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ORIGINAL ARTICLE

Decreased antioxidant capacity and increased oxidative stress in patients with juvenile idiopathic arthritis

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Abstract:
Purpose: *Juvenile idiopathic arthritis (JIA) is a common rheumatic disease in children which has three types. Systemic type goes with fever oligoarticular type involving joints are less than five and the polyarticular type more than five joints involved. Reactive oxygen species (ROS) have been implicated in its pathogenesis. The ROS generated damage proteins, lipids and serve to amplify the signaling pathways sustaining the synovitis. Enzymes such as superoxide dismutase (SOD) and catalase protect cellular systems from ROS. Our hypothesis is; patients with JIA could have defective defense mechanisms against ROS, which can vary from one type to other.*
Patients and Methods: *We investigated antioxidant status including plasma SOD, catalase and serum ceruloplasmin levels in 25 JIA patients. Also, malondialdehyde (MDA), a product generated by the oxygenation of arachidonic acid, levels were measured.*
Results: *Three patients had systemic; 10 with oligoarticular and 12 with polyarticular JİA and control subject number is 20. Plasma SOD and catalase levels were lower ($p<0.001$), ceruloplasmin and MDA levels were higher ($p<0.001$ and $p<0.05$, respectively) were higher in the study group than in controls. There were a negative correlation between catalase, MDA and SOD levels in patients (respectively $p=0.039$ and $p=0.003$). In between JIA types; the lowest catalase and ceruloplasmin levels were found in oligoarticular type. Conclusively, present study suggested that patients with JIA have decreased antioxidant capacity and defective defense mechanism against ROS and this could be more evident in patients with oligoarticular JIA. In addition, elevated ceruloplasmin levels do not seem to protect against ROS in JIA.*

Keywords: *Juvenile idiopathic arthritis, super oxide dismutase, catalase, ceruloplasmin, reactive oxygen species*
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Introduction

Juvenile idiopathic arthritis (JIA) is a chronic syndrome of unknown etiology and is characterized by non-specific inflammation of the peripheral joints with swelling, morning stiffness, destruction of articular tissues and joint deformities. Prevalence of JIA is about 16 - 150 per 100000 children [1]. Pathogenesis of JIA is characterized by prolonged chronic inflammation of the synovial membrane accompanied by morphological alterations and the recruitment of mononuclear and polymorphonuclear cells into the synovial fluid. Apart from the immunological reaction, there is another biological process, based on the injurious activity of reactive oxygen species (ROS), playing a role in the pathogenesis. ROS damage cellular elements in cartilage directly and damage components of the extracellular matrix either directly or indirectly by up regulating mediators of matrix degradation [2]. Reactive oxygen and nitrogen species directly damage DNA and impair DNA repair mechanisms. This damage can occur in the form of DNA strand breakage or individual nucleotide base damage [3]. Also, oxidative stress that occurs during inflammation can cause non enzymatically damage of immunoglobulins by glycoxidation. This process ultimately results in the generation of advanced glycation endproducts (AGE). Antibodies to AGE - IgG are specifically associated with arthritis that the actual formation of AGE-IgG is related to the intensity of the

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systemic inflammatory response [4]. We hypothesized that patients with JIA have defective defense mechanisms against ROS and these mechanisms vary according to the types of JIA. We, therefore, investigated antioxidant status including super oxide dismutase (SOD), catalase (CAT), malondialdehyde (MDA) and ceruloplasmin in 25 patients with systemic, oligoarticular and polyarticular JIA and 20 healthy age-matched controls.

Material and Methods

Patients and Controls:

25 patients with active course of JIA and 20 healthy controls were included in this study. JIA was diagnosed in these patients on the basis of criteria given by the American College of Rheumatology [5]. Active JIA was determined for patients with active arthritis (using the American College of Rheumatology definition of "active joint") with age at onset < 16 and duration of disease > 6 weeks and / or fever, rash, serositis, splenomegaly or generalized lymphadenopathy attributable to JIA and / or active uveitis. Type of JIA defined by characteristics at onset: oligoarticular JIA < 5 joints; polyarticular≥ 5 joints with rheumatoid factor positive or negative; systemic JIA: arthritis with characteristic fever. Macrophage activation syndrome (MAS), as a complication of the JIA, was defined according to the Histiocyte Society which stipulates that diagnosis of HLH should meet the following criteria: fever, cytopenia, splenomegaly, hypertriglyceridemia and/or hypofibrinogemia, and hemophagocytosis [6]. Our 3 patients who had concurrent JIA and MAS had fever, pancytopenia, splenomegaly, hemophagocytosis in bone marrow, high ferritin (> 10000 µg/dl), LDH, triglyceride and sCD25 levels. JIA with MAS was successfully treated with pulse steroid (30 mg/kg/day-3 day), intravenous immuno globulin (0.4 g/kg-5 day) and cyclosporine (5 mg/kg/day 3 month) in our patients.

