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LIPID PEROXIDATION AND OXIDATIVE PROTEIN PRODUCTS IN CHILDREN WITH EPISODIC FEVER OF UNKNOWN ORIGIN

LIPIDNA PEROKSIDACIJA I OKSIDATIVNI PROTEINSKI PRODUKTI KOD DECE SA EPIZODIČNOM GROZNICOM NEPOZNATOG UZROKA

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Summary

Background: Episodic fever syndromes are commonly seen in pediatric practice. Episodic fever of unknown origin (FUO) lasts for a few days or weeks and is followed by a fever-free period with a sense of well-being. In this condition, activated neutrophils and monocytes intensively generate reactive oxidative species that may further damage various molecules. The aim of the study was to evaluate oxidative stress biomarkers, lipid peroxidation in erythrocytes and plasma, and advanced oxidation protein products (AOPP) in children with episodic FUO.
Methods: The study enrolled 25 children with episodic FUO in afebrile phase and 25 healthy children as controls. Lipid peroxidation was evaluated by measuring malondialdehyde (MDA) production with the thiobarbituric-acid-reactive substances (TBARS) assay in erythrocytes and plasma. Oxidative modification of proteins was measured spectrophotometrically by the determination of AOPP in plasma.
Results: Mean duration of episodic fevers was 3.96±2.8 years. Erythrocyte MDA levels were higher in children with FUO than in controls (86.26±10.75 vs. 78.0±3.21 nmol/g hemoglobin), although not significantly (p=0.202). The MDA plasma concentrations were similar (2.42±0.35 vs. 2.41±0.39 μmol/L) between the groups (p=0.732). Unexpectedly, levels of AOPP were significantly lower in children with FUO than in healthy controls (18.8±5.04 vs. 25.1±3.35 μmol/L, p=0.047).

Kratak sadržaj

Uvod: Sindromi epizodične ili rekurentne groznice često se sreću u pedijatrijskoj praksi. Epizodična groznica nepoznatog uzroka (FUO) traje nekoliko dana do nekoliko nedelja, nakon čega sledi miran period bez povišene temperature uz osećaj potpunog zdravlja. U ovim stanjima, aktivirani neutrofili i monociti intenzivno produkuju reaktivne kiseonične vrste koje naknadno mogu oštetiti različite molekule. Cilj našeg rada bio je oceniti biomarkere oksidativnog stresa, odnosno lipidnu peroksidaciju u eritrocitima i plazmi, kao i uznapredovale oksidativne proteinske produkte (AOPP) kod dece sa epizodičnom FUO.
Metode: U istraživanje je uključeno 25 dece sa epizodičnom groznicom u afebrilnoj fazi i 25 zdrave dece. Lipidna peroksidacija je utvrđena merenjem produkcije malondialdehida (MDA) korišćenjem testa tiobarbiturnih reagujućih supstanci (TBARS) u eritrocitima i plazmi. Oksidativna modifikacija proteina je određivana spektrofotometrijski, merenjem AOPP-a u plazmi.
Rezultati: Srednje vreme trajanja epizodičnih groznica bilo je 3,96±2,8 godina. Vrednosti MDA u eritrocitima su bile više kod dece sa epizodičnom FUO nego kod zdrave dece (86,26 ±0,75 vs. 78,0±3,21 nmol/g hemoglobina), iako ne statistički značajno (p=0,202). Koncentracije MDA u plazmi su bile slične kod ove dve grupe dece (2,42±0,35 vs. 2,41± 0,39 μmol/L, p=0,732). Neočekivano, nivoi AOPP-a su značajno bili manji kod dece sa FUO nego kod zdravih kontrolnih subjekata (18,8±5,04 vs. 25,1±3,35 μmol/L, p=0,047).

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List of non-standard abbreviations: AID, autoinflammatory diseases; AOPP, advanced oxidation protein products; FUO, fever of unknown origin; MDA, malondialdehyde; TBARS, thiobarbituric-acid-reactive substances.

Conclusions: Episodic fevers of unknown origin with an average duration of 3.96±2.8 years do not cause significant oxidative modifications of lipids and proteins in children.

Keywords: blood proteins, fever of unknown origin, malondialdehyde, pediatrics, reactive oxygen species

Zaključak: Epizodične groznice nepoznatog uzroka u trajanju od 3,96±2,8 godina ne izazivaju značajnu oksidativnu modifikaciju lipida i proteina kod dece.

