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Plasma level of myeloperoxidase in children with juvenile idiopathic arthritis (a pilot study)

Research Article

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Abstract: To examine the plasma levels of MPO in oligoarthritis and polyarthritis subtypes of JIA in comparison with healthy age-matched controls. Thirty-eight JIA patients (25 girls and 13 boys) aged 9.1-11.8 years and 23 healthy controls (8 girls and 15 boys) participated in the study. Twenty-one patients had oligoarthritis (8 with extended oligoarthritis) and 17 had polyarthritis (among them three were seropositive). The plasma concentration of MPO was measured by the ELISA technique (OxisResearchTM, BIOXYTECH® MPO-EIATM, Portland, OR USA). The mean plasma concentration of MPO in the JIA group was significantly higher than in the control group (76.6±24.8 μg/L versus 62.7±15.6 μg/L; p=0.01). Patients with polyarthritis presented a significantly higher mean plasma MPO level than patients with oligoarthritis (81.3±25.6 μg/L and 62.1±27.1 μg/L, respectively; p=0.02). Different subtypes of JIA may have different MPO-related backgrounds. MPO is a new potent inflammatory marker. Patients with polyarthritis have higher mean plasma MPO levels than patients with oligoarthritis and may therefore have an enhanced risk for subclinical oxidative stress-related atherogenic promotion.

Keywords: *Juvenile idiopathic arthritis • Myeloperoxidase*

1. Introduction

Juvenile idiopathic arthritis (JIA) is the most frequent systemic connective tissue disease in children. JIA is considered to be a heterogeneous group of diseases with different etiopathologies, clinical presentation, course, and prognosis. JIA begins before the 16th birthday and is defined as a sterile inflammation in at least one joint that is persistent for at least six weeks, and for which there is no defined diagnosis (traumatic arthritis, rheumatic fever, septic arthritis, reactive arthritis, neoplasma, etc.) [1].

Many JIA patients do not present high levels of classic inflammatory markers – C-reactive protein (CRP) and/or erythrocyte sedimentation rate (ESR) – especially those with oligoarthritis, which forms approximately 70% of the entire group of patients. Among new inflammatory markers, the enzyme myeloperoxidase (MPO) has been recently accentuated [2,3]. However, its plasma level and/or changes in level in the case of inflammation-driven diseases like JIA remains to be elucidated. It is known that MPO is stored in granules in polymorphonuclear neutrophils (PMN) and is released from PMN and monocytes at inflammatory *loci* [4]. MPO produces the hypochlorous acid (HOCl) – a potent oxidant – from the chloride ion (Cl^-) and hydrogen

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Table 1. The presence of RF, ANA and HLA-B27 antigen in JIA patients.

Subtype of JIA	RF	ANA	HLA-B27
Persistent oligo	0	5	3
Extended oligo	1	4	1
Seroneg poly	0	5	3
Seropos poly	3	1	0
Total of pos patients	4	15	7

peroxide (H_2O_2). HOCl belongs to reactive oxygen species (ROS). Cells that are present in inflamed joints (macrophages, neutrophils, lymphocytes, and endothelial cells) are capable of producing ROS, which damage the articular cartilage [5]. Plasma MPO levels are accepted as a new marker for both inflammation and oxidative stress (OxS), whereas both conditions have an impact on vascular dysfunctionalities [6]. HOCl or nitric dioxide (NO_2), both produced by MPO, oxidizes low-density lipoprotein (LDL) to inflammatory atherogenic oxidized LDL [7]. Thus, MPO is assumed to be involved in the pathology of different diseases, particularly cardiovascular and neurodegenerative diseases [8-10]. The production of ROS like HOCl can lead to tissue damage; the process can play a significant role in chronic inflammation such as JIA. So far there are very few studies on the role of OxS in JIA. JIA patients present high concentrations of lipid peroxidation products, oxidative damage of proteins, changes in activity of antioxidative enzymes, decreased antioxidative glutathione levels and enhanced nitrite/nitrate production in the joints [5,11]. As the etiopathology of JIA varies between the subtypes, the role of MPO in different JIA subtypes may also be different. The measuring of plasma MPO (a new inflammation marker and potent atherogenic factor) in different subtypes of JIA has not been performed earlier.

