 0.045^*$	114.5 41–160 $p = 0.014^*$	84 48–177 $p = 0.174^*$	112 45–162 $p = 0.042^*$	892 722–1171 $p = 0.047^*$	10.3 3.4–25.8 $p < 0.001^*$	12.6 9.1–18.7 $p < 0.013^*$
Past phase (n = 27)	21.6 18.1–36.1 $p = 1.0^\#$ $p = 0.001^\wedge$	13.0 12–19 $p = 0.878^\#$ $p < 0.001^\wedge$	27.0 26–39 $p = 0.436^\#$ $p < 0.001^\wedge$	96.0 17.0–175 $p = 0.480^\#$ $p = 0.870^\wedge$	352.5 302.5–431.5 $p = 1.0^\#$ $p = 0.002^\wedge$	17.0 2.1–40.7 $p < 0.001^\#$ $p = 1.0^\wedge$	10.2 7–14.1 $p = 1.0^\#$ $p = 0.118^\wedge$

ALT – alanine aminotransferase, AST – aspartate aminotransferase, CRP – C-reactive protein, GGT – γ -glutamyltransferase, IL-18 – interleukin-18, LDH – lactate dehydrogenase, WBC – white blood cells. The results are presented as medians and quartiles (Q1, Q2). Differences between the groups were compared using the Kruskal-Wallis one-way analysis of variance with multiple comparisons using the rank sums post hoc test. For all tests, differences were considered significant at $p < 0.05$.

* When comparing acute phase subgroup and controls

^\# When comparing past phase subgroup and controls

^\wedge When comparing acute and past phase subgroups

TABLE 2. Correlation between interleukin-18 and other laboratory parameters in different phases of mononucleosis

Subgroups	CRP	WBC	AST	ALT	GGT	LDH
Acute phase	R = -0.089 $p = 0.664$	R = 0.359 $p = 0.0777$	R = -0.326 $p = 0.201$	R = -0.228 $p = 0.305$	R = -0.771 $p = 0.072$	R = -0.285 $p = 0.534$
Past phase	R = 0.339 $p = 0.112$	R = 0.289 $p = 0.167$	R = 0.091 $p = 0.789$	R = 0.282 $p = 0.255$	R = 0.175 $p = 0.268$	R = -0.119 $p = 0.778$

ALT – alanine aminotransferase, AST – aspartate aminotransferase, CRP – C-reactive protein, GGT – γ -glutamyltransferase, LDH – lactate dehydrogenase, R – Spearman correlation coefficient, WBC – white blood cells

* Statistically significant correlation when $p < 0.05$

were significantly higher in the acute phase in comparison with those in the healthy subjects (114.5 vs. 10.5 IU/l, $p = 0.014$; 84 v 18.0 IU/ml, $p = 0.174$; 112 vs. 37 IU/l, $p = 0.042$; 892 vs. 388 IU/l, $p = 0.047$; 10.3 vs. 1.9 mg/l, $p < 0.001$; 12.6 vs. $9.1 \times 10^3/\mu\text{l}$, $p = 0.013$, respectively). In the past phase of disease only CRP level was higher than the value in the control group (17 vs. 1.9 mg/l, $p < 0.001$, respectively). In the acute phase of the disease, serum activities of ALT, AST, GGT, LDH, CRP and WBC were also found to be significantly increased compared to those in the past stage IM (114.5 vs. 13 IU/l, $p < 0.001$; 84 vs. 27 IU/l, $p < 0.001$; 112 vs. 96 IU/ml, $p = 0.870$; 892 vs. 352.5 IU/l, $p = 0.002$; 10.3 vs. 17.0 mg/l, $p = 1.0$; 12.6 vs. $10.2 \times 10^3/\mu\text{l}$, $p = 0.118$, respectively). Table 2 shows correlation coefficients between IL-18 and other laboratory tests in different stages of mononucleosis. The serum IL-18 did not correlate with laboratory parameters in the acute and past phase of the disease.

DISCUSSION

In the acute phase, anti-VCA IgM and heterophilic antibodies were predominant. Some children in this phase had only anti-VCA IgM and no heterophilic antibodies were found. In the past phase IM, patients showed the presence of anti-VCA IgG and anti-EBNA IgG. Trzcińska *et al.* [5] stated that the presence of anti-VCA

IgM and the absence of anti-EBNA IgG indicate primary infection in the body. The reactivation of the viral infection is characterized by the presence of anti-EA IgG and anti-VCA IgG or anti-EBNA IgG. Other researchers concluded that the presence of IgM antibodies against VCA may suggest an ongoing inflammatory process of the disease or the virus reactivation in the body [6].