Study Protocol:

We measured (SOD), CAT, MDA and ceruloplasmin levels in patients with active stage of JIA and controls. Leukocyte and platelet count, hemoglobin, serum C reactive protein (CRP), albumin, immunoglobulin G, A and M, complement C3 and C4, fibrinogen, anti nuclear antibody (ANA), rheumatoid factor (RF), triglycerides and total cholesterol levels and erythrocyte sedimentation rate (ESR) were studied. Plasma (EDTA-treated) samples were stored at -70°C refrigerator until they began to be analyzed. Biochemical and hematological variables were determined on the same day the samples had been drawn.

CAT levels were measured by the spectrophotometric (240 nm) assay in plasma samples [7]. In brief, the measurement was based on reduction of absorbance values of hydrogen peroxide with CAT. We determined CAT activity (enzyme unit) by reducing absorbance values of hydrogen peroxide within the first 60 seconds by means of a spectrophotometer.

SOD was measured by the spectrophotometric (490 nm) assay in plasma samples [8]. In short, the measurement was based on the effects of SOD on auto-oxidation of 6 - hydroxydopamine. One enzyme unit was determined for SOD, which was achieved through measuring the reduction in the speed of enzyme levels as much as 50% including the onset of 6-hydroxydopamine auto-oxidation within the first 60 seconds by means of a spectrophotometer.

MDA was measured via a spectrophotometer (532 nm) by Thiobarbituric acid test [9].

Statistical Analysis:

Results have been presented as mean ± standard deviation and median. Mann-Whitney U, t test and Pearson correlation tests were used. Statistical significance was considered as a value of $p < 0.05$. These statistical analyses were conducted using SPSS 13.0 software.

Ethics:

The present study gained approval from the Research Ethics Committee of Faculty of Medicine at Eskisehir Osmangazi University. Informed consent was obtained

Table 1. Demographic and clinical features of JIA (n=25).

Age	8.1 ± 2.9
Female / male	19 / 13
Disease duration (year)	3.2 ± 1.4
Morning stiffness	20 (80%)
Drug used	
NSAID	4 (16%)
Methotrexate	10 (50%)
Corticosteroids	2 (8%)
Anti-TNF	4 (16%)
Types of JIA	
Oligoarticular	**10 (40%)**
ANA (+)	3 (12%)
Polyarticular	**12 (48%)**
RF (+)	4 (33.3%)
RF (-)	7 (58.3%)
ANA (+)	1 (8.3%)
Systemic onset	**3 (12%)**
Uveitis	3 (12%)
Lymphadenopathy	2 (8%)
Hepatosplenomegaly	2 (8%)
Fever	2 (8%)
Macrophage activation syndrome	3 (12%)

JIA, Juvenile idiopathic arthritis; NSAI, non-steroid anti-inflammatory drugs; TNF, tumor necrosis factor; ANA, anti-nuclear anti-body; RF, rheumatoid factor

from all study group and control group patients or from their parents/guardians.

Results:

Disease duration of our patients was 3.2 ± 1.4 years. While collecting blood samples, our patients have been treated with non-steroid anti-inflammatory drugs (NSAID) (18.8%), methotrexate (62.5%), corticosteroids (6.2%) and etanercept (12.5%). Demographic and clinical findings of our patients have been presented shown in Table 1. 13 of 32 (39.3%) patients had oligoarticular JIA, 16 of 32 (50%) patients had poliarticular JIA and 3 (9.4%) had systemic JIA. Also, we diagnosed psoriatic arthritis in one patient (3.1%), uveitis in 3 patients as an associated condition (9.4%) and MAS in another 3 patients with systemic JIA (9.4%). Our patients with MAS indicate that patients who have systemic JIA should be carefully examined for the possibility of MAS. CRP, IgG, complement C3, fibrinogen and hemoglobin levels, ESR, platelet and leukocyte counts were significantly higher in patients with JIA than those of healthy subjects (Table 2).

There is not a significant difference in serum albumin, uric acid, triglyceride, total cholesterol, complement C4, immunoglobulin M and immunoglobulin A levels in JIA patients and control group($p > 0.05$).

Plasma SOD and catalase levels were lower in JIA patients compared to controls ($p<0.001$). Ceruloplasmin and MDA levels were higher in patients with JIA than in control group ($p<0.001$and $p<0.05$ respectively, Table 3).