The aim of this study was to examine the plasma level of MPO in oligoarthritis and polyarthritis subtypes of JIA in comparison with healthy age-matched controls.

2. Material and Methods

MPO was determined in the plasma of 38 patients with JIA (25 girls and 13 boys) aged 9.1-11.8 years (a mean age of 10.4 years). Among the patients there were 21 with oligoarthritis (8 of them with extended oligoarthritis) and 17 with polyarthritis (among them three with seropositive polyarthritis). In 29 out of 38 patients (76%) the blood for measuring MPO concentration was collected at diagnosis or before treatment had begun (the first visit to a paediatric rheumatologist). In nine patients the blood was collected later (five months to one-and-a-half years). Blood samples from JIA patients were collected during an epidemiologic study carried out in Estonia during the years 1998-2000. The control group was formed from 23 healthy children (8 girls and 15 boys) aged 4-15 years (a mean age of 11.7 years). Patients were tested for markers of inflammation (ESR; normal value 4-12 mm/h and CRP; normal below 5 mg/l), rheumatoid factor (RF, by a semiquantitative latextest; a titre of 20 IU/ml or more was considered as positive), and antinuclear antibodies (ANA, by an indirect immunofluorescence method; with positive titres in children from 1:10) at the time the MPO level was determined. HLA B27 antigen was detected using a polymerase chain reaction.

The plasma concentration of MPO was measured by the ELISA technique (Oxis Research™, BIOXYTECH® MPO-EIA™, Portland, USA) as described earlier [12]. The MPO-EIA assay system is a "sandwich" ELISA. Antigen captured by a solid phase monoclonal antibody is detected with a biotin-labeled goat polyclonal anti-MPO. An avidine alkaline phosphatase conjugate then binds to the biotinylated antibody. The alkaline phosphatase substrate p-nitrophenyl phosphate is added and the yellow product (p-nitrophenol) is monitored at 405 nm.

The study was approved by the Ethics Committee of Tartu University. All parents gave their informed consent for participation in the study.

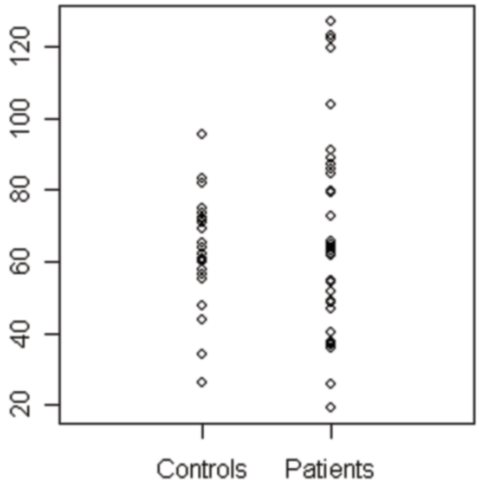
2.1. Statistical analysis

Statistical analysis was performed using the statistical package SAS Version 8.02. Continuous variables are presented as mean values with standard deviation. To compare proportions, the Chi-square test or the Fisher's Exact test (when expected values were <5%) were used. Odds ratios (OR) and 95% CI were used to estimate relative risk. Kolmogorov-Smirnov criterion was used for the assessment of normality. Differences between groups were studied with the nonparametric Mann-Whitney U test. Statistical significance was set at the 95% level ($p<0.05$).

Table 2. Mean plasma concentration of MPO in patients with different JIA subtypes in comparison with healthy controls (62.7 ± 15.6 μg/L).

Subtype of JIA	No of patients	MPO, μg/L (mean ± SD)	p-value
oligoarthritis	21	62.1 (27.1)	0.66
polyarthritis	17	81.3 (25.6)	0.01
all patients	38	76.6 (24.8)	0.01
girls	25	69.5 (28.1)	
boys	13	71.3 (28.2)	

Figure 1. The plasma values of myeloperoxidase in 38 JIA patients and 23 controls (μg/L).



