# ZNAČAJ OKSIDATIVNOG STRESA U PATOGENEZI OVARIJALNOG KARCINOMA

PREGLEDNI RAD

REVIEW ARTICLE

# THE ROLE OF OXIDATIVE STRESS IN OVARIAN CANCER PATHOGENESIS

**Petar Simić[1], Marija Plješa Ercegovac[2,3,4], Vesna Ćorić[2,3,4], Vladimir Pažin[1,2]**

[1] Ginekološko akušerska Klinika „Narodni front", Beograd, Srbija
[2] Univerzitet u Beogradu, Medicinski fakultet, Beograd, Srbija
[3] Institut za medicinsku i kliničku biohemiju, Beograd, Srbija
[4] Centar izvrsnosti za Redoks medicinu, Beograd, Srbija

[1] *Obstetrics and Gynecology Clinic Narodni Front, Belgrade, Serbia*
[2] *University of Belgrade, Faculty of Medicine, Belgrade, Serbia*
[3] *Institute of Medical and Clinical Biochemistry, Belgrade, Serbia*
[4] *Center of Excellence for Redox Medicine, Belgrade, Serbia*

## SAŽETAK

Ovarijalni karcinom predstavlja najsmrtonosniji ginekološki malignitet. Nespecifične tegobe, uz otkrivanje bolesti u uznapredovalom stadijumu, predstavljaju osnovne faktore loše prognoze sa ukupnim petogodišnjim preživljavanjem od 45%. Za sada, terapiju prvog izbora u tretmanu ovarijalnog karcinoma predstavlja hirurško lečenje sa ciljem potpune eliminacije makroskopske bolesti, uz adjuvantnu primenu hemioterapije. Važnost rane dijagnoze se ogleda u brojnim sprovedenim prospektivnim istraživanjima, koja nisu identifikovala efikasan način rane detekcije ove bolesti. Oksidativni stres, kao disbalans u produkciji i eliminaciji reaktivnih jedinjenja kiseonika, dokazani je faktor karcinogeneze kod mnogih karcinoma. Reaktivne kiseonične vrste imaju svoj uticaj, kako na patološke, tako i na fiziološke procese. Kada je u pitanju oksidativni stres, literaturni podaci ukazuju na njegov značaj u patogenezi ovarijalnog karcinoma kroz sledeća četiri aspekta ćelijskog funkcionisanja: (i) genetske alteracije, (ii) signalni putevi, (iii) uticaj na transkripcione faktore, kao i na (iv) tumorsko mikro-okruženje. Reaktivne kiseonične vrste mogu da dovedu do nastanka mutacija, što prouzrokuje inicijalnu genezu, rast i progresiju tumora tkiva. Takođe, dugoročno povećana koncentracija slobodnih radikala može imati citotoksični efekat, koji nastaje usled modulacije redoks-zavisnih puteva apoptoze. Pod uticajem slobodnih radikala, dolazi do oštećenja biomolekula i formiranja produkata ove interakcije, što za posledicu ima pojavu ovih jedinjenja u tkivu, plazmi, urinu kao i u mnogim drugim odeljcima u kojima se mogu identifikovati. S obzirom na izrazito nepoznatu etiologiju, kao i heterogenost ovarijalnog karcinoma, a uz do sada sprovedena istraživanja koja su najvećim delom bila fokusirana na operativno ili medikamentozno zbrinjavanje, cilj ovog preglednog rada je da se čitaocima približi značajna uloga oksidativnog stresa u procesu patogeneze ovarijalnog karcinoma.

**Ključne reči:** ovarijalni karcinom, oksidativni stres, patogeneza ovarijalnog karcinoma

## ABSTRACT

Ovarian cancer is the deadliest form of gynecological malignancy. The presence of non-specific symptoms, together with the identification of the disease in an advanced stage, are the primary determinants of an unfavorable prognosis, resulting in an overall five-year survival rate of 45%. Currently, the treatment of choice for ovarian cancer is surgery aimed at completely removing the illness macroscopically, along with adjuvant chemotherapy. The significance of early diagnosis is evident in the several conducted prospective studies, which have not identified a successful method for early identification of this disease. Oxidative stress (OS) is a well-established contributor to carcinogenesis in several types of malignancies. It occurs when there is an imbalance between the production and clearance of reactive oxygen species. These molecules affect both pathological and physiological processes. Literature findings indicate that oxidative stress has a role in the development and pathophysiology of ovarian cancer by affecting the following four areas of cellular functioning: (i) genetic alterations, (ii) signaling pathways, (iii) transcription factors, and (iv) tumor microenvironment. Reactive oxygen species can induce mutations, which initiate the formation, proliferation, and maturation of tumor tissue. Also, prolonged elevation of the concentration of free radicals harmfully affects cells, causing the initiation of apoptotic pathways. Specifically, the presence of free radicals leads to the impairment of biomolecules and the creation of byproducts from this interaction. Consequently, these compounds may be found in various body structures and products such as tissue, plasma, urine, and other, where they can be detected. The study aims to enhance the readers' understanding of the pathogenesis of ovarian cancer, which is characterized by its undetermined cause and diverse nature. Previous research has primarily focused on surgical or medicamentous treatment, therefore the present study aims to shed light on the role of oxidative stress in this process.

**Keywords:** ovarian cancer, oxidative stress, pathogenesis of ovarian cancer

Autor za korespondenciju:
Petar Simić
Ginekološko akušerska klinika „Narodni front", Beograd, Srbija
Kraljice Natalije 62, 11000 Beograd, Srbija
Elektronska adresa: simicp93@gmail.com

*Corresponding author:*
*Petar Simić*
*Obstetrics and Gynecology Clinic Narodni front, Belgrade, Serbia*
*62 Kraljice Natalije Street, 11000 Belgrade, Serbia*
*E-mail:* simicp93@gmail.com

**Primljeno • *Received*:** April 12, 2024; **Revidirano • *Revised*:** April 29, 2024; **Prihvaćeno • *Accepted*:** May 13, 2024; ***Online first*:** June 25, 2024

DOI: 10.5937/smclk5-50413

## UVOD

Ovarijalni karcinom predstavlja najsmrtonosniji ginekološki malignitet. Godišnje, širom sveta, kod 230.000 žena se dijagnostikuje karcinom jajnika, dok njih 150.000 izgubi život usled posledica ove bolesti [1]. Bez obzira na velike napore naučne i akademske zajednice da se razjasne mehanizmi koji su u osnovi ove bolesti, karcinom jajnika predstavlja sedmi najčešće dijagnostikovani karcinom u ženskoj populaciji, dok petogodišnje preživljanje trenutno iznosi tek oko 45% [2]. Kasno otkrivanje bolesti, uz nespecifične tegobe koje se javljaju u uznapredovalim stadijumima bolesti, osnovni su uzrok lošeg ishoda.

Histološki, ovarijalni tumori mogu voditi poreklo od sva tri klicina lista. Na prvom mestu po učestalosti se nalazi epitelni karcinom jajnika, dok ne-epitelni tumori jajnika čine značajno manji procenat ovog oboljenja. Faktori rizika za javljanje ovarijalnog karcinoma uključuju benigna ginekološka stanja poput endometrioze, sindroma policističnih jajnika, te pelvične inflamatorne bolesti, uz nizak paritet, ranu menarhu i kasnu menopauzu. Prvu liniju terapije predstavlja primarna debulking (engl. *debulking*) hirurgija koja za cilj ima postizanje nulte rezidualne bolesti u trbuhu, dok nakon toga uobičajeno sledi sistemska hemioterapija alkilirajućim agensima i preparatima porekla taksana [3].

## EPIDEMIOLOGIJA OVARIJALNOG KARCINOMA

Karcinom ovarijuma spada u grupu retkih bolesti sa incidencijom od 12 slučajeva na 100.000 žena [4]. Epitelni ovarijalni karcinom čini većinu dijagnostikovanih slučajeva u odnosnu na germinativne i tumore porekla gonadalne vrpce, sa učestalošću od oko 90% u populaciji žena obolelih od tumora jajnika. Uobičajeni profil žene obolele od karcinoma jajnika karakteriše starosno doba od 50 godina uz nuliparitet, izostanak perioda laktacije i dugačak period neprekidnih ovulacija. Većina slučajeva ovarijalnog karcinoma se javlja sporadično, dok 10% – 15% slučajeva ima naslednu komponentu [5]. U okviru grupe pacijentkinja obolelih od naslednih formi oboljenja, 65% – 75% slučajeva je prouzrokovano *BRCA 1* i *BRCA 2* genskom mutacijom, uz mali procenat naslednih formi koje su povezane sa Linčovim (engl. *Lynch*) sindromom [6].

## VAŽNOST RANE DIJAGNOSTIKE

S obzirom da se bolest najčešće dijagnostikuje u uznapredovalom stadijumu, sa već udaljenim metastatskim promenama na površini creva, kapsuli jetre i u limfnim čvorovima, uloženo je dosta napora u cilju otkrivanja efikasnih metoda skrininga karcinoma jajnika. Nažalost, pretpostavka da se ranim otkrivanjem bolesti

## INTRODUCTION

Ovarian cancer is the deadliest gynecological malignancy. Worldwide, 230,000 women are diagnosed with ovarian cancer annually, while 150,000 women lose their lives as a result of this disease [1]. Regardless of the great efforts of the scientific and academic community to fully understand the underlying mechanisms of this disease, ovarian cancer is the seventh most frequently diagnosed cancer in the female population, while the five-year survival rate is currently only about 45% [2]. Late detection and non-specific complaints that occur in the advanced stages of the disease are the main cause of an unfavorable outcome.

