

# State of biochemical homeostasis in women with course of pregnancy complicated by miscarriage

V.G. Syusyuka<sup>1</sup>, V.O. Potapov<sup>2</sup>, I.F. Belenichev<sup>1</sup>, A.V. Abramov<sup>1</sup>, N.M. Soloviova<sup>1</sup>

<sup>1</sup>State Medical University of Zaporozhye

<sup>2</sup>State Institution «Dnipropetrovska Medical Academy of Ministry of Health of Ukraine»

*The objective:* estimate oxidative-antioxidant homeostasis in women with pregnancy course complicated by miscarriage.

*Materials and methods.* 90 women in II and at the beginning of III trimesters of pregnancy were examined. In order to estimate peculiarities of biochemical homeostasis in women with pregnancy course complicated by miscarriage there were studied 44 women with pregnancy course complicated by miscarriage (the main group) and 46 women without clinical manifestations of miscarriage during pregnancy (comparison group). Withdrawal criteria were diseases of cardiovascular, urinary systems and endocrine pathology. Average age of pregnant women in the main group was 27.3±1.5 years and 27.4±1.1 years – in comparison group (p>0.05). Markers of oxidative modification of proteins (OMP) in blood serum were analyzed by spectrophotometric method. State of antioxidative system of protection was determined in accord with level of activity of superoxide dismutase, catalase, glutathione reductase, glutathione peroxidase and glutathione-S-transferase. Variational and statistical processing of results was made using STATISTICA 13 – license standard application program packages for multidimensional statistical analysis.

*Results.* During study of oxidation-reduction processes in pregnant women who had pregnancy complicated by miscarriage there was revealed that level of OMP exceeded the similar indicators among women without clinical manifestations of miscarriage during current pregnancy (p<0.05). Investigations of superoxide dismutase revealed statistically significant (p<0.05) decrease of activity of enzymes in women with pregnancy course complicated by miscarriage compared to pregnant women without such complication. It should be noted that statistically significant (p>0.05) difference between groups during analyzing of catalase activity was not revealed. Studies of condition of thiol-disulfide system namely activity of erythrocytic glutathione reductase and glutathione peroxidase indicate decrease of their level in women with pregnancy course complicated by miscarriage compared to women without such complication but statistically significant difference (p>0.05) was not found.

*Conclusion.* Analysis of biochemical investigations shows that even in conditions of keeping and progression of pregnancy in women with complication such as miscarriage the antioxidant protection is shifted along with activation of peroxidation processes. Such features are the manifestation of oxidative stress in the present group of pregnant women along with deprivation of enzymic and non-enzymic links of protective antioxidant system.

*Key words:* pregnancy, complications of pregnancy, miscarriage, oxidative modification of proteins, antioxidative system of protection.

## Стан біохімічного гомеостазу у жінок, перебіг вагітності яких ускладнився невиношуванням

В.Г. Сюсюка, В.О. Потапов, І.Ф. Беленічев, А.В. Абрамов, Н.М. Соловійова

*Мета дослідження:* оцінювання окиснювально-антиоксидантного гомеостазу у жінок, перебіг вагітності у яких ускладнився невиношуванням.

*Матеріали та методи.* Обстежено 90 жінок у II та на початку III триместрів вагітності. Для оцінювання особливостей біохімічного гомеостазу у жінок з ускладненим перебігом вагітності було проведено дослідження у 44 жінок (основна група), перебіг вагітності яких ускладнився невиношуванням вагітності (НВ), та у 46 жінок без клінічних проявів НВ у період даної вагітності (група порівняння). Критерієм виключення були захворювання серцево-судинної, сечової систем та ендокринна патологія. Середній вік вагітних в основній групі становив 27,3±1,5 року і 27,4±1,1 року – у групі порівняння (p>0,05).

Дослідження маркерів окиснювальної модифікації білків (ОМБ) оцінювали у сироватці крові спектрофотометричним методом. Стан антиоксидантної системи захисту визначали за рівнем активності супероксиддисмутази, каталази, глутатіонредуктази, глутатіонпероксидази та глутатіон-S-трансферази. Варіаційно-статистичне оброблення результатів здійснювали з використанням ліцензованих стандартних пакетів прикладних програм багатовимірного статистичного аналізу STATISTICA 13.

*Результати.* Під час вивчення окисно-відновлювальних процесів у жінок, перебіг вагітності у яких ускладнився НВ, встановлено, що рівень ОМБ перевищував аналогічні показники жінок без клінічних проявів НВ у період даної вагітності (p<0,05). Дослідження супероксиддисмутази виявило достовірне (p<0,05) зниження активності фермента у жінок, перебіг у яких ускладнився НВ, порівняно з вагітними, у яких такого ускладнення не було. Слід зазначити, що статистично достовірної (p>0,05) різниці між групами під час оцінювання активності каталази не виявлено. Вивчення стану показників тиол-дисульфідної системи, а саме – активності еритроцитарної глутатіонредуктази та глутатіонпероксидази, свідчить про зниження їхнього рівня у жінок, перебіг вагітності у яких ускладнився НВ, порівняно з вагітними, у яких такого ускладнення не було. Проте статистично достовірної різниці (p>0,05) не встановлено.

*Заключення.* Аналіз біохімічних досліджень свідчить про те, що навіть в умовах збереження та прогресування вагітності у жінок, у яких перебіг гестації ускладнився невиношуванням, виявляли порушення антиоксидантного захисту на тлі активації процесів пероксидації. Такі особливості є проявом оксидативного стресу у даного контингенту вагітних на тлі депривації як ферментативної, так і неферментативної ланки антиоксидантної системи захисту.

*Ключові слова:* вагітність, ускладнення вагітності, невиношування, окиснювальна модифікація білків, антиоксидантна система захисту.

## Состояние биохимического гомеостаза у женщин, течение беременности которых осложнилось невынашиванием

В.Г. Сюсюка, В.А. Потапов, И.Ф. Беленичев, А.В. Абрамов, Н.Н. Соловьева

*Цель исследования:* оценка окислительно-антиоксидантного гомеостаза у женщин, течение беременности которых осложнилось невынашиванием.

*Материалы и методы.* Обследовано 90 женщин во II и в начале III триместров беременности. Для оценки особенностей биохимического гомеостаза у женщин с осложненным течением беременности было проведено исследование у 44 женщин (основная группа), течение беременности которых осложнилось невынашиванием (НВ), и у 46 женщин без клинических проявлений НВ в период данной беремен-

ности (группа сравнения). Критерием исключения были заболевания сердечно-сосудистой, мочевой систем и эндокринная патология. Средний возраст беременных в основной группе составил  $27,3 \pm 1,5$  года и  $27,4 \pm 1,1$  года – в группе сравнения ( $p > 0,05$ ).

Исследование маркеров окислительной модификации белков (ОМБ) оценивали в сыворотке крови спектрофотометрическим методом. Состояние антиоксидантной системы защиты определяли по уровню активности супероксиддисмутазы, каталазы, глутатионредуктазы, глутатионпероксидазы и глутатион-S-трансферазы. Вариационно-статистическую обработку результатов осуществляли с использованием лицензированных стандартных пакетов прикладных программ многомерного статистического анализа STATISTICA 13.

**Результаты.** При изучении окислительно-восстановительных процессов у женщин, течение беременности у которых осложнилось НБ, установлено, что уровень ОМБ превышал аналогичные показатели женщин без клинических проявлений НБ в период данной беременности ( $p < 0,05$ ). Исследование супероксиддисмутазы выявило достоверное ( $p < 0,05$ ) снижение активности фермента у женщин, течение беременности которых осложнилось НБ, по сравнению с беременными, у которых данного осложнения не было. Следует отметить, что статистически достоверной ( $p > 0,05$ ) разницы между группами при оценке активности каталазы не обнаружено. Изучение состояния показателей тиол-дисульфидной системы, а именно – активности эритроцитарной глутатионредуктазы и глутатионпероксидазы, свидетельствует о снижении их уровня у женщин, течение беременности которых осложнилось НБ, по сравнению с беременными, у которых такого осложнения не было. Однако статистически достоверной разницы ( $p > 0,05$ ) не установлено.

**Заключение.** Анализ биохимических исследований свидетельствует о том, что даже в условиях сохранения и прогрессирования беременности у женщин, у которых течение гестации осложнилось невынашиванием, выявляли сдвиг антиоксидантной защиты на фоне активации процессов перекисидации. Такие особенности являются проявлением оксидативного стресса у данного контингента беременных на фоне депривации как ферментативного, так и неферментативного звеньев антиоксидантной системы защиты.

**Ключевые слова:** беременность, осложнения беременности, невынашивание, окислительная модификация белков, антиоксидантная система защиты.

Miscarriage as one of the important problems of saving of reproductive potential requires detailed study in order to prevent natural abortions, premature birth and birth of low weight babies. Preterm infants are the main «contribution» to indicators of perinatal disease and death rate. Such pathology covers 60–75% of such infants [1, 14]. Investigation of miscarriage problems and understanding of the main links of its pathogenesis are one of priority directions of modern obstetrics. Rate of this pathology makes almost 30% and doesn't trend to decrease. It has direct influence on demographic situation in the country [7, 9]. Miscarriage is included into group of obstetrical syndromes which responsible for high level of mother and perinatal diseases and death rate. Now they obtained name «great obstetrical syndromes» and they consist of preeclampsia, fetus growth inhibition syndrome, premature break of birth membranes, premature birth, fetus death, late spontaneous abortions [8].

Tissue hypoxia which is considerably connected with processes of free radical peroxidation of lipids and mechanism of antioxidant protection is very important in miscarriage pathogenesis. Lack of antioxidants for support of homeostasis in women with miscarriage leads to exhaustion of blood antioxidant protective system that, in its turn, causes significant increase of radical formation processes [12]. Oxidative stress can have destructive influence on cellular structures of placenta. During investigation of placenta tissues it was found that in case of complicated pregnancy course (threatening miscarriage, gestosis, urogenital infection) the morphological changes, which consist of damage of biomembranes and change of immune homeostasis, are obvious in placenta tissues [9]. Long activation of free-radical oxidation processes is the base of pathogenesis of different pathological conditions. Besides the connection between nature of pathological process and changes of markers of organism protective antioxidant system is found [4]. In case of miscarriage the development of oxidative stress in post-mitochondrial fraction of placenta is connected with change of activity of enzymes of glutathione dependent link of antioxidant protection [9]. In case of miscarriage insufficiency of antioxidants for support of homeostasis in women causes the exhaustion of antioxidant protective system in blood that, in its turn, enables significant increase of radical formation processes [12].

Numerical data as to antioxidant status and also efficiency of use of different antioxidants in case of miscarriage are available in literature. However the question of further adaptation of woman's organism including biochemical adaptation for women with pregnancy course complicated by miscarriage is still unsolved.

The objective: estimate oxidative-antioxidant homeostasis in women with pregnancy course complicated by miscarriage.

## MATERIALS AND METHODS

90 women in II and at the beginning of III trimesters of pregnancy were examined. In order to estimate peculiarities of biochemical homeostasis in women with pregnancy course complicated by miscarriage there were studied 44 women with pregnancy course complicated by miscarriage (the main group) and 46 women without clinical manifestations of miscarriage during pregnancy (comparison group).

Withdrawal criteria were diseases of cardiovascular, urinary systems and endocrine pathology. Average age of pregnant women in the main group was  $27,3 \pm 1,5$  years and  $27,4 \pm 1,1$  years – in comparison group ( $p > 0,05$ ). Significant differences according to social and professional structure were not revealed.

Markers of oxidative modification of proteins in blood serum were analyzed by spectrophotometric method with wave length of 270 nm (aliphatic aldehydedinitrophenylhydrazones of the main aminoacid residues – APH) and 363 nm (carbonyl dinitrophenylhydrazones of the main aminoacid residues – CPH). Analysis of oxidative modification of proteins was made according to the method of B. Halliwell and its level was expressed in standard units per 1 gram of protein (s.u./g protein). Markers of oxidative destruction of proteins are the most informative markers of oxidative damage of functional macromolecules. They are random and special and products of this destruction are the markers of early oxidative stress. Protein oxidative modification causes decrease of protein function. Oxidated proteins can be the source of free radicals and exhaust reserves of cell antioxidants and mediate oxidative damages of DNA [10].

Level of catalase was determined by spectrophotometric method with wave length of 410 nm against test sample. Activity of superoxide dismutase was determined by method of Chevary S. and it was expressed in s. u./mg protein/minutes. Activity of glutathione reductase, glutathione peroxidase and glutathione-S-transferase was registered spectrophotometrically with wave length of 340 nm and expressed in  $\mu\text{mol NDP/gHb}$ ,  $\text{ME/gHb}$ ,  $\text{ME/gHb}$  and in  $\text{mmol/minutes/gHb}$  correspondingly [5, 6, 10, 13].

Each pregnant woman was interviewed as to expediency of additional research methods and the consent for research was obtained. Research meets modern requirements of ethical and moral principles with respect to ICH / GCP rules of Declaration of Helsinki (1964), Conference of Council of Europe on human rights and biomedicine and provisions of legal acts of Ukraine.

Variational and statistical processing of results was made using STATISTICA 13 – license standard application program packages for multidimensional statistical analysis.

Table 1

**Indicators of oxidative modification of proteins in blood serum in pregnant women of groups under investigation, Me (Q25; Q75)**

Indicators	Pregnant women with miscarriage, n=44	Pregnant women without miscarriage manifestations, n=46	P
APH (spontaneous), s.u./g protein	4.2 (3.4; 4.8)	3.2 (2.1; 3.9)	p<0.05
CPH (spontaneous), s.u./g protein	3,3 (2.5; 4.4)	2.3 (1.7; 3.3)	p<0.05
APH (stimulated), s.u./g protein	5,8 (5.0; 7.1)	4.5 (3.2; 5.6)	p<0.05
CPH (stimulated), s.u./g protein	4.0 (3.1; 6.2)	3,3 (2.8; 4.1)	p<0.05

Table 2

**Activity of superoxide dismutase and catalase in pregnant women of groups under investigation, Me (Q25; Q75)**

Indicators	Pregnant women with miscarriage, n=44	Pregnant women without miscarriage manifestations, n=46	P
Superoxide dismutase, s.u./mg protein/minutes	7.7 (5.8; 10.2)	9.5 (6.7; 16.3)	p < 0.05
Catalase, µcat/l	11.5 (9.6; 14.2)	11.4 (9.4; 14.4)	p > 0.05

Table 3

**Indicators of thiol-disulfide system in pregnant women of group under investigation, Me (Q25; Q75)**

Indicators	Pregnant women with miscarriage, n=44	Pregnant women without miscarriage manifestations, n=46	P
Glutathione reductase, µmol/ NDP/ g Hb	5.3 (4.,0; 7.7)	5,9 (4.1; 9.1)	p > 0. 05
Glutathione peroxidase, ME/g Hb	8.4 (6.2; 14.6)	11.2 (6.7; 24.7)	p > 0.05
Glutathione-S-transferase mmol/minutes/g Hb	10.3 (8.7; 12.3)	10.0 (7.6; 11.6)	p > 0.05

### RESEARCH RESULTS AND THEIR DISCUSSION

Markers of oxidative destruction of proteins are the most informative markers of oxidative damage of functional macromolecules. They are random and special and products of this destruction are the markers of early oxidative stress. Protein oxidative modification causes decrease of protein function. Oxidated proteins can be the source of free radicals and exhaust reserves of cell antioxidants and mediate oxidative damages of DNA [10]. During study of oxidation-reduction processes in pregnant women who had pregnancy complicated by miscarriage there was revealed that level of APH and CPH during spontaneous course of oxidation process exceeded the similar indicators among women without clinical manifestations of miscarriage during current pregnancy (Table 1).

Stimulation of oxidation process with Fenton medium showed that adaptative antioxidant reserves of blood serum are stipulated by functioning of antioxidant protective system. Research results of metal-catalyzed oxidative modification of proteins of serum indicate the rise of content of markers of blood protein (APH and CPH) oxidative damage in pregnant women of both groups. However increase of APH and CPH content in pregnant women without clinical manifestations of miscarriage was less significant and indicators were statistically and significantly (p<0.05) lower compared to group of group of pregnant women with pregnancy course complicated by miscarriage.

Study of adaptation mechanism of pregnant woman organism to oxidative stress stipulates determination of activity of the main enzymes of antioxidant protection. In conditions of increase of oxygen radicals the expression of redox-sensitive gens among which there are gens of glutathione peroxidase, catalase, superoxide dismutase, glutathione reductase etc. Protective antioxidant system is very important in regulation of organism vital activity. In case of different diseases the disorder of functioning of antioxidant system occurs [2]. The reason of oxidative stress can be not only the rise of free radicals but also decrease of efficiency of antioxidant systems of organism [11].

Investigations of superoxide dismutase revealed statistically significant (p<0.05) decrease of activity of enzymes in women with pregnancy course complicated by miscarriage compared to pregnant women without such complication. It should be noted that statistically significant (p>0.05) difference between groups during analyzing of catalase activity was not revealed (Table 2).

Estimation results of indicators of thiol-disulfide system in women with miscarriage and without its manifestations are presented in Table 3.

Studies of condition of thiol-disulfide system namely activity of erythrocytic glutathione reductase and glutathione peroxidase indicate decrease of their level in women with pregnancy course complicated by miscarriage compared to women without such complication but statistically significant difference (p>0.05) was not found.

Markers indicating decrease of antioxidant ability of blood serum in pregnant women are the reserves of reduced glutathione which

content was statistically and significantly ( $p < 0.05$ ) lower in women with pregnancy course complicated by miscarriage. Total content of thiol compounds was also decreased but statistically significant ( $p > 0.05$ ) difference was not revealed.

### CONCLUSION

Analysis of biochemical investigations shows that even

in conditions of keeping and progression of pregnancy in women with complication such as miscarriage the antioxidant protection is shifted along with activation of peroxidation processes. Such features are the manifestation of oxidative stress in the present group of pregnant women along with deprivation of enzymic and non-enzymic links of protective antioxidant system.

### Сведения об авторах

**Сюсюка Владимир Григорьевич** – Кафедра акушерства и гинекологии Запорожского государственного медицинского университета, 69035, г. Запорожье, просп. Маяковского, 26; тел.: (099) 098-82-55, (098) 735-83-82. E-mail: [svg.zp.ua@gmail.com](mailto:svg.zp.ua@gmail.com), [svg.zp@i.ua](mailto:svg.zp@i.ua)

**Потапов Валентин Александрович** – Кафедра акушерства и гинекологии ГУ «Днепропетровская медицинская академия МЗ Украины», 49044, г. Днепр, ул. В.Вернадского, 9

**Беленичев Игорь Федорович** – Кафедра фармакологии и медицинской рецептуры Запорожского государственного медицинского университета, 69035, г. Запорожье, ул. Сталеваров, 31

**Абрамов Андрей Владимирович** – Кафедра патологической физиологии Запорожского государственного медицинского университета, 69035, г. Запорожье, ул. Сталеваров, 31

**Соловьева Наталия Николаевна** – Кафедра акушерства и гинекологии Запорожского государственного медицинского университета, 69035, г. Запорожье, просп. Маяