

## DIAGNOSTIC AND THERAPEUTIC SIGNIFICANCE OF THE OXIDATIVE STRESS PARAMETERS IN CHILDREN

DIJAGNOSTIČKI I TERAPIJSKI ZNAČAJ PARAMETARA OKSIDATIVNOG STRESA KOD DECE

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**Summary:** Pharmacotherapy of pediatric diseases represents a major challenge considering that the majority of medicines in everyday practice have not been pediatrically evaluated. The efficacy of therapy depends to a large extent on the knowledge of pathophysiological processes in the children organism at different ages. Therefore, research in that direction is of the utmost importance. An imbalance in the production of free oxygen/nitrogen species and parameters of antioxidative protection is a significant factor in many diseases (e.g. heart failure, pulmonary hypertension, asthma, neonatal sepsis, cancer etc.) in children of different age groups. Reactive oxygen/nitrogen species serve as cell signaling molecules for normal biologic processes. An increase in their generation can cause damages which can disrupt normal physiological cellular processes and eventually cause cell death. This review outlines the previous assessments of oxidative stress parameters in children of different ages for some diseases. Also, the potential diagnostic and therapeutic possibilities for the oxydative stress parameters in children have been considered.

**Keywords:** oxidative stress parameters, antioxidants, children

### Introduction

The efficacy of a therapy depends to a large extent on the knowledge of the pathophysiological

**Kratka sadržaj:** Farmakoterapija oboljenja kod dece predstavlja veliki izazov s obzirom da najveći broj lekova koji se svakodnevno koristi nije pedijatrijski evaluiran. Efikasnost terapije zavisi u velikoj meri od poznavanja patofizioloških procesa u organizmu dece različitih uzrasta te istraživanja u tom pravcu predstavljaju imperativ. Narušen balans u produkciji slobodnih kiseoničkih/azotnih vrsta i parametara antioksidantne zaštite značajan je patofiziološki činilac brojnih oboljenja (npr. srčana insuficijencija, plućna hipertenzija, astma, neonatalna sepsa, karcinom i dr.) u različitim dečijim uzrastnim periodima. Reaktivne kiseonične/azotne vrste imaju funkciju signalnih molekula u normalnim fiziološkim procesima. Njihova povećana produkcija može izazvati oštećenja koja mogu narušiti normalne fiziološke procese u ćeliji i u krajnjem ishodu izazvati ćelijsku smrt. U ovom radu dat je pregledni prikaz dosadašnjih ispitivanja parametara oksidativnog stresa kod dece različite starosne dobi za pojedina oboljenja. Takođe, razmotrili smo sve potencijalne dijagnostičke i terapijske mogućnosti parametara oksidativnog stesa u dečijem uzrastu.

**Ključne reči:** parametri oksidativnog stresa, antioksidansi, deca.

processes in the organisms of children of different ages (1–3). Numerous disorders in childhood have been linked to oxidative damage. The role of reactive oxygen species (ROS) in the pathogenesis and progression of these diseases has been only partially defined. As oxidative conditions have been suggested to be of importance in developmental and maturational processes, parameters of oxidation may need to be differently interpreted in infancy and adulthood. Therefore, the research in that direction is of the utmost importance.

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### Oxidative stress in pediatric diseases

Results from numerous studies published in the past decade have suggested that many pediatric diseases are linked to oxidative damage attributable to ROS in their pathogenesis and progression. In the newborn period oxidative damage has been implicated in the pathogenesis of numerous diseases and entities, such as maternal preeclampsia (4), premature birth (5–7), asphyxia (8, 9), neonatal respiratory distress syndrome (10–12), retinopathy (7, 10).

Numerous factors can influence the creation and development of cardiovascular diseases in pediatrics (13). There is enough evidence that oxidative stress plays an important role in the development and progression of cardiovascular diseases in children, such as essential hypertension (14), dilated cardiomyopathy, chronic heart failure (CHF) (15), cardiopulmonary bypass (16), cardiac transplantation (17) and surgery (18).

Diabetes mellitus (19–23), glutathione synthetase deficiency (24), hyperthyroidism (25), iodine-deficient goiter (26), mitochondrial disorder (27, 28), multimetabolic syndrome (29), phenylketonuria (30) and X-linked adrenoleukodystrophy (31) are endocrinologic/metabolic diseases in children where the link with oxidative stress has also been shown.