Histologically, ovarian tumors can originate from all three germ layers. Epithelial ovarian cancer ranks first in terms of frequency, while non-epithelial ovarian tumors make up a significantly smaller percentage of this disease. Risk factors for ovarian cancer include benign gynecological conditions such as endometriosis, polycystic ovary syndrome, and pelvic inflammatory disease, as well as low parity, early menarche, and late menopause. The first-line therapy is primary debulking surgery, which aims to achieve zero residual disease in the abdomen. It is usually followed by systemic chemotherapy composed of alkylating agents and taxane-based preparations [3].

## EPIDEMIOLOGY OF OVARIAN CANCER

Ovarian cancer belongs to the group of rare diseases with an incidence of 12 cases per 100,000 women [4]. Epithelial ovarian cancer makes up most of the diagnosed cases, as compared to germinal and gonadal cord tumors, with a frequency of about 90% in the population of women suffering from ovarian tumors. The characteristics of the usual profile of women suffering from ovarian cancer are the following: 50 years of age, nulliparity, the absence of a lactation period, and a long period of continuous ovulation. Most cases of ovarian cancer occur sporadically, while 10% - 15% of cases have a hereditary component [5]. Within the group of patients suffering from hereditary forms of the disease, 65% - 75% of cases are caused by BRCA 1 and BRCA 2 gene mutations, with a small percentage of hereditary forms associated with Lynch syndrome [6].

## IMPORTANCE OF EARLY DIAGNOSIS

Given that the disease is most often diagnosed in an advanced stage, with already existing distant metastases on the surface of the intestine, the liver capsule, and within the lymph nodes, a lot of effort has been put into discovering effective screening methods for ovarian cancer. Unfortunately, the assumption that early de-

može smanjiti mortalitet i morbiditet, brzo je odbačena, zbog rezultata velike kohortne studije sprovedene u Ujedinjenom Kraljevstvu, u kojoj je preko 200.000 žena randomizovano u tri grupe. Prva grupa je praćena određivanjem serumskog nivoa karcinoembrionalnog antigena 125 (*Ca-125*), druga grupa je praćena putem redovnih godišnjih ultrazvučnih pregleda, dok je treća grupa pasivno praćena. Tokom perioda trajanja studije, zabeleženo je smanjenje mortaliteta od 15% i 11%, za grupe pacijentkinja praćenih uz pomoć algoritma za procenu rizika od karcinoma jajnika (*Risk of ovarian cancer algorithm – ROCA*) i pacijentkinja određenih za redovne ultrazvučne kontrole, u odnosu na populaciju žena koje nisu praćene specifičnim skriningom [7]. Međutim, ovi rezultati nisu bili statistički značajni.

Za razliku od skrininga u populaciji zdravih žena, istraživanje sprovedeno u Americi uključilo je 2,359 žena sa pozitivnom porodičnom anamnezom na karcinom dojke ili jajnika ili postojanjem *BRCA 1* i *BRCA 2* genske mutacije. Osnovni cilj ove studije bilo je određivanje pozitivne prediktivne vrednosti i specifičnosti testova radi prevencije ovarijalnog karcinoma. Pacijentkinje su praćene devet godina uz pomoć *ROCA* testa. Primenom ovog testa, u ispitivanoj kohorti je otkriveno 18 od ukupno 19 slučajeva karcinoma jajnika, pri čemu je izmerena specifičnost bila 90% u opštoj populaciji, odnosno 92% u populaciji pacijentkinja visokog rizika [8]. Trenutno, jedina preporuka Evropskog udruženja ginekoloških onkologa (engl. *European Society of Gynaecological Oncology – ESGO*) za sprečavanje pojave tumora i za ranu detekciju bolesti predstavlja primena profilaktičkog ili rizik-redukujućeg uklanjanja jajovoda obostrano, u skladu sa hipotezom da se epitelni ovarijalni karcinom prenosi sa fimbrijalnog dela tuba na površinu jajnika i potom širi dalje po trbušnoj duplji.

## PATOGENEZA OVARIJALNOG KARCINOMA

Dualistički model epitelne ovarijalne karcinogeneze, koji je zasnovan na molekularno-genetičkoj osnovi, za razliku od uobičajene morfološke, sada već beleži dve decenije od kada je uspostavljen [9]. Pokušaj da se napravi određeno grupisanje, u inače ekstremno heterogenoj grupi tumora, već dugo predstavlja veliki naučni izazov. Štaviše, u literaturi su opisani brojni pokušaji klasifikacije tumora jajnika na osnovu strukturnog, patohistološkog, potom molekularnog, genetskog, a zatim i antigenskog sastava. Ova dualistička klasifikacija opisuje dve populacije tumora. Karakteristike karcinoma tipa I su: unilateralna pojava, cističnost, velike dimenzije i histološki opis tumora niskog gradusa. Sa druge strane, karcinomi tipa II se uobičajeno prezentuju u kasnom stadijumu, čine preko 75% slučajeva, histološki ih karakteriše visoki gradus kao i teška klinička slika bolesti [10].

tection can reduce mortality and morbidity was quickly refuted by a large cohort study conducted in the United Kingdom, wherein over 200,000 women were randomized into three groups. The first group was monitored by determining the serum level of carcinoembryonic antigen 125 (Ca-125); the second group was monitored with regular annual ultrasound examinations, while the third group was monitored passively. Throughout the study, a reduction in mortality by 15% and 11% was recorded for the groups of patients monitored with the Risk of Ovarian Cancer Algorithm (ROCA) and for patients designated for regular ultrasound follow-ups, as compared to the population of women who were not monitored with any specific screening [7]. However, these results were not statistically significant.

As opposed to screening performed in the population of healthy women, a study conducted in America included 2,359 women with a positive family history of breast or ovarian cancer or the existence of BRCA 1 and BRCA 2 gene mutations. The primary aim of this study was to determine the positive predictive value and specificity of the tests for the prevention of ovarian cancer. The patients were followed up for nine years with the help of the ROCA test. Using this test, 18 out of a total of 19 cases of ovarian cancer were detected in the examined cohort, with the measured specificity being 90% in the general population, i.e., 92% in the high-risk patient population [8]. Currently, the only recommendation of the European Society of Gynaecological Oncology (ESGO) for preventing the development of tumors and for early detection of the disease is the application of prophylactic or risk-reducing removal of the fallopian tubes on both sides, in accordance with the hypothesis that epithelial ovarian cancer is transferred from the fimbrial part of the tubes to the surface of the ovary and then spreads further within the abdominal cavity.

## PATHOGENESIS OF OVARIAN CANCER

The dualistic model of epithelial ovarian carcinogenesis, based on a molecular-genetic basis, as opposed to the usual morphological one, has been established for two decades [9]. Attempting to make a more definitive grouping, in an otherwise extremely heterogeneous group of tumors, has long been a major scientific challenge. Moreover, literature describes numerous attempts to classify ovarian tumors based on structural, pathohistological, molecular, genetic, and antigenic composition. This dualistic classification describes two tumor populations. The characteristics of Type I carcinoma are the following: unilateral occurrence, cystic nature, large size, and low-grade histology of the tumor. On the other hand, Type II carcinomas usually present at a late stage, they account for over 75% of cases, and are

Pored razlike u agresivnosti i invazivnosti bolesti, jedna od glavnih karakteristika principa koji stoji iza dualističke podele zasniva se na prisustvu prekursorskih lezija. Naime, kod karcinoma tipa I, prisutna je sekvencijalna kaskada, odnosno pojava *borderline* ili atipičnih promena, slične etiopatogeneze kao kod karcinoma grlića materice ili kolona. Nasuprot tome, tip II karcinoma karakteriše razvoj *de novo* tumorskih promena, prema najnovijim istraživanjima, uobičajeno lociranih na fimbrijalnom delu jajovoda, koji su označeni kao intraepitelne karcinomske lezije [10].

## GENETSKI FAKTORI

Mutacije *KRAS*, *BRAF* i *ERBB2* gena imaju za posledicu aktivaciju MAP kinaznog signalnog puta (mitogenom-aktivirana protein kinaza); (engl. *mitogen-activated protein kinase*) [11]. MAP kinazni put igra ključnu ulogu u prenosu signala indukovanih faktorima rasta, što posledično dovodi do neoplastične transformacije. Mutacije *KRAS* i *ERBB2* dešavaju se relativno rano u onkogenezi, i upravo one dovode do ushodne regulacije glukoznog transportera, sa posledičnim povećanjem intenziteta metabolizma glukoze, što je neophodno za malignu transformaciju [12].

Promenom tehnika resekcije i patohistološkog pregleda preparata jajovoda kod pacijentkinja sa *BRCA* mutacijom, uočeno je prisustvo prekursorskih lezija seroznog epitelnog karcinoma jajnika visokog gradusa. Za razliku od karcinoma tipa I, tumore visokog malignog potencijala karakteriše mutacija na *TP53*, *BRCA* i *PTEN* onkogenima, uz rasprostranjene strukturne genomske varijacije [13].

## HORMONSKI FAKTORI

Jajnici su primarni reproduktivni organi u kojima se odvija produkcija estrogena, progesterona i testosterona [14]. Estrogeni utiču na brojne fiziološke i patofiziološke procese. Postoje klasični (*Era, Erβ*) i neklasični (estrogenski receptor udružen sa G proteinom 1; engl. *G protein-coupled estrogen receptor – GPER1*) estrogenski receptori [15]. Uprkos uobičajenom uverenju da hormoni koji vode poreklo od holesterola, usled svoje liposolubilnosti, lako prodiru kroz ćelijsku membranu i imaju receptore u citoplazmi, mnogobrojna istraživanja pokazala su prisustvo receptora za estrogen udruženog sa G proteinom na površini ćelijske membrane. Nakon vezivanja estradiola za *GPER1* receptor, pokreće se nishodna signalna kaskada koja dovodi do aktivacije *PI3K/Akt* i *ERK/MAPK* signalnih puteva, uključenih u proces onkogeneze [16]. Jan i saradnici su, u istraživanju sprovedenom 2005. godine, pokazali da izlaganje selektivnim agonistima *GPER1* receptora dovodi do modulacije i proliferacije ćelijskih linija putem produkcije i aktivacije matriksne metaloproteinaze 9 (MMP-9) [17].

histologically characterized by a high grade as well as a severe clinical presentation of the disease [10].

