Koncentracije malondialdehida i antioksidativnih vitamina iz krvi majke i pupkovine kod majki oboljelih od preeklampsije i zdravih majki

Maternal and cord blood malondialdehyde and antioxidant vitamin levels in normal and preeclamptic women

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Sažetak

Uvod: Cilj nam je bio odrediti odnos između peroksidacije lipoproteina i antioksidacijskog kapaciteta krvi majke i krvi iz pupkovine te ispitati razlikuju li se koncentracije malondialdehida i vitamina E, A i C u plazmi majke i pupkovine između trudnica oboljelih od preeklampsije i zdravih trudnica.

Metode: Usporedili smo oksidacijski i antioksidacijski sustav u krvi majke i krvi iz pupkovine kod dviju skupina majki i njihove novorođenčadi. Njihove su koncentracije malondialdehida (engl. *malondialdehyde*, MDA) i antioksidacijskih vitamina E, A i C u krvi određene i uspoređene, kako bi se procijenilo razlikuje li se prooksidacijski status majke s preeklampsijom od statusa u krvi iz pupkovine kod odgovarajućeg novorođenčeta.

Rezultati: Koncentracija MDA u plazmi majki s preeklampsijom bila je značajno viša ($P < 0,001$) nego kod kontrolne skupine, no njegova je koncentracija bila značajno niža u krvi iz pupkovine kod majki s preeklampsijom. Koncentracije MDA bile su značajno niže ($P < 0,001$) u plazmi iz pupkovine u usporedbi s koncentracijom u plazmi majke. Koncentracija vitamina E bila je niža u plazmi majki s težim oblikom preeklampsije ($P = 0,048$). Koncentracija vitamina A bila je statistički značajno niža ($P = 0,009$) u plazmi majki s preeklampsijom u usporedbi s kontrolnom skupinom. Zanimljivo je da nije bilo statistički značajne razlike u koncentracijama u krvi majke i krvi iz pupkovine kod bolesnica s težim oblikom preeklampsije. Međutim, u plazmi iz pupkovine, izmjerena je znatno niža koncentracija vitamina A nego u plazmi majke. Za razliku od vitamina E i A, koncentracija vitamina C u plazmi krvi iz pupkovine bila je znatno viša kod obiju skupina.

Zaključak: Pretpostavljamo da je antioksidacijski kapacitet krvi iz pupkovine dovoljan te da je placentalna barijera odgovarajuća za zaštitu fetusa od oksidacijskog oštećenja izazvanog oksidacijskim stresom kod majki s preeklampsijom. Stoga zaključujemo da je status oksidacijskog stresa u krvi novorođenčeta nizak u usporedbi s njegovim statusom kod pripadajuće majke s preeklampsijom. Potrebna su daljnja istraživanja kako bi se ispitala strategija održavanja normalne koncentracije antioksidacijskih vitamina radi suzbijanja preeklampsije kod žena koje su u visokorizičnim skupinama.

Ključne riječi: majčina krv; krv iz pupkovine; malondialdehid; antioksidacijski vitamini; trudnoća; preeklampsija

Abstract

Introduction: We aimed to assess the relationship between lipoperoxidation and maternal/cord blood antioxidant capacity and to examine whether the maternal and cord plasma concentrations of malondialdehyde and of vitamins E, A, and C were different between preeclamptic and healthy pregnant women.

Methods: We compared oxidative and anti-oxidative system in maternal and cord blood of two groups of pair-matched mothers and neonates. Their blood malondialdehyde (MDA) and antioxidant vitamins E, A, and C were estimated and compared to evaluate if pro-oxidative status of preeclampsia differs from the status in the cord blood of pair-matched neonate.

Results: MDA content in preeclamptic maternal plasma was significantly higher ($P < 0.001$) compared to control, but its level was significantly lower in the preeclamptic cord. The concentrations of MDA were significantly lower ($P < 0.001$) in the cord plasma of preeclamptics compared to maternal plasma. Vitamin E concentration was lower in severely preeclamptic maternal plasma ($P = 0.048$). Vitamin A concentration was significantly lower ($P = 0.009$) in preeclamptic plasma compared to normotensive group. Interestingly, there was no significant difference between maternal and cord levels in severely preeclamptic patients. However, the cord plasma had significantly lower concentrations of vitamin A compared with those of maternal plasma. Contrary to vitamins E and A, vitamin C in the cord plasma collected from both groups showed significantly higher concentrations.

Conclusions: We hypothesize that antioxidant capacity of the cord blood is sufficient, and placental barrier is adequate, to shield the fetus from oxidative injury due to increased oxidative stress of a preeclamptic mother. Thus, we conclude that the oxidative stress status is low in the blood of neonates compared to its level in the pair-matched preeclamptic mothers. Further studies are needed to explore strategies so that the normal levels of antioxidant vitamins are maintained to combat preeclampsia in women at high risk.

Key words: maternal plasma; cord plasma; malondialdehyde; anti-oxidant vitamins; pregnancy; preeclampsia