The assessment of the level of IL-18 in the serum of children suffering from infectious mononucleosis was an important part of our study. The findings revealed that the level of IL-18 in the acute phase was nearly two-fold higher than in the past phase ($p = 0.011$) and between the acute phase and control group ($p = 0.045$). Further analysis did not show any differences in the level of IL-18 in the acute phase or chronic phase as compared to the respective control groups. Setsuda *et al.* [7] observed a markedly higher level of IL-18 in the lymphatic tissue in patients with infectious mononucleosis as compared to patients suffering from post-transplant lymphoproliferative disease. This is probably associated with the body response to the action of the virus in host cells [7]. A study conducted by Liu *et al.* [8] clearly showed that the level of IL-18 in the acute phase was significantly higher than in the control group ($p < 0.05$). Van de Veerdonk *et al.* [9] reported that the level of IL-18 in the acute phase of the disease was markedly higher than in the control group. They also found a significant negative correlation

of IL-18 with the disease duration and a positive correlation with symptom intensity. The findings demonstrate that IL-18 can be used as an auxiliary marker to determine the phase of infectious mononucleosis [9]. Yoshimori *et al.* [10] reported that the body response to EBV infection can be controlled by a specific immunological response. A major role in this process could be ascribed to monocytes, in which EBV infection may lead to inflammatory accumulation and caspase-1 activation, which ultimately leads to the production of IL-18 and IL-1 β . Interleukin-18 as an immunoregulatory cytokine is capable of stimulating the population of NK cells and T-cells to produce IFN- γ . The effect of IL-18 may play a crucial role in the host response to the EBV infection [10].

Ou *et al.* [11] compared the serum levels of 34 cytokines of children with hemophagocytic lymphohistiocytosis (HLH). In this study children with HLH were divided into subtypes: Epstein-Barr virus-associated HLH (EBV-HLH), chronic active EBV-associated HLH (CAEBV-HLH), malignant-associated HLH, rheumatological HLH, familial HLH. The levels of IFN- γ and IL-18 increased in more than 90% of patients, while MIP-1 α , SDF-1 α , IP-10, IL-10, IL-8, IL-10, IL-1 RA and TNF- α increased at different levels in more than 50% of patients. EBV-HLH had significantly increased IL-10 levels, followed by IFN- γ and IL-18, while IL-10 and IFN- γ were slightly higher in CAEBV-HLH.

The results of white blood cell (WBC) count determined in the current study indicate their markedly elevated level in the acute phase of the disease as compared to the past phase. The study conducted by Son *et al.* [12] observed increased levels of leukocytes mainly in younger patients suffering from infectious mononucleosis [12]. Topp *et al.* [13] found marked lymphocytosis in patients aged 5–15 years as compared to those under 5 years of age. Chan *et al.* [14] noted an increased leukocyte count and the presence of atypical lymphocytes – virocytes – in mononucleosis patients in all age groups.

The analysis of acute phase protein CRP level showed that it was statistically significantly higher in the acute phase of the disease as compared to the control group ($p < 0.001$). It was also significantly higher in the chronic phase than in the control group. Similar results were reported by Weber *et al.* [15], who observed a markedly elevated CRP level in more than 50% of patients suffering from infectious mononucleosis. Van de Veerdonk *et al.* [9] reported that CRP was either low or below the method sensitivity.

Our study revealed markedly higher activities of the hepatic enzymes AST, ALT and LDH in the acute phase of infectious mononucleosis as compared to the past phase. We obtained similar results comparing the activity of the enzymes between the acute phase and the control. Our findings are consistent with data reported by other authors. Lihua *et al.* [16] observed substantially elevated activities of AST and ALT in as many as 57.4% of patients with infectious mononucleosis as

compared to the other two groups of patients suffering from acute hepatitis and respiratory tract infection. Moreover, the researchers indicated the correlation between the increased AST and ALT activities and the occurrence of atypical lymphocytes in blood smear, which may help to diagnose the disease. Also Tsai *et al.* [17] reported increased activities of aminotransferases. Chan *et al.* [14] found elevated AST and ALT activities in 42 out of 71 children suffering from infectious mononucleosis. They were markedly higher in older children as compared to infants. Hemophagocytic lymphohistiocytosis is an independent risk factor for central nervous system involvement in patients with CAEBV. Ou *et al.* reported that compared to the non-central nervous system (CNS) group, blood EBV-DNA loads and CD4⁺/CD8⁺ ratio of T lymphocytes in the CNS group were higher, while fibrinogen levels and NK were lower. Children with CAEBV were more likely to develop CNS diseases with low NK cell or high ALT levels [18].

Our study showed that the activity of GGT in the acute phase of the disease was statistically significantly higher as compared to the control group. Similar results have been obtained by other researchers. Zhang *et al.* [19] reported substantially increased activity of GGT in patients suffering from infectious mononucleosis in comparison with the control group.

In the acute and chronic phase, no correlations were noted with biochemical or hematological parameters.

The study results suggest that the level of IL-18 increases markedly, in the acute phase of infectious mononucleosis. Hence, it seems that IL-18 can be an essential prognostic marker in the course of infectious mononucleosis.

CONCLUSIONS

Interleukin-18 can be a useful new marker to differentiate between the acute and past phase of the disease. The determination of IL-18 with the activities of AST, ALT, LDH GGT, leukocytosis (WBC) and the level of acute phase protein (CRP) may help assess the phase of the disease.

DISCLOSURE

The authors declare no conflict of interest.

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