

CORRELATION BETWEEN REACTIVE OXYGEN SPECIES AND HOMOCYSTEINE LEVELS IN NORMAL PREGNANCY

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Abstract

The purpose of this study was to determine and to compare the modifications of homocysteine (Hcy), malondialdehyde (MDA) ceruloplasmin and iron concentrations in the second and third trimester of normal pregnancy. The study was performed on 53 women being under surveillance at the Obstetric Gynecology Clinical Hospital Oradea and divided in the following groups: (1) second trimester of pregnancy (n=16), (2) third trimester of pregnancy (n=17), (3) non-pregnant women (n =20). We measured the homocysteine, MDA and the ceruloplasmin levels, in the venous blood samples collected from all the studied groups. Homocysteine concentrations were determined by an enzymatic method, MDA using a method with thiobarbituric acid and ceruloplasmin using the Ravin method. The results were compared with the non-pregnant control group. Supplementary biochemical determinations such as C Reactive Protein (CRP), iron, uric acid, the enzymatic activities of aspartate transaminase (AST) and alanine transaminase (ALT) were also performed. In the studied groups 1 and 2 the concentration of homocysteine was low compared to the control group. Malondialdehyde and ceruloplasmin levels were high compared with those of the non-pregnant women. The conclusions of our research were: the pregnant women in the second and third trimester present a high level of oxidative stress and a low level of homocysteine.

Rezumat

Scopul lucrării a fost determinarea și compararea modificărilor concentrației de homocisteină (Hcy), malondialdehidă (MDA), ceruloplasmină precum și a sideremiei în trimestrul II și III de sarcină. Studiul a cuprins 53 de femei de la Spitalul de Obstetrică Ginecologie Oradea, împărțite în trei loturi: lotul 1- gravide în trimestrul II de sarcină (n=16), lotul 2- gravide în trimestrul II de sarcină (n=17), lotul 3-femei sănătoase non-gestaționale (n=20). La grupurile luate în studiu s-a evaluat concentrația de homocisteină, MDA și ceruloplasmină din ser. Homocisteina a fost determinată printr-o metodă

enzimatică, MDA prin metoda cu acid tiobarbituric, iar ceruloplasmina cu ajutorul metodei Ravin. Rezultatele obținute au fost comparate cu grupul de control. S-au mai dozat suplimentar proteina C reactivă, sideremia, acidul uric, activitățile enzimice ale aspartat transaminazei (AST) și alanin transaminazei (ALT). Concentrația homocisteinei la grupurile 1 și 2 a fost scăzută comparativ cu lotul martor. Concentrațiile serice ale MDA și ceruloplasminei au fost crescute la grupurile studiate comparativ cu lotul de control. Din rezultatele obținute reiese că la gravidele cercetate s-a pus în evidență existența unui stres oxidativ accentuat și o concentrație scăzută de homocisteină.

Keywords: Reactive oxygen species, homocysteine, pregnancy

Introduction

Oxygen is absolutely necessary for the survival of body cells. Under aerobic conditions reactive oxygen species (ROS) are formed permanently. However, reactive oxygen species such as the superoxide radical, the hydroxyl radical and hydrogen peroxide are generated from oxygen also in damaged cells.

The level of serum ROS is controlled by antioxidant enzymes and small-molecule antioxidants [10, 12].

Oxidative stress is an imbalance between the reactive oxygen species production and the level of antioxidant factors [20, 10].

There are strong evidences that hyperhomocysteinemia is associated with the production of ROS in endothelial and smooth muscle cells. The mechanism may be due to either a highly reactive thiol group of homocysteine which is oxidized, or by an excessive formation of intracellular ROS associated with a concomitant inhibition of the synthesis of cellular antioxidant enzymes such as superoxide dismutase and glutathione peroxidase [21].

Homocysteine, an intermediate product of methionine metabolism is removed through two pathways: the re-methylation pathway which regenerates methionine, and the trans-sulfuration pathway which degrades homocysteine into cysteine and then taurine. Folate, vitamin B12, and vitamin B6 are required for homocysteine re-methylation and deficiencies of these vitamins result in an increased total serum homocysteine concentration.