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Uvod

Antioksidacijski obrambeni mehanizmi tijela uključuju stanične i izvanstanične enzime te čistače slobodnih radikala (kao što su npr. vitamini C, E i A). Antioksidacijski kapacitet krvi iz pupkovine rezultat je cjelokupnog intrauterinog sustava. Genetička varijabilnost, oksidacijski stres u krvi majke i antioksidacijski kapacitet u krvi majke mogu promijeniti antioksidacijski kapacitet u krvi pupkovine. Nakon rođenja će povišeni oksidacijski stres rezultirati smanjenjem antioksidacijskog kapaciteta. Jednom kada se prekorači antioksidacijski kapacitet, tada se može očekivati povećanje koncentracije oksidacijskih produkata lipida, proteina i nukleinskih kiselina, što rezultira oštećenjem tkiva. Kliničke manifestacije bolesti uzrokovanih radikalima kisika, ovise o ravnoteži između oštećenja tkiva i njegove obnove. Naš je cilj bio utvrditi povezanost između lipidne peroksidacije i antioksidacijskog kapaciteta krvi majke, odnosno krvi iz pupkovine, te ispitati ima li krv iz pupkovine, zahvaćena bolešću koju uzrokuju kisikovi radikali, različit ukupni antioksidacijski kapacitet od krvi bez preeklampsije, poremećaja kojeg karakterizira visoki krvni tlak uzrokovan trudnoćom (≥ 140 mmHg sistolički i / ili ≥ 90 mmHg dijastolički), prva pojava proteinurije (≥ 300 mg proteina/dan) i edem koji se pojavljuje u drugoj polovini trudnoće.

Neki autori izvještavaju o značajno povišenoj koncentraciji malondialdehida (engl. *malondialdehyde*, MDA) u krvi iz pupkovine kod odgovarajuće majke s preeklampsijom (1,2), drugi pak govore o sniženju koncentracije (3) ili promjeni koja nije statistički značajna (4), dok Karabulut i sur. (5) iz svojih rezultata zaključuju da je došlo do povišenja koncentracije MDA i u krvi majke i u krvi iz pupkovine kod majki s preeklampsijom za razliku od zdravih majki. Tijekom normalne trudnoće nekoliko je istraživanja ukazalo na niže koncentracije vitamina A i E u krvi iz pupkovine (6,7), no Kanishtha i sur. (8) izvještavaju o povišenoj koncentraciji vitamina A u plazmi iz pupkovine. Postoje izvješća (9,10) koja bilježe značajno višu koncentraciju vitamina E u krvi iz pupkovine kod majki s preeklampsijom, dok druga (2) izvještavaju o značajno nižoj koncentraciji vitamina E u serumu iz pupkovine u usporedbi s krvi majki s preeklampsijom. Slično tome postoje i proturječnosti oko koncentracije vitamina C u krvi iz pupkovine majki s preeklampsijom. Neki autori (12,13) izvještavaju o povišenoj koncentraciji vitamina C u plazmi majki s preeklampsijom za razliku od niskih koncentracija u krvi iz pupkovine (12,13), dok drugi (14) izvještavaju o nepromijenjenoj koncentraciji vitamina C kod majki s preeklampsijom.

Proturječnosti ovih izvješća navele su nas na ovo istraživanje, kako bismo razumjeli i objasnili povećava li se ili smanjuje ukupni antioksidacijski kapacitet tijekom preeklampsije. Nadalje, slažemo se s Chappellom i suradnicima (15) da postoji potreba za većim, multicentričnim ispitivanjem kako bi se (a) izmjerile koncentracije biljega oksidacijskog

Introduction

Antioxidant defense mechanisms of the body include cellular and extracellular enzymes and free radical quenchers (like vitamins C, E, and A). Cord blood antioxidant capacity is the result of overall intrauterine experience. Genetic variability, maternal oxidative stress, and maternal antioxidant capacity are likely to alter the cord antioxidant capacity. After birth, increased oxidative stress will result in a decreased antioxidant capacity. Once the antioxidant capacity is overwhelmed, then an increase in oxidation products of lipids, protein, and nucleic acids is expected, resulting in tissue damage. Clinical manifestations of oxygen radical diseases depend on a balance between tissue damage and repair. Our aim was to assess the relationship between lipid peroxidation and maternal/cord blood antioxidant capacity and to examine whether the cord blood with oxygen radical disease had different total antioxidant capacity than the one without preeclampsia, a disorder of pregnancy characterized by pregnancy-induced hypertension (≥140 mmHg systolic and / or ≥ 90 mmHg diastolic blood pressure), new-onset proteinuria (≥ 300mg protein/day), and edema occurring in the second half of pregnancy.

Significant elevation of malondialdehyde (MDA) levels in the cord of pair-matched preeclamptic mother has been reported (1,2), whereas some authors observed a decline in its level (3) or no significant change (4). Karabulut *et al.* (5), however, inferred elevation of MDA levels both in the cord and mother during preeclamptic development compared to normal pregnancy. During normal pregnancy, several reports showed lower cord vitamins A, E contents (6,7) but Kanishtha *et al.* (8) reported higher vitamin A level in the cord plasma. There are reports (9,10) showing significantly higher vitamin E level in the cord plasma of the preeclamptic mother, whereas some authors observed (2) significantly lower cord serum vitamin E level as compared to maternal preeclamptics. Similarly, contradictions exist about the level of vitamin C in the cord blood of preeclamptic mothers. Some authors (12,13) reported higher contents of vitamin C in preeclamptic plasma in contrast to lower levels in the cord (11,2), whereas others (14) reported no change in the vitamin C level of a preeclamptic mother.

Inconsistency in these reports has led us to take up the present study in order to understand and elaborate if total antioxidant capacity increases or decreases during preeclampsia. Further, we agree with the advocacy of Chappell *et al.* (15) that there is a need to perform large multicenter trials to (a) measure the levels of oxidative stress markers and antioxidants, (b) administer antioxidant therapy to all women with abnormal levels, and (c) determine whether these levels can be improved with antioxidant therapy. We have recently reported our fin-

stresa i antioksidanata, (b) propisala antioksidacijska terapija svim ženama s patološkim koncentracijama i (c) odredilo mogu li se te koncentracije poboljšati antioksidacijskom terapijom. Nedavno smo objavili naše rezultate na tom polju zajedno s učincima vitamina E i C (16,17).
Cilj ovog istraživanja bio je odrediti povezanost između peroksidacije lipoproteina i antioksidacijskog kapaciteta krvi majke/krvi iz pupkovine te ispitati razlikuju se koncentracije MDA, vitamina E, A i C u majčinoj plazmi i plazmi iz pupkovine između skupine trudnica s preeklampsijom i skupine zdravih trudnica.

