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ORIGINAL ARTICLE

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Circulating biomarkers of oxidative stress in complicated pregnancies

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Abstract Increased lipid peroxidation (LPO) and reduced antioxidant activity may contribute to the development of complications in pregnancy. The present study discusses the possibility of LPO and antioxidant activity in both maternal and umbilical cord blood as an indicator of oxygen radical activity. For this aim, pregnancies with hypertension and pre-eclampsia, diabetes mellitus (insulin dependent diabetes mellitus and gestational diabetes mellitus), oligohydramnios and abruptio placentae, as well as a healthy control group, were subjected in the present study. Simultaneous determination of glutathione S-transferase (GST), selenium dependent glutathione peroxidase (Se-GPx), catalase (CAT) activities and thiobarbituric acid reactive-substances (TBARs) levels were carried out in maternal erythrocyte and plasma in the antenatal period (in the third trimester) and immediately after the delivery. The same oxidative stress-related parameters were determined in umbilical cord blood as well. Erythrocyte GST activity was significantly increased in insulin-dependent diabetic pregnancy (IDDP) when compared to the control ($P<0.05$). Erythrocyte Se-GPx activity was found to be significantly increased in hypertensive preeclamptic pregnancy (HPP) ($P<0.05$) and in IDDP ($P<0.05$). Alterations in enzyme activities were accompanied by a simultaneous significant increase in the levels of TBARs in plasma samples of HPP ($P<0.05$), and IDDP ($P<0.05$). Enzyme activities were found to be significantly lower in cord blood samples than the maternal values, except GST. This enzyme represents about two- to threefold higher activity than those of the maternal activity in uncomplicated and complicated groups. Cord blood erythrocyte and plasma Se-GPx and CAT activities were decreased significantly in the HPP group when compared to the maternal value ($P<0.05$). Cord blood erythrocyte CAT activity was significantly decreased in the HPP group compared to the control ($P<0.05$). Cord blood TBARs levels were significantly lower than the before deliveries maternal value in the HPP group ($P<0.05$). No difference was detected between umbilical cord blood and maternal blood TBARs levels after delivery. The results of the present study suggest that oxidative stress and subsequent lipid peroxidation accompany the complications of hypertension, preeclampsia and diabetes mellitus in pregnancy. Maternal erythrocyte GST activity seems to be a sensitive indicator of oxidative stress in IDDP before delivery. The same enzyme can be used in cord blood as a biomarker of oxidative stress upon a sudden increase in oxygenation during delivery. These multiparameter biomarkers can also be used in monitoring the efficiency of antioxidant supplementation in complicated pregnant women, as has recently been suggested for diabetic and preeclamptic pregnancies.

Keywords Pregnancy · Antioxidant enzymes · Cord blood · Erythrocyte · Complication

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Introduction

Lipid peroxidation has been aetiologically involved in a variety of physiological, pathological and clinical conditions, including pregnancy and its complications, mainly in preeclampsia and diabetes [2, 8, 12, 26]. In normal pregnancy, an increase in malondialdehyde levels is associated with an increase in total serum lipids, indicating that the ratio of lipid peroxide to total lipid is not changed. Compared with women having normal pregnancies, women with preeclampsia display an increase in serum total lipid and triglyceride fractions [8]. It has been suggested that uncontrolled lipid peroxidation may

play an important role in the pathophysiology of preeclampsia by causing vascular endothelial cell dysfunction [6].

The role of oxidative stress in pathogenesis in insulin-dependent diabetes mellitus has been implicated in several studies [9, 19, 24]. Increased lipid peroxidation products and altered antioxidative enzyme activities were also reported in non-insulin-dependent diabetes mellitus [10]. However, there are limited numbers of investigations in diabetic pregnancy [2, 11, 14].

Oligohydramnios is characterised by an extremely limited volume of amniotic fluid surrounding the fetus [5]. In this complication, fetal urination cannot take place, as in instances of renal agenesis or atresia of the urethra. But, in most of the cases, the conditions frequently associated with oligohydramnios include hypertension and preeclampsia, in which oxidative stress is implicated in their aetiology [5].

The last investigated complication in the present study is abruptio placentae, which is characterised by the early separation of placenta before the delivery of the fetus. Several factors, such as impairment in connective tissue or localised inflammation of placenta, may cause this phenomena.

In the present study, our aim was to investigate the role of oxidative stress in the above-mentioned complications in pregnancy. Further, to investigate the possibility of both maternal and umbilical cord blood LPO status and antioxidant activity as an indicator of oxygen radical activity in those complications. These parameters also have the potential to show the efficiency of using antioxidants such as vitamin E and/or vitamin C to reduce the malformation risk especially in the diabetic [3, 25] and in preeclamptic pregnancies [1, 4, 31]. Studies in oxidative stress-related complications in pregnancy have been focused on maternal parameters. In comparison, few studies were carried out on placenta, fetus and/or umbilical cord blood [16, 18, 29]. Therefore, we have undertaken such a study on the levels of TBARs, which is an index of lipid peroxidation, and antioxidant enzymes' activities in maternal (in the third trimester of antenatal period, and after the delivery) and in umbilical cord blood (during the delivery). Red blood cells (RBC) were chosen as a biologic specimen since they are particularly sensitive to oxidative stress and, like other cells of aerobic organism, are supplied with protective antioxidant mechanism in order to counteract the toxic action of free radicals.

Materials and methods

Chemicals

All chemicals used in this study were of analytical grade.

Patients

In this prospective study, samples were obtained from complicated and uncomplicated pregnant women who came to the outpatient clinic of the Obstetric and Gynecology Department at Hacettepe University Hospital. They were divided into groups of healthy Control (n=16, 27.5±3.8 [mean age±standard deviation]), Hypertensive and Pre-eclamptic Pregnancy (HPP; n=9, 32.1±5.3), Insulin-Dependent Diabetes mellitus in Pregnancy (IDDP; n=3, 29.3±2.9), Gestational Diabetes mellitus in Pregnancy (GDP; n=3, 31.3±2.5), Oligohydramniotic Pregnancy (OP; n=3, 21±5.3) and Pregnancy with Abruptio Placentae (PAP; n=2, ages 30 and 34). Hypertension was defined according to the criteria of the Committee on Terminology of the American College of Obstetricians and Gynecologists, which has defined hypertension as a blood pressure greater than 140/90 mmHg on more than two occasions; greater than 42 days' postpartum is also classified as chronic hypertension. Pre-eclampsia is defined as blood pressure of 140/90 mmHg or greater along with proteinuria, edema or both after 20 weeks' gestation, except in the presence of trophoblastic disease or multiple gestation, in which cases preeclampsia may appear earlier. We defined insulin dependent diabetes diagnosed when not pregnant as Type I diabetes mellitus according to National Diabetes Data Group Classification [17]. Gestational diabetes mellitus is defined as any degree of glucose intolerance with the onset or first recognition during pregnancy. The diagnosis of the disease was made when two or more of the following plasma glucose concentrations were met or exceeded: fasting, 105 mg/dl; 1 h, 190 mg/dl; 2 h, 165 mg/dl; and 3 h, 145 mg/dl. All pregnant women were screened for glucose intolerance with 50 g oral glucose load (glucose challenge test [GCT]). A venous plasma cutoff of 140 mg/dl (7.8 mmol/L) 1 h after the glucose load was recommended as the cutoff for the screening test. We performed a diagnostic 100 g oral glucose tolerance test (OGTT) on that subset of women exceeding the glucose threshold value on the GCT. Amniotic fluid that is abnormally low for gestational age is termed oligohydramnios. We evaluated the amniotic fluid with transabdominal ultrasonography by using the four-quadrant amniotic fluid index calculation. Abruptio placentae is defined as a premature separation of the normally implanted placenta from the uterine wall.

Blood samples

Blood samples were collected by venopuncture in heparinised tubes in the third trimester of antenatal period and 1–2 days after the delivery from uncomplicated and four groups of complicated pregnant women. Umbilical cord blood samples were obtained from the cord immediately after the delivery into heparinised tubes. Fresh blood samples were centrifuged for 15 min at 2000 *xg*. After removal of plasma and buffy coats, the red cells were washed twice with two volumes of phosphate buffered saline (PBS) of pH 7.00. Plasma was stored for further measurements at –35°C. Hemolysates were prepared by addition of two volumes of cold distilled water to erythrocytes. Cellular debris was removed by centrifugation at 4000 *xg* for 30 min.

Enzyme activities

Erythrocyte GST, Se-GPx, CAT activities and plasma Se-GPx activity were determined as described in previous studies [20, 21, 22].

TBARs levels

Thiobarbituric acid reactive substances' levels of blood samples were analysed according to the published procedure [22].

Clinical parameters

Blood biochemical markers of the subjects were determined by routine biochemistry laboratory of the University Hospitals.

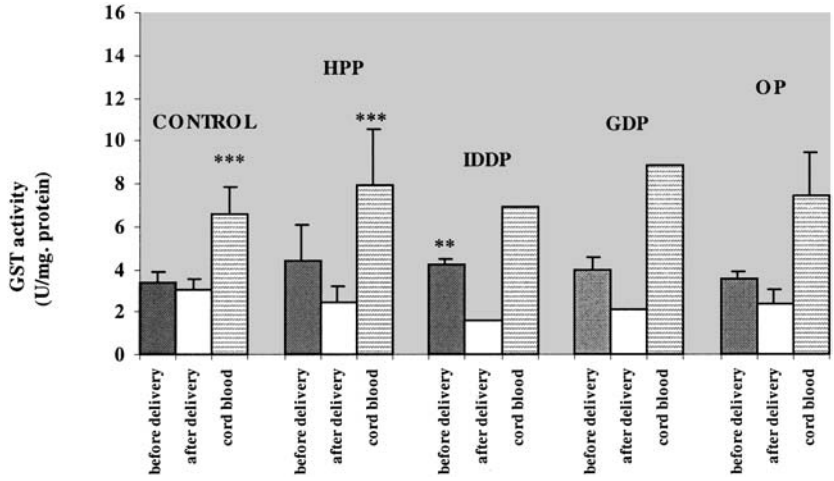

Fig. 1 Erythrocyte glutathione S-transferase activity in the groups. Results are expressed in terms of Unit/mg of protein and represent means±SD. Key: (**) significantly different (P<0.05) from the corresponding control group's activity, and (***) significantly different (P<0.05) from the corresponding before deliveries maternal activity

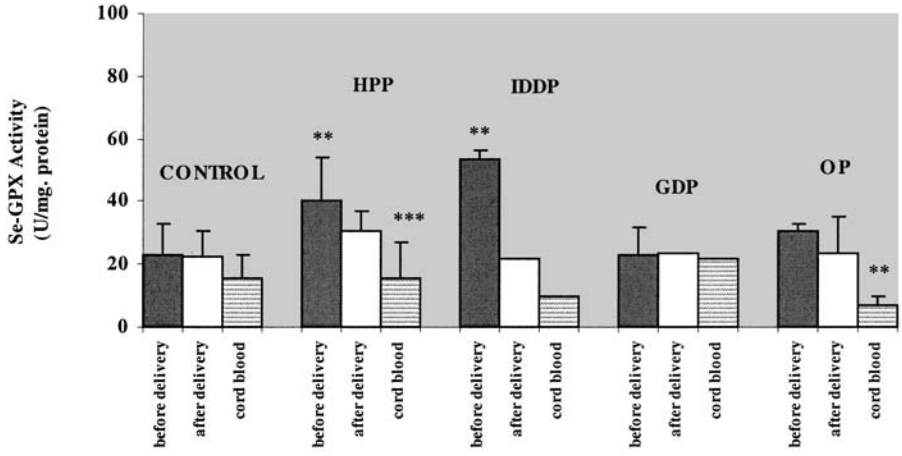

Fig. 2 Erythrocyte selenium dependent glutathione peroxidase activity in the groups. Results are expressed in terms of Unit/mg of protein and represent means±SD. Key: (**) significantly different (P<0.05) from the corresponding control group's activity, and (***) significantly different (P<0.05) from the corresponding before deliveries maternal activity

Protein

Protein content of the samples was determined according to the method of Lowry modified by Miller [15].

Statistical analysis

The results are expressed as mean±standard deviation. Mann Whitney U test was used for pairwise comparison. A P value of less than 0.05 was considered significant. The PAP group was excluded from statistical analyses because of an inadequate subject number (n=2). IDDP and GDP maternal values after delivery and their cord blood values were excluded as well, since not all the women in these groups delivered during the course of the study.

Results

Figures 1–5 represent the blood antioxidant enzyme activities and TBARs levels of each group in the third trimester of antenatal period and after delivery. Same parameters in corresponding cord blood samples were shown beside each group's column.

Erythrocyte glutathione S-transferase activity was found to be significantly higher in the IDDP group in the antenatal period when compared to the control value (Fig. 1; P<0.05). Cord blood erythrocyte GST activity was two- to threefold higher in the HPP and in the IDDP groups than the corresponding maternal activity before delivery, also in the control group (P<0.05).

Erythrocyte Se-GPx activity was significantly higher in HPP and in IDDP groups in the antenatal period when compared to the corresponding control (Fig. 2; P<0.05 for both). There was no significant alteration in this enzyme activity compared to the control after the delivery. Cord blood erythrocytic Se-GPx activity was significantly lower than the before delivery maternal value in HPP group (P<0.05).

Erythrocyte CAT activity was not different in any group when corresponded to the control in the antenatal period (Fig. 3). Cord blood erythrocytic CAT activity was found to be significantly lower (P<0.05) than it before delivery maternal value in HPP group.

Plasma Se-GPx activity was not different in the groups in the antenatal period when compared to the control, and after the delivery when compared to the corresponding antenatal period values (Fig. 4). This enzyme activity was significantly lower in HPP group cord blood samples when compared to their maternal activity (P<0.05).

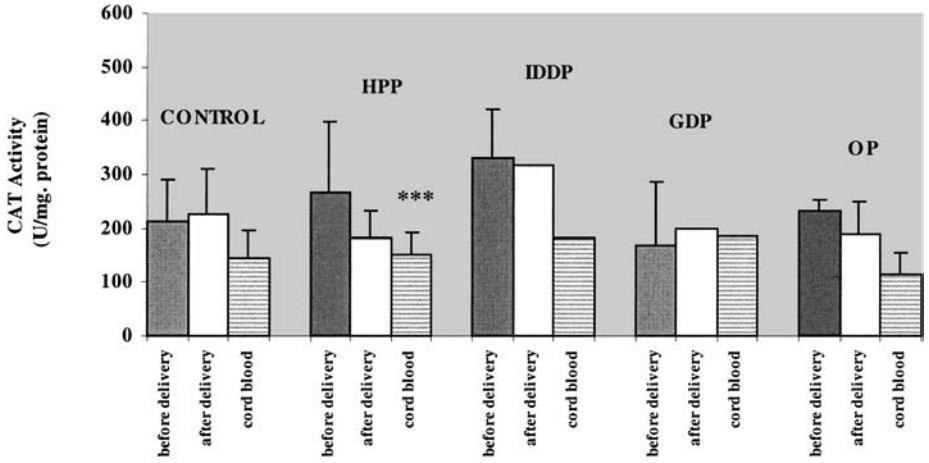

Fig. 3 Erythrocyte catalase activity in the groups. Results are expressed in terms of Unit/mg of protein and represent means±SD. Key: (**) significantly different (P<0.05) from the corresponding control group's activity, and (***) significantly different (P<0.05) from the corresponding before deliveries maternal activity

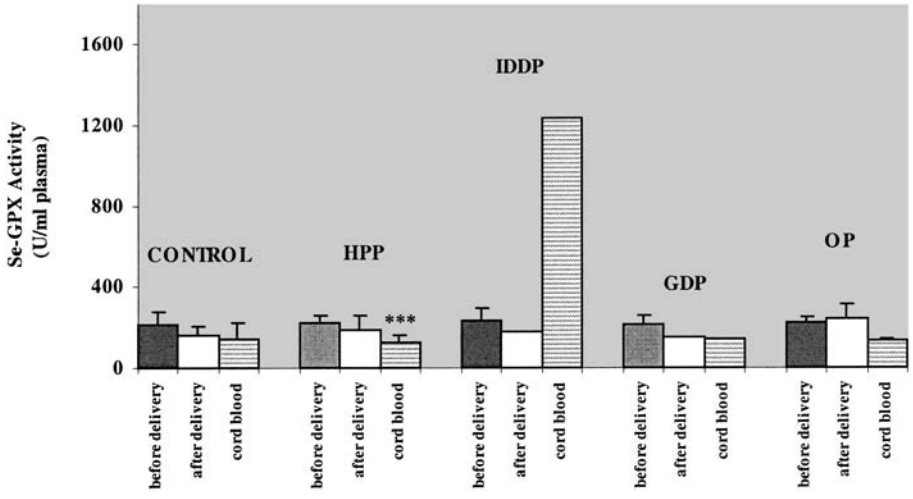

Fig. 4 Plasma selenium-dependent glutathione peroxidase activity in the groups. Results are expressed in terms of Unit/ml of plasma and represent means±SD. Key: (**) significantly different (P<0.05) from the corresponding control group's activity, and (***) significantly different (P<0.05) from the corresponding before deliveries maternal activity.

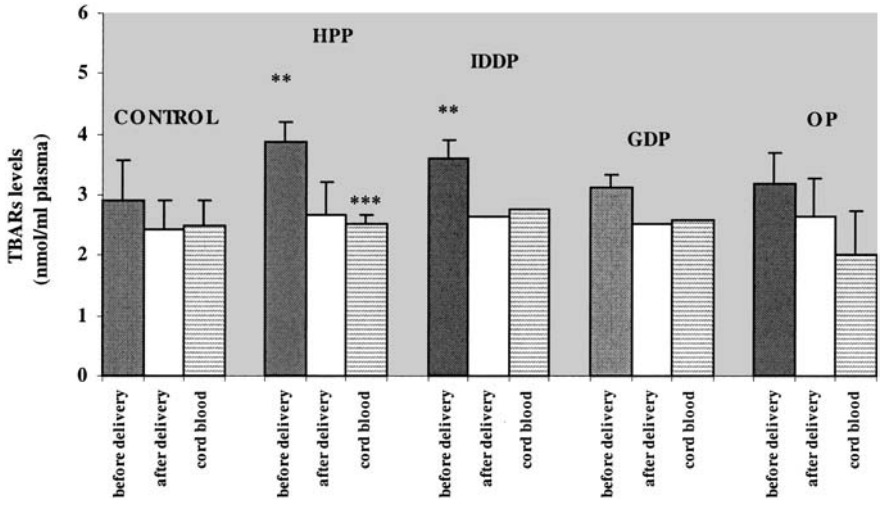

Fig. 5 Plasma TBARs levels in the groups. Results are expressed in terms of nmol/ml of plasma and represent means±SD. Key: (**) significantly different (P<0.05) from the corresponding control group's value, and (***) significantly different (P<0.05) from the corresponding before deliveries maternal value.

Plasma TBARs levels were significantly higher than the control in the HPP and in the IDDP groups in the antenatal period (Fig. 5; P<0.05 for both). Cord blood erythrocytic TBARs levels were significantly lower than the corresponding maternal value in HPP group (P<0.05). Only two patients of abruptio placentae could be subjected in the present study, since this complication is quite rare. Although statistical analyses can not be performed, the results of two patients can be seen in Table 1. In contrast to the other complication groups, erythrocyte GST was lower than the control in the antenatal period of PAP patients. This reduced GST activity

Table 1 Oxidative Stress-related parameters of two patients of pregnancy with abruptio placentae. * The gestation was not termed for this patient during the course of the study; *Before* before delivery maternal blood sample during third trimester; *After* after delivery maternal blood sample; *Cord* umbilical cord blood sample immediately after the delivery

	Control (n=16)	PAP	
		Patient 1	Patient 2*
Erythrocyte GST Activity (U/mg protein)			
Before	3.4±0.5	2.5	1.93
After	3.0±0.5	3.9	–
Cord	6.5±1.3	6.03	–
Erythrocyte Se-GPx Activity (U/mg protein)			
Before	22.9±10	17.3	24.4
After	22.1±8	18.0	–
Cord	15.4±7	21.2	–
Erythrocyte CAT Activity (U/mg protein)			
Before	211±80	192	221
After	225±84	225	–
Cord	146±48	134	–
Plasma Se-GPx Activity (U/L plasma)			
Before	218±57	192	221
After	165±42	138	–
Cord	141±84	82	–
Plasma TBARs (nmol/ml plasma)			
Before	2.9±0.7	2.7	3.04
After	2.4±0.5	2.5	–
Cord	2.5±0.4	2.5	–

Data are presented as mean±standard deviation

was increased near to control value after delivery in one patient. As seen in other groups, cord blood GST activity was about two- to threefold higher than the maternal activity. Other parameters seemed to be relatively unchanged.

Clinical biochemical parameters of groups in the antenatal period can be seen in Table 2. Triglyceride (TG) and very low density lipoprotein (VLDL) levels were significantly lower than the control values in the IDDP group.

Discussion

The supplementation of antioxidants, such as vitamin E and/or C, to prevent eclampsia and diabetes mellitus during pregnancy has been suggested recently and several animal model and clinical trials have been conducted [3, 4, 25]. Analysing reliable, sensitive and specific biomarkers is the essential need in such studies. Our aim in the present study was to investigate whether LPO plays a role in aetiology and/or the onset of the investigated complications during pregnancy on one hand, and to show the possibility of using the measured parameters as an indicator of oxidative stress. Our present data implied that lipid peroxidation/antioxidative mechanism's balance is changed in investigated complications. This was accompanied by simultaneous alterations of the same parameters in umbilical cord blood of newborn.

We observed that maternal erythrocyte GST activity is higher than the control in the IDDP group. On the other hand, it has been reported that erythrocyte GST is vulnerable to oxidative stress as it is deactivated by the oxidation of a cysteine (47-Cys) residue near to the active centre of the enzyme [27]. The reason for the discrepancy between that and our present data for GST remains unclear. This unexplained behavior of erythrocyte GST has been observed by us previously in vitro as well [23]. It can be assumed that at low levels of oxidants the enzyme is deactivated, but after a certain level, enzyme might be activated by same oxidant(s). Another explanation is consistent with the finding of [7], that two different forms of human erythrocyte GST Pi exist, and that the one that is more heat stable contains older erythrocytes. One form could be vulnerable to oxidants and deactivated, while the other form might be activated at high levels of oxidants.

Erythrocyte Se-GPx was also higher in the HPP and IDDP groups in the antenatal period. This result was in accordance with previous reports [28, 30]. However, there are a number of conflicting reports as well [8, 32]. This increase in Se-GPx activity with an increase in TBARs in same groups can be interpreted as a compensatory mechanism of the enzyme to defend against the increased substrate (H_2O_2). In contrast, cord blood Se-GPx activity of the HPP and OP groups has been found to be diminished with those of maternal value.

Erythrocyte CAT activity was not different than the control in the complication groups during antenatal period. This may be explained by physiological properties of enzymes Se-GPx and CAT. Hydrogen peroxide, which is formed at relatively low levels during normal metabolic routes or mild oxidative status, is decomposed by Se-GPx. CAT shows the same activity only at very high levels of hydrogen peroxide. Our present observation that no affection for CAT shows that lipid peroxides and the product hydrogen peroxide are increased in HPP and IDDP groups, but the levels can still be maintained at a reasonable state. Cord blood CAT activity of HPP group was lower than the corresponding maternal value. Together with the decrease in the activity of Se-GPx and CAT, this may show the circulating hydrogen peroxide in cord blood is much higher when compared to that of maternal blood. This is an expected outcome during the delivery of fetus, since high oxygen challenge occurring at birth might lead to increased formation of reactive oxygen species and, subsequently, hydrogen peroxide.

Plasma Se-GPx activity was unchanged in the maternal samples of all groups; however, there was a significantly lower activity in cord blood samples of the HPP group. This observation was in accordance with the decrease in erythrocyte Se-GPx and erythrocyte CAT activity.

Although statistical analyses cannot be performed in the PAP group, these preliminary results imply that GST activity might be affected under these disease conditions.

Table 2 Results of blood and urine biochemical markers for individual patients. *St. dev.*: Standard deviation; *Hb* hemoglobin; *Htc* hematocrit, *Uri. Prot* urinary protein; *BUN* blood urea nitrogen; *Cre* creatinine; *Ca* calcium; *ALT* alanine transaminase; *AST* aspartate transaminase; *TG* triglyceride; *COL* cholesterol; *HDL* high density lipoprotein; *LDL* low density lipoprotein; *VLDL* very low density lipoprotein; *m.d.*: missing data

Groups Control	Hb g/dl	Htc %	Uri prot mg/ml	BUN mg/dl	Cre mg/dl	Ca mg/dl	ALT U/L	AST U/L	TG mg/dl	COL mg/dl	HDL mg/dl	LDL mg/dl	*VLDL mg/dl*
AE	11.2	33.6	–	11	0.7	9.1	17	13	203	187	41	120	26
DÇ	11.6	31.5	–	7	0.7	10.1	24	19	166	194	60	108	33
EE	12.2	35.4	–	8	0.8	9.0	9	14	403	290	43	166	81
CK	12.1	36.9	–	11	0.8	10	8	25	227	380	76	259	45
GÇ	10.9	29.8	–	15	1.1	7.5	23	23	241	262	50	101	39
HK	12.3	35.6	75	5	0.7	9.8	11	22	294	337	100	178	59
GT	12.8	36.7	–	5	0.7	8.1	29	46	200	206	60	106	40
TÜ	m.d.	m.d.	–	m.d.	m.d.	m.d.	m.d.	m.d.	m.d.	m.d.	m.d.	m.d.	m.d.
SG	9.7	29.3	25	8	1	7.9	79	52	250	206	47	109	50
ÖA	12.5	37.3	25	8	0.7	9.2	10	16	272	163	65	43	44
FS	m.d.	m.d.	–	m.d.	m.d.	m.d.	m.d.	m.d.	m.d.	m.d.	m.d.	m.d.	m.d.
SY	10.7	30.9	–	8	0.7	9.2	27	24	270	214	71	156	43
HE	11	33.5	–	12	0.6	6.3	20	37	163	210	64	113	33
SY	11.5	30.8	–	11	0.6	8.5	13	17	217	181	41	113	27
SB	11.6	35.1	25	18	0.9	11.8	19	27	252	163	45	68	50
GP	11	32.8	25	8	0.6	9.1	16	20	169	321	110	177	34
Average	11.5	33.5		9.6	0.7	9.0	21.8	25.3	237	237	62.3	130	43.1
St. dev.	±0.8	±2.8		±3.6	±0.2	±1.3	±18	±12	±63	±69	±21	±54	±14
HPP													
LK	13.8	38.8	25	4	0.6	8.3	23	27	326	273	47	161	65
SY	11.3	34.1	75	18	1.0	10.3	12	18	532	260	50	104	106
PK	11.9	33.8	75	10	0.8	8.7	21	32	257	212	22	139	51
NA	12	34	25	8	0.7	7.7	12	29	180	187	56	104	36
IA	12	34.3	25	7	0.6	8.8	11	10	198	184	46	98	40
ÖB	12.4	36.6	25	6	0.5	8.7	10	20	192	284	61	185	38
NS	12.2	33.3	–	10	0.9	9.3	10	24	218	203	66	93	44
MA	11.9	35.5	–	9	0.8	9.2	12	28	183	191	52	102	37
KA	11.4	32.8	–	11	0.7	8.6	26	46	177	172	49	88	49
Average	12.1	34.8		9.2	0.7	8.8	15.2	26	251	218	50	119	52
St. dev.	±0.7	±2		±4	±0.2	±0.7	±6.2	±10	±115	±42	±12	±34	±22
IDDP													
GK	11.8	33.7	–	6	0.7	10.7	13	6	176	153	53	48	35
BA	13.1	38.5	–	10	0.7	9.5	13	13	115	179	53	103	23
TI	13.1	37.9	–	10	0.9	9.8	25	33	82	256	84	156	16
Average	12.7	36.7		8.7	0.77	10	17	17.3	124	196	63.3	102	24.7
St. dev.	±0.7	±2.6		±2.3	±0.1	±0.6	±6.9	±14	±48*	±53	±18	±54	±9**
GDP													
GY	12.3	34	25	13	0.5	9.4	23	19	382	257	46	137	76
NS	12.2	33.3	–	10	0.9	9.3	10	24	218	203	66	93	44
HD	12.5	33.7	–	8	0.7	9.2	8	14	462	228	58	78	92
Average	12.3	33.6		10.3	0.7	9.3	13.7	19	354	229	56.7	102	70
St. dev.	±0.1	±0.3		±2.5	±0.2	±0.1	±8.1	±5	±124	±27	±10	±31	±24
OP													
HA	12	35.4	–	8	0.7	8.3	10	27	231	173	52	75	46
GB	10.2	29.9	–	10	0.6	9.2	20	15	295	217	52	106	59
GD	11.9	34.1	25	3	0.6	8.4	18	58	147	202	50	125	29
Average	11.3	33.1		7	0.63	8.6	16	33.3	224	197	51.3	102	44.7
St. dev.	±1	±2.9		±3.6	±0.1	±0.5	±5.3	±22	±74	±22	±1.1	±25	±15

(*) $P<0.05$, and (**) $P<0.02$, statistically significant when compared to corresponding control value

Clinical biochemical parameters, such as TG and VLDL, were different only in the IDDP group when compared to the control. The significantly low TG and VLDL levels of these patients seem to be a result of a diet with limited carbohydrate and fat.

Though hypertriglyceridemia is a well-characterised feature of preeclampsia [13], we did not find an antepartum increase in plasma triglyceride or VLDL levels of the HPP group when compared to the controls. As seen in Table 2, the HPP group consists of true preeclampsia (new gestational hypertension with significant proteinuria) and of gestational hypertension without proteinuria. The low triglyceride and VLDL levels of the latter three patients caused a decrease in the total average.

In conclusion, the present study shows that oxidative stress/lipid peroxidation accompanies the complications of hypertension-preeclampsia, insulin-dependent diabetes mellitus and oligohydramnios during pregnancy. Beside TBARs, erythrocyte GST and erythrocyte Se-GPx seem to be appropriate biomarkers for showing the status of oxidative stress in these diseases. The validation of these biomarkers for monitoring the efficiency of antioxidant supplementation in complications should be further investigated. This supplementation may provide the prevention and/or attenuation of malformation risks in investigated complications.

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