SOD levels were ranked according to the type of JIA as follows: Systemic>oligoarticular> polyarticular. CAT levels were ranked according to the type of JIA as follows: polyarticular> systemic>oligoarticular. MDA levels were ranked according to the type of JIA as follows: systemic>polyarticular>oligoarticular. Ceruloplasmine levels were ranked according to the type of JIA as follows: systemic>polyarticular>oligoarticular (Table 3). Although, CAT levels were higher in the control group than were in the JIA group, these levels proved to be higher in polyarticular JIA than they were in oligoarticular JIA ($p<0.0001$). It is interesting to notice that we determined the lowest CAT levels for oligoarticular JIA ($p<0.0001$), whereas the highest MDA levels were determined for systemic JIA (Table 3). MDA levels were higher in polyarticular JIA than they were in oligoarticular JIA ($p<0.001$). We also determined that ceruloplasmin increased to the highest levels in systemic JIA (Table 3). Although ceruloplasmin levels did not vary significantly between systemic JIA and oligoarticular JIA and polyarticular JIA ($p>0.05$), the lowest ceruloplasmin levels were found in oligoarticular JIA (Table 3).

There were significant negative correlations between catalase and MDA ($r = -0.600$ and $p< 0.039$) and SOD ($p<0.003$, $r = - 0.573$; Figure 1) levels. Also, there were negative correlation between ceruloplasmine and CAT levels ($r = -323$ and $p = 0.025$). There were positive correlations between ceruloplasmin and MDA levels ($r = 0.746$ and $p=0.0001$) ESR, CRP and platelet counts.

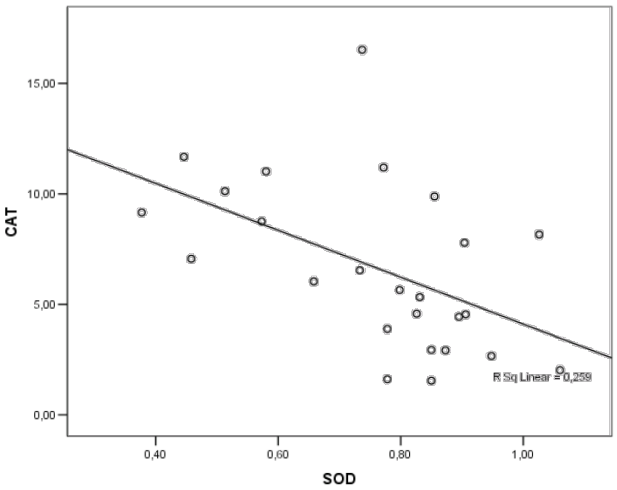

Figure 1. Negative correlations between catalase (CAT) and super oxide dismutase (SOD) levels ($p < 0.003$, $r= -0.573$).

Discussion

JIA describes a clinically heterogeneous group of arthritis of unknown cause. Antigen-specific T cells play a central role in the pathogenesis of JIA, including the predominance of T lymphocytes in the synovial infiltrate, as indicated by expression of CD25, CD45RO, CD69, MHC class II, and several activation-dependent chemokine receptors [10, 11]. On the other hand, there are some studies indicating the pathogenesis of chronic inflammation is associated with the excess ROS production [12,13].

Pro-inflammatory factors such as cytokines and prostaglandins are released at inflammation sites together with ROS [2]. Also, pro-inflammatory cytokines such as IL-1β and TNF-α are involved in the formation of toxic peroxynitrite by increasing the activity of nitric oxide synthase [14]. ROS cause oxidative changes on proteins which causes fragmentation or increased vulnerability to proteases; fragment DNA, lipid structures and matrix components [15].

In the neutralization process of those ROS, SOD, a catalyst which represents the first line of defense against superoxide radicals, and CAT are key enzymes. Altered activity of blood antioxidant enzymes including glutathione peroxidase, SOD and catalase has been reported in the literature. While Ashour et al reported SOD activity to decrease Renke et al's study showed SOD activity to be higher in oligoarticular JIA than in controls [16, 17]. Gotia et al. showed that CAT levels were low at the time of diagnosis of JIA [18]. We found that SOD levels were decreased in all types of JIA. CAT levels were lower in all types of JIA than it was in controls although

Table 2. Laboratory findings of patients with JIA and controls

	Patients (n = 25)	**Controls (n = 20)**	**p**
Hemoglobin (g/dl)	10.1 ± 1.2	13.4 ± 1.1	0.02
Leukocyte count (mm^3, $x10^3$)	17.4 ± 2.3	6.4 ± 3.8	0.01
Thrombocyte count (mm^3, $x10^5$)	64.6 ± 4.7	23.6 ± 6.5	0.0001
ESR (mm/hour)	56.3 ± 20.4	12.1 ± 3.9	0.0001
CRP (mg/dl)	5.5 ± 2.3	0.3 ± 0.05	0.0001
Fibrinogen (mg/dl)	423 ± 45.4	230 ± 24.6	0.01
Complement C3 (mg/dl)	132.1 ± 28.7	64.8 ± 11.5	0.02
IgG (mg/dl)	1450.5 ± 504.4	864.4 ± 266.8	0.003