Ključne reči: proteini krvi, groznica nepoznatog uzroka, malondialdehid, pedijatrija, reaktivne kiseonične vrste

Introduction

Fever is one of the most common signs in pediatric practice. Children, especially in the first years of life, have about 10 self-limited viral illnesses accompanied with fever. Sometimes the origin of fever remains unknown, and if the diagnosis is uncertain after 1 week of intensive evaluation, the fever is designated as a fever of unknown origin (FUO). FUO may occur as a single illness, where fever ≥38.0 °C lasts at least 3 weeks. On the other hand, recurrent or episodic FUOs are defined as three or more fevers ≥38.0 °C, that last for a few days to a few weeks, with a fever-free period and a sense of well-being (1–3). These syndromes represent a diagnostic challenge and usually the right diagnosis is delayed. According to the results of various studies, the main causes of FUO are infections. However, there are numerous noninfectious FUO. The second most frequent single FUOs are of inflammatory and malignant origin or due to collagen vascular disease (2, 4, 5). Beside infections, other causes of episodic FUO are more commonly hereditary, autoinflammatory and sometimes autoimmune diseases (2–4).

The most prevalent reasons of recurrent or episodic fever syndromes in pediatric practice are autoinflammatory diseases (AID). This is a group of hereditary diseases, monogenic or multifactorial (*Table I*), characterized by seemingly unprovoked episodes of fever and localized inflammation (peritonitis or pleuritis, synovitis, skin rash, fatigue, etc.) (1, 2, 5). Most of the AID are caused by mutations in the genes coding inflammasome sequences or its regulatory molecules in the cells of the innate immune system. A defect in the inflammasome control leads to interleukin-1 (IL-1) pathway disorders, without involvement of a distinct pathogen or the adaptive immune system. The caspase-1 inflammasome dysregulation results in diminished control over the IL-1 precursor processing and increased secretion of this proinflammatory cytokine (1, 6).

In chronic episodic FUO there is a cyclic activation of leukocytes, mostly neutrophils, monocytes, macrophages and dendritic cells, that now intensively secrete lymphokines and endogenous pyrogenic cytokines, such as IL-1β, IL-6, TNF-alpha (7). Interleukin-1β plays an important part in the inflammatory response. It resets the hypothalamic thermoregulatory center causing fever, induces leukocytosis, cachexia, hyperalgesia, increases acute-phase protein synthesis, etc. Beside cytokines, activated neutrophils and monocytes produce significant amounts of reactive oxygen species (ROS) and reactive nitrogen intermediates via the NADPH oxidase complex and myeloperoxidase system during phagocyte oxidative burst (8). In these instances, reactive oxygen/nitrogen species may cause cell injury via the membrane lipid peroxidation and oxidative modification of carbohydrates, proteins and nucleic acids.

A positive feedback loop between IL-1β secretion, phagocyte activation and ROS production may aggravate inflammation and oxidative stress. Interleukin-1β induces neutrophilia and activation of phagocytic NADPH oxidase (6, 9, 10). Activated phagocytes in turn produce the proinflammatory cytokines (IL-1, TNF-α, and IL-6) (8), while ROS regulate IL-1β production through the inflammasome and nuclear factor-κ B activation, and recruitment of phagocytes into the affected tissues (11). Because of

Table I The monogenic and multifactorial autoinflammatory diseases.

Monogenic	Polygenic/Multifactorial
Blau's syndrome	Adult onset Still's disease
Cyclic neutropenia syndrome and periodic fever	Behçet's disease
Familial Cold Autoinflammatory Syndrome (FCAS)*	Chronic recurrent multifocal osteomyelitis
Familial Mediterranean Fever (FMF)	Crohn's disease
Mevalonate Kinase Deficiency (MKD)	Periodic Fever, Aphthous stomatitis, Pharyngitis, cervical Adenitis syndrome (PFAPA)
Muckle-Wells Syndrome (MWS)*	Pseudo-gout
Neonatal-Onset Multisystem Inflammatory Disease (NOMID)*	Systemic onset juvenile idiopathic arthritis
Tumor necrosis factor Receptor-Associated Periodic Syndrome (TRAPS)	Urate crystal arthritis (gout)
Pyogenic Arthritis, Pyoderma gangrenosum and Acne syndrome (PAPA)	

* belong to the Cryopyrin-Associated Periodic fever Syndromes (CAPS).

this, ROS formation may be prolonged in episodic FUO syndromes even in the fever-free periods. The role of oxidative stress in inflammation has been described in the pathogenesis of many diseases, including the autoinflammatory (8, 9, 12). In some patients with AID subclinical inflammation and oxidative stress have been reported during fever-free periods (13–15). Considering all the abovementioned, there is perhaps enhanced oxidant production and accumulation of oxidative stress damage in persons with episodic FUO.

The goal of our study was to determine oxidative stress biomarkers in children with episodic fever of unknown origin, through the assessment of lipid peroxidation, both in erythrocytes and plasma, as well as advanced oxidation protein products. Our intention was to generally assess oxidative stress damage in these persons, when they are not directly affected by a febrile phase.

Patients and Methods

The study was conducted in compliance with the Declaration of Helsinki and Good Clinical Practice (GCP) Guidelines. The relevant study documents have been approved by an Independent Ethics Committee. The patients' informed consent forms were provided in both written and oral form and signed by parents and, when possible, the patients.

The study group comprised 25 eligible children with episodic FUO referred to the Department of Pediatric Rheumatology, Clinical Center in Niš, Serbia. There were 14 (56%) boys and 11 (44%) girls with episodic FUO, with febrile episodes recurring for at least one year, without a febrile episode in the previous two weeks. During the thorough diagnostic procedure, infectious, autoimmune, metabolic and potentially malignant diseases were excluded. The children were not on drug therapy in the afebrile period when the blood samples were collected. A total of 25 healthy children who accepted to donate blood samples, taken for regular health checkups, comprised the control group. They were age and sex-matched to the children with episodic FUO.

We collected the whole blood samples with EDTA, after which plasma and washed erythrocytes were separated by a centrifuge. Lipid peroxidation (LP) was evaluated by measuring malondialdehyde (MDA) production with the thiobarbituric acid reactive substances (TBARS) assay in plasma and washed erythrocytes. Protein oxidation was measured by the determination of advanced oxidation protein products (AOPP) in plasma.

TBARS in erythrocytes were assessed spectrophotometrically according to the Jain et al. (16) method. Trichloroacetic acid and tertiary butyl alcohol were added to erythrocytes in phosphate buffer (pH – 7.4) forming the chromogen. The absorption was measured at 532 nm wavelength. MDA concentration was expressed as nanomoles per gram of hemoglobin. TBARS concentration in plasma was determined spectrophotometrically according to the Andreeva et al. (17) method. The method is based on the reaction of MDA with thiobarbituric acid, at a high temperature and low pH. Measurement of MDA-TBA 2 chromogen is then assessed at 532 nm wavelength.

AOPP in plasma were determined spectrophotometrically according to chloramine T solution, which in the presence of potassium iodide absorbs at 340 nm (18).

Complete blood count, erythrocyte sedimentation rate, and C-reactive protein (CRP) and albumin concentrations were measured at the Biochemical Laboratory of the Clinic of Children's Internal Disease in Niš, Serbia.

Complete blood count parameters (erythrocytes, leukocytes, platelets and hemoglobin) were assessed using the COULTER® AcT Diff Analyzer (Beckman Coulter Corporation, Hialeah, FL, USA), an automated hematology analyzer with a complete reagent system and authentic Coulter technology: triplicate counting, proven Coulter histogram differential, patented sweep flow technology and extended platelet counting.

For CRP and albumins concentration measurement, we used a fully automated Erba Mannheim XL600 analyzer with photometric tests and commercially available reagents of the manufacturer (ERBA Diagnostics Mannheim Gmbh, Baden-Wurttemberg, Germany).

Erythrocyte sedimentation rate was assessed using the Westengard ESR method (19).

The results were expressed as mean ± standard deviation or median ± interquartile range values, as appropriate. Statistical analysis of biochemical parameters was conducted with the Mann-Whitney U-test or Students t-test, with statistical significance at $p<0.05$. For correlation between the parameters, we used Spearman's rank order correlation. Statistical analysis was performed using the SPSS 17.0 (SPSS, Chicago, IL, USA) statistical program.

Results

Average age of the children with episodic FUO was 10.7±4.0 years, and 11.2±3.9 years that of the healthy children. Mean duration of a fever episode was 3.53±1.26 days, and the average duration of recurrent episodes was 3.96±2.8 years (range: 1– 6.7).

The children with episodic FUO reported occasionally other symptoms, and among the most frequent were: abdominal pain (70%), lymphadenopathy (62.5%), headache (45.8%), polyarthralgia (33.3%),

Table II The results of MDA and AOPP levels in the study group and controls.

	MDA erythrocytes (nmol/g Hgb)	MDA plasma (μmol/L)	AOPP (μmol/L)
Children with FUO	86.26±10.75	2.42±0.35	18.8±5.04
Healthy children	78.0±3.21	2.41±0.39	25.1±3.35*
* statistically significant for $p<0.05$ compared to study group			

AOPP – advanced oxidation protein products; Hgb – hemoglobin; MDA – malondialdehyde.

Table III Demographic data, blood count and inflammatory parameters.

	Children with FUO (n=25)	Healthy children (n=25)	*p* value
Age (years)	10.7±4.0	11.2±3.9	–
BMI (kg/m^2)	16.7±2.1	17.2±3.3	–
RBC (×10^{12}/L)	4.583±0.228	4.602±0.188	0.76
HGB (g/L)	133.80±14.4	135.50±10.17	0.35
WBC (×10^9/L)	7.08±3.17	7.34±2.24	0.84
PLT (×10^9/L)	332.43±80.6	291.2±57.5	0.51
ESR	9±8.8	9.1±9.9	0.98
CRP (mg/L)	1.93±2.78	0.68±1.9	0.27
Albumins (g/L)	47.28±3.75	45.18±5.04	0.22

CRP – C-reactive protein; ESR – erythrocyte sedimentation rate; PLT – thrombocyte count; RBC – erythrocyte count; WBC – leukocyte count.

urticarial skin rash (33.3%), myalgia (29.2%), malaise (29.2%), oligoarthralgia (20.8%) and monoarthralgia (16.7%).

Levels of erythrocyte MDA were higher in children with episodic FUO than in healthy children, but without statistical significance ($p=0.202$). There was no difference in MDA concentrations in plasma ($p=0.732$) between the groups. Interestingly, levels of AOPP were significantly lower in children with episodic FUO than in controls ($p=0.047$). The levels of MDA and AOPP are shown in *Table II*.

Correlation between the examined oxidative parameters was low (0.1 to 0.4), except for MDA erythrocytes and AOPP levels in children with FUO that showed a moderate but statistically significant positive correlation ($rs=0.516$, $p=0.02$).

Also, erythrocyte MDA levels correlated positively with the albumin levels in the children with episodic FUO ($rs=0.476$, $p=0.046$). There were no other significant correlations between the oxidative stress biomarkers and C-reactive protein, albumins or leukocytes (*Table III*).

There was no significant difference in the complete blood count between the groups. Demographic data, complete blood count and inflammatory parameters are shown in *Table III*.

Discussion

Fever is an important adaptive mechanism in the fight against infections. However, in the innate immune system disorders with spontaneous inflammasome activity, prolonged fever and inflammation may cause damage to the biomolecules and cells (7).

Malondialdehyde is one of the LP end-products and, since it is not generated exclusively through LP, it is considered as a general indicator of oxidative stress (8, 12). One of the often used and valid models for studying the effects of oxidative stress and lipid peroxidation are erythrocyte membranes (20). Although the levels of erythrocyte MDA in our study were higher in the children with episodic FUO than in controls, the difference did not reach statistical significance. This result most probably reflects an effective erythrocytes' antioxidative system response and sufficient protective concentrations of plasma antioxidants in these conditions (12, 21).

Plasma MDA originates from peroxidation of plasma lipids, thrombocytes, endothelial and other cells. Its level has been used as an indirect indicator of tissue LP, as lipid peroxidation products diffuse from damaged tissues and sites of inflammation into the circulation (12, 22, 23). Plasma MDA values were very similar between our groups, suggesting no significant tissue damage in children with episodic FUO. It is important to note that both erythrocyte and plasma MDA values were assessed in the afebrile phase, because lipid peroxidation products are often found in inflammatory diseases and show significantly higher values in the acute period of disease than in remission (12, 24, 25). The significant positive correlation of erythrocyte MDA with AOPP and albumins in our study may indicate elevated ROS levels in the febrile phase of episodic FUO and their modifying effects on lipids and proteins in this phase.

Albumins provide very important antioxidant defense activity in the plasma, especially their reduced thiol groups, that protect other macromolecules from oxidative injury (26). Advanced oxidation protein products are oxidative stress biomarkers in plasma, mostly derived from albumins, and to a lesser extent from fibrinogen and lipoproteins. They are normally formed in small amounts and their concen-

trations steadily increase with age (27, 28). AOPP are also recognized as markers of inflammation, with high levels being reported in diseases such as diabetes, chronic renal failure, hyperlipidemia, etc. (29, 30). Neutrophil oxidative potential was shown to be directly involved in plasma AOPP formation through the activity of myeloperoxidase, that is mostly abundant in these cells (8, 27, 28).

The higher values of AOPP in healthy children were unexpected, although the probability value was borderline significant ($p=0.047$). AOPP concentrations were not increased in the afebrile phase in the children with episodic FUO. It has been determined that AOPP levels are significantly higher in the attack period of inflammatory diseases than in remission and in controls (14, 28, 31). In the study of Keskin et al. (28), levels of AOPP were significantly higher in the active stage of Henoch–Schonlein purpura, a systemic inflammation of the blood vessels that commonly affects children, than in remission stage and in healthy controls. Also, the AOPP levels were similar in the remission stage and in controls.

We showed that episodic fevers that persist for approximately four years are not accompanied by significant AOPP accumulation, unlike in chronic inflammatory diseases. In the fever-free period, AOPP are normally cleared from plasma by the mononuclear phagocyte system (16, 27, 28). Also, free oxygen radicals, that are gradually and episodically released during the phases of inflammation, may increase the antioxidant defense capacity by the activation of enzymatic defense systems and better counteract ROS effects. This is opposite to prolonged or continuous inflammation in chronic diseases, where the total antioxidant capacity becomes reduced due to the high consumption (32, 33).

In conclusion, our study shows that episodic fevers with an average duration of four years do not cause significant oxidative modifications of lipids and proteins, and there is no substantial oxidative stress in children with these conditions. Among the examined parameters, erythrocyte membranes were the most vulnerable to ROS, and these oxidative changes endure the longest in the circulation. Decreased AOPP values in episodic FUO could be the result of the compensating, higher antioxidative capacity of the blood, induced by reactive molecular species.

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Conflict of interest statement

The authors stated that there are no conflicts of interest regarding the publication of this article.

References

1. Ozen S, Frenkel J, Ruperto N, Gattorno M; Eurofever Project. The Eurofever Project: towards better care for autoinflammatory diseases. Eur J Pediatr 2011; 170(4): 445–52.
2. Long SS. Distinguishing among prolonged, recurrent, and periodic fever syndromes: approach of a pediatric infectious diseases subspecialist. Pediatr Clin North Am 2005; 52(3): 811–35, vii.
3. Chandy CJ, Gilsdorf JR. Recurrent fever in children. Pediatr Infect Dis J 2002; 21: 1071–80.
4. Majeed HA. Differential diagnosis of fever of unknown origin in children. Curr Opin Rheumatol 2000; 12(5): 439–44.
5. Knockaert DC. Recurrent fevers of unknown origin. Infect Dis Clin N Am 2007; 21: 1189–211.
6. Papin S, Cuenin S, Agostini L, Martinon F, Werner S, Beer H-D, et al. The SPRY domain of Pyrin, mutated in familial Mediterranean fever patients, interacts with inflammasome components and inhibits proIL-1b processing. Cell Death Differ 2007; 14: 1457–66.
7. Sarkisian T, Emerit I, Arutyunyan R, Levy A, Cernjavski L, Filipe P. Familial Mediterranean fever: clastogenic plasma factors correlated with increased $O_2^{(-)}$ – production by neutrophils. Human Genetics 1997; 101: 238–42.
8. Witko-Sarsat V, Gausson V, Descamps-Latscha B. Are advanced oxidation protein products potential uremic toxins? Kidney Int Suppl 2003; (84): S11–14.
9. Hou CC, Lin H, Chang CP, Huang WT, Lin MT. Oxidative stress and pyrogenic fever pathogenesis. Eur J Pharmacol 2011; 667(1–3): 6–12.
10. Chae JJ, Aksentijevich I, Kastner DL. Advances in the understanding of familial Mediterranean fever and possibilities for targeted therapy. Br J Haematol 2009; 146(5): 467–78.
11. Meissner F, Molawi K, Zychlinsky A. Superoxide dismutase 1 regulates caspase-1 and endotoxic shock. Nature Immunology 2008; 9: 866–72.
12. Gürel A, Armutçu F, Damatoğlu S, Unalacak M, Demircan N. Evaluation of erythrocyte Na^+, K^+-ATPase and superoxide dismutase activities and malondialdehyde level alteration in coal miners. Eur J Gen Med 2004; 1(4): 22–8.
13. Duzova A, Bakkaloglu A, Besbas N, Topaloglu R, Ozen S, Ozaltin F, et al. Role of A-SAA in monitoring subclinical

inflammation and in colchicine dosage in familial Mediterranean fever. Clin Exp Rheumatol 2003; 21: 509–14.

14. Guzel S, Andican G, Seven A, Aslan M, Bolayirli M, Celik Guzel E, et al. Acute phase response and oxidative stress status in familial Mediterranean fever (FMF). Mod Rheumatol 2012; 22: 431–7.
15. Touitou I, Koné-Paut I. Autoinflammatory diseases. Best Pract Res Clin Rheumatol 2008; 22(5): 811–29.
16. Jain SK, McVie R, Duett J, Herbst JJ. Erythrocyte membrane lipid peroxidation and glycosylated hemoglobin in diabetes. Diabetes 1989; 38: 1539–43.
17. Andreeva JL, Kozjemakin AL, Kiskun AA. Modifikacija metoda opredelnija perekisej lipidov testes tiobarbiturovoj kisloti. Lab Delo 1988; 11: 41–3. Russian.
18. Witko-Sarsat V, Friedlander M, Capeillère-Blandin C, Nguyen-Khoa T, Nguyen AT, Zingraff J, et al. Advanced oxidation protein products as a novel marker of oxidative stress in uremia. Kidney Int 1996; 49(5): 1304–13.
19. Thomas RD, Westengard JC, Hay KL, et al. Calibration and validation for erythrocyte sedimentation tests: role of the International Committee on Standardization in Hematology reference procedure. Arch Pathol Lab Med 1993; 117: 719–23.
20. Eritsland J. Safety considerations of polyunsaturated fatty acids. Am J Clin Nutr 2000; 71: 197S–201S.
21. Kolanjiappan K, Manoharan S, Kayalvizhi M. Measurement of erythrocyte lipids, lipid peroxidation, antioxidants and osmotic fragility in cervical cancer patients. Clinica Chimica Acta 2002; 326: 143–9.
22. Frankel EN, Neff WE. Formation of malonaldehyde from lipid oxidation products (Lipid oxidation, malonaldehyde synthesis, thiobarbituric acid). Elsevier Biomedical Press 1983; 754(3): 264–70.
23. Kwiatkowsha S, Piasecka G, Zieba M, Piotrowski W, Nowak D. Increased serum concentrations of conjugated dienes and malondialdehyde in patients with pulmonary tuberculosis. Respir Med 1999; 93: 272–6.
24. Erdoğan Ö, Öner A, Aydın A, Işimer A, Demircin G, Bülbül M. Effect of vitamin E treatment on the oxidative damage occurring in Henoch–Schönlein purpura. Acta Paediatr 2003; 92: 546–50.
25. Ece A, Kelekçi S, Kocamaz H, Hekimoğlu A, Balik H, Yolbaş I, et al. Antioxidant enzyme activities, lipid peroxidation, and total antioxidant status in children with Henoch-Schönlein purpura. Clin Rheumatol 2008; 27: 163–9.
26. Halliwell B. Albumin – An important extracellular antioxidant? Biochem Pharmacol 1998; 37: 569–71.
27. Piwowar A. Advanced oxidation protein products. Part I. Mechanism of the formation, characteristics and property. Pol Merkur Lekarski 2010; 28(164): 166–9. Polish.
28. Keskin N, Civilibal M, Elevli M, Koldas M, Duru NS, Ozturk H. Elevated plasma advanced oxidation protein products in children with Henoch-Schonlein purpura. Pediatr Nephrol 2011; 26(11): 1989–93.
29. Hübner-Woźniak, Okecka-Szymańska J, Stupnicki R, Malara M, Kozdron E. Age-related blood antioxidant capacity in men and women. J Med Biochem 2011; 30: 103–8.
30. Hamad M, Awadallah S, Nasr H. The relationship between haptoglobin polymorphism and oxidative stress in hemodialysis patients. J Med Biochem 2013; 32: 220–6.
31. Yazici C, Köse K, Caliş M, Demlr M, Kirnap M, Ate F. Increased advanced oxidation protein products in Behçet's disease: a new activity marker? Br J Dermatol 2004; 151(1): 105–11.
32. Ozdogan M, Devay AO, Gurer A, Ersoy E, Devay SD, Kulacoglu H, et al. Plasma total anti-oxidant capacity correlates inversely with the extent of acute appendicitis: a case control study. World J Emerg Surg 2006; 1: 6.
33. Koltuksuz U, Uz E, Ozen S, Aydinc M, Karaman A, Akyol O. Plasma superoxide dismutase activity and malondialdehyde level correlate with the extent of acute appendicitis. Pediatr Surg Int 2000; 16: 559–61.

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