In addition to the difference in the aggressiveness and invasiveness of the disease, one of the main characteristics of the principle behind the dualistic categorization of the tumors is based on the presence of precursor lesions. Namely, in Type I carcinoma, there is a sequential cascade, i.e., the development of borderline or atypical lesions, which are of similar etiopathogenesis as in cervical or colon cancer. In contrast, Type II carcinoma is characterized by the development of *de novo* tumor lesions, which are, according to the latest research, usually located on the fimbrial part of the fallopian tube, designated as intraepithelial carcinoma lesions [10].

## GENETIC FACTORS

Mutations in the KRAS, BRAF, and ERBB2 genes result in the activation of the MAP kinase signaling pathway (mitogen-activated protein kinase) [11]. The MAP kinase pathway plays a key role in transmitting signals induced by growth factors, which consequently leads to neoplastic transformation. KRAS and ERBB2 mutations occur relatively early in oncogenesis, and they lead to the upregulation of the glucose transporter, with a consequent increase in the intensity of the glucose metabolism, which is necessary for malignant transformation [12].

By changing resection techniques and the techniques of the pathohistological examination of fallopian tube preparations in patients with the BRCA mutation, the presence of precursor lesions of high-grade serous epithelial ovarian cancer has been observed. In contrast to Type I cancers, tumors of high malignant potential are characterized by mutations in the TP53, BRCA, and PTEN oncogenes, with widespread structural genomic variations [13].

## HORMONAL FACTORS

The ovaries are the primary reproductive organs wherein estrogen, progesterone, and testosterone production occurs [14]. Estrogens affect numerous physiological and pathophysiological processes. There are classical (Erα, Erβ) and non-classical (G protein-coupled estrogen receptor – GPER1) estrogen receptors [15]. Despite the common belief that cholesterol-derived hormones, due to their liposolubility, easily penetrate the cell membrane and have receptors in the cytoplasm, numerous studies have shown the presence of a G protein-coupled estrogen receptor on the surface of the cell membrane. After the binding of estradiol to the GPER1 receptor, a downstream signaling cascade is triggered that leads

## FAKTORI SREDINE

Supstance koje su prisutne u životnoj sredini kao posledica zagađenja, ili se nalaze u sastavu različitih kozmetičkih ili prehrambenih aditiva, mogu da utiču na hormonski zavisna tkiva, zbog strukturne i funkcionalne sličnosti sa prirodnim estrogenima. Ove supstance se nazivaju ksenoestrogeni i mogu se direktno ili indirektno vezati za estrogenske receptore. Brojna istraživanja sprovedena na različitim ćelijskim linijama opisuju različiti uticaj sredinskih faktora na ćelije karcinoma jajnika. Genistein pripada grupi izoflavonoida, koji vrše aktivaciju signalnih puteva povezanih sa ER receptorima [18]. Jedinjenja poput fitoestrogena dovode do izmenjene modulacije, aktivacije i ekspresije *Erβ* receptora, koja rezultira izmenjenim odgovorom *PI3K/AKT* signalnog puta [19]. Pokazano je i da ubikvitarno prisutno jedinjenja bisfenol A, koje se oslobađa prilikom zagrevanja plastične ambalaže, indukuje proliferaciju ovarijalnih karcinomskih ćelijskih linija pomoću regulacije *Era* signalnog puta [20].

## OKSIDATIVNI STRES

Ključna karakteristika slobodnih radikala je postojanje nesparenih elektrona u molekulima, atomima i jonima, usled čega su oni veoma reaktivni. Prisustvo nesparenog elektrona je dokazao još Fenton davne 1894. godine, opisujući reakciju pri kojoj dolazi do oslobađanja manje količine ljubičaste svetlosti u prisustvu jona gvožđa [21]. Reaktivne kiseonične vrste (superoksidni anjon, vodonik peroksid i hidroksi radikal) imaju svoj uticaj kako na fiziološke, tako i na patološke procese. Još 2007. godine, pokazano je da redoks-zavisni signalni putevi utiču na aktivnost transkripcionih faktora i na taj način menjaju odgovor ćelija. Pokazano je da kada na ćeliju deluje odgovarajući stimulus dolazi do stvaranja male količine vodonik peroksida, koji zatim modulira aktivnost pojedinih signalnih puteva, a što ukazuje da reaktivne kiseonične vrste, pored svoje osnovne uloge, imaju i ulogu sekundarnih glasnika u ćeliji [22]. Naime, slobodni radikali i vodonik peroksid mogu dovesti do aktivacije tirozin kinaza i nishodne stimulacije MAP (engl. *mitogen activated protein kinase*) kinaza i fosfolipaze C. Prema jednoj teoriji, kao posledica aktivacije i produkcije veće količine slobodnih radikala u unutrašnjosti ćelije, dolazi do aktivacije transkripcionih faktora koji mogu biti značajni za proces onkogeneze.

## DEFINICIJA OKSIDATIVNOG STRESA I NJEGOV UTICAJ NA ORGANIZAM

Oksidativni stres se definiše kao poremećaj ravnoteže između produkcije slobodnih radikala i njihove eliminacije od strane protektivnih mehanizama, odnosno

to the activation of PI3K/Akt and ERK/MAPK signaling pathways, involved in the process of oncogenesis [16]. Yan et al., in a 2005 study, showed that exposure to selective agonists of GPER1 receptors leads to the modulation and proliferation of cell lines through the production and activation of matrix metalloproteinase 9 (MMP-9) [17].

## ENVIRONMENTAL FACTORS

Substances present in the environment as the result of pollution, or those that make up various cosmetic or food additives, can affect hormone-dependent tissues due to their structural and functional similarity to natural estrogens. These substances are called xenoestrogens and can bind directly or indirectly to estrogen receptors. Numerous studies conducted on different cell lines describe the different effects of environmental factors on ovarian cancer cells. Genistein belongs to the group of isoflavonoids, which activate signaling pathways associated with ER receptors [18]. Compounds such as phytoestrogens lead to the altered modulation, activation, and expression of the Erβ receptor, which results in an altered response of the PI3K/AKT signaling pathway [19]. It has also been shown that the ubiquitous compound bisphenol A, which is released when plastic packaging is heated, induces the proliferation of ovarian cancer cell lines by regulating the Erα signaling pathway [20].

## OXIDATIVE STRESS

A key characteristic of free radicals is the existence of unpaired electrons in molecules, atoms, and ions, which makes them highly reactive. The presence of an unpaired electron was proved by Fenton, as early as 1894, when he described a reaction wherein a small amount of violet light is released in the presence of iron ions [21]. Reactive oxygen species (superoxide anion, hydrogen peroxide, and the hydroxyl radical) influence both physiological and pathological processes. As early as 2007, it was shown that redox-dependent signaling pathways influence the activity of transcription factors and thereby alter the cellular response. It has been shown that when a suitable stimulus acts on the cell, a small amount of hydrogen peroxide is created, which then modulates the activity of certain signaling pathways, thus indicating that reactive oxygen species, in addition to their basic role, also play the role of secondary messengers in the cell [22]. Namely, free radicals and hydrogen peroxide can lead to the activation of tyrosine kinases and the subsequent stimulation of MAP (mitogen-activated protein kinase) kinases and phospholipase C. According to one theory, as a consequence of the activation and production of a

antioksidanasa. Ovaj disbalans dovodi do pojave oštećenja na biomolekulima i ćelijama, sa potencijalnim štetnim efektom na ceo organizam [23]. Atomi, molekuli ili joni sa jednim ili više nesparenih elektrona u svojoj strukturi jako su nestabilni i veoma reaktivni, zbog čega mogu biti prouzrokovači lančane reakcije nastanka novih slobodnih radikala u organizmu. Nestabilnost ovih jedinjenja proizilazi iz potrebe da se nespareni elektron, odnosno elektroni spare, pri čemu se elektroni „ukradu" od drugih jedinjenja, stvarajući tako nove radikale i oštećujući različite ćelijske strukture.

Prema svom poreklu, slobodni radikali u organizmu se mogu podeliti u dve velike grupe. Prvoj grupi pripadaju reaktivne kiseonične vrste nastale na mitohondrijalnom respiratornom lancu, dok drugoj grupi pripadaju radikali koji potiču iz metabolizma ksenobiotika. Tokom endogenih aerobnih metaboličkih reakcija, u mitohondrijama dolazi do produkcije reaktivnih vrsta kiseonika (engl. *reactive oxygen species – ROS*), kao što su superoksid anjon i hidroksil radikal, koji predstavljaju proizvode parcijalne redukcije molekulskog kiseonika [24].

Brojni pozitivni efekti malih koncentracija *ROS*-a omogućavaju pravilno funkcionisanje organizma. Međutim, postoji posebno interesovanje naučne zajednice za povezanost oksidativnog stresa sa inflamacijom, što predstavlja jedan od glavnih mehanizama nastanka mnogih hroničnih bolesti. Premda se ranije na oksidativni stres gledalo isključivo kao na patološki proces, danas je perspektiva promenjena ka otkrivanju sve većeg broja fizioloških uloga slobodnih radikala.

Antioksidativni mehanizmi zaštite organizma od oksidativnog stresa mogu biti endogeni i egzogeni. Endogeni sistemi antioksidativne zaštite mogu biti enzimski i neenzimski. Endogeni enzimski sistem antioksidativne zaštite proizvode ćelije organizma i njihova uloga je odbrana od neželjenih dejstava primarnih parcijalnih produkata redukcije kiseonika (superoksidnog anjona i vodonik peroksida), a obuhvata antioksidantne enzime superoksid dismutazu, katalazu i glutation peroksidazu. Glavni endogeni neenzimski antioksidans je glutation, unutarćelijsko tiol jedinjenje koje sprečava oksidaciju tiol grupa enzima i proteina.

## OKSIDATIVNI STRES KAO FAKTOR RIZIKA ZA OVARIJALNI KARCINOM

Uloga oksidativnog stresa u patogenezi ovarijalnog karcinoma je dualne prirodne. Reaktivne vrste kiseonika mogu da dovedu do nastanka mutacija, što prouzrokuje inicijaciju, rast i progresiju tumora. Dugoročno povećana koncentracija slobodnih radikala ima citotoksični efekat, koji dovodi do aktivacije puteva apoptoze [25]. Usled visokog stepena aktivnosti i proliferacija

larger quantity of free radicals inside the cell, activation of transcription factors occurs, which can be significant for the process of oncogenesis.

## DEFINITION OF OXIDATIVE STRESS AND ITS EFFECT ON THE BODY

Oxidative stress is defined as a disruption of the balance between the production of free radicals and their elimination by protective mechanisms, i.e. antioxidants. This imbalance leads to the damage of biomolecules and cells, with a potentially harmful effect on the whole body [23]. Atoms, molecules, or ions with one or more unpaired electrons in their structure are very unstable and very reactive, which is why they can cause a chain reaction of new free radicals in the body. The instability of these compounds stems from the need for the unpaired electron or electrons to become paired, whereby electrons are "stolen" from other compounds, thus creating new radicals and damaging various cell structures.

According to their origin, free radicals in the body can be divided into two large groups. The first group includes reactive oxygen species formed on the mitochondrial respiratory chain, while the second group includes radicals originating from the metabolism of xenobiotics. During endogenous aerobic metabolic reactions, reactive oxygen species (ROS) are produced in the mitochondria, such as superoxide anion and hydroxyl radical, which are products of partial reduction of molecular oxygen [24].

Numerous positive effects of small concentrations of ROS enable the proper functioning of the organism. However, the scientific community is especially interested in the connection between oxidative stress and inflammation, which is one of the main mechanisms of many chronic diseases. Although oxidative stress was previously viewed exclusively as a pathological process, today the focus has shifted towards discovering an increasing number of physiological roles of free radicals.

Antioxidative mechanisms of protecting the organism from oxidative stress can be endogenous and exogenous. Endogenous systems of antioxidant protection can be enzymatic and non-enzymatic. The endogenous enzymatic system of antioxidant protection is produced by the cells of the organism and their role is to defend against the unwanted effects of the primary partial products of oxygen reduction (superoxide anion and hydrogen peroxide) and includes the antioxidant enzymes superoxide dismutase, catalase, and glutathione peroxidase. The main endogenous non-enzymatic antioxidant is glutathione, an intracellular thiol compound that prevents oxidation of the thiol groups of enzymes and proteins.

cije, tumorske ćelije moraju da pronađu načine da se izbore sa velikom koncentracijom reaktivnih molekula, odnosno da izbegnu neželjena dejstva reaktivnih kiseoničnih vrsta koja bi dovela do pokretanja apoptoze ili feroptoze [26].

Oksidativni stres učestvuje u etiopatogenezi ovarijalnog karcinoma kroz četiri aspekta: (i) nastanak genetske alteracije, (ii) modifikacija signalnih puteva, (iii) aktivnost transkripcionih faktora, (iv) tumorsko mikrookruženje [27]. Oštećenje DNK lanca i genetska nestabilnost koja nastaje kao posledica oksidativnog stresa igraju ključnu ulogu u nastanku i razvoju ovarijalnog karcinoma. Najčešći produkt oksidativnog oštećenja DNK je 8-hidroksi-2-deoksiguanozin (8-OHdG), koji nastaje oksidacijom guanina [28]. Naime, interakcijom hidroksilnog radikala sa nukleobazama DNK lanca, dolazi do formiranja oštećenja koja dovode do pojave promutagenih lezija, koje doprinose procesu karcinogeneze, što je potvrđeno kod pacijenata sa tumorima mokraćne bešike, bubrega i drugim tumorima [29,30]. Pored toga, Valavanidis i saradnici su pokazali prististvo 8-OHdG u urinu kao mogući marker oksidativnog oštećenja DNK kod pacijentikanja sa ovarijalnim karcinomom [31].

## UTICAJ OKSIDATIVNOG STRESA NA PROLIFERACIJU, INVAZIJU I METASTAZIRANJE OVARIJALNOG KARCINOMA

Mitohondrijalna DNK (mtDNK) je posebno značajna u procesu oksidativnog stresa. Štaviše, istraživanja su pokazala da prisustvo slobodnih elektrona koji vode poreklo iz respiratornog lanca, kao i izostanak uobičajenih mehanizama za popravku DNK lanca čini mtDNK posebno osetljivom na oksidativni stres. Ni i saradnici su pokazali da serozni ovarijalni karcinom visokog gradusa sa mutacijama na mtDNK ima veću učestalost rezistencije na platinu kao i incidenciju relapsa bolesti u odnosu na grupu pacijentkinja bez somatskih mutacija na mtDNK [32]. Analizom isečaka tumorskog tkiva i ispitivanjem somatskih mutacija nastalih kao posledica oksidativnog oštećenja, može se dobiti slika o izloženosti tumorskih ćelija oksidativnom stresu. Tumorsko mikrookruženje predstavlja prostor gde tumorske ćelije interaguju sa ćelijama domaćina, odnosno ćelijama tkivne strome, uključujući fibroblaste, endotelne ćelije i različite ćelije porekla imunskog sistema, a oksidativni stres na njega deluje posredstvom neutrofila i makrofaga povezanih sa tumorom, T regulatornih ćelija, kao i mnogih drugih komponenti vanćelijskog matriksa [27,33].

Ascites, odnosno prisustvo slobodne tečnosti u peritonealnoj duplji, predstavlja ključni faktor tumorskog mikrookruženja kod ovarijalnog karcinoma. Putem tečnosti, dolazi do karakterističnog širenja bolesti po

## OXIDATIVE STRESS AS A RISK FACTOR FOR OVARIAN CANCER

The role of oxidative stress in the pathogenesis of ovarian cancer is dualistic in nature. Reactive oxygen species can lead to mutations that cause tumor initiation, growth, and progression. Long-term increased concentration of free radicals has a cytotoxic effect, which leads to the activation of apoptosis pathways [25]. Due to the high degree of activity and proliferation, tumor cells have to find ways to deal with the high concentration of reactive molecules, i.e., to avoid the unwanted effects of reactive oxygen species that would lead to the initiation of apoptosis or ferroptosis [26].

Oxidative stress participates in the etiopathogenesis of ovarian cancer through four aspects: (i) development of a genetic alteration, (ii) modification of signaling pathways, (iii) activity of transcription factors, (iv) tumor microenvironment [27]. DNA strand damage and genetic instability that occur as the result of oxidative stress play a key role in the onset and development of ovarian cancer. The most common product of oxidative DNA damage is 8-hydroxy-2-deoxyguanosine (8-OHdG), which is formed by the oxidation of guanine [28]. Namely, the interaction of the hydroxyl radical with the nucleobases of the DNA chain leads to damage that causes the formation of promutagenic lesions, which contribute to the process of carcinogenesis, confirmed in patients with bladder, kidney, and other tumors [29,30]. In addition, Valavanidis et al. showed the presence of 8-OHdG in urine as a possible marker of oxidative DNA damage in patients with ovarian cancer [31].

## EFFECT OF OXIDATIVE STRESS ON PROLIFERATION, INVASION, AND METASTASIS OF OVARIAN CARCINOMA

Mitochondrial DNA (mtDNA) is particularly important in the process of oxidative stress. Furthermore, research has shown that the presence of free electrons originating from the respiratory chain (electron transport chain), as well as the absence of the usual DNA chain repair mechanisms, makes mtDNA particularly sensitive to oxidative stress. Ni et al. showed that high-grade serous ovarian cancer with mtDNA mutations has a higher frequency of platinum resistance as well as a higher incidence of disease relapse, as compared to the group of patients without somatic mtDNA mutations [32]. By analyzing tumor tissue sections and examining somatic mutations resulting from oxidative damage, insight into the exposure of tumor cells to oxidative stress can be obtained. The tumor microenvironment is a space where tumor cells interact with

trbuhu u smeru suprotnom kretanju kazaljki na satu. Naime, Pakula i saradnici su pokazali da maligni ascites porekla seroznog ovarijalnog tumora dovodi do oksidativnog stresa u peritonealnim mezotelnim ćelijama, kroz indukciju citohrom c oksidaze i *NADPH* dehidrogenaze, što rezultuje starenjem i inaktivacijom ovih ćelija. Gubitkom ovih ćelija dolazi do pojave priraslica, proliferacije i generalne migracije ćelija ovarijalnog karcinoma u trbuhu [34].

## OKSIDATIVNI STRES I TERAPIJSKE IMPLIKACIJE

Kao što je ranije napomenuto, uloga oksidativnog stresa kod ovarijalnog karcinoma je dualne prirode. S jedne strane se oksidativni stres, zbog promutagenog potencijala i mogućnosti nastanka oksidativnog oštećenja makromolekula, dovodi u vezu sa procesom kancerogeneze, što ukazuje i da povećanje antioksidativnog kapaciteta i smanjenje oksidativnog stresa mogu uticati na prevenciju ovarijalnog karcinoma. S druge strane, terapijski pristup kod pacijentkinja koje su obolele od ovarijalnog karcinoma usmeren je, pre svega, na pojačanu produkciju slobodnih radikala i povećani oksidativni stres u cilju indukovanja apoptoze u ćelijama tumora, a u cilju prevencije širenja i progresije bolesti u trbuhu.

Upotreba hemioterapijskih lekova zasnovanih na promeni redoks balansa tumorskih ćelija nije nov koncept [27]. Poznato je da pripadnici ove grupe lekova utiču na povećanje intenziteta oksidativnog stresa i inflamacije i dovode do indukcije apoptoze u tumorskim ćelijama. Osnovni terapijski izbor prilikom lečenja ovarijalnog karcinoma jeste kombinovana upotreba preparata platine i taksana. Za platinu je poznato da indukuje produkciju reaktivnih vrsta kiseonika, dok taksani imaju manji uticaj na produkciju ovih jedinjenja [35].

Lekovi poznati pod nazivom PARP (poli-ADP-riboza polimeraza) inhibitori utiču na mogućnost tumorske ćelije da popravi oštećenja nastala na svom DNK lancu. Primena lekova iz ove grupe za sada je ograničena samo na pacijentkinje sa *BRCA* mutacijama. Pored svog osnovnog mehanizma inhibiranja enzima za popravku DNK lanca, ovi lekovi dovode i do ushodne regulacije *NADPH* oksidaze 1 i 4, što dovodi do antitumorskog efekata povećanjem oksidativnog stresa u ćelijama karcinoma [36].

Poznato je da se u komercijalne svrhe na tržištu nalazi mnogo preparata sa manje ili više dokazanim antioksidantnim efektima. Brojni prirodni ekstrakti biljnog porekla se koriste radi povećanja antioksidantnog kapaciteta. Pokazano je da *Ganoderma lucidum* dovodi do indukcije antioksidantnih enzima superoksid dizmutaze, katalaze i glutation S-trasferaze P1 posredstvom NRF-2 signalnog puta, a u cilju prevencije promutagenih efekata oksidativnog stresa [27].

host cells, i.e., cells of the tissue stroma, including fibroblasts, endothelial cells, and various cells originating from the immune system. Oxidative stress acts on this microenvironment through tumor-associated neutrophils and macrophages, T regulatory cells, as well as many other components of the extracellular matrix [27,33].

Ascites, i.e. the presence of free fluid in the peritoneal cavity, is a key factor in the tumor microenvironment in ovarian cancer. Via the liquid, the disease characteristically spreads throughout the abdomen, counterclockwise. Namely, Pakula et al. have shown that malignant ascites originating from a serous ovarian tumor leads to oxidative stress in peritoneal mesothelial cells, through the induction of cytochrome c oxidase and NADPH dehydrogenase, resulting in the aging and inactivation of these cells. The loss of these cells leads to the development of adhesions, as well as to the proliferation and general migration of ovarian cancer cells in the abdomen [34].

## OXIDATIVE STRESS AND TREATMENT IMPLICATIONS

As noted earlier, the role of oxidative stress in ovarian cancer is dualistic in its nature. On the one hand, oxidative stress, due to its promutagenic potential and the possibility of oxidative damage to macromolecules, is linked to the process of carcinogenesis, which indicates that an increase in antioxidant capacity and a decrease in oxidative stress can affect the prevention of ovarian cancer. On the other hand, the treatment approach in patients suffering from ovarian cancer is aimed primarily at increased production of free radicals and increased oxidative stress, for the purpose of inducing apoptosis in tumor cells and preventing the spread and progression of the disease in the abdomen.

The use of chemotherapy drugs whose action is based on changing the redox balance of tumor cells is not a new concept [27]. Medications belonging to this group of drugs are known to increase the intensity of oxidative stress and inflammation and lead to the induction of apoptosis in tumor cells. The treatment of choice for ovarian cancer is the combined use of platinum preparations and taxanes. Platinum is known to induce the production of reactive oxygen species, while taxanes have a lesser effect on the production of these compounds [35].

Drugs known as PARP (poly-ADP-ribose polymerase) inhibitors affect the ability of the tumor cell to repair damage to its DNA strand. The use of this group of drugs is currently limited only to patients with BRCA mutations. In addition to their basic mechanism of inhibiting DNA strand repair enzymes, these drugs also

Ovarijalni karcinom i dalje predstavlja veliki izazov na svim poljima, kako prevencije i dijagnostike, tako i terapije. Ulažu se veliki napori radi otkrivanja novih pristupa u lečenju ove teške bolesti. Naime, sprovedena su istraživanja o ulozi nanopartikula kao terapijskog modaliteta kod karcinoma ovarijuma, pre svega zbog malih dimenzija i uniforme distribucije. Bai i saradnici su opisali povezanost između primene cink-oksid nanočestica i procesa apoptoze i autofagije u ćelijskoj liniji karcinoma ovarijuma. Veličina čestica je u proseku bila 20 nm, što je omogućilo efikasno prodiranje i ostvarivanje značajnog stepena citotoksičnosti putem dramatičnog smanjenja količine intracelularnog glutationa sa posledičnim smanjenjem antioksidantnog potencijala, što je za posledicu imalo značajno povećanje osetljivosti ovih ćelija na oksidativni stres [37].

Moguće je pretpostaviti da u patogenezi ovarijalnog karcinoma postoji presudan momenat, nakon čega dolazi do sloma mehanizama zaštite od promocije tumora. Smatra se da oksidativni stres ima veoma značajnu ulogu u različitim mehanizmima koji tom momentu doprinose, uključujući nastanak mutacija na DNK, hipometilaciju određenih regiona DNK, somatske mutacije mtDNK, kao i promene u aktivnosti velikog broja redoks-zavisnih signalnih puteva.

## BIOMARKERI OKSIDATIVNOG STRESA U OVARIJALNOM KARCINOMU

Biomarkeri su određene karakteristike koje se mere kao pokazatelji fizioloških i patoloških procesa ili odgovora na izloženost ili intervenciju, uključujući i odgovor na terapiju. Biomarkeri mogu obuhvatati molekularne, histološke, radiografske ili fiziološke pokazatelje, ali ne obuhvataju procenu subjektivnog osećaja pacijenta. Većina biomarkera koji se dovode u vezu sa oksidativnim stresom ispunjava osnovni kriterijum, odnosno da se mogu izmeriti. Rezultati brojnih istraživanja su doveli u vezu oksidativni stres sa velikim brojem oboljenja kod ljudi. Međutim, da bi određena jedinjenja bila klasifikovana kao klinički relevantni biomarkeri, neophodno je da ispune određene kriterijume kao što su: (i) prisustvo prognostičke vrednosti, (ii) jasna korelacija sa progresijom bolesti i (iii) specifičnost, odnosno dijagnostička vrednost za određenu bolest [38].

*Đang* i saradnici su sproveli istraživanje o razvoju sistema bodovanja za model rizika zasnovanog na nivou ekspresije gena povezanih sa oksidativnim stresom. Naime, podaci dobijeni iz baze sekvencionirane jednoćelijske RNK, korišćeni su za formiranje 12 klastera koji su potom evaluirani regresionom analizom. Od inicijalnih 151 gena, analizom je detektovano devet prognostičkih genskih markera, na osnovu kojih su konstruisani modeli bodovanja rizika. Pokazano je da lead to the up-regulation of NADPH oxidase 1 and 4, leading to antitumor effects by increasing oxidative stress in cancer cells [36].

It is known that there are many preparations on the market with more or less proven antioxidant effects. Numerous natural plant extracts are used to increase antioxidant capacity. It has been shown that *Ganoderma lucidum* leads to the induction of antioxidant enzymes superoxide dismutase, catalase, and glutathione S-transferase P1 via the NRF-2 signaling pathway, preventing the promutagenic effects of oxidative stress [27].

Ovarian cancer still represents a great challenge in all respects – prevention, diagnosis, and therapy. Great efforts are being made to discover new approaches to the treatment of this serious disease. Namely, research was conducted regarding the role of nanoparticles as a therapeutic modality in ovarian cancer, primarily due to their small dimensions and uniform distribution. Bai et al. described the association between the application of zinc oxide nanoparticles and the process of apoptosis and autophagy in an ovarian cancer cell line. The size of the particles was 20 nm on average, which enabled efficient penetration and a significant degree of cytotoxicity through a dramatic decrease in the amount of intracellular glutathione with a consequent decrease in antioxidant potential, which resulted in a significant increase in the sensitivity of these cells to oxidative stress [37].

It is possible to assume that there is a decisive moment in the pathogenesis of ovarian cancer, after which the mechanisms of protection against tumor promotion break down. Oxidative stress is considered to play a very significant role in various mechanisms that contribute to that moment, including the occurrence of DNA mutations, hypomethylation of certain DNA regions, somatic mutations of mtDNA, as well as changes in the activity of a large number of redox-dependent signaling pathways.

