Effect of acute brucellosis on total oxidant and antioxidant status in children

Çocuklarda akut brusellozun total oksidan ve antioksidan denge üzerine etkileri

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Abstract

Background: Infectious agent can alter organism oxidative/antioxidative balance. In this study, we investigate the effect of acute brucellosis on total oxidant and antioxidant status in children.

Methods: Forty-six children with acute brucellosis (age range, 3 to 12 years; mean, 7.6 years) and 40 healthy children (age range, 3 to 12 years; mean, 7.4 years) were included in the study. The subjects' serum catalase concentrations were measured using commercial kits (Abbott), ascorbic acid concentrations were measured FRASC method, thiol contents were measured using dithionitrobenzoic acid; ceruloplasmin, total antioxidant capacity (TAC), and total oxidant status (TOS) levels were measured by Erel's methods; lipid hydroperoxide (LOOH) concentrations were measured using the automated ferric-xylenol orange method, and oxidative stress indexes (OSI) were calculated before acute brucellosis treatment.

Results: Statistically significant differences were noted between the acute brucellosis and the control groups for their serum catalase, 4375±1781 kU/L versus 5626±2610 kU/L (P = 0.029), ascorbic acid levels were 0.82±0.99 mg/dL versus 1.3±1.2 mg/dL (P = 0.046), thiol levels were 0.38±0.03 mmol/L versus 0.42±0.03 mmol/L (P < 0.001), TAC were 0.98±0.1 mmol versus 1.2±0.12 mmolTrolox Equiv./L (P < 0.001), which were significantly decreased in children with acute brucellosis than in controls (P < 0.05). In contrast, serum ceruloplasmin levels were 42.3±5 mg/dL versus 32.9±6 mg/dL (P = 0.02), LOOH levels were 6.3±1.5 µmol versus 5.5±1.1 µmol H2O2/L (P = 0.027), TOS were 9.5±3 µmol versus 6.2±2.6 µmol H2O2/L (P < 0.001), OSI 9.8±3.1 Arbitrary Unit versus 5.8±2.6 Arbitrary Unit (P < 0.001), which were significantly elevated in study group than in controls (P < 0.05).

Conclusion: We found an increased oxidative status and decreased antioxidant status in children with acute brucellosis before treatment.

Key words: antioxidants, brucellosis, children, oxidants, oxidative stress

Özet

Amaç: Enfeksiyon etmenlerinin organizmanın oksidatif ve antioxidatif dengesi üzerine önemli etkiler yaptığı bilinmektedir. Bu çalışmada akut brusellozun çocukların oksidatif/antioksidatif dengeleri üzerine etkilerini araştırdık.

Materyal ve metod: Akut brusellozlu 46 çocuk hasta (yaş aralığı 3-12 yaş, ortalama yaş 7.6 yıl) ile 40 sağlıklı çocukta ve (yaş aralığı 3-12 yaş, ortalama yaş 7.4 yıl) çalışmaya alındı. Çalışmada akut bruselloz tedavisi öncesi serum katalaz konsantrasyonu ticari kit (Abbott) ile, askorbik asit konsantrasyonu FRASC metodu ile, thiol seviyesi dinitrobenzoik asit kullanılarak, seruloplazmin, total antioxidan kapasite (TAK) ve total oksidan seviyeleri (TOS) Erel metodları ile, lipid hidroperoksit (LOOH) konsantrasyonu otomatik ferrik-ksilenol oranj metodu ile ölçüldu ve oksidatif stress indeksleri (OSİ) hesaplandı.

Bulgular: Akut brusellalı çocuk grubu ve kontrol grubunda sırası ile serum katalaz düzeyi 4375±1781 kU/L ve 5626±2610 kU/L (P = 0.029), askorbik asit düzeyi 0.82±0.99 mg/dL, 1.3±1.2 mg/dL (P = 0.046), thiol düzeyi 0.38±0.03 mmol/L ve 0.42±0.03 mmol/L (P < 0.001) ve TAK 0.98±0.1 mmolTrolox Equiv./L ve 1.2±0.12 mmolTrolox Equiv./L (P < 0.001) olarak anlamlı derecede düşük olduğu gözlendi (P < 0.05). Diğer taraftan yine bruselloz ve kontrol grubunda sırası ile serum seruloplazmin düzeyi 42.3±5 mg/dL ve 32.9±6 mg/dL (P = 0.02), LOOH düzeyi 6.3±1.5 µmol H2O2/L ve 5.5±1.1 µmol H2O2/L (P = 0.027), TOS 9.5±3 µmol H2O2/L, 6.2±2.6 µmol H2O2/L (P < 0.001), OSI 9.8±3.1, 5.8±2.6 Arbitrary Unit (P < 0.001) değerleri ile bruselloz grubunda istatistiksel olarak anlamlı derecede yüksek bulundu (P < 0.05).

Sonuç: Akut brusellozda tedavi öncesinde antioxidant seviye düşmekte, oxidatif seviye ise yükselmektedir.

Anahtar kelimeler: antioksidant, brusellozis, çocuk, oksidan, oksidatif stress

Introduction

Reactive oxygen species (ROS) plays a key role in host defense against infections (1). They are produced during an inflammatory response and are an important part of host-defense strategies of organisms to kill the microorganism. In infections, the host produces oxidative stress and maintains it as a defense mechanism (2-4). When oxidative stress lasts for a long time or antioxidants are insufficient due to increased oxidants, proteins and some other structures,

lipid structures of the cell in particular are oxidized. Oxidization of the membrane lipids causes reduces the fluidity and permeability of the cell membrane and increases its fragility, and enhances the aging and death of the cell. Recently, it also became evident that ROS play an important role in the pathophysiology of granulomatous infections, including leishmaniasis, malaria, tuberculosis and brucellosis (1-5).

To the best of our knowledge, all of the published studies related to the oxidant/antioxidant effects of brucellosis are about adults and rat model (6-8). Also, the effects of brucellosis on superoxide dismutase, nitric oxide and malondialdehyde (MDA) have been investigated previously (4,5). In this study, serum catalase, ceruloplasmin, ascorbic acid, thiol (total-SH), total antioxidant capacity (TAC), lipid hydroperoxide (LOOH), and total oxidant status (TOS) levels were investigated in children with acute brucellosis.

Material and methods

Subjects:

Forty-six children suffering from acute brucellosis and 40 healthy children age and sex matched were enrolled in this study. In all subjects a detailed history was taken concerning passive smoking, antioxidant medication and fruit juice consumption. The local ethics committee approved this study. Children with partially treated brucellosis or chronic brucellosis or with neurodevelopmental delay or severe malnutrition, and those that had taken any antioxidant medications (ascorbic acid, vitamin E, etc.) within one week prior to participation in this study were excluded. Acute brucellosis was diagnosed on the basis of the clinical symptoms and signs, as well as standard slide agglutination tests (Rose Bengal Plate Test, Veterinary Control and Research Institute, Pendik, Istanbul, Turkey) as demonstrated by at least one of the following: (1) positive standard tube agglutination test (Veterinary Control and Research Institute, Pendik, Istanbul, Turkey) for brucella (at least 1/160 dilution), or (2) positive blood culture (9-11). The local scientific ethics committee approved this study.

Methods of analyzing the oxidants and antioxidants

Venous blood was withdrawn into normal tubes and serum was separated from the cells by centrifugation at 1500 x g for 10 min, followed by decanting into clean tubes and storage at -80 °C until analysis.

The serum catalase concentrations were analyzed with commercial kits (Abbott). Serum ascorbic acid concentrations were measured using the ferric reducing/antioxidant and ascorbic acid concentration (FRASC) method (12). Serum thiol (total –SH group) contents were measured using dithionitrobenzoic acid (DTNB) (13). Ceruloplasmin, TAC, and TOS levels were measured by Erel's methods (14-16). In addition, LOOH concentrations of the serum were measured using the automated ferric-xylenol orange method (17). All parameters were analyzed by chemistry analyzer (Aeroset, Abbott). The percentage of TOS level to TAC level was regarded as the OSI (18). To perform the calculation, the unit of TAC, mmol Trolox equivalent/L, was changed to mol Trolox equivalent/L, and the OSI value was calculated as follows: OSI= [(TOS, mol/L)/(TAC, mol Trolox equivalent/L) X 100] (18).

Statistical analysis

Normality plots and Kolmogorov-Smirnov tests were used to test the normality of data distribution.

Table 1: Comparison of demographic data

	Brucellosis (*n*= 46)	Controls (*n*= 40)	*P*
Age (years)	7.6 ± 2.2	7.4 ± 2.7	0.687[a]
Height (cm)	124.1 ± 5.9	123.8 ± 6.3	0.884[a]
Body weight (kg)	22.4 ± 4.4	23.7 ± 4.2	0.572[a]
Body mass index	14.5 ± 1.2	15.6 ± 1.1	0.456[a]
Sex (M/F)	24/22	21/19	0.868[b]

[a]Student's *t*-test [b]Chi-square test

Table 2: Comparison of serum antioxidants and oxidants

	Brucellosis (*n*= 46)	Control (*n* = 40)	*P**
Catalase (kU/L)	4375 ± 1781	5626 ± 2610	0.029
Ceruloplasmin (mg/dL)	42.3 ± 5	32.9 ± 6	0.020
Ascorbic acid (mg/dL)	0.82 ± 0.99	1.3 ± 1.2	0.046
Total –SH group (thiol) mmol/L	0.38 ± 0.03	0.42 ± 0.03	< 0.001
TAC (mmol Trolox Equiv. /L)	0.98 ± 0.1	1.2 ± 0.12	< 0.001
Lipid hydroperoxide (µmol H_2O_2 /L)	6.3 ± 1.5	5.5 ± 1.1	0.027
TOS (µmol H_2O_2 /L)	9.5 ± 3	6.2 ± 2.6	< 0.001
OSI (Arbitrary unit)	9.8 ± 3.1	5.8 ± 2.6	< 0.001

TOS, total oxidant status; TAC, total antixoidant capacity, OSI, oxidative stress index
* Student's *t* test

Student's t test was used for normally distributed data; otherwise the non-parametric U-test (Mann-Whitney U test) was used for comparisons between groups. The chi-square test was used to determine male/female distribution differences between the groups. The data were expressed as arithmetic means and standard deviations. A P value < 0.05 denoted statistical significance. Statistical analyses were performed using SPSS for Windows Release 11.5 (SPSS Inc.).

Results

We found similarities between the groups with regard to male/female distribution, mean age, height, weight, body mass index values and exposure to passive smoke per day (Table 1) (P > 0.05). Statistically significant differences were noted between the acute brucellosis and the control groups for their serum catalase, 4375±1781 kU/L versus 5626±2610 kU/L (P = 0.029), ascorbic acid levels were 0.82±0.99 mg/dL versus 1.3±1.2 mg/dL (P = 0.046), thiol levels were 0.38±0.03 mmol/L versus 0.42±0.03 mmol/L (P < 0.001), TAC were 0.98±0.1 mmol versus 1.2±0.12 mmolTrolox Equiv./L (P < 0.001), which were significantly decreased in children with acute brucellosis than in controls (P < 0.05). In contrast, serum ceruloplasmin levels were 42.3±5 mg/dL versus 32.9±6 mg/dL (P = 0.02), LOOH levels were 6.3±1.5 µmol versus 5.5±1.1 µmol H2O2/L(P = 0.027), TOS were 9.5±3 µmol versus 6.2±2.6 µmol H2O2/L (P < 0.001), OSI 9.8±3.1 Arbitrary Unit versus 5.8±2.6 Arbitrary Unit (P < 0.001), which were significantly elevated in study group than in controls (P < 0.05) (Table 2). We also found significant positive correlation between serum catalase and TAC levels in study group and controls (r = 0.756, P < 0.001) (r = 0.564, P < 0.001, respectively). A negative correlation between serum catalase and TOS levels in study group and controls (r= -0.446, P = 0.012, r = -0.371, P = 0.04, respectively).

Discussion

The major findings of this study are as follows: serum antioxidant status was significantly lower, and serum oxidant status was significantly higher in the children with brucellosis than in the healthy children. However, serum ceruloplasmin levels were significantly higher in the brucellosis group. This is the first report showing an association between low serum thiol and TAC, and high TOS and OSI in children with acute brucellosis.

All aerobic organisms have mechanisms that protect them against oxidative compounds (14). Pathogenic bacteria possess adaptive and defensive mechanisms that allow survival in the hostile phagocyte environment (19). Direct inactivation of superoxide and hydrogen peroxide would seem to be an effective strategy for pathogenic bacteria. Brucella is resistant to damage from polymorphonuclear cells owing to suppression of the myeloperoxidase–hydrogen peroxide-halide system and the production of catalase, which is well documented to play an important role in protecting cells from oxidative stress (19,20). In particular, pathogenic bacteria seem to use this enzyme as a defensive tool against attack by the host. Therefore, ROS is an important part of the host-defense strategies of organisms against brucellosis (1). Upon infection, phagocytes suddenly increase oxygen consumption, and produce oxygen intermediates, such as H2O2, superoxide, hydroxyl radical, and singlet oxygen (2,3,20). By increasing the oxidant process producing reactive oxygen species, decreasing the activity of enzymes such as catalase and superoxide dismutase removing reactive oxygen species, and by the insufficiency of antioxidants the oxidant potential dominates antioxidant potential; as a result, oxidative stress occurs. In our study, serum catalase, thiol and TAC levels were lower; TOS and OSI levels were higher in the brucellosis group than in the controls.

Ceruloplasmin is an acute phase protein, with a response of intermediate magnitude compared with other acute phase proteins; the plasma concentration is increased two- to threefold during inflammation and after traumatic injury, including surgery (16). It is reported that ceruloplasmin is increased in infections and other inflammatory diseases (22,23). Serum ceruloplasmin levels were significantly higher in the brucellosis group than those in the controls. This indicates that children with brucellosis are exposed to oxidative stress due to the generation of reactive oxygen species and peroxides as a consequence of acute inflammation.

Since ascorbic acid is a critical component of the body's antioxidant defense mechanisms, it has been investigated in studies assessing a range of biomarkers of oxidative stress, and may help protect against ROS-related diseases (24,25). Under oxidative stress, ascorbic acid is the first antioxidant to be consumed in human blood serum. Clinical observations of a number of infections accompanied by fever show a decreased blood level of ascorbic acid (23). Our results showed that, in terms of oxidative stress, ascorbic acid concentrations were significantly lower in brucellosis.

It's reported that plasma MDA levels were significantly higher in patients with acute brucellosis than in the healthy subjects (4,7,8). They also reported

that plasma MDA levels were significantly decreased after brucellosis (4). Similarly, in this study, LOOH levels were significantly higher in the brucellosis group than in the controls. Both MDA and LOOH are lipid peroxidation products. Kilic et al. investigated lipid peroxide levels in terms of MDA levels (TBARs) in brucellosis, whereas we investigated LOOH levels because it is known that the TBARs method is not specific for lipid peroxide measurement and it is positively affected especially by bilirubin and some aldehyde structures (15). Moreover, MDA is an end product of the lipid peroxidation process, but LOOH, which is our parameter, is an early marker of the oxidation chain of lipids. We also found that serum levels of TOS and OSI, which are other oxidative stress markers, were significantly higher in children with brucellosis than in the controls.

It's reported that plasma TOS and OSI levels were significantly higher in adult with acute brucellosis as compared to the controls (6,7). Similarly, in this study, serum TOS and OSI levels were significantly higher in children with brucellosis than in the controls. Karaagac et al. also reported that TOS and OSI levels were significantly higher after treatment (6).

Karaagac et al. reported that plasma TAC levels were sign ificantly lower in adult with acute brucellosis as compared to the controls (6). Karabulut et al. reported that leukocyte activities of superoxide dismutase, which is another antioxidant enzyme, were lower in patients with acute brucellosis (5). They reported that the use of an antioxidant agent in addition to classical antimicrobial therapy for acute brucellosis might be a therapeutic approach. They also stated that antioxidant therapy may shorten treatment duration or may prevent chronicity. Similarly, in this study, serum catalase and TAC levels were significantly lower, but we recommend avoiding its use in brucella treatment, because it was stated that decreasing of almost all of the reducing capacity and increasing of the oxidative stress may be caused by the host, especially as a defense mechanism against infections (1,25-27). It was suggested that replacement of antioxidants during brucella infection might interrupt the defense mechanism of the host. In particular, pathogenic bacteria seem to use this enzyme as a defensive tool against attack by the host.

In conclusion, an increased oxidative status and decreased antioxidant status in children with acute brucellosis before treatment.

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