The increased level of the total serum homocysteine is a well known risk factor of vascular disease such as coronary heart disease, stroke, peripheral vascular disease, and atherosclerosis. Elevated concentrations of Hcy are frequently observed in patients with chronic renal disease [2].

The high serum concentration of homocysteine acts on the blood vessel walls, inducing changes in the endothelial cells with endothelial dysfunction, especially within the placental vasculature [11]. These

placental vascular changes are thought to be related to recurrent pregnancy loss, preeclampsia, and placenta abruption.

Normal pregnancy is a physiological condition characterized by an increase in the production of reactive oxygen species [4]. Human placenta exerts a lot of influence upon maternal homeostasis including the involvement in the oxidative stress processes. One of the explanations is the abundance of mitochondria within the placenta and the high value of the oxygen partial pressure in the pregnant women.

The purpose of this study was to examine the relationships between homocysteine, MDA concentrations and ceruloplasmin and the iron status in the last part of physiological pregnancy.

Materials and Methods

We studied 53 women enrolled in the following groups: (1) second trimester of pregnancy (n=16), (2) third trimester of pregnancy (n=17), (3) non-pregnant women (n=20). Written informed consent was obtained from all participants, prior to enrollment and the study was approved by the institutional ethical committee.

The normal pregnant women having the age 18-39 years came to the Obstetric Gynecology Clinical Hospital Oradea for their routine antenatal visit. Diagnosis was made on clinical and ultrasonography examinations. Both systolic and diastolic blood pressures were recorded on two occasions separated by an interval of six hours. Maternal venous samples were collected after overnight fasting in vacutainer tubes. Specimens were transported to the laboratory within 30 min, and centrifuged at 1500g, 4 °C, for 10 min to separate serum, which was stored at -20 °C until analysis.

The results were compared to a control group (group 3) matched with the study groups for age, BMI (body mass index), blood pressure.

We measured serum homocysteine concentrations using an enzymatic method, Axis-Shield Enzymatic Homocysteine Assay Cat No.FHER100, on Hitachi 912, Roche. C Reactive Protein (CRP) was determined by immunoturbidimetry (reagent Cat. No.CPTXL-H00: Diagam, Belgium), on Hitachi 912. The other biochemical parameters (Iron, ALT, AST) were assessed on the same device with Diasys reagents.

For proving the oxidative stress we determined the level of malondialdehyde (MDA) using a method with thiobarbituric acid (TBA) mixed up with serum and heated in a boiling water bath for 30 min. After cooling, the resulting chromogen was extracted with n-butyl alcohol and the absorbance of the extracted and centrifuged liquid was evaluated at the wavelength of 530 nm [18].

As the most important plasmatic antioxidant factor, the level of ceruloplasmin was determined using the Ravin method.

Data were expressed as mean $\pm$ SD (standard deviation). The significance of the results was assessed by the Student's test. A p value < 0.05 was considered statistically significant. The correlation between parameters was performed using Pearson's correlation coefficient (r).

Results and Discussion

There was no significant difference in age, body weight, height and blood pressure between pregnant women and non-pregnant controls at the time of recruitment, as shown in Table I.

Table I
Characteristics of the study and control groups

	Group 1	Group 2	Control group	p*	p**	p***
n	16	17	20			
Age (years)	27.5 $\pm$ 3.8	29.1 $\pm$ 5.3	27.9 $\pm$ 4.8	p>0.05	p>0.05	p>0.05
BMI (body mass index)	24.08 $\pm$ 2.91	28.06 $\pm$ 2.81	27.72 $\pm$ 2.83	p>0.05	p>0.05	p>0.05
Arterial pressure (mmHg)	108.5 $\pm$ 7.1	112.9 $\pm$ 6.8	110.3 $\pm$ 7.2	p>0.05	p>0.05	p>0.05
AST (U/L)	16.61 $\pm$ 2.11	15.17 $\pm$ 4.23	15.88 $\pm$ 3.82	p>0.05	p>0.05	p>0.05
ALT (U/L)	11.11 $\pm$ 2.12	10.72 $\pm$ 2.97	12.18 $\pm$ 2.78	p>0.05	p>0.05	p>0.05
Uric acid (mg/dL)	2.56 $\pm$ 0.38	3.23 $\pm$ 0.64	2.62 $\pm$ 0.62	p>0.05	p>0.05	p>0.05
Iron (μ g/dL)	96.04 $\pm$ 11.67	83.29 $\pm$ 27.59	100.65 $\pm$ 21.71	p>0.05	p>0.05	p>0.05
CRP (mg/L)	3.02 $\pm$ 2.10	2.61 $\pm$ 1.59	1.40 $\pm$ 0.11	p<0.001	p<0.001	p>0.05
Homocysteine (μ mol/L)	4.10 $\pm$ 0.76	4.01 $\pm$ 1.01	7.83 $\pm$ 0.98	p<0.001	p<0.001	p>0.05
Malondialdehyde (nmol/mL)	3.08 $\pm$ 0.61	2.59 $\pm$ 0.71	1.91 $\pm$ 0.33	p<0.001	p<0.001	p>0.05
Ceruloplasmin (mg/dL)	44.77 $\pm$ 2.68	47.82 $\pm$ 6.29	32.11 $\pm$ 1.62	p<0.001	p<0.001	p>0.05

* group 1 compared to control group,** group 2 compared to control group,*** group 1 compared to group 2

Group 1 - second trimester of pregnancy, Group 2 – third trimester of pregnancy, Control group – non-pregnant women

There were no significant differences among the groups regarding AST, ALT, uric acid, iron levels (table I).

Plasma homocysteine concentrations were significantly lower in pregnant women groups compared with non-pregnant controls (p <0.001). No statistic difference was observed in pregnant women in the second trimester compared to pregnant women in the third trimester (p>0.05) (Figure 1).

The mean levels of MDA were significantly higher in pregnant women groups than in control group (p< 0.001). No significant differences

were obtained between the pregnant women in the second trimester and pregnant women in the third trimester ($p>0.05$) (Figure 2).

The concentration of ceruloplasmin in pregnant women in the second and the last trimester of pregnancy is higher than in the reference group ($p<0.001$). There was no significant variation of the serum ceruloplasmin concentration between group 1 and group 2 ($p>0.05$) (see Figure 3).

No correlation between iron and ceruloplasmin values in normal pregnancy was established (Pearson's coefficient of correlation was $r=0.04$). Figure 4 presents the association between the level of CRP and homocysteine $r=-0.40$, $p<0.01$. Figure 5 presents the correlation between homocysteine and MDA $r=0.65$, $p<0.01$. The mean level of CRP is significantly higher in group 1 and group 2 compared to control group ($p<0.001$) and no difference was registered between group 1 and group 2 ($p>0.05$) (Figure 6). The levels of uric acid, iron, AST, ALT are shown in table I.