## BIOMARKERS OF OXIDATIVE STRESS IN OVARIAN CANCER

Biomarkers are certain characteristics that are measured as indicators of physiological and pathological processes or responses to exposure or a procedure, including response to treatment. Biomarkers may include molecular, histological, radiographic, or physiological indicators, but do not include an assessment of the patient's subjective symptoms. Most of the biomarkers associated with oxidative stress meet the basic criterion of being measurable. The results of numerous studies have linked oxidative stress to a large number of human diseases. However, for certain compounds to be classi-

vrednost skora u okviru ovog modela rizika predviđa ishod primene imunoterapijskih lekova, kao i da može da predstavlja nezavisni prognostički faktor za ovarijalni karcinom [39].

U poslednje vreme, analiza gena koji se dovode u vezu sa oksidativnim stresom veoma privlači pažnju naučne zajednice. Sve više resursa se ulaže u analizu i evaluaciju velikih baza podataka, u pokušaju da se dobiju odgovori na brojna pitanja koja se tiču ovarijalnog karcinoma. Jedno takvo istraživanje su sproveli Liu i saradnici, koji su, analizom *Omnibus* genske baze podataka identifikovali 34 gena koji se dovode u vezu sa prognozom ovarijalnog karcinoma, a povezani su sa oksidativnim stresom. U cilju procene rizika, generisan je skor u koji je uključeno 14 gena i pokazano je da pacijentkinje sa niskim skorom mogu imati bolji odgovor na terapiju u odnosu na grupu pacijentkinja sa visokim skorom [40].

Poznato je da pod uticajem slobodnih radikala dolazi do oksidativnog oštećenja biomolekula i stvaranja pokazatelja oksidativnog oštećenja, što za posledicu može imati pojavu ovih jedinjenja u tkivu, plazmi, urinu, kao i u drugim telesnim tečnostima, u kojima se mogu identifikovati i određivati.

Lipidna peroksidacija predstavlja proces u kome hidroksil radikal reaguje sa lipidima, pri čemu nastaju lipidni radikal i voda. Proizvodi lipidne peroksidacije se detektuju u krvnoj plazmi i koriste se kao biomarkeri oksidativnog stresa. Najčešće se određuje koncentracija malondialdehida, markera oksidativnog oštećenja polinezasićenih masnih kiselina.

I oksidovani lipoproteini male gustine (*LDL*) se kao biomarkeri oksidativnog stresa pojavljuju u literaturi još od postavljanja inicijalne hipoteze o značaju oksidativnog stresa u nastanku ateroskleroze. Naime, oksidovana forma *LDL*-a (*oxLDL*), kao potencijalni biomarker za kardiovaskularne bolesti, analizirana je u brojnim istraživanjima [41]. Pokazano je da *oxLDL* najverovatnije ne može da predstavlja specifičnu meru stepena oksidativnog stresa, usled limitacije eseja koji se koriste u određivanju koncentracije ovog markera [42].

Stepen oštećenja DNK lanaca usled prisustva slobodnih radikala je povezan sa efikasnošću mehanizama popravke DNK. U uslovima kada je koncentracija slobodnih radikala niska, dolazi do potpune reparacije struktura DNK, što omogućava pravilno funkcionisanje ćelije. Međutim, u stanju redoks disbalansa nastaju različite lezije DNK lanca, od jednostrukih ili dvostrukih prekida do pojave tzv. oksidativno generisanih klasterizovanih lezija DNK lanca [43]. Kao što je već pomenuto, jedan od osnovnih biomarkera oksidativnog oštećenja DNK lanca je 8-hidroksi-2-deoksiguanozin (8-OHdG). Jelić i saradnici su ispitivali postojanje veze

fied as clinically relevant biomarkers, they must meet certain criteria such as: (i) prognostic value, (ii) clear correlation with disease progression, and (iii) specificity, i.e. diagnostic value for a specific disease [38].

Zheng et al. conducted research on the development of a scoring system for a risk model based on the expression level of genes associated with oxidative stress. Namely, the data obtained from the database on sequenced single-cell RNA were used to form 12 clusters, which were then evaluated by regression analysis. Among the initial 151 genes, the analysis detected nine prognostic gene markers, based on which risk scoring models were constructed. It was shown that the value of the score within this risk model predicted the outcome of the application of immunotherapy drugs, as well as that it could represent an independent prognostic factor for ovarian cancer [39].

Recently, the analysis of genes associated with oxidative stress has attracted considerable attention from the scientific community. More and more resources are being invested in the analysis and evaluation of large databases, in an attempt to obtain answers to the many questions regarding ovarian cancer. One such study was conducted by Liu et al., who analyzed the *Omnibus* gene database and identified 34 genes associated with the prognosis of ovarian cancer and oxidative stress. To assess the risk, a score was generated wherein 14 genes were included, showing that patients with a low score could have a better response to therapy, as compared to the group of patients who had a high score [40].

It is known that under the influence of free radicals, oxidative damage to biomolecules occurs and indicators of oxidative damage develop, which can result in the appearance of these compounds in tissue, plasma, urine, and other body fluids, where they can be identified and measured.

Lipid peroxidation is a process wherein the hydroxyl radical reacts with lipids, resulting in a lipid radical and water. Lipid peroxidation products are detected in blood plasma and used as oxidative stress biomarkers. Most commonly, the concentration of malondialdehyde, a marker of oxidative damage to polyunsaturated fatty acids, is measured.

Oxidized low-density lipoproteins (LDL) have also been identified in literature as biomarkers of oxidative stress, ever since the initial hypothesis about the importance of oxidative stress in the development of atherosclerosis was put forward. Namely, the oxidized form of LDL (oxLDL), as a potential biomarker for cardiovascular diseases, has been analyzed in numerous studies [41]. It has been shown that oxLDL most likely cannot represent a specific measure of the degree of oxidative stress,

između oksidativnog stresa i cervikalnog karcinoma. U istraživanju koje je uključivalo 153 pacijentkinje, određivali su aktivnost antioksidantnih enzima, kao i koncentraciju 8-OHdG. Pokazano je da su pacijentkinje u uznapredovalom stadijumu bolesti imale statistički značajno veću aktivnost katalaze, superoksid-dismutaze i glutation-S-transferaze, kao i da su bili prisutni viši nivoi 8-OHdG u urinu [44].

U Finskoj je grupa autora, 2015. godine, sprovela retrospektivno istraživanje u kom su analizirane serumske vrednosti 8-OHdG kod pacijentkinja obolelih od karcinoma jajnika. Istraživanje je sprovedeno na 112 ispitanica i pokazano je da su pacijentkinje sa višim nivoima 8-OHdG imale značajno kraće ukupno preživljavanje, kao i kraći period bez bolesti uz veću učestalost rezistencije na platinu, kao i suboptimalno hirurško lečenje [45]. Međutim, grupa autora iz Japana je analizirala nivo 8-OHdG i aktivnost hem oksigenaze-1, kao i ukupni antioksidantni kapacitet u grupi pacijentkinja obolelih od benigne endometrioze i endometrioze povezane sa ovarijalnim karcinomom. Ovo istraživanje je pokazalo da su vrednosti 8-OHdG bile statistički značajno niže u grupi pacijentkinja sa ovarijalnim karcinomom u odnosu na grupu sa benignom endometriozom, što ukazuje na postojanje kontradiktornosti po pitanju uloge 8-OHdG u karcinogenezi ovarijalnog karcinoma [46].

Većina procesa modifikacije proteina izazvanih oksidativnim stresom zasniva se na karbonilaciji, glikaciji i stvaranju krajnjih proizvoda uznapredovale glikozilacije (engl. *advanced glycositaion produsts – AGEs*). Oksidativna deaminacija lizina i glutaminske kiseline može da ima za posledicu formiranje proteinskih karbonilnih grupa. S obzirom da karbonilne grupe mogu nastati putem različitih mehanizama, njihova koncentracija je obično viša u odnosu na druge biomarkere oksidativnog stresa [47].

Iako u literaturi ne postoje direktni podaci o prognostičkom značaju produkata oksidacije proteina kod ovarijalnog karcinoma, postoje rezultati istraživanja za druge karcinome. Naime, Gil i saradnici su pokazali značajno više nivoe proteinskih karbonilnih grupa kod pacijenata sa hepatocelularnim karcinom u odnosu na grupu pacijenata sa hepatitisom B [48]. Takođe, grupa naučnika je, 2004. godine, analizirala povezanost nivoa androgena kod pacijentkinja obolelih od sindroma policističnih jajnika (engl. *polycystic ovary syndrome – PCOS*) sa količinom *AGEs*. Pokazano je da su nivoi androgena bili značajno veći u grupi pacijentkinja sa povišenim vrednostima *AGEs* [49]. Pored toga, Mažibrada i saradnici su ispitivali vrednosti visoko senzitivnog C reaktivnog proteina i fibrinogena u grupi normoinsulinemičnih, negojaznih adolescentkinja sa *PCOS*-om.

due to the limitations of the assays used in determining the concentration of this marker [42].

The degree of damage to DNA strands due to the presence of free radicals is related to the efficiency of DNA repair mechanisms. In conditions when the concentration of free radicals is low, there is a complete repair of DNA structures, which enables the proper functioning of the cell. However, in a state of redox imbalance, various lesions of the DNA chain occur, ranging from single-strand or double-strand breaks to the so-called oxidatively generated clustered DNA strand lesions [43]. As previously mentioned, one of the basic biomarkers of oxidative DNA strand damage is 8-hydroxy-2-deoxyguanosine (8-OHdG). Jelić et al. investigated the existence of a link between oxidative stress and cervical cancer. In a study including 153 female patients, they investigated the activity of antioxidant enzymes, as well as the concentration of 8-OHdG. It was shown that patients in the advanced stage of the disease had a statistically significantly higher activity of catalase, superoxide dismutase, and glutathione-S-transferase, as well as higher levels of 8-OHdG in the urine [44].

In Finland, in 2015, a group of authors conducted a retrospective study wherein serum 8-OHdG levels were analyzed in patients with ovarian cancer. The study was conducted on 112 subjects and showed that patients with higher 8-OHdG levels had significantly shorter overall survival, as well as a shorter disease-free period with a higher frequency of platinum resistance, as well as suboptimal surgical treatment [45]. However, a group of authors from Japan analyzed the level of 8-OHdG and heme oxygenase-1 activity, as well as total antioxidant capacity in a group of patients with benign endometriosis and endometriosis associated with ovarian cancer. This study showed that the values of 8-OHdG were statistically significantly lower in the group of patients with ovarian cancer, as compared to the group with benign endometriosis, which indicates contradictions regarding the role of 8-OHdG in the carcinogenesis of ovarian cancer [46].

Most of the protein modification processes caused by oxidative stress are based on carbonylation, glycation, and the production of advanced glycosylation products (AGEs). Oxidative deamination of lysine and glutamic acid can result in the formation of protein carbonyl groups. Since carbonyl groups can be formed through different mechanisms, their concentration is usually higher, as compared with other biomarkers of oxidative stress [47].

Although there are no direct data in literature on the prognostic significance of protein oxidation products in ovarian cancer, there are research results for other

Istraživanje je pokazalo da inflamatorni markeri mogu biti korisni u praćenju adolescentkinja normalne telesne težine sa *PCOS*-om u nastojanju da se na vreme spreče nepovoljne promene telesne težine i lipidnog profila, što može korelirati sa rezultatima prethodno navedenih istraživanja [50].

## ZAKLJUČAK

S obzirom na izraziti stepen heterogenosti ovarijalnog karcinoma, kako iz genetske i molekularne perspektive, tako i iz ugla kliničkog i terapijskog ponašanja ovih tumora, prisutno je i dalje dosta kontroverzi u pogledu patogeneze. Između ostalog, oksidativni stres može da započne kaskadu događaja zasnovanu na oštećenju DNK lanca, promenama tumorskog mikrookruženja, kao i na redoks-zavisnim signalnim putevima i sekundarnim glasnicima unutar ćelije, koji mogu rezultirati malignom alteracijom tkiva jajnika. Trenutno dostupni vidovi prevencije ovarijalnog karcinoma zasnivaju se na redovnom praćenju žena koje su pod povećanim rizikom za nastanak ove bolesti, uz eventualno profilaktičko operativno lečenje. Sve ove činjenice su podstrek za buduća istraživanja uloge oksidativnog stresa u ovarijalnom karcinomu, kroz dalje pojedinačno razjašnjavanje brojnih komponenti ove kompleksne bolesti.

**Sukob interesa:** Nije prijavljen.

cancers. Namely, Gil et al. reported significantly higher levels of protein carbonyl groups in patients with hepatocellular carcinoma, as compared to the group of patients with hepatitis B [48]. Also, in 2004, a group of researchers analyzed the relationship between androgen levels in patients with polycystic ovary syndrome (PCOS) and the levels of AGEs. It was shown that androgen levels were significantly higher in the group of female patients with elevated AGEs [49]. In addition, Mažibrada et al. examined the levels of high-sensitivity C-reactive protein and fibrinogen in a group of normoinsulinemic, non-obese adolescent girls with PCOS. Research has shown that inflammatory markers can be useful in the follow-up of adolescent girls of normal body weight with PCOS, for the purpose of the timely prevention of unfavorable changes in body weight and the lipid profile, which may be in correlation with the results of previously mentioned studies [50].

## CONCLUSION

Given the distinct degree of heterogeneity of ovarian cancer, both from a genetic and molecular perspective, as well as from the point of view of clinical presentation and response to treatment of these tumors, there is still a lot of controversy regarding pathogenesis. Among other things, oxidative stress can initiate a cascade of events based on DNA strand damage, changes in the tumor microenvironment, as well as on redox-dependent signaling pathways, and intracellular second messengers, which can result in malignant alteration of ovarian tissue. The currently available methods of ovarian cancer prevention are based on regular follow-up of women who are at increased risk of developing this disease, with possible prophylactic surgical treatment. All these facts are an incentive for future research into the role of oxidative stress in ovarian cancer, through further individual clarification of the numerous components of this complex disease.

**Conflict of interest:** None declared.

## LITERATURA / *REFERENCES*

1. Doherty JA, Peres LC, Wang C, Way GP, Greene CS, Schildkraut JM. Challenges and Opportunities in Studying the Epidemiology of Ovarian Cancer Subtypes. Curr Epidemiol Rep. 2017 Sep;4(3):211-220. doi: 10.1007/s40471-017-0115-y.
2. Reid BM, Permuth JB, Sellers TA. Epidemiology of ovarian cancer: a review. Cancer Biol Med. 2017 Feb;14(1):9-32. doi: 10.20892/j.issn.2095-3941.2016.0084.
3. Harter P, Hilpert F, Mahner S, Kommoss S, Heitz F, du Bois A. Role of cytoreductive surgery in recurrent ovarian cancer. Expert Rev Anticancer Ther. 2009 Jul;9(7):917-22. doi: 10.1586/era.09.49.
4. Howlader N, Morton LM, Feuer EJ, Besson C, Engels EA. Contributions of Subtypes of Non-Hodgkin Lymphoma to Mortality Trends. Cancer Epidemiol Biomarkers Prev. 2016 Jan;25(1):174-9. doi: 10.1158/1055-9965.EPI-15-0921.
5. Lheureux S, Gourley C, Vergote I, Oza AM. Epithelial ovarian cancer. Lancet. 2019 Mar 23;393(10177):1240-1253. doi: 10.1016/S0140-6736(18)32552-2.
6. Bewtra C, Watson P, Conway T, Read-Hippee C, Lynch HT. Hereditary ovarian cancer: a clinicopathological study. Int J Gynecol Pathol. 1992 Jul;11(3):180-7. doi: 10.1097/00004347-199207000-00003.
7. Jacobs IJ, Menon U, Ryan A, Gentry-Maharaj A, Burnell M, Kalsi JK, et al. Ovarian cancer screening and mortality in the UK Collaborative Trial of Ovarian Cancer Screening (UKCTOCS): a randomised controlled trial. Lancet Lond Engl. 2016 Mar 5;387(10022):945–56. doi: 10.1016/S0140-6736(15)01224-6.

8. Skates SJ, Greene MH, Buys SS, Mai PL, Brown P, Piedmonte M, et al. Early Detection of Ovarian Cancer using the Risk of Ovarian Cancer Algorithm with Frequent CA125 Testing in Women at Increased Familial Risk – Combined Results from Two Screening Trials. Clin Cancer Res. 2017 Jul 15;23(14):3628–37. doi: 10.1158/1078-0432.CCR-15-2750.
9. Shih IeM, Kurman RJ. Ovarian tumorigenesis: a proposed model based on morphological and molecular genetic analysis. Am J Pathol. 2004 May;164(5):1511-8. doi: 10.1016/s0002-9440(10)63708-x.
10. Kurman RJ, Shih IeM. The Dualistic Model of Ovarian Carcinogenesis: Revisited, Revised, and Expanded. Am J Pathol. 2016 Apr;186(4):733-47. doi: 10.1016/j.ajpath.2015.11.011.
11. Singer CA, Baker KJ, McCaffrey A, AuCoin DP, Dechert MA, Gerthoffer WT. p38 MAPK and NF-kappaB mediate COX-2 expression in human airway myocytes. Am J Physiol Lung Cell Mol Physiol. 2003 Nov;285(5):L1087-98. doi: 10.1152/ajplung.00409.2002.
12. Sheu JJC, Lee CC, Hua CH, Li CI, Lai MT, Lee SC, et al. LRIG1 modulates aggressiveness of head and neck cancers by regulating EGFR-MAPK-SPHK1 signaling and extracellular matrix remodeling. Oncogene. 2014 Mar 13;33(11):1375–84. doi: 10.1038/onc.2013.98.
13. Vang R, Levine DA, Soslow RA, Zaloudek C, Shih IM, Kurman RJ. Molecular Alterations of TP53 are a Defining Feature of Ovarian High-Grade Serous Carcinoma: A Rereview of Cases Lacking TP53 Mutations in The Cancer Genome Atlas Ovarian Study. Int J Gynecol Pathol Off J Int Soc Gynecol Pathol. 2016 Jan;35(1):48–55. doi: 10.1097/PGP.0000000000000207.
14. Boon WC, Chow JD, Simpson ER. The multiple roles of estrogens and the enzyme aromatase. Prog Brain Res. 2010;181:209-32. doi: 10.1016/S0079-6123(08)81012-6.
15. Chen P, Li B, Ou-Yang L. Role of estrogen receptors in health and disease. Front Endocrinol (Lausanne). 2022 Aug 18;13:839005. doi: 10.3389/fendo.2022.839005.
16. Revankar CM, Cimino DF, Sklar LA, Arterburn JB, Prossnitz ER. A transmembrane intracellular estrogen receptor mediates rapid cell signaling. Science. 2005 Mar 11;307(5715):1625-30. doi: 10.1126/science.1106943.
17. Yan Y, Liu H, Wen H, Jiang X, Cao X, Zhang G, et al. The novel estrogen receptor GPER regulates the migration and invasion of ovarian cancer cells. Mol Cell Biochem. 2013 Jun;378(1-2):1-7. doi: 10.1007/s11010-013-1579-9.
18. Goh YX, Jalil J, Lam KW, Husain K, Premakumar CM. Genistein: A Review on its Anti-Inflammatory Properties. Front Pharmacol. 2022 Jan 24;13:820969. doi: 10.3389/fphar.2022.820969.
19. Chan N, Toppmeyer DL. The Final Verdict: Chemotherapy Benefits Estrogen Receptor-Negative Isolated Local Recurrence. J Clin Oncol. 2018 Apr 10;36(11):1058-1059. doi: 10.1200/JCO.2017.77.4877.
20. Sang C, Song Y, Jin TW, Zhang S, Fu L, Zhao Y, et al. Bisphenol A induces ovarian cancer cell proliferation and metastasis through estrogen receptor-α pathways. Environ Sci Pollut Res Int. 2021 Jul;28(27):36060–8. doi: 10.1007/s11356-021-13267-0.
21. Di Meo S, Venditti P. Evolution of the Knowledge of Free Radicals and Other Oxidants. Oxid Med Cell Longev. 2020;2020:9829176. doi: 10.1155/2020/9829176.
22. Kamata H, Hirata H. Redox regulation of cellular signalling. Cell Signal. 1999 Jan;11(1):1-14. doi: 10.1016/s0898-6568(98)00037-0.
23. Jabs T. Reactive oxygen intermediates as mediators of programmed cell death in plants and animals. Biochem Pharmacol. 1999 Feb 1;57(3):231–45. doi: 10.1016/s0006-2952(98)00227-5.
24. Fridovich I. Superoxide radicals, superoxide dismutases and the aerobic lifestyle. Photochem Photobiol. 1978 Oct-Nov;28(4-5):733-41. doi: 10.1111/j.1751-1097.1978.tb07009.x.
25. Reuter S, Gupta SC, Chaturvedi MM, Aggarwal BB. Oxidative stress, inflammation, and cancer: how are they linked? Free Radic Biol Med. 2010 Dec 1;49(11):1603–16. doi: 10.1016/j.freeradbiomed.2010.09.006.
26. Redza-Dutordoir M, Averill-Bates DA. Activation of apoptosis signalling pathways by reactive oxygen species. Biochim Biophys Acta. 2016 Dec;1863(12):2977–92. doi: 10.1016/j.bbamcr.2016.09.012.
27. Ding DN, Xie LZ, Shen Y, Li J, Guo Y, Fu Y, et al. Insights into the Role of Oxidative Stress in Ovarian Cancer. Oxid Med Cell Longev. 2021;2021:8388258. doi: 10.1155/2021/8388258.
28. Ye M, Dewi L, Liao YC, Nicholls A, Huang CY, Kuo CH. DNA oxidation after exercise: a systematic review and meta-analysis. Front Physiol. 2023;14:1275867. doi: 10.3389/fphys.2023.1275867.
29. Djukic T, Simic T, Radic T, Matic M, Pljesa-Ercegovac M, Suvakov S, et al. GSTO1*C/GSTO2*G haplotype is associated with risk of transitional cell carcinoma of urinary bladder. Int Urol Nephrol. 2015 Apr;47(4):625–30. doi: 10.1007/s11255-015-0933-0.
30. Bumbasirevic U, Bojanic N, Pljesa-Ercegovac M, Zivkovic M, Djukic T, Zekovic M, et al. The Polymorphisms of Genes Encoding Catalytic Antioxidant Proteins Modulate the Susceptibility and Progression of Testicular Germ Cell Tumor. Cancers. 2022 Feb 20;14(4):1068. doi: 10.3390/cancers14041068.
31. Valavanidis A, Vlachogianni T, Fiotakis C. 8-hydroxy-2' -deoxyguanosine (8-OHdG): A critical biomarker of oxidative stress and carcinogenesis. J Environ Sci Health Part C Environ Carcinog Ecotoxicol Rev. 2009 Apr;27(2):120–39. doi: 10.1080/10590500902885684.
32. Ni J, Wang J. [Mitochondrial genome variants and neurodegenerative diseases]. Zhonghua Yi Xue Yi Chuan Xue Za Zhi Zhonghua Yixue Yichuanxue Zazhi Chin J Med Genet. 2020 Aug 10;37(8):898–902. doi: 10.3760/cma.j.issn.1003-9406.2020.08.023.
33. Mijatović S, Savić-Radojević A, Plješa-Ercegovac M, Simić T, Nicoletti F, Maksimović-Ivanić D. The Double-Faced Role of Nitric Oxide and Reactive Oxygen Species in Solid Tumors. Antioxid Basel Switz. 2020 Apr 30;9(5):374. doi: 10.3390/antiox9050374.
34. Pakuła M, Mikuła-Pietrasik J, Stryczyński Ł, Uruski P, Szubert S, Moszyński R, et al. Mitochondria-related oxidative stress contributes to ovarian cancer-promoting activity of mesothelial cells subjected to malignant ascites. Int J Biochem Cell Biol. 2018 May;98:82–8. doi: 10.1016/j.biocel.2018.03.011.
35. Yang X, Yu Y, Huang X, Chen Q, Wu H, Wang R, et al. Delivery of platinum (II) drugs with bulky ligands in trans-geometry for overcoming cisplatin drug resistance. Mater Sci Eng C Mater Biol Appl. 2019 Mar;96:96–104. doi: 10.1016/j.msec.2018.10.092.
36. Hou L, Wang K, Zhang C, Sun F, Che Y, Zhao X, et al. Complement receptor 3 mediates NADPH oxidase activation and dopaminergic neurodegeneration through a Src-Erk-dependent pathway. Redox Biol. 2018 Apr;14:250–60. doi: 10.1016/j.redox.2017.09.017.
37. Bai X, Liu F, Liu Y, Li C, Wang S, Zhou H, et al. Toward a systematic exploration of nano-bio interactions. Toxicol Appl Pharmacol. 2017 May 15;323:66–73. doi: 10.1016/j.taap.2017.03.011.
38. Frijhoff J, Winyard PG, Zarkovic N, Davies SS, Stocker R, Cheng D, et al. Clinical Relevance of Biomarkers of Oxidative Stress. Antioxid Redox Signal. 2015 Nov 10;23(14):1144–70. doi: 10.1089/ars.2015.6317.
39. Zheng M, Hu Y, Liu O, Li S, Wang Y, Li X, et al. Oxidative Stress Response Biomarkers of Ovarian Cancer Based on Single-Cell and Bulk RNA Sequencing. Oxid Med Cell Longev. 2023;2023:1261039. doi: 10.1155/2023/1261039.
40. Liu Q, Yang X, Yin Y, Zhang H, Yin F, Guo P, et al. Identifying the Role of Oxidative Stress-Related Genes as Prognostic Biomarkers and Predicting the Response of Immunotherapy and Chemotherapy in Ovarian Cancer. Oxid Med Cell Longev. 2022;2022:6575534. doi: 10.1155/2022/6575534.

41. Stocker R, Keaney JF. Role of oxidative modifications in atherosclerosis. Physiol Rev. 2004 Oct;84(4):1381–478. doi: 10.1152/physrev.00047.2003.
42. Wu D, Zhai Q, Shi X. Alcohol-induced oxidative stress and cell responses. J Gastroenterol Hepatol. 2006 Oct;21 Suppl 3:S26-29. doi: 10.1111/j.1440-1746.2006.04589.x.
43. Kryston TB, Georgiev AB, Pissis P, Georgakilas AG. Role of oxidative stress and DNA damage in human carcinogenesis. Mutat Res. 2011 Jun 3;711(1–2):193–201. doi: 10.1016/j.mrfmmm.2010.12.016.
44. Jelić M, Mandić A, Kladar N, Sudji J, Božin B, Srdjenović B. Lipid Peroxidation, Antioxidative Defense and Level of 8-Hydroxy-2-Deoxyguanosine in Cervical Cancer Patients. J Med Biochem. 2018 Jul 1;37(3):336–45. doi: 10.1515/jomb-2017-0053
45. Pylväs-Eerola M, Karihtala P, Puistola U. Preoperative serum 8-hydroxydeoxyguanosine is associated with chemoresistance and is a powerful prognostic factor in endometrioid-type epithelial ovarian cancer. BMC Cancer. 2015 Jul 2;15:493. doi: 10.1186/s12885-015-1504-6.
46. Fujimoto Y, Imanaka S, Yamada Y, Ogawa K, Ito F, Kawahara N, et al. Comparison of redox parameters in ovarian endometrioma and its malignant transformation. Oncol Lett [Internet]. 2018 Jul 31 [cited 2024 Mar 31]; Available from: http://www.spandidos-publications.com/10.3892/ol.2018.9242
47. Dean RT, Fu S, Stocker R, Davies MJ. Biochemistry and pathology of radical-mediated protein oxidation. Biochem J. 1997 May 15;324 ( Pt 1)(Pt 1):1–18. doi: 10.1042/bj3240001.
48. Gil L, Siems W, Mazurek B, Gross J, Schroeder P, Voss P, et al. Age-associated analysis of oxidative stress parameters in human plasma and erythrocytes. Free Radic Res. 2006 May;40(5):495–505. doi: 10.1080/10715760600592962.
49. Davies SS, Bodine C, Matafonova E, Pantazides BG, Bernoud-Hubac N, Harrison FE, et al. Treatment with a γ-ketoaldehyde scavenger prevents working memory deficits in hApoE4 mice. J Alzheimers Dis JAD. 2011;27(1):49–59. doi: 10.3233/JAD-2011-102118.
50. Mažibrada I, Djukić T, Perović S, Plješa-Ercegovac M, Plavšić L, Bojanin D, et al. The association of hs-CRP and fibrinogen with anthropometric and lipid parameters in non-obese adolescent girls with polycystic ovary syndrome. J Pediatr Endocrinol Metab [Internet]. 2018 Oct 18 [cited 2024 Apr 9];0(0). Available from: https://www.degruyter.com/document/doi/10.1515/jpem-2017-0511/html