Materijali i metode

Ispitanici

U ispitivanje su bile uključene trudnice (N = 21) s normalnim krvnim tlakom i trudnice s teškim oblikom preeklampsije (N = 21), primljene u našu bolnicu, od kojih su neke bile na liječenju, neke nisu, a neke su s uputnicom upućene iz privatne prakse i centara primarne zdravstvene zaštite. Ispitanice obiju skupina nisu dobile nikakve multivitaminske dodatke prehrani. Sve su ispitanice bile unutar raspona od 18-34 godine starosti. Trudnice s preeklampsijom imale su krvni tlak ≥ 160/110, izlučivanje proteina mokraćom > 0,8 g/24 sata, te edem. Istraživanje je provedeno uz prethodno odobrenje lokalnog etičkog odbora. Sve su ispitanice pismenim putem dale svoj informirani pristanak te su im prije samog ispitivanja detaljno objašnjeni ciljevi istraživanja. Kliničkim su pretragama i uvidom u anamnezu ispitanica iz istraživanja isključene one koje puše, boluju od šećerne bolesti, od ishemijske bolesti srca, koje su pretrpile moždani udar, imaju poremećaj rada bubrega ili boluju od bilo kojeg drugog poremećaja poznatih etiologija slobodnih radikala.

Uzorci

Osoblje laboratorija vršilo je uzorkovanje majčine krvi tijekom poroda te krvi iz pupkovine odmah nakon poroda iz umbilikalne vene nakon rezanja pupčane vrpce. Svakoj je ispitanici uzeto 10 mL krvi i stavljeno u epruvete s natrij-heparinatom kao antikoagulansom; uzorci su pohranjeni na 4 °C do obrade. Postupak s uzorcma krvi majke i krvi iz pupkovine bio je identičan, svi su uzorci obrađeni unutar 20 sati od uzorkovanja i uzorci plazme pohranjeni su na -70 °C do određivanja koncentracije vitamina. Prije pohranjivanja svim je uzorcima plazme dodan jednak volumen metafosforne kiseline (10%), kako bi se plazma deproteinizirala i tako stabilizirala koncentracija vitamina C.
Proizvođači za sve potrebne reagense bili su E. Merck (Mumbai, Indija), BDH ili SISCO Chemicals (Mumbai, Indija).

dings on these aspects along with the effects of vitamins E and C (16,17). The aim of the present study was to assess the relationship between lipoperoxidation and maternal/cord blood antioxidant capacity and to examine whether the maternal and cord plasma concentrations of malondialdehyde, vitamins E, A, and C differ between preeclamptic and healthy pregnant women.

Materials and methods

Subjects

The patients in our study included pregnant women (N = 21) with normal blood pressure, severely preeclamptic women (N = 21) admitted to our hospital, who either had or not been under regular care, and those who were referred from private sectors or primary health centers. The subjects of both groups were not administered any multivitamin supplementation. All the participants were within the age range of 18-34 years. The preeclamptic women had the blood pressure ≥ 160/110, with urinary protein excretion over 0.8 g/24 hours and edema. The present study was carried out with the prior approval of the local ethic committee. All the patients mentioned above gave their consent in writing, and the objectives of the study were fully explained to them in detail prior to signing consent. Clinical examination and history taking excluded pregnant women addicted to tobacco smokers, patients with diabetes, ischemic heart disease and a history of stroke, kidney disorders or other conditions of known free radical etiology.

Samples

Blood samples were collected from mothers at delivery. The cord blood was obtained immediately post partum from the umbilical vein after clamping of the cord by labor ward staff. In each case, 10 mL blood were drawn into a sodium heparin vacutainer tube for separating plasma and stored at 4 °C until processed. Maternal and umbilical cord blood samples were handled identically, all samples were processed within 20 hours of sampling, and plasma samples were stored at -70 °C until required for vitamin analyses. Before storage, an equal volume of metaphosphoric acid (10%) was added to plasma samples designated for vitamin C analysis in order to deproteinize the plasma and stabilize vitamin C content.
All the chemicals were of analytical grade from either E. Merck (Mumbai, India), BDH or SISCO Chemicals (Mumbai, India).

Metode

Određivanje lipidne peroksidacije

Kvantifikacija lipidne peroksidacije izvršena je prema metodi Jaina i suradnika (18). Za određivanje koncentracije malondialdehida (MDA) kao reaktivne supstance tiobarbiturne kiseline (TBARS) korišteno je 0,2 mL sedimenta eritrocita. Alikvoti od 0,2 mL dobro su pomiješani s 0,8 mL otopine puferirane fosfatom (pH 7,4) i 25 µL otopine butil-hidroksitoluena. Nakon dodavanja 0,5 mL tridesetpostotne triklorocetne kiseline, uzorci su stavljeni na dva sata u ledenu kupku te su nakon toga 15 minuta centrifugirani na 2000 g i 25 °C. 1 mL supernatanta pomiješano je s 75 µL 0,1 M EDTA i 250 µL jednopostotne tiobarbiturne kiseline u 0,05 M NaOH i ostavljeno 15 minuta iznad kipuće vode. Nakon što se ta smjesa ohladila na sobnu temperaturu, izmjerena je absorbancija na 532 nm. Koncentracija MDA izražena je kao nmol/g Hb**.**
Linearnost postignuta za koncentraciju MDA imala je raspon od 0,2-6 µmol/L, postotak točnosti/iskorištenja bio je 90-95%, koeficijent varijacije (CV) bio je 5% (unutar serije) i 12% (iz dana u dan). Granice detekcije (engl. *limit of detection*, LOD) i granice kvantifikacije (engl. *limit of quantification*, LOQ) bile su 0,05 µmol/L i 0,09 µmol/L.