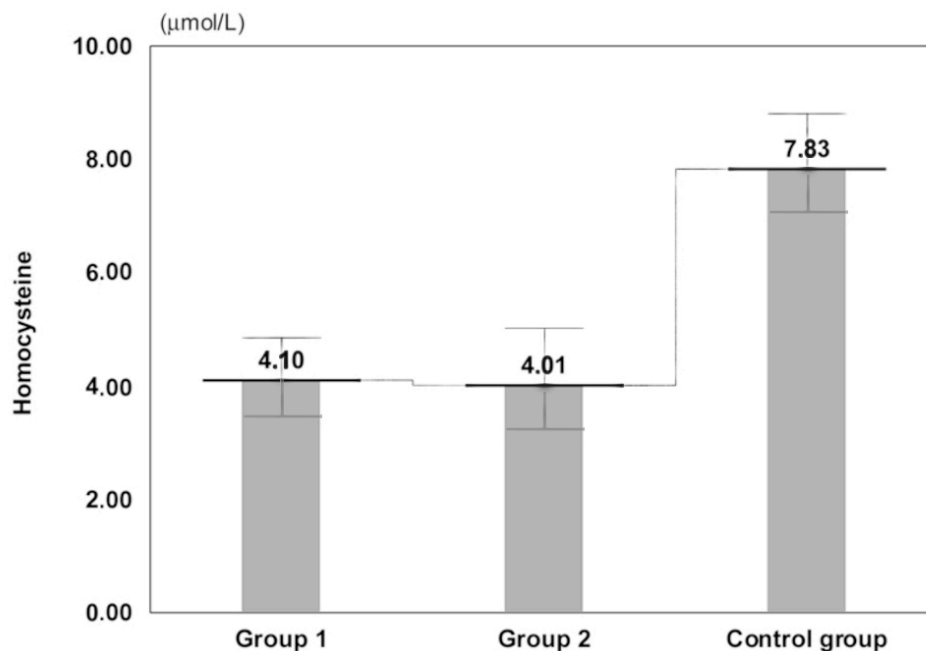
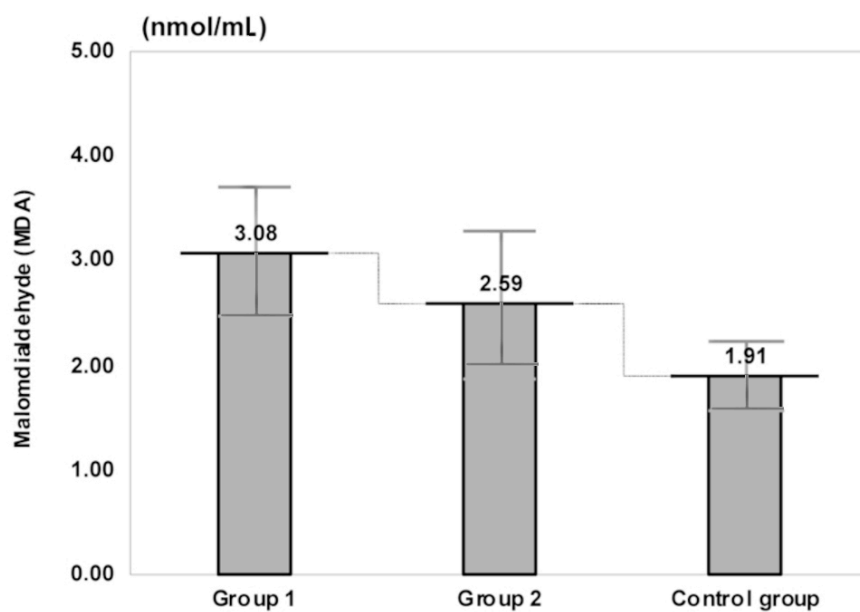
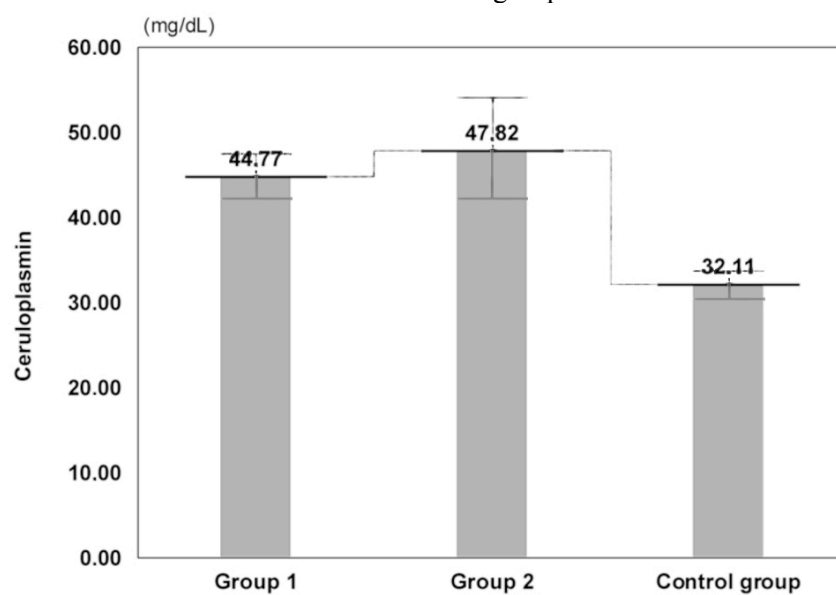


Figure 1

Values of homocysteine in pregnant women in the second and third trimester compared with the control group

