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Endogenous enzymatic antioxidants and oxidative damages predetermine in intracellular redox defense in normotensive pregnant women

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Normotensive pregnancy is a high-energy physiological state punctuate by increased necessity of oxygen. The increased oxygen intake would lead to the formation of residual reactive oxygen species and increased oxidative stress damages.

Sixty- five women from Stara Zagora, Bulgaria were carefully clinically selected to determine the plasma levels of superoxide dismutase (SOD), catalase (CAT), glutathione (GSH), glutathione peroxidase (GPx_1), and malondialdehyde (MDA) changes during the Ist, IInd, IIIrd trimesters of pregnancy and separated into two groups: 1. The control group (CG) consisted of total n=25 non-pregnant, 17-36 years old, non-pregnant, healthy volunteers, normotensive, nulliparous, non-smokers and had similar weights; and 2. The normotensive pregnancy (NP) group consisted of 40 pregnant women with a singlet pregnancy, healthy, nulliparous, non-smokers, with similar weights and normal diastolic and systolic blood pressure (mmHg), and ages ranging from 17- 36 years. The NP woman had uncomplicated singleton pregnancies and delivered vaginally without anesthesia between 38 and 40 weeks of pregnancy. SOD, CAT, GSH, GPx_1 activities ($p > 0.05$) were higher in the third trimester than in the first trimester. MDA level ($p > 0.05$) was lower in the third trimester of pregnancy.

These results suggest the importance of the balance between the generation of toxic ROS, compensation mechanism of antioxidant systems, and reduction of intracellular oxidative stress in NP group.

Key words: NP, endogenous-enzymatic antioxidants, redox defense

INTRODUCTION

Normal pregnancy is not a sickness state but still is stressful condition for the maternal organism with significant changes in metabolic and physiological infringements [1]. Under physiological processes in the organism generated reactive oxygen species (ROS) playing an important regulatory role through various intracellular signaling pathways in pregnancy. Increased ROS (including hydroxyl radicals (•OH), superoxide anions (O^{2-}), and hydrogen peroxide (H_2O_2)) generation leads to oxidative stress damages (OSD). As a result, cells developed enzymatic antioxidant protective mechanisms that prevent the imbalance between ROS, OSD and pathological changes in DNA structure, proteins oxidation and lipid peroxidation [1, 2, 3]. Enhanced ROS directly attack membranes lipids, resulting in cellular damage, abnormal lipid peroxidation, endothelial dysfunction, reduced antioxidant protection, and oxidative disorders of normotensive pregnancy [4, 5].

The reducing effect of the antioxidant defense system and the increased superoxide radical formation (O_2^-) are typical cellular dysfunctions, even in normotensive pregnancy (NP). Many studies report that the OSD increased during the early stages of NP pregnancy since the high metabolic percent of the placental oxygen consumption, free radicals generation and lipids oxidation (MDA) levels [5, 6, 7]. The free-radical scavenging mechanisms include enzymatic antioxidant such as Glutathione (GSH), Glutathione peroxidase (GPx_1), Superoxide dismutase (SOD) and Catalase (CAT), who lead to restriction of cellular ROS concentration and protect of placental OSD [8].

The aim of the present investigation was to determine levels of endogenous antioxidants and lipid peroxidation, in normotensive pregnant (NP) women and healthy controls (CG) and to influence the effect on intracellular redox changes during the I^{st}, II^{nd} and III^{rd} trimesters of pregnancy

EXPERIMENTAL
MATERIALS AND METHODS
NP patients and healthy controls

Sixty-five women from the Department of Obstetrics and Gynecology at UMBAL ''St. Kirkovich'' Stara Zagora, Bulgaria were included in the study. The pregnant women and control participants were randomly examined after admission to the hospital. Written agreement was received from all participants and the study was observed by ethical committee (2017/18). The personal participants' data were pre-protected.

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Each woman was carefully selected after clinical evaluation, and separated into two groups:

1. **The control group (CG)** consisted of total n=25 non-pregnant, 17-36 years old, non-pregnant, healthy volunteers, normotensive, nulliparous, non-smokers and had similar weights. In CG did not present previous disease and without any pharmacological therapy during the experiment;

2. **The normotensive pregnancy (NP) group** consisted of 40 pregnant women (I^{st}, II^{nd}, III^{rd} trimesters) with a singlet pregnancy, healthy, nulliparous, non-smokers, with similar weights and normal diastolic and systolic blood pressure (mmHg), and ages ranging from 17-36 years (Table 1). The NP woman had uncomplicated singleton pregnancies and delivered vaginally without anesthesia between 38 and 40 weeks of pregnancy and had no a history of other relevant disease states.

Table 1.

Characteristics	Control group (n= 25)	Normotensive pregnancy (n=40)	*p
Age, years	26.3 ± 2.4	27.8 ± 3.4	0.066
Smokers	Non	Non	-
Height, cm	168 ± 11.4	172 ± 9.4	≤0.031
Schooling	57%	22%	≤0.021
Family history of diabetes	9 (21,08)	11(25.3)	≤0.001
Body mass index, kg/m^2	39.34 ± 7.11	37.5 ± 2.7	0.33
Gestational age, weeks/ range	**NA**	35.5 ± 1.12	-
Systolic blood pressure (SBP, mmHg)	126.2 ± 7.0	119.2 ± 11	≤0.001
Diastolic blood pressure (DBP, mmHg)	76.8 ± 9.1	69.1± 6.0	≤0.001
Mean arterial pressure (MAP, mmHg)	99.712 ± 9.1	93.51 ± 2.2	≤0.2
Pulse pressure	64 ± 7	62.2 ± 8	≤0.001
Chronic hypertension	0.36 %	0.22%	≤0.031
Data presented as mean ± SD	NA- not applicable		p*- comparison bet. groups

Collection of blood samples

Twenty milliliters of fresh peripheral blood was collected directly by venous puncture from the ante-cubital region, in both groups. NP group blood samples were obtained in the first (9–12), second (22–25), and third trimester (36–37.5 weeks) of gestation. Whole blood samples and blood/ containing EDTA anticoagulant samples were cumulated into plastic tubes, and centrifuged at 4000 rpm for 10 min at 4°C. 1 ml of plasma samples was separated and stored at –20 °C until further assay was done. In additional plastic tube with serum clot activator without anticoagulant were taken samples for the separation of blood fractions.

Determination of hematological parameters and instruments

The number of erythrocytes (RBC, 10ˇ12/L), hemoglobin (HGB, g/L) and hematocrit (HCT, L/L) both in CG and NP woman was performed on Pentra-ES 60 (Japan) automatic cell counter (with impedance and photometric method). The method provide 28 reading hematological parameter. The biochemical analyses were performed at UV–VIS spectrophotometer-400 (TERMO Sci., RS232C, Stratagene, USA) and ELISA spectrophotometer (Urиd-660 A, Germany).

Determination of SOD and CAT activities

Plasma samples were used and analyzed to determine SOD and CAT activities using method described by Sun *et al.* [9] and by Aebi H. [10], respectively.

Determination of lipid peroxidation

Plasma lipid peroxidation was estimated using thiobarbituric acid (85%), by the method of Plaser *et al*. [11] which measure the concentration in nmoles of malondialdehyde (MDA) reactive products.

Determination of the GSH and GPx$_1$ activities

GSH and GPx$_1$ activities in plasma were measured with ELISA commercial kits (Sigma Aldrich Company, Catalog No. CS0260, 2–8^{0}C) and (EH0826, C6-323, Biolake, 1–4^{0}C), respectively. Enzyme activities were expressed as nmoles/ ml and ng/ml concentration.

Statistical analysis

Statistical analysis was performed using Statistica 8.0, Stasoft, Inc.,one-way ANOVA,

Student- t- test to determine significant difference among data groups. The results were expressed as means ± standard error (SE). A value of $p > 0.05$ was considered statistically. To define which groups are different from each other we have used Turkey HSD post hoc test

RESULTS AND DISCUSSION

Trough period of pregnancy in the mother's body a number of anatomical, physiological, biochemical, ROS production, antioxidant status and oxidative changes were carried out, witch lead to protection the pregnant woman from risks of gestation and promote healthy growth and development of the embryo [12, 13]. Yüksel and Yiğit [12] and Toescu *et al*. [14] reported that by reason of increased mitochondrial oxygen consumption by the placenta, the OS levels increased.

Hematological parameters

RBC, HGB and HCT metering is mandatory during normal pregnancy with reference HGB levels of 12-16 grams and HCT levels (36-48%) for women of childbearing age. Lu *et al*. [15] commend that the complete determination of the erythrocyte volume, the hematocrit appears as precise parameter than hemoglobin.

With respect to haematological parameters, a statistical difference between the NP women and CG groups was observed. The number of RBC, HGB and HCT were significantly reduced in the NP group in IIIrd tremester, compared to CG (Table 2).

Decreased RBC, HGB and HCT levels during NP were probably caused by previous anemia or pregnant woman predisposing to infections or intensification of OS damages [16, 17, 18] or socio-economic status of NP woman, compared to non-pregnant individuals. Slightly elevated HGB and HCT levels during the IInd trimester of NP group is presumably decrease in the physiological blood thinning in several of NP mothers [18].

Table 2.

Groups	Erythrocytes (RBC) ($10^{<}12$/L)	Hemoglobin (HGB) (g/L)	Hematocrit (HCT) (L/L)
Non- pregnant women (n = 25)	5.12 ± 0.08	14.06 ± 0.29	42.91 ± 0.99
Normotensive pregnancy (n=40)			
Ist Trimester n =13	3.42 ± 0.1*	13.76 ± 0.68	42.06 ± 1.59
IInd Trimester n =13	3.97 ± 0.82	14.39 ± 0.04	43.11 ± 1.41
IIIrd Trimester n =14	4.02 ± 1.01	11.83 ± 0.32*	36.53 ± 0.84$^{\#}$
	p<0.002	p<0.004	p<0.002

Determination of SOD and CAT activities and levels of lipid peroxidation

Tissue reoxygenation leads to increased lipid peroxidation and ROS overproduction, which causes oxidative changes in the maternal organism. As a consequence, endogenous antioxidant enzymatic activity is increased which seeks to scavenged and deactivate ROS and significantly lowered lipid peroxidation [12, 19]. The antioxidant enzymes SOD and CAT work in concert in the cells to the complete metabolism and elimination of toxic ROS and the transformation of harmful oxygen forms into innoxious molecules [5, 20].

The SOD activity (Fig. 1) was significantly increased in the NP group in IIIrd trimester compared to the CG (1317.12 ± 35.1 vs. 1006.7 ± 38.5 IU/gHb, $p<0.05$). The CAT activity (Fig. 1) was also increased in the NP group in IIIrd trimester compared to the CG (33927.81 ± 339.6 vs. 28723.12 ± 354.3, IU/gHb, $p<0.05$).

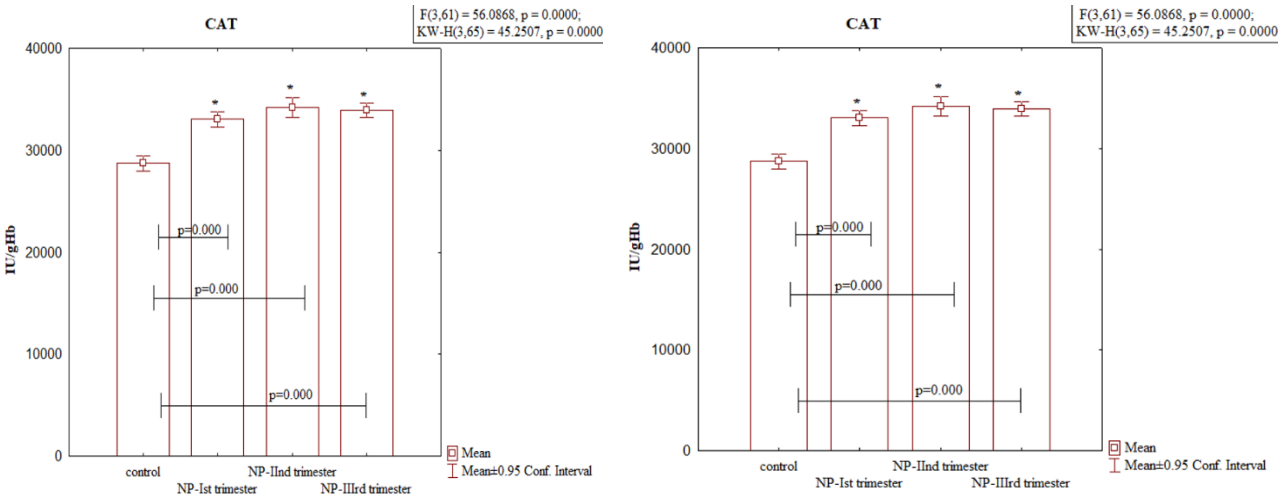

Figure 1. Plasma SOD (a) and CAT (b) activity in different trimesters of pregnancy (n=40). *considered statistically significant NP groups vs CG ($p<0.05$); (t-test).

In contrast, MDA levels (**Fig. 2)** were lower in the IIIrd trimester compared to the Ist, IInd trimesters ($p<0.03$). Decreased levels of lipid peroxidation was observed in the IInd and IIIrd trimester compared the CG (5.531±0.74 IIIrd trimester vs. 11.14±0.33 CG, IU/gHb, $p<0.05$).

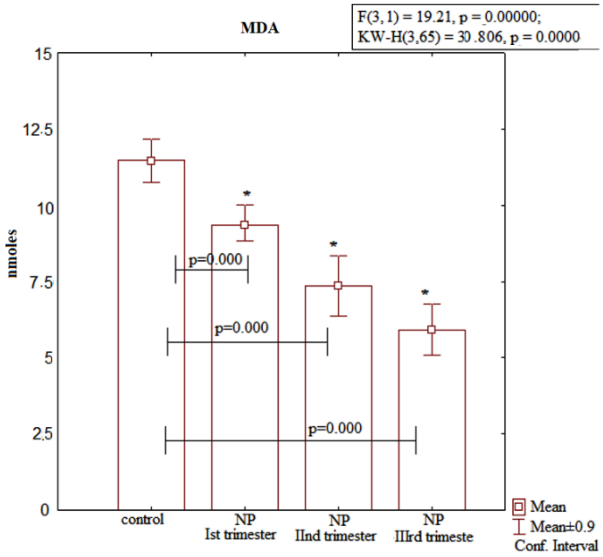

Figure 2. MDA levels in plasma in different trimesters of pregnancy (n = 40).* $p<0.05$ compared to CG; #p < 0.03 compared to IIIrd trimester.

The results showed that the MDA levels decreased in NP group during the IIIrd trimester of gestation in comparison to the non- pregnant controls. In parallel with this change, there was an increase in the amounts of the endogenous antioxidants SOD and CAT during the same period. Previous studies reported, that the changes of the endogenous antioxidants might be related to the process of NP, oxygen absorption and the placental circulation [9, 12, 13] affecting OS -changes in pregnancy progression. In normotensive pregnancy, the higher production of placental lipid peroxides (MDA levels) is controlled and neutralized by the activation of endogenous antioxidant system [12, 21]. Qanungo *et al.* [22], Utola *et al.* [23] and Yüksel and Yiğit [12] in previous reports showed significant decreased in plasmatic MDA levels, in accordance with our results.

Determination of the GSH and GPx$_1$ activities

Prominent part of the endogenous protective antioxidants is loan of Glutathione and glutathione-related enzymes. The tripeptide GSH and related enzymes directly interact and inactivated ROS (*such as superoxide anion radicals ($^{\cdot}O_2^-$), hydrogen radicals ($^{\cdot}OH$)*), protected cells of oxidative and electrophilic stress [24, 25] and reacted as substances of cytosolic CSH-redox cycle [24]. GPx$_1$ increased antioxidant protection by reduction of lipid and DNA hydroperoxides [24] and reduced hydrogen peroxide to non-toxic water molecules [26]. The results clearly demonstrated that GSH activity (**Fig. 3**) (0.315 ± 0.003 IIIrd trimester vs. 0.197±0.01, nmol/ml, $p<0.05$) and GPx$_1$ levels (3.08 ± 0.05 IIIrd trimester vs. 1.29 ± 0.03, ng/ml, $p<0.05$) (**Fig. 4**) in NP group was significantly two-fold higher in IIIrd trimester, compared to CG.

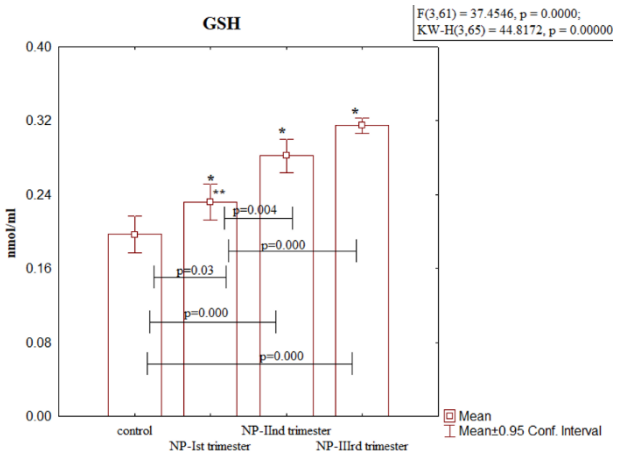

Figure 3. GSH activity in different trimesters of pregnancy (n=40); (t-test). *considered statistically significant control vs NP groups (p<0.05); **considered statistically significant NP Ist trimester vs NP IInd and IIIrd trimester (p<0.05).

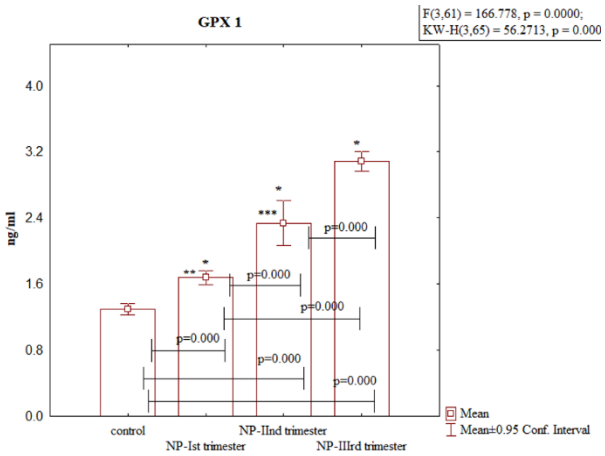

Figure 4. GPx_1 activity in different trimesters of pregnancy (n=40); (t-test). *considered statistically significant control vs NP groups (p<0.05); **considered statistically significant NP I^{st} trimester vs NP II^{nd} and III^{rd} trimesters (p<0.05); *** considered statistically significant NP II^{nd} trimester vs III^{rd} trimester (p<0.05).

Increased GSH and GPx_1 activity in the IInd and IIIrd trimester is consistent with the findings of other investigators [12, 22, 23, 27], who reported that GSH and GPx_1 levels increased during late stages of pregnancy. These higher enzymatic levels in the III^{rd} trimester protected normotensive pregnant woman against hydrogen peroxide and free-radical toxins or protect fetus against highly reactive stress compounds [12, 24]. Additionally, our results suggest that increased SOD, CAT, GSH and GPx_1 activities suppressed the lipid peroxidation levels in the late pregnancy. Moreover, antioxidant peptides prevent direct inactivation of ROS/RNS molecules, leading to normalization of the intracellular redox status, provides the antioxidant- prooxidant balance, promoting normal fetal development [5, 12, 23] and reduced the OS- detoxification processes.

CONCLUSION

Together, our results are of the importance of the balance between the generation of toxic ROS and antioxidant systems protection in normal pregnancy and in healthy individuals. The reduced intracellular OS in NP group probably indicates the antioxidant system compensation mechanism and simultaneous protection of the mother and the embryo from toxic free- radical formations.

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Conflict of interests. *The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.*

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