

# Lipofuscin, homocysteine and tissue polypeptide specific antigen in gestational hypertension

R. PARTYKA<sup>1</sup>, B. CHMIEL<sup>2</sup>, J. SIKORA<sup>2</sup>, T. GRABOWSKA<sup>1</sup>, B. WRÓBEL<sup>2</sup>,  
A. PRAISNER<sup>2</sup>, A. PIÓRO<sup>1</sup>, P. JAŁOWIEICKI<sup>1</sup> & D. KOKOCIŃSKA<sup>1</sup>

1. Dept. of Clinical Immunodiagnosics, Medical University of Silesia, Katowice, Poland

2. Dept. of General, Transplantation and Vascular Surgery, Medical University of Silesia, Katowice, Poland

*Correspondence to:* Bogdan Chmiel,  
Department of General, Vascular and Transplantation Surgery,  
Medical University of Silesia, ul. Francuska 20/24, 40-027, Katowice, Poland  
FAX: +48322555052  
EMAIL: chmiel@slam.katowice.pl

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## Abstract

**OBJECTIVE:** The aim of this study were to assess the levels of lipofuscin (parameter of oxidative stress), homocysteine (as a marker of vascular injury) and tissue specific antigen – TPS – (as a marker of cell proliferation) in relation to arterial pressure of pregnant woman.

**STUDY DESIGN:** Healthy pregnant women (n=18), women with mild 140/90= $\leq$ RR<160/100 (n=19), and severe 160/100= $\leq$ RR (n=23) gestational hypertension were enrolled. Lipofuscin has been determined by fluorescence spectroscopy, homocysteine by MEIA commercial kit from Abbott IMx, TPS by EIA method using kit from BEKI.

**RESULTS:** Mean duration of gestation was 34 $\pm$ 5 weeks, and there were no differences between groups. Serum lipofuscin levels in mild form of pregnancy-induced hypertension were decreased comparing to normal pregnancy. Homocysteine levels were decreased and TPS levels increased in both mild and severe gestational hypertension.

**CONCLUSION:** Our results suggest overestimation of the role of oxidative stress and hyperhomocysteinemia in gestational hypertension.

## INTRODUCTION

Gestational hypertension (GH) is placenta-mediated disease and complicates about 10% of pregnancies [1].

The etiology of GH is not fully understood, but excess free radical formation and accumulation of lipid peroxidation products have been cited [2]. Lipofuscin (LIP) is believed to be the last product of lipid peroxidation. Some evidence suggest, that

lipofuscin is a species of reaction of malonyldialdehyde with aminoacids, proteins and phosphatidylethanolamine [3]. Elevated serum lipofuscin levels in many situations associated with oxidative stress are reported [4,5].

Homocysteine is a one of the risk factor of atherosclerosis and hypertension [6]. Hyperhomocysteinemia has been linked to impaired endothelial function at several levels, including reduced nitric oxide synthesis, enhanced oxidative vascular

injury, and increased vascular smooth muscle cell proliferation [7]. Pregnant woman revealed lower levels of homocysteine comparing to non-pregnant [8], but data about homocysteine concentration in preeclampsia are conflicting. No difference in serum homocysteine level between healthy pregnant and preeclamptic women has been observed [9], but in early pregnancy (15<sup>th</sup> week) serum homocysteine levels from woman, which developed preeclampsia in the future, is higher comparing to normal pregnant women [10]. Another studies revealed association of elevated homocysteine concentration with the higher risk for preeclampsia, prematurity and very low birth weight [11]. Homocysteine can be a marker of placental disorders [12].

Tissue polypeptide specific antigen (TPS) is a component of cytokeratine 18, and is a marker of cell proliferation especially in oncology [13]. Elevated serum levels of TPS have been detected not only in cancer, but in inflammatory diseases such ulcerative colitis and hepatitis too [14]. After delivery both in mother and child TPS concentration are higher comparing to non pregnant women and decreases with time [15].

Taken together, oxidative stress, homocysteine metabolism disturbances and cell proliferation are involved in pathophysiology of GH. Since the aim of this study was to assess the relationship of the serum levels of lipofuscin, homocysteine and TPS according to arterial pressure of pregnant woman.

**Table 1.** Mean standard deviation, median, lower and upper quartile of duration of gestation (weeks).

	N	MEAN	STD	MEDIAN	Q25	Q75
Healthy pregnant women	18	35.4	6.08	38.0	32.0	40.0
Mild GH	20	33.0	3.87	33.0	31.0	36.0
Severe GH	22	33.2	4.5	34.0	29.0	37.0

No significant difference (ANOVA).

**Table 2.** Mean and standard deviation of creatinine, lipofuscin, homocysteine and TPS concentration in the serum of pregnant women.

	Creatinine [mg/100ml]	Lipofuscin [a.u.]	Homocysteine umol/l	TPS unit/l
Healthy pregnant women	0.71±0.07	240±6.5	7.78±1.11	60.5±12.2
Mild GH	0.72±0.07	226±14.9 ***	5.04±1.1 ***	78.2±19.5 *
Severe GH	0.82±0.07 ***	236±6.3	5.01±1.05 ***	155.9±45.0 ***

Significant increase in concentration of creatinine in severe form of pregnancy induced hypertension. Significant decrease of lipofuscin level in mild form of gestational hypertension. Significant decrease in homocysteine concentration and increase in TPS level in both mild and severe form of gestational hypertension. ANOVA and post hoc Tukey test; \* p<0.05; \*\*\* p<0.001

## MATERIAL AND METHODS

Healthy pregnant women (n=18), women with mild (n=20), and severe (n=22) gestational hypertension were enrolled. Women with arterial hypertension before pregnancy, abnormal hematologic, hepatic or renal test were excluded. Gestational hypertension was defined according to National High Blood Pressure Education Program Working Group [16]. Mild pregnancy induced hypertension was diagnosed when sustained elevation of arterial pressure greater or equal than 140/90 of mm Hg was detected. Severe pregnancy induced hypertension was defined when diastolic pressure (DP) was higher or equal than 110 or systolic pressure (SP) was higher or equal than 160 of mm of Hg. Arterial pressure was measured by manual mercury sphygmomanometer. Diastolic pressure was determined according to Korotkoff IV sound. If initial arterial pressure was higher than normal, measurement was repeated after four hours. Arterial pressure measuring and blood taking was performed during routine visits in out-patient clinic. Mean arterial pressure (MAP) was defined as follows: MAP=DP+1/3 (SP-DP).

Lipofuscin (LIP) has been determined according to Roumen [3]. Briefly, 150 µl of serum was added to 5 ml ethanol-ether (3:1 vol/vol) vortexed and centrifugated (5 min 3 000 g). Supernatant was removed and the sediment washed again with 5 ml ethanol-ether (3:1 vol/vol). The obtained sediment was dissolved in 3 ml phosphate buffer solution pH 7.0. The fluorescence intensity of the solution was measured at excitation wave length of 345 nm and emission wave length of 430 nm, both slits 5 nm with Perkin-Elmer MPF-44 fluorescence spectrophotometer. Concentrations of lipofuscin were expressed as arbitrary units [a.u.]. By definition: 100 arbitrary units of lipofuscin is a fluorescence intensity of 0.1 µg/ml quinine sulfate in 0.1 N sulfuric acid solution at ex/em 345/430.

Homocysteine was assessed by MEIA commercial kit from Abbott IMx (USA). Limit detection of this assay is 0.05 µmol/l. TPS was measured by EIA method using kit from BEKI (Sweden) with limit detection of 20 unit/l.

Statistical analysis was performed by ANOVA with post hoc Tukey test, or Kolmogoroff-Smirnoff non parametrical test when appropriate. For quantifying the relationships between variables Pearson correlation coefficient, and multiple linear regression was used.

## RESULTS

Average duration of gestation at the time of blood collection was 33.8 weeks (min 21.0, max 41.0), and there were no differences between groups (Table 1). Serum creatinine concentration in women fall in normal range, but in women with severe form of GH was significantly higher comparing to normal pregnant women (Table 2). Lipofuscin concentration in plasma of women with mild GH was significantly lower comparing to normal pregnancy. A decrease in lipofuscin concentration in

plasma from severe GH did not reach a significance. Both forms of GH revealed significantly lower plasma levels of homocysteine comparing to normal gestation. Opposite, women with GH revealed significant increase in TPS concentration in serum comparing to healthy pregnant women (Table 2). Mean arterial pressure was positively correlated with TPS and creatinine, and negatively with homocysteine and lipofuscin. TPS was negatively correlated with homocysteine and positively with creatinine. Homocysteine was positively correlated with lipofuscin (Table 3).

Multiple linear regression analysis shown that mean arterial pressure was associated with TPS (beta 0.56), homocysteine (beta -0.3), and creatinine (beta 0.18) concentration, but not with lipofuscin (Table 4).

## DISCUSSION

The main findings of this study is an increase in TPS and decrease in lipofuscin and homocysteine concentration in gestation induced hypertension comparing to healthy pregnant women. Our results confirm the other investigations. Bancher-Todesca and coworkers [17] revealed increase in serum TPS concentration of woman with gestational induced hypertension comparing to normal pregnancy. TPS concentration was positively correlated with disease severity. Elevated TPS concentration in the serum of women with GH and positive correlation between TPS and mean arterial pressure suggest more important influence of placenta, because placenta can be a source of cytokeratine[18]. Assessment of cytokeratin 18 neoepitope formation within tissue of the placenta is a marker of apoptosis[19]. Experiments on cancer cell culture revealed after induction of apoptosis more than fourfold increase in concentration of TPS within the medium [20]. Therefore it is likely elevated levels of TPS in GH can reflect an increase in the apoptosis rate within placenta.

A decrease in lipofuscin level in mild GH suggest absence of oxidative stress in this condition. Measurements of end product of lipid peroxidation in the serum of pregnant women have shown conflicted results. Gratacos [2] revealed increase in MDA level in preeclamptic comparing to normal pregnant women but Davidge [21] did not show any differences. Another team revealed normal level of lipid peroxidation products in preeclampsia but elevated in woman with HELLP (hemolysis, elevated liver enzymes, and low platelet count) syndrome – the most severe form of preeclampsia [22]. Zusterzeel *et al.* [23] using high specific method of measuring of oxidative stress by exhaled ethane revealed no changes both in GH and preeclampsia. Authors conclude that GH and preeclampsia are associated with minor alterations in antioxidant levels without signs of oxidative stress [23]. Recent data suggest that supplementation of antioxidants to pregnant women can even increase a risk of being admitted antenatally for hypertension and being prescribed antihypertensive drug [24].

**Table 3.** Spearman's rank correlation's coefficients of investigated parameters.

	Mean arterial pressure	Tps	Homocysteine	Creatinine
Tps	0.77 p=0.000			
Homocysteine	-0.57 p=0.000	-0.48 p=.0001		
Creatinine	0.54 p=0.00002	0.4 p=0.001	-0.13 p=0.34	
Lipofuscin	-0.26 p=0.042	-0.14 p=0.3	0.53 p=0.00001	0.06 p=0.642

**Table 4.** Multiple regression analysis. Independent: TPS, homocysteine, creatinine, lipofuscin; dependent – mean arterial pressure.

	Beta	St. error of Beta	B	St. error of B	p-value
Intercept			91.66	26.5	0.001
TPS	0.56	0.093	0.179	0.03	0.000000
Homocysteine	-0.30	0.089	-2.989	0.9	0.0013
Creatinine	0.18	0.089	35.689	17.2	0.043
Lipofuscin	-0.038	0.084	-0.055	0.1	0.65

R=0.83 R<sup>2</sup>= 0.70; corrected R<sup>2</sup>= 0.68; F(4,55)=32.66 p<0.00000 st. err. of est.: 9.75.

We observed a decrease in homocysteine concentration in GH comparing to normal pregnancy. Suggestion exist that decreased level of homocysteine during pregnancy is not depend on folic acid supplementation, hemodilution, and a decrease in serum albumin. The reasons of decreased homocysteine levels in GH comparing to normotensive pregnancy are not clear. It is well established that estrogen concentration in woman is negatively correlated with homocysteine concentration [25] and can prevent homocysteine metabolic disturbances and decrease concentration of this compound [26]. But estrogens revealed no influence on maternal blood pressure during pregnancy [27]. Hyperhomocysteinemia can reflect oxidative stress. We found a decrease in lipofuscin and homocysteine level in GH, together with significant correlations between this parameters. It suggest a presence of shift from oxidative stress toward antioxidative state in GH.

Taken together, our results suggest in gestation induced hypertension overestimation of the oxidative stress and homocysteine metabolism disturbances and underestimation of inappropriate cell proliferation within placenta.

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