**Figure 2**

Values of MDA in pregnant women in the second and the third trimester compared with the control group

**Figure 3**

Values of ceruloplasmin in pregnant women in the second and third trimester compared with the control group

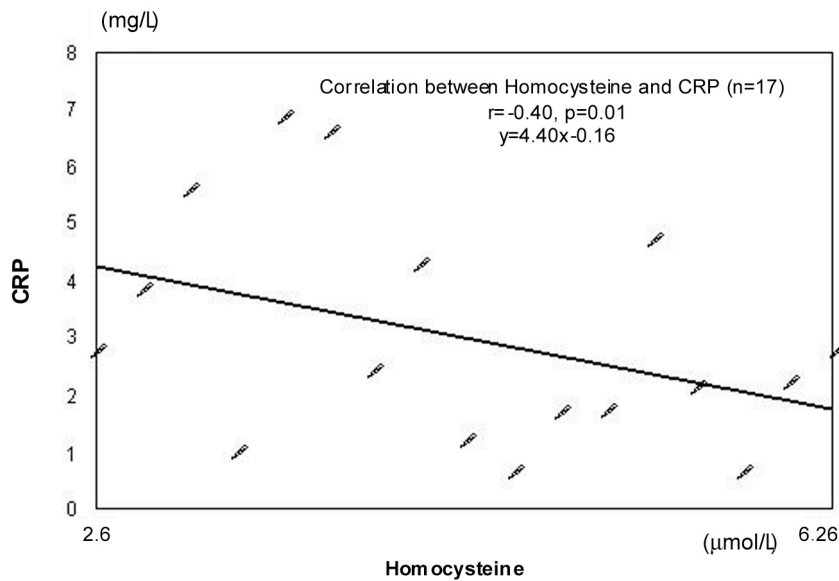


Figure 4
Correlation between the levels of CRP and homocysteine

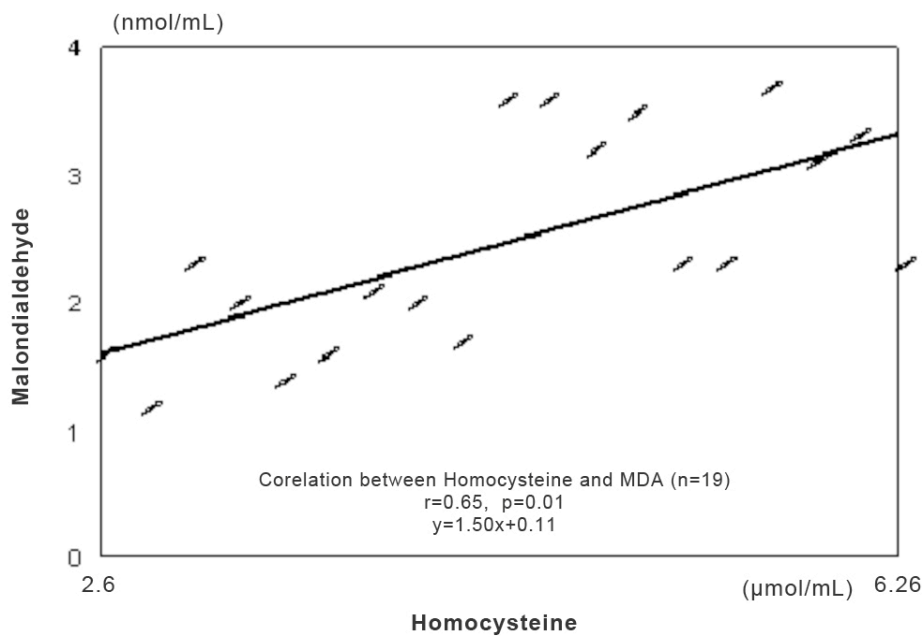


Figure 5
Correlation between the levels homocysteine and MDA

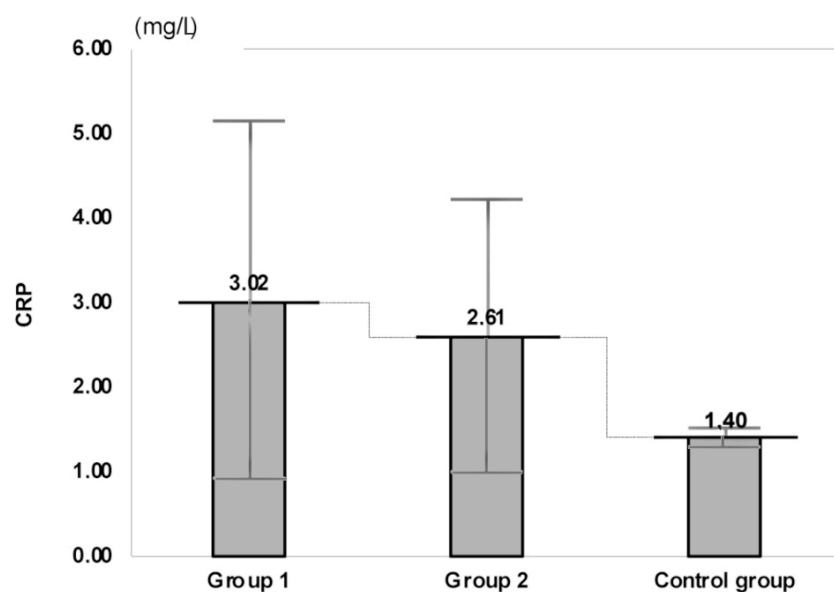


Figure 6

Values of CRP in pregnant women in the second and third trimester compared with control group

In many countries, for the prevention of neural tube defects women are recommended to take a daily 400 mg folic acid supplement before conception until the end of the 12th week of gestation. The low folate serum level is associated with an increased concentration of plasma total homocysteine, a risk factor associated with pregnancy complications for example preeclampsia [9].

Our findings show that homocysteine concentrations were significantly lower in pregnant women (second and third trimester) compared to the non-pregnant controls ($p < 0.001$). There was no significant difference between the two studied groups ($p > 0.05$). We observed, as others researchers, lower homocysteine concentrations in the pregnant state. The lower concentration of homocysteine in the pregnant women is related to altered maternal aminoacid metabolism due to fetal requirements. The precise mechanism remains unclear [9].

Holmes [8] found that plasma total homocysteine was significantly diminished throughout pregnancy compared with non-pregnant controls, with the lowest values in the second trimester, before increasing toward non-pregnant values in the third trimester. In pregnant women that received folic acid supplement, homocysteine concentrations were below the level of the untreated women, an effect that reached significance in the third trimester [9]. Folate is essential for DNA and RNA biosynthesis and is

required for homocysteine metabolism. The relationship between maternal folate status and homocysteine concentrations during pregnancy is more complex.

Linda Dodds [3] confirmed the findings that a high homocysteine level in early pregnancy is a risk factor for pregnancy loss and preeclampsia. This is consistent with the hypothesis that increased homocysteine results from abnormalities of the placental vasculature (3).

Estradiol concentration increases linearly throughout pregnancy, and a few researches suggest that its association with homocysteine is evident only up to 20 weeks of gestation [13].

It has been speculated whether homocysteine plays a role in regulating the hemostasis during pregnancy and the myometrial contractility at labor [8].

Homocysteine has been shown to induce vascular inflammation by enhancing the expression of pro-inflammatory cytokines such as monocyte chemoattractant protein 1 (MCP-1) [15]. In the same time it decreases the bioavailability of nitric oxide (NO), one of the major endothelium-dependent vasodilators [16]. Wu, in 2007 [22] underlined that inflammation enhances the synthesis of nitric oxide, which stimulates the production of a hyperhomocysteinemia, through binding with vitamin B₁₂. Consequently, varying degrees of hyperhomocysteinemia are detectable in all inflammatory diseases (may be considered as a risk factor for inflammatory diseases including cardiovascular disease, stroke, renal failure and cancer). It should be noted that hyperhomocysteinemia does not result only secondary to an inflammation, but the oxidative stress generated by hyperhomocysteinemia will again promote inflammation. Several anti-inflammatory compounds such as resveratrol, acetylsalicylic acid, salicylic acid and atorvastatin have all been shown to down-regulate the release of homocysteine from stimulated human peripheral blood mononuclear cells [19].

The results of this study clearly show that the levels of MDA, were statistically elevated in both groups of second and third trimester of pregnancy ($p < 0.001$) compared to the control group. No significant changes were observed in the second trimester of pregnancy compared to the third trimester of pregnancy MDA ($p > 0.05$). The body counteracts the oxidative stress in normal pregnancy through the induction of some enzymes such as, catalase, SOD, glutathione peroxidase, as well as through non-enzymatic free radicals scavengers i.e., vitamins C and E, uric acid proteins thiols. Pregnancy is a physiological condition in which women are more prone to oxidative stress, due to an imbalance between the prooxidant-antioxidant

factors [1]. A proper balance between oxidative stress and antioxidant systems during pregnancy is important. The involvement of hypoxia/oxidative stress in the pathophysiology of a variety of pregnancy complications including preterm labor miscarriage, fetal growth restriction and preeclampsia was reported [20].

Our determinations showed that the concentration of ceruloplasmin was high compared to the non-pregnant controls ($p < 0.001$). We found no difference between the groups of the second and third trimester pregnancy ($p > 0.05$). Elevated concentrations of ceruloplasmin in pregnancy represent perhaps a response to an oxidative injury.

Ceruloplasmin was first described as a member of a copper-containing oxidase family of enzymes and identified in liver and several other tissues including spleen, lung, brain, testis, and placenta.

It has an important function as a ferroxidase enzyme because even trace amounts of iron can produce hydroxyl radicals through the Fenton reaction which can destroy cellular architecture. Ferroxidatic activity of ceruloplasmin is known to convert toxic ferrous iron to less toxic ferric iron, which reduces oxidative damage to lipids, proteins, and DNA [7].

Guller et. al. suggest that syncytial ceruloplasmin and its associated ferroxidase activity, induced by the hypoxia, accompanying severe preeclampsia, is important in an endogenous cellular program to mitigate the damaging effects of subsequent reperfusion injury at this site [6]. Ceruloplasmin expression in placenta has also been shown to increase under hypoxic conditions [17].

Using soluble transferrin receptor (sTfR) Cedric Fosset et.al were able to demonstrate an inverse linear relationship between iron status and ceruloplasmin. Their data support the results showing the regulation of ceruloplasmin by iron and also suggest that the interactions between iron and ceruloplasmin should be considered when iron supplementation is recommended [5]. We observed no correlations between iron and ceruloplasmin. Pearson's correlation coefficient between iron and ceruloplasmin was $r = 0.04$. Ceruloplasmin may be a sensitive marker of hypoxia and limits oxidative damage in pregnancies.

The risk of hyperhomocysteinemia is not limited to heart disease but it is also registered in pregnancy complications, neural tube defects and osteoporotic fracture [14].

Wu *et al.* [22] found that elevated homocysteine and inflammation markers are frequently being detected at the same time but are not correlated with each other. Because of their different inflammatory pathways, measuring simultaneously inflammation markers will improve the overall

sensitivity of detection [22]. In our study we wanted to demonstrate if there is any correlation between homocysteine and inflammation markers (in this case CRP), in the physiological pregnancy. CRP results showed no significant difference in the two groups of pregnancy ($p > 0.05$) but a significant one compared with the control group ($p < 0.001$). Pearson's correlation coefficient between CRP and homocysteine is $r = -0.40$, $p = 0.01$. There was a negative and significant statistic correlation. The presence of oxidative stress may be considered as a promoter of inflammation. In our study we found a positive correlation between homocysteine and MDA suggesting that a high level of MDA also increases homocysteine ($r = 0.65$, $p < 0.01$).

Conclusions

The pregnant women in the second and third trimester are subjected to oxidative stress, demonstrated by the high concentration of MDA.

The level of total serum homocysteine was low, with no significant differences between the pregnant women in the second trimester and pregnant women in the third trimester.

There is a significant correlation between MDA and homocysteine, and also between CRP and homocysteine in pregnant women.

Therefore we consider important to determine together with other biochemical investigations also homocysteine and MDA plasmatic levels.

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