JIA, Juvenile idiopathic arthritis; ESR, erythrocyte sedimentation rate; CRP, C reactive protein: Ig, immunoglobulin

Table 3. SOD, Catalase, MDA and ceruloplasmine levels in JIA and controls (mean ± SD)

	JIA Patients					
	All Patients (n = 25)	**Systemic (n = 3)**	**Oligo articular (n = 10)**	**Poly articular (n = 12)**	**Controls (n = 20)**	**p**
SOD (enzyme unit / mg)	0.8 ± 0.2	1 ± 0.09	0.8 ± 0.1	0.7± 0.2	0.9 ± 0.06	p1= 0.0001 p2 = 0.001 p3 = 0.005 p4= 0.001
Catalase (enzyme unit / mg)	6.8 ± 3.9	5.8 ± 2.4	3.6 ± 1.7	5.6 ± 3.4	13.8 ± 7	p3 = 0.01 p4 = 0.001 p5 = 0.0001 p6 = 0.0001 p7 = 0.026
Ceruloplasmine (mg / dl)	74.6 ± 5.2	61 ± 14.7	51.7 ± 16.9	53 ± 14.6	28.3 ± 4.9	p1= 0.0001 p4 = 0.001 p5 = 0.0001 p6 = 0.0001
MDA (nmol / ml)	3.4 ± 1.3	4.7 ± 0.2	3.9 ± 0.4	4.4 ± 0.4	2.5 ± 0.7	p4 = 0.03 p5 = 0.001

MDA, malondialdehyde; SOD, super oxide dismutase

P, significance levels for comparison between parameters; (p1, patients with polyarticular JIA vs. controls; p2, patients with systemic JIA vs. patients with polygorticular; p3, patients with oligoarticular JIA vs. patients with polyarticular JIA; p4, all patients vs. controls; p5, patients with systemic JIA vs. controls; p6, patients with oligoarticular JIA vs. controls; p7, patients with systemic JIA vs. patients with oligoarticular JIA)

MDA, a product generated during enzyme oxygenation of arachidonic acid, as well as being a product of the oxidative degradation of lipids, levels were determined to increase.

According to our study results, oxidative stress is more often seen in systemic JIA patients in relation to other types of JIA despite a concomitant increase in MDA levels.

We found that SOD levels were decreased in patients and CAT and ceruloplasmin levels were lower in oligoarticular JIA when compared to systemic JIA led us to conclude that deficiency of mechanisms reacting against ROS could be more evident in oligoarticular JIA patients.

Therefore, the present study shows that high levels of MDA and mal-adaptative state (decreased SOD and CAT levels) while exposed to oxidative stress contribute to development of active course of JIA. Possible reasons for decreased SOD and CAT levels and increased MDA levels in our patients: (i) disease itself may inhibit the activity of SOD and / or reduce the synthesis of SOD and CAT [19]. (ii) Although we did not measure any nutrient levels, the increased MDALab levels may be due to the lower antioxidant nutrients intake. In fact, decreased plasma concentrations of vitamin C and E and ß-carotene have been reported in chronic arthritis [16, 20].

Ceruloplasmin is considered to be the main plasma and synovial antioxidant in adult rheumatoid arthritis, being responsible for up to 70% of the protective capacity against superoxide free radicals which have been shown to be directly related to the pathogenesis of the inflamed joint in adult rheumatoid arthritis, and the related increases in lipid peroxidation, ascorbate depletion and hyaluronate degradation [21, 22]. In the present study, serum ceruloplasmin levels were significantly increased in JIA than in controls. According to the best of our knowledge, decreased ceruloplasmin levels have been reported in JIA but an increased ceruloplasmin level in the active course of JIA has not been reported yet [16]. Although our patients with active JIA have elevated ceruloplasmin, antioxidant effects of ceruloplasmin are not protected against oxidative

stress because significant inactivation of ceruloplasmin with ROS occurs during oxidative stress. It is likely that this is why an alteration of the ceruloplasmin can lead to the extension of ROS-mediated dysfunction to other molecules such as thioredoxine, essential component of the cellular response to oxidative stress, also exposed to oxidative stress [23, 24].

In conclusion, these results provide some evidence for a potential role of decreased catalase and SOD in JIA by its inflammatory character. The patients with JIA have defective defense mechanism against ROS and this defective defense could be more evident in oligoarticular JIA. Also, active JIA patients seem to have elevated ceruloplasmin with inadequate antioxidant effects.

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