An imbalance in the production of free oxygen/nitrogen species and the parameters of antioxidative protection is a significant factor in children of different age-ranges in many diseases:

- gastrointestinal and hepatologic (autoimmune hepatitis (32), chronic constipation (33), inflammatory bowel disease (34), nonalcoholic fatty liver disease (35, 36), viral hepatitis (37), Wilson disease (38)),
- hematologic (acute leukemia (39–41), thalassemia (42), erythropoietic protoporphyria (43), Fanconi anemia (44), sickle cell anemia (45)),
- infectious (acute bronchiolitis (46), acute infectious mononucleosis (47), acute otitis media (48), acute tonsillitis (48), adenovirus infection (47), chronic nail candidiasis (49), chronic otitis media (50), chronic tonsillitis (51, 52), cutaneous leishmaniasis (53), HIV infection (54, 55), measles encephalitis (56), meningitis (57, 58), septic shock (59)),
- neurologic and muscular (ataxia telangiectasia (60), attention deficit hyperactivity disorder (61), autism (62–64), cerebral organic acid disorder (65), cerebral palsy (66), congenital muscular dystrophy (67–69), epilepsy (27, 70, 71), Friedreich ataxia (72), inflammatory myopathy (73), selenium-deficient skeletal muscle disorder (73), spinal muscular atrophy (74), traumatic brain injury (75, 76), migraine (77)),
- renal (glomerulonephritis (78), nephrotic syndrome (79), renal insufficiency/failure (78–80), urinary tract infection (78)),

- respiratory (chronic pulmonary disease (81), cystic fibrosis (82, 83)),
- allergic and immunologic (atopic dermatitis (84, 85), bronchial asthma (86–88), chronic arthritis (89, 90), Henoch-Schonlein purpura (91), Kawasaki disease (47), systemic lupus erythematosus (92), vasculitis syndrome (93)).

Some genetic disorders: Cockayne syndrome (94), Down syndrome (95,96), Zellweger syndrome (97), as well as obesity (98), hyperlipidemia (99) and kwashiorkor (100–102) are also linked with oxidative stress.

Oxidative stress might also contribute to tissue damage in childhood induced by certain drugs (analgesics (103), anticancer drugs (103–107), immunosuppressive drugs (108)) and environmental and toxicologic factors (total body irradiation (109), carcinogenic metal (chromium, arsenic) exposure (110), ozone exposure (88), passive smoking (111), urban residence (112)).

Unfortunately, studies of oxidative stress in children had limited sample sizes and not enough convincing evidence to prove the causal relation between oxidative stress and disease conditions. There is also a plethora of other factors which might be responsible for the pathogenesis and progression of diseases. Therefore, the extent to which oxidative stress contributes to the etiology of pediatric pathologies remains difficult to determine.

### Diagnostic significance of oxidative stress parameters in children

This review outlines the recent achievements of oxidative stress parameter determination in pediatric medicine. So far, the published studies have determined the oxidative stress status *in vivo* in:

- samples of blood (serum, plasma, erythrocytes, granulocytes and lymphocytes),
- urine (5, 10, 20, 23, 44, 64, 84, 85, 97)
- other body fluids (cerebrospinal fluid (57, 58, 75), bronchoalveolar lavage fluid (80, 83), joint fluid (90), nasal lavage fluid (88) and middle-ear fluid (50)),
- tissues (18, 28, 41, 50, 51, 52, 56, 67, 69, 74),
- exhaled breath (71, 82, 86).

The use of fluorescent probes might enable detection of ROS generation in human live cells, however, it is not applicable yet in the routine clinical practice (113–115).

Oxidative stress parameters are important for predicting the consequences of oxidation, and for providing a basis for designing appropriate interventions to prevent or alleviate injury. There are two categories of these parameters:

1. Formation of modified molecules by ROS. Molecules are subjected to either scission, cross-linking or covalent modification in these reactions. Therefore, the increased ROS activity also increases the amount of these molecules. Membrane lipids, proteins, nucleic acids and carbohydrates are the major targets of ROS in the molecular components of cells. Clinically applicable parameters for estimating lipid membrane damage during lipid peroxidation are malondialdehyde-lysine, 4-hydroxy-2-nonenal-lysine, and F2-isoprostane. Parameter for oxidative DNA damage is: 8-hydroxy-2'-deoxyguanosine (113, 116). Acrolein-lysine is a sensitive marker of lipid peroxidation and oxidative protein damage (116, 117). Parameters of glyco-oxidation are carboxymethyl-lysine, pentosidine, argpyrimidine, methylglyoxal, and of nitro-oxidation: nitrotyrosine, nitrite/nitrate;
2. Consumption or induction of enzymes or antioxidants. Oxidative stress results from an imbalance of ROS and endogenous antioxidant defense mechanisms, enzymatic and non-enzymatic. Balanced and coordinated antioxidant defense enzyme activities are essential for physiologic function and shielding against pathologic conditions. Superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), glutathione reductase (GR), glutathione-S-transferase (GST), thioredoxin reductase and heme oxygenase are important antioxidant enzymes. Erythrocytes are particularly vulnerable to oxidative stress, due to their constant exposure to oxygen radicals that are endogenously generated via the auto-oxidation of hemoglobin and from plasma, particularly nitric oxide (NO) and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>). SOD catalyzes the dismutation of the superoxide radical to H<sub>2</sub>O<sub>2</sub>, which is then independently converted to water by CAT or GSH-Px. GR catalyzes the reduction of oxidized GSH back to GSH, the latter being the co-substrate of GSH-Px. When the enzymatic activity of the first step (SOD) and the second step (CAT and/or GSH-Px) are balanced, the risk for cell damage is lower. Proteins (albumin, ferritin, transferrin, lactoferrin, ceruloplasmin, thioredoxin, L-type fatty acid binding protein) and low molecular weight molecules (tocopherols, ascorbate, carotenoids, bilirubin, ubiquinol/ubiquinone, glutathione, cysteine, urate, nitrite/nitrate, selenium) belong to the biologically active antioxidants.

### Therapeutic implications

The therapeutic implications of oxidative stress determination in childhood are questionable. Some data suggest that decreasing exposure to ROS or augmenting antioxidant defenses might be beneficial as adjunctive therapy for oxidative-stress related pediatric pathological conditions. Our results and the results of recently published studies suggest that

**Table I** Therapeutic antioxidant strategies in pediatric pathological conditions.

Antioxidant agents	Pathological conditions
Tocopherols and ascorbate	endothelial dysfunction in hyperlipidemia (100)
L-arginine	endothelial dysfunction in cardiac transplantation (17)
Angiotensin converting enzyme inhibitors and angiotensin II type-1 receptor antagonists	endothelial dysfunction in diabetes mellitus (22)
Carvedilol	dilated cardiomyopathy, chronic heart failure (15)
Tocopherols and ubiquinol/ubiquinone	Friedreich ataxia (72)
Tocopherols, carotenoids and ascorbate	immunosuppressive drug use (cyclosporine A, tacrolimus) (102)
Ubiquinol /ubiquinone	anthracycline use (104)
Selenium	skeletal muscle disorder in selenium deficiency (73)
Corticosteroids	bronchial asthma (86)
	bacterial meningitis (54)
Melatonin	neonatal asphyxia (8)
	epilepsy (70)
Folinic acid, betaine and methylcobalamin	autism (63)

establishment of redox balance using antioxidant agents could be therapeutically beneficial (*Table I*).

On the other hand, in the few disease conditions in which treatment with antioxidants has been evaluated, results have been disappointing. For example, adjunct antioxidant chelating cocktail in the treatment of neonatal hemochromatosis has not resulted in significant improvement. In 14 infants, the antioxidant cocktail did not improve outcome and only a successful orthotopic liver transplant afforded a cure (118). In children with cystic fibrosis, selenium supplementation did not affect lipid peroxidation markers (119). Similarly, in children suffering from kwashiorkor, vitamin A had no effect on the duration of edema or weight gain (120).

Recently published studies suggest that high dose antioxidant therapy may be damaging. For example, alpha tocopherol supplementation increased the risk for colorectal adenomas (121). Recent *in vitro* and *in vivo* studies have suggested that although beta-carotene itself may act as an anticarcinogen, its oxidized products may facilitate carcinogenesis (122, 123). A report by the Food and Nutrition Board of the American National Academy of

Sciences warns against injudicious, excessive intake of antioxidants such as vitamin E, vitamin C, selenium and carotenoids (124).

The antioxidant therapy has shown beneficial effects in some pediatric diseases. However, before making the decision to apply antioxidant therapy or nutritional antioxidant supplementation one should be aware that under certain conditions antioxidant agents may exhibit prooxidant properties and even worsen general progression of the disease.

## Conclusion

Further fundamental investigation of the basic science may be necessary to understand the oxidative processes during childhood. In order to achieve this, methods need to be developed which would comply with the rigorous ethical requirements for pediatric

research. Currently, the determination of oxidative stress parameters in urine samples has the most far-reaching potential for the monitoring of oxidative stress related diseases in pediatric medicine. Safe and efficient antioxidant therapy and optimal nutritional antioxidant supplementation in children should be based on the knowledge of oxidative processes in pediatric diseases and on randomized, placebo-controlled trials with large sample sizes.

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## Conflict of interest statement

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

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