3. Results

The plasma MPO level was determined in two main subtypes of JIA: oligo- and polyarthritis.

In the study group, rheumatoid factor (RF) was positive in four patients (10.5%) (Table 1). Antinuclear antibodies (ANA) were present in 15 patients (39.5%). HLA B27 antigen was determined in 35 patients (92%); it was present in seven patients (Table 1).

The mean plasma concentration of MPO in the JIA group was significantly higher than in the control group (76.6±24.8 μg/L versus 62.7±15.6 μg/L; p=0.01) (Figure 1). There was no gender difference within or between the groups (Table 2). In the patients with polyarthritis, the mean plasma MPO level was significantly higher (81.3±25.6 μg/L) when compared to the patients with oligoarthritis (62.1±27.1 μg/L), p=0.02 (Table 2). The MPO plasma level was higher than that of the control group in 13 of 17 (76%) patients with polyarthritis (in all, 3 patients with seropositive polyarthritis) and in 8 of 21 (38%) oligoarthritis patients (p=0.04).

No significant correlations were found between the levels of inflammatory markers (ESR and CRP) and plasma MPO concentration. There was no statistically significant difference in the mean MPO level when comparing the patients according to their CRP levels – 85.5±33.7 μg/L in the group with elevated CRP (n = 6) and 67.9±26.3 μg/L in the group with normal CRP values (n = 32) – or ESR levels – 74.1±30.5 μg/L in the group with elevated ESR (n = 18) and 67.6±25.6 μg/L in the group with normal ESR values (n = 20). Mean plasma MPO concentration was not different between the groups by ANA or HLA B27 status. In ANA positive patients the mean MPO level was 68.1±26.8 μg/L, in ANA negative patients 73.6±28.8 μg/L (p=0.57); in HLA B27 positive and negative patients the values were 80.5±29.5 μg/L and 70.0±28.2 μg/L, respectively (p=0.39).

4. Discussion

There are some published literature concerning the levels of some markers of OxS in juvenile arthritis patients and in different subtypes as well (5,13). Ramos *et al.* (2000) found that OxS markers such as plasma levels of hydroperoxide and malondialdehyde were significantly higher in patients with JRA when compared with the controls, especially in the systemic sybgroup, which is considered to be the most severe form of the disease [5]. Renke *et al.* (2007) found an increased level of plasma protein carbonyls at diagnosis and after a year of treatment in JIA patients as compared to controls. The blood total antioxidant status level was initially comparable to controls and rose significantly after a year of treatment. The authors concluded that the analysis of plasma OxS markers and blood antioxidant potential might be helpful in monitoring the treatment of JIA [13]. As ROS are thought to have an influence in the development of inflammation of the joints and the damage of cartilage, it is clear that the role of the enzyme that catalyzes their production – MPO – cannot be overlooked. A comparison between the different subtypes of JIA on the basis of plasma MPO has not been performed earlier.

ROS are key mediators of signalling pathways that underlie vascular inflammation in atherogenesis. Many data support the notion that ROS released by MPO have a causatory role in atherosclerosis and other vascular

diseases [14]. Thus, the plasma MPO level is accepted as a new marker for high-grade OxS as well as for inflammation, having an impact on the pathogenesis of vascular dysfunctionalities [6,15].

The mean concentration of MPO was elevated in the whole JIA group compared with controls, and in patients with polyarthritis in whom the inflammatory process is often more active (especially in the seropositive ones), such an elevation was clearly evident.

No relation between gender and MPO activity was found in JIA patients. However, gender-dependent differences in MPO activity have been described in connection with MPO genetic polymorphisms [16]. The 463G/A MPO promoter polymorphism has been found to have an effect on MPO activity, resulting in more expressed clinical findings and poorer outcome [17-19].

In case of JIA, oxidative stress and inflammation are supposed to be the clear factors causing the increase in the level of MPO. Genotypic polymorphisms of MPO in JIA patients are a subject for future research.

The results of our study refer to the need of future investigations using MPO as a marker for both the role of OxS-linked inflammation and the very early signals for OxS-driven subclinical atherosclerosis in patients with JIA.

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