Određivanje koncentracije vitamina C u plazmi

Koncentracija vitamina C u plazmi izmjerena je metodom prema Jagota i Dani (19). Precipitirano je 0,2 mL plazme 5 minuta na ledu s 0,8 mL trikloroctene kiseline te nakon toga centrifugirano sljedećih 5 minuta na 1000 g. Ukupno je 0,5 mL supernatanta razrijeđeno destiliranom vodom u volumenu 2 mL. Uzorcima je dodano 200 µL reagensa Folin-Ciocalteau razrijeđenog u omjeru 1:10 destiliranom vodom i odmah pomiješano. Nakon 10 minuta spektrofotometrijski je izmjerena absorbancija na 760 nm. Koncentracije uzoraka uspoređene su sa standardnim uzorcima askorbinske kiseline pripremljenim u destiliranoj vodi.
Linearnost određena za koncentracije vitamina C je bila u rasponu od 2-40 mg/mL, postotak točnosti/iskorištenja bio je 93-100%, CV je iznosio 2,5% (unutar serije) i 4,5% (iz dana u dan). LOD je bila 0,5 mg/L i LOQ 1 mg/L.

Određivanje koncentracije vitamina A u plazmi

Koncentracija vitamina A u plazmi odredila se prema postupku Sobela i Snowa (20), 1 mL 95%-tnog etanola dodano je 1 mL plazme i laganim je potresanjem promiješan sadržaj epruvete. Dodano je 2 mL analitičkog reagensa benzina te se sadržaj epruvete miješao 10 minuta. Nakon miješanja sadržaj je centrifugiran oko 30 sekundi. Supernatant benzina usisan je i stavljen u epruvetu. S drugih 2mL benzina i mućkanjem u trajanju od samo 5 minuta ponovljen je postupak ekstrakcije. Ekstrakt je dobiven isparavanjem u kupelji na 40-50 °C i puštanjem struje dušika preko smjese. Kako bi se iz suhog ekstrakta napravi-

Methods

Estimation of lipid peroxidation

Lipid peroxidation was quantified was carried out following the method of Jain *et al*. (18). Packed red cells (0.2 mL) were used for the quantification of malondialdehyde (MDA) as thiobarbituric acid reactive substances (TBARS). Aliquots of 0.2 mL were mixed thoroughly with 0.8 mL of phosphate buffered saline (pH 7.4) and 25 µL of butylated hydroxytoluene solution. After adding 0.5 mL of 30% trichloroacetic acid, the samples were placed on ice-bath for 2 hrs and then centrifuged at 2000 g at 25 ˚C for 15 min. One mL of supernatant was mixed with 75 µL of 0.1 M EDTA and 250 µL of 1% thiobarbituric acid in 0.05 M NaOH and placed on boiling water for 15 min. After cooling to room temperature, absorbance was measured at 532 nm. MDA contents were expressed as nmol/gHb**.**
The linearity established for MDA concentrations ranged from 0.2-6 µmol/L**,** accuracy/recovery percentage was 90-95%, precision coefficient of variation (CV) values were 5% (intraday) and 12% (inter-days). LOD (limit of detection) and LOQ (limit of quantification) were 0.05 µmol/L and 0.09 µmol/L, respectively.

Estimation of plasma vitamin C

Vitamin C concentrations were determined in plasma using the method of Jagota and Dani (19). 0.2 mL of plasma was precipitated on ice with 0.8 mL of trichloroacetic acid for 5 minutes and then centrifuged at 1000 g for 5 min. A total of 0.5 mL of the supernatant was diluted with distilled water to the volume of 2 mL. 200 µL of Folin-Ciocalteau's solution were diluted 1:10 in distilled water and added to the samples which were immediately mixed. After 10 min, the absorbance at 760 nm was measured spectrophotometrically. The sample values were compared with values of standard samples of ascorbic acid prepared in distilled water.
The linearity established for vitamin C concentrations ranged from 2-40 mg/mL, accuracy/recovery percentage was 93-100%, precision CV values were 2.5% (intraday) and 4.5% (inter-days). LOD and LOQ were 0.5 mg/L and 1 mg/L, respectively.

Estimation of plasma vitamin A

This estimation was performed following the procedure of Sobel and Snow (20). 1 mL of 95 per cent ethanol was added to 1 mL of plasma, and the contents of the tube mixed by tapping. 2 mL of analytical reagent petroleum ether was added, and the tube was shaken for 10 min. After shaking, the tube was centrifuged for about 30 seconds. The supernatant petroleum ether was aspirated and placed in a test tube. With another 2 mL of the petroleum ether, and shaking for only 5 min, the extraction procedure was repeated. The extract was evaporated to dryness

la otopina, suhi je ekstrakt otopljen u 1 mL kloroforma. Dodano je 4 mL glicerol-dehidrogenaze (GDH). Otopine s kloroformom i GDH su pomiješane te je nakon 2 minute izmjerena apsorbancija prvo na 550 nm, a kao slijepa proba upotrijebljena je otopina 4 mL GDH i 1 mL kloroforma. Linearnost određena za koncentracije vitamina A bila je u rasponu od 50-1200 µg/L, postotak točnosti/iskorištenja bio je 87-96%, vrijednosti CV bile su 7,5% (unutar serije) i 10,8% (iz dana u dan). LOD je bila 9 µg/L, a LOQ 20 µg/L.

Određivanje koncentracije vitamina E u plazmi

0,8 mL plazme pipetirano je u epruvetu te je dodan jednak volumen pročišćenog apsolutnog etanola za precipitaciju proteina. Sastojci su odmah dobro izmiješani. Nakon toga je u epruvetu dodano 0,8 mL ksilena te je sadržaj promiješan minimalno 30 sekundi i centrifugiran 5-10 minuta na 1000 g. Nakon centrifugiranja gornji je sloj ksilena, koji sadrži ekstrahiran tokoferol, premješten medicinskom kapaljkom u malu epruvetu. Kako bi se spriječilo isparavanje epruvete su prekrivene parafilmom. U epruvetu s 0,2 mL 4,7-difenil-1, 10-fenantrolina (batofenantrolina, BA) dodano je 0,4 mL ekstrakta ksilena iz plazme. Dodano je 0,2 mL željezo (III)-klorida te nakon toga 0,2 mL ortofosforne kiseline. Nakon svakog dodavanja reagensa sadržaj epruvete dobro je izmiješan. Redoslijed dodavanja reagensa izuzetno je važan. Absorbancija je izmjerena spektrometrom na 536 nm nakon prilagodbe instrumenta na nultu absorbanciju slijepom probom (pripremljenom s 0,4 mL ksilena umjesto ekstrakta ksilena iz plazme).
Linearnost određena za koncentracije vitamina E bila je u rasponu od 0,8-20 mg/L, postotak točnosti/iskorištenja bio je 85-90%, vrijednosti CV bile su 6% (unutar serije) i 11% (iz dana u dan). LOD je bila 0,4 mg/L, a LOQ 0,6 mg/L. Koncentracija vitamina E izražena je kao µmol/L (21).

Statistička analiza

Za analizu podataka različitih parametara korišten je programski paket SPSS, verzija 15.0, za operacijski sustav Windows (SPSS Inc., Chicago, IL, SAD). Vrijednosti parametara u obje vrste uzoraka između normotenzivnih trudnica i trudnica sa preeklampsijom uspoređene su t-testom. Vrijednosti parametara u parovima majčine plazme i odgovarajuće krvi iz pupkovine uspoređene su parnim t-testom. Vrijednosti $P \leq 0,05$ smatrane su statistički značajnima. Vrijednosti su izražene kao postotak i srednja vrijednost ± standardna devijacija.

Rezultati

Usporedili smo parametre oksidacijskog i antioksidacijskog sustava u krvi majke i u krvi iz pupkovine kod dviju skupina majki i njihove novorođenčadi. Skupina A je uključivala 21 normotenzivnu trudnicu, bez komplikacija (kontrolna skupina), a skupina B, 21 bolesnu trudnicu s preeklampsijom. Sve su trudnoće bile trudnoće s jednim

by placing the tube in a 40-50 °C water bath and running a stream of nitrogen over it. 1 mL of analytical reagent grade chloroform was added to bring the dried extract into solution. 4 mL of glycerol dichlorohydrin (GDH) was added. The chloroform solution and the GDH were mixed and after 2 min, absorption was measured first at 550 nm against a blank consisting of 4 mL of GDH and 1 mL of chloroform.
The linearity established for vitamin A concentrations ranged from 50–1200 µg/L**,** accuracy/recovery percentage was 87-96%, precision CV values were 7.5% (intraday) and 10.8% (inter-days). LOD and LOQ were 9 µg/L and 20 µg/L, respectively.

Estimation of plasma vitamin E

A volume of 0.8 mL of plasma was pipetted into a test tube and an equal volume of purified absolute ethanol was added to the tube for protein precipitation. The contents were immediately mixed with a vortex mixer. Then, 0.8 mL of xylene was added and the test tube was mixed for at least 30 sec and centrifuged for 5-10 min at 1000 g. After centrifugation, the upper xylene layer, which contained the extracted tocopherol, was collected with a medicinal dropper and transferred to a small tube. The tubes were covered with parafilm to avoid evaporation. Added 0.4 mL of plasma-xylene extract to the test tube containing 0.2 mL of 4, 7-diphenyl-1, 10-phenanthroline (bathophenanthroline, BA). A volume of 0.2 mL ferric chloride was added, followed by 0.2 mL of orthophosphoric acid. The contents of the tube were mixed thoroughly using a vortex mixer after every addition of reagents. The order of reagent addition is critical. Absorbance was read in the spectrophotometer at 536 nm after setting the instrument to zero absorbance with a blank (prepared by using 0.4 mL xylene instead of plasma-xylene extract).
The linearity established for vitamin E concentrations ranged from 0.8–20 mg/L**,** accuracy/recovery percentage was 85-90%, precision CV values were 6% (intraday) and 11% (inter-days). LOD and LOQ were 0.4 mg/L and 0.6 mg/L, respectively. Vitamin E contents were expressed as µmol/L (21).

Statistical analysis

SPSS version 15.0 for Windows (SPSS Inc., Chicago, IL, USA) software package was used to analyze the data of various parameters. The results were statistically analyzed using t-test to compare both maternal/cord blood of normotensive pregnant and preeclamptic patient groups. Sample pairs (maternal plasma and cord blood) in both normotensive and preeclamptic patient groups were compared using paired t-test. The statistical significance was set at $P \leq 0.05$. Values were expressed as percentage and mean ± standard deviation.

Tablica 1. Značajke skupina ispitanika

Table 1. Characteristics of study groups

Parameters	Normotensive control group	Severely preeclamptic group	P*
Number of maternal/neonatal pairs	21	21	/
Maternal age (years)	27 ± 6	29 ± 5	0.584
BMI at delivery (kg/m^2)	21.2 ± 3.2	19.8 ± 3.4	0.177
Gestational age (weeks)	35 ± 3	33 ± 4	0.133
BP at delivery, systolic (mm/Hg)	111.6 ± 12.4	176.8 ± 13.2	< 0.001
BP at delivery, diastolic (mm/Hg)	66.8 ± 12.2	108.3 ± 13.8	< 0.001
Pulse rate (beats/min)	72 ± 3	73 ± 3	0.102
Birth weight (g)	3052 ± 410	1926 ± 378	< 0.001
Proteinuria (g/day)	/	1.32 ± 0.79	/
Edema	/	++ in all cases	

Values are expressed as mean ± SD; BMI- body mass index; BP- blood pressure
* - t-test

plodom. U skupini A bile su tri, a u skupini B četiri operacije carskog reza, a sve zbog produljenog poroda. Značajke obiju skupina prikazane su u Tablici 1.

Srednje vrijednosti parametara u majčinoj krvi i odgovarajućoj krvi iz pupkovine kod normotenzivne skupine majki i skupine majki s preeklampsijom prikazane su u Tablici 2.

Koncentracija MDA u plazmi majki s preeklampsijom je 30,6% viša nego kod kontrolne skupine. Nije nađena statistički značajna razlika između koncentracija u krvi majke i krvi iz pupkovine u kontrolnoj skupini. Koncentracija

Results

We compared the parameters of the oxidative and anti-oxidative system in maternal and cord blood of two groups of pair matched mothers and neonates. Group A consisted of 21 uncomplicated, normotensive- pregnancies (called controls) and group B comprised 21 severely preeclamptic patients, all singleton pregnancies. Group A had three, whereas group B had four caesarian sections because of prolonged labor. The characteristics of these groups are shown in Table 1.

Tablica 2. Koncentracija malonaldehida, vitamina E, A i C u plazmi majke i odgovarajućoj plazmi iz pupkovine kod normotenzivne skupine te skupine s preeklampsijom.

Table 2. Concentrations of malondialdehyde, vitamins E, A, and C in pair-matched normotensive and preeclamptic maternal and cord plasma.

Parameters	Normotensive		Preeclamptic		P*		P**	
	Maternal (N = 21)	Cord (N = 21)	Maternal (N = 21)	Cord (N = 21)	Maternal	Cord	Normotensive	Preeclamptic
MDA (nmol/gHb)	7.08 ± 1.20	7.11± 1.46	9.25 ± 1.19	7.62 ± 0.93	< 0.001	0.129	0.959	< 0.001
Vitamin E (µmol/L)	28.19 ± 4.81	4.76 ± 1.48	25.53 ± 4.81	7.78 ± 3.02	0.048	< 0.001	< 0.001	< 0.001
Vitamin A (µmol/L)	0.86 ± 0.08	0.64 ± 0.19	0.75 ± 0.15	0.65 ± 0.29	0.009	0.945	< 0.001	0.185
Vitamin C (µmol/L)	48.51 ± 12.70	106.49 ± 14.66	34.78 ± 13.53	73.90 ± 15.12	0.004	< 0.001	< 0.001	< 0.001

Values are expressed as mean ± SD; MDA = malondialdehyde.
P* normotensive vs. preeclamptic; t-test, P** maternal vs. cord difference; paired t-test

MDA u krvi majke kod skupine s preeklampsijom bila je statistički značajno viša (P < 0,001) u usporedbi s rezultatima kontrolne skupine. Međutim, koncentracija MDA u plazmi iz pupkovine kod skupine s preeklampsijom u usporedbi s krvi majke bila je statistički značajno niža (P < 0,001), kao što prikazuje Tablica 2.

Koncentracija vitamina E bila je niža u plazmi majki iz skupine s teškim oblikom preeklampsije u usporedbi s normotenzivnom skupinom, te je bila 9,4% niža nego u kontrolnoj skupini. U plazmi iz pupkovine izmjerena koncentracija je bila statistički značajno niža (P < 0,001) kod obje skupine.

Koncentracija vitamina A bila je statistički značajno niža (P = 0,009) u plazmi majke u skupini s preeklampsijom u usporedbi s normotenzivnom skupinom. Zanimljivo je da nije bilo statistički značajne razlike između koncentracija u krvi majke i krvi iz pupkovine kod skupine s teškim oblikom preeklampsije. Međutim, koncentracija vitamina A u plazmi iz pupkovine bila je statistički značajno niža u usporedbi s koncentracijama u plazmi majke. Tablica 2. prikazuje varijacije srednjih vrijednosti za obje skupine.

Suprotno vitaminima A i E, koncentracija vitamina C u plazmi iz pupkovine uzeta od obiju skupina bila je statistički značajno viša (P < 0,001) u usporedbi s odgovarajućom plazmom majke. Njegove su koncentracije bile statistički značajno niže (P = 0,004) u plazmi majke kod skupine s preeklampsijom u usporedbi s normotenzivnom skupinom, no u plazmi iz pupkovine kod skupine s preeklampsijom koncentracija je bila statistički značajno viša (P < 0,001). Koncentracija vitamina C bila je statistički značajno viša (P < 0,001) u krvi iz pupkovine kod normotenzivne skupine nego kod skupine s preeklampsijom.

Rasprava

Kumulativni rezultati iz prošlih godina, zajedno s našim rezultatima, pokazuju da se biokemijska neravnoteža kod preeklampsije događa s povećanim oksidacijskim stresom te nedovoljnom antioksidacijskom zaštitom (16,17,22). Antioksidacijski obrambeni sustav uključuje antioksidanse koji cijepaju lance kao što su vitamin C i vitamin E te antioksidacijske enzime. Antioksidansi topivi u mastima, od kojih je vjerojatno najvažniji vitamin E (23), vežu radikale na membranama i na lipoproteinskim česticama te su ključni u prevenciji lipidne peroksidacije. Antioksidansi topivi u vodi izravno vežu radikale prisutne u vodenom odjeljku. Vitamin C ili askorbat najvažniji je antioksidans topiv u vodi (24). Dobro je poznato da postoji sinergija između vitamina C i E. Ta interakcija između vitamina C i E potvrđena je i *in vivo* (25) u radovima koji izvještavaju da se uzimanjem askorbinske kiseline kao dodatka prehrani kod zdravih odraslih osoba povećavaju koncentracije askorbinske kiseline i α-tokoferola (standardizirano prema lipidima), a da je uzimanje α-tokoferola povezano s povi-

The mean values along with statistical significant differences of the normotensive and preeclamptic pair-matched maternal and cord plasma are shown in Table 2.

The MDA content in preeclamptic maternal plasma was found to be 30.6% higher than that of control. There was no significant difference between control maternal and cord plasma levels. The MDA content in preeclamptic maternal plasma was significantly high (P < 0.001) compared to control. However, its content in preeclamptic cord plasma compared to maternal plasma was significantly low (P < 0.001) as evident from Table 2.

Vitamin E level was low in severely preeclamptic maternal plasma compared to normotensive mother which amounted to be by 9.4% lower than in control. The concentrations were significantly lower (P < 0.001) in both the cord plasma of controls, as well as those from preeclamptics.

Vitamin A amount was significantly low (P = 0.009) in preeclamptic maternal plasma compared to normotensive group. Interestingly, there was no significant difference between maternal and cord levels in severely preeclamptic patients. However, the cord plasma had significantly lower concentrations of vitamin A compared with those of maternal plasma. Table 2 depicts the variation in the mean values of both pair-matched groups.

Contrary to vitamins E and A, the concentrations of vitamin C in the cord plasma collected from both groups showed significantly higher (P < 0.001) levels compared to pair-matched maternal plasma. Its concentrations were significantly low (P = 0.004) in preeclamptic maternal plasma compared with those of normotensive subjects, but in preeclamptic cord plasma the level was significantly (P < 0.001) high. Its level was significantly (P < 0.001) higher in the normotensive cord compared to the preeclamptic cord.

Discussion

Cumulative evidence in recent years that a biochemical imbalance in preeclampsia occurs with an increase of oxidative stress and a deficient antioxidant protection (16,17,22). Antioxidant defense systems include the chain-breaking antioxidants, such as vitamin C and vitamin E, and antioxidant enzymes. Lipid-phase chain-breaking antioxidants, the most important of which is probably vitamin E (23), scavenge radicals in membranes and lipoprotein particles and are central to the prevention of lipid peroxidation. Aqueous-phase chain-breaking antioxidants directly scavenge radicals present in the aqueous compartment. Vitamin C or ascorbate is the most important aqueous phase chain-breaking antioxidant (24). It is well established that there is synergy between vitamins C and E. This interaction between vitamin C and vitamin E has been confirmed *in vivo* by authors (25) who have

šenom koncentracijom askorbinske kiseline u plazmi kao i višom koncentracijom vitamina E.

Naši su rezultati pokazali statistički značajno nižu koncentraciju vitamina E u plazmi iz pupkovine u usporedbi s rezultatima iz odgovarajuće plazme majke kod skupine s preeklampsijom, što se slaže s rezultatima drugih istraživanja (12). Isto se tako naši rezultati, vezani za statistički značajno niže koncentracije vitamina A u plazmi iz pupkovine normotenzivne skupine, slažu s rezultatima drugih istraživanja (6,7,11), no nismo našli značajnu promjenu u skupini s preeklampsijom između plazme majke i plazme iz pupkovine. Zanimljive smo rezultate dobili promatrajući koncentracije vitamina C, koje su bile statistički značajno više u plazmi iz pupkovine kod obje skupine (normotenzivne i skupine s preeklampsijom), što se također slaže s rezultatima drugih istraživanja (6,12). Međutim, statistički značajno niža ($P < 0,001$) koncentracija u skupini majki s preeklampsijom može se objasniti visokom koncentracijom MDA koji je vjerojatno potrošio više vitamina C, što je rezultiralo nižom koncentracijom vitamina C.

MDA, produkt lipidne peroksidacije inducirane reaktivnim kisikovim spojevima (engl. *reactive oxygen species*, ROS), u čvrstoj je uzajamnoj vezi sa stupnjem lipidne peroksidacije (16,26). Naši rezultati pokazali su 30,6% višu koncentraciju MDA kod skupine s preeklampsijom, nego u plazmi majki u normotenzivnoj skupini. Ranije se izvještavalo (27) da posteljica trudnice s preeklampsijom sadrži više MDA nego posteljica zdrave trudnice. Međutim, izmjerili smo 17,6% nižu koncentraciju MDA u plazmi iz pupkovine u usporedbi s odgovarajućom plazmom majki, što bi moglo biti povezano s pojačanim uzimanjem vitamina C. Ovi rezultati koncentracije MDA slažu se s rezultatima iz naših prijašnjih izvješća (16) te izvješća drugih autora (1,2,5).

Kao što je to već prije spomenuto, antioksidacijske aktivnosti plazme progresivno se mijenjaju tijekom trudnoće. Antioksidacijski vitamini svojom sposobnošću stabiliziranja visoko reaktivnih slobodnih radikala djeluju kao prva linija obrane protiv slobodnih radikala i lipidne peroksidacije. Vitamini E (α-tokoferol) i C na različit način doprinose stvaranju antioksidacijskog potencijala; dok je vitamin E veliki antioksidans topiv u lipidima u staničnoj membrani, vitamin C je važan antioksidans topiv u vodi. Antioksidansi mogu djelovati i sinergijski, na primjer kada vitamin C stvara α-tokoferol iz radikala tokoferola (28) taj „žrtveni" antioksidans djeluje više tako da pohrani vitamin E nego da ga samoobnavlja (24). Pretpostavlja se da je važna uloga vitamina C kod preeklampsije u tome da promjene njegove koncentracije mogu utjecati na podložnost vaskularnog endotela toksičnosti kisika (29). Stoga naše istraživanje koncentracije vitamina C može poslužiti kao način procjene ukupnog kapaciteta antioksidansa koji cijepaju lance u sprječavanju lipidne peroksidacije u plazmi i moglo bi u ograničenom opsegu biti važno kod procjene uči-

reported that supplementation of healthy adults with ascorbic acid increases ascorbic acid and lipid-standardized α-tocopherol levels in plasma, and that supplementation with α-tocopherol is associated with increased plasma ascorbic acid concentration, as well as improved vitamin E status.

Our findings showed significantly low vitamin E levels in the cord plasma compared to pair-matched preeclamptic maternal plasma, which is consistent to other reports (12). Similarly, our results on significantly low concentrations of vitamin A in the cord plasma of pair-matched normotensive mother are consistent with other reports (6, 7,11), but no significant change was observed in the case of preeclamptic maternal and cord plasma. Interesting results were observed with the vitamin C levels which were significantly higher in both the cords of normotensive as well as preeclamptic mothers, which is in harmony with other reports (6,12). However, the significantly low ($P < 0.001$) level in pair-matched preeclamptic mother can be explained because of high MDA concentration in preeclamptic mother, which might have consumed more of vitamin C, consequently resulting in the low amount of vitamin C.

MDA, a product of lipid peroxidation induced by ROS, is well correlated with the degree of lipid peroxidation (16,26). Our results show 30.6% higher MDA content in preeclamptics compared to normotensive maternal plasma. Earlier, it has been reported (27) that preeclamptic placenta contains higher MDA than those from normal pregnancies. However, 17.6% lower concentration of MDA in the cord plasma of preeclamptics compared to their pair-matched maternal plasma might be due to increased consumption of vitamin C. Present results on MDA content were consistent with our previous report (16) along with those by other authors (1,2,5).

Plasma antioxidant activities alter progressively throughout pregnancy, as mentioned earlier. Antioxidant vitamins, with the ability to stabilize highly reactive free radicals, act as the first line of defense against free radical attack and lipid peroxidation. Vitamins E (α-tocopherol) and C show differences in the contribution they make to antioxidant potential, as vitamin E is the major lipid soluble chain-breaking antioxidant in cell membranes while vitamin C is an important aqueous phase antioxidant. Antioxidants may act synergistically; for instance, when vitamin C regenerates α-tocopherol from the tocopherol radical (28) this 'sacrificial' antioxidant acts more by sparing vitamin E than by recycling it (24). The important role of vitamin C in preeclampsia suggests that changes in its concentration may influence susceptibility of vascular endothelium to oxygen toxicity (29). Thus, our present study on vitamin C concentration may provide a means of assessing the total capacity of the chain-breaking antioxidants to prevent lipid peroxidation in plasma and it

nkovitosti potencijalnog antioksidacijskog obrambenog sustava.

U našem smo prethodnom istraživanju zaključili da su antioksidacijski dodaci prehrani kod žena s rizikom od preeklampsije povezani s poboljšanom antioksidativne aktivnosti, te da se uzimanjem vitamina C i E mogu kontrolirati neki važni biološki pokazatelji tijekom razvoja preeklampsije (17). Sadašnji se rezultati slažu s rezultatima iz prošlog istraživanja o ulozi vitamina C i E kod teškog oblika preeklampsije. Zaključujemo da postoji neravnoteža između lipidne oksidacije i koncentracije antioksidacijskih vitamina tijekom trudnoće, što je češće kod težeg oblika preeklampsije. Ukupni oksidacijski status u plazmi iz pupkovine kod obiju je skupina gotovo identičan. Stoga je vjerojatnije da će majka s preeklampsijom prije doživjeti oksidacijski stres nego njeno novorođenče. Pretpostavljamo da je antioksidacijski kapacitet krvi iz pupkovine dovoljan i da placentalna barijera pruža odgovarajuću zaštitu fetusa od oksidacijskog oštećenja zbog povećanog oksidacijskog stresa kod majki s preeklampsijom. Stoga zaključujemo da je status oksidacijskog stresa u krvi novorođenčeta nizak u usporedbi sa statusom kod njegove majke s preeklamsijom. Potrebna su daljnja istraživanja kako bi se istražile strategije očuvanja normalnih koncentracija antioksidacijskih vitamina u zaštiti od preeklampsije kod žena s visokim rizikom od te bolesti.

Zahvala

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might be important to evaluate the effectiveness of potential antioxidant defense systems on a limited scale.

In our previous report, we had concluded that antioxidant supplementation in women who were at risk of preeclampsia was associated with improvement in the activity of antioxidants, and use of vitamins C and E showed control of certain important biochemical indices during the development of preeclampsia (17). Further, present results are in harmony with our previous report on the role of vitamins C and E in severe preeclampsia. We infer that there is an imbalance between lipooxidation and antioxidant vitamins levels during pregnancy which is increased in severe preeclampsia, and overall oxidative status in the cord plasma of both pair-matched mothers are nearly the same. Thus, the preeclamptic mother is under oxidative stress rather than the pair-matched neonate. We hypothesize that the antioxidant capacity of the cord blood is sufficient, and placental barrier is adequate, to shield the fetus from oxidative injury due to increased oxidative stress of preeclamptic mother. Thus, we conclude that the oxidative stress status is low in the blood of neonates compared to its level in the pair-matched preeclamptic mothers. Further studies are needed to explore strategies to be applied so that the normal levels of antioxidant vitamins are maintained to combat preeclampsia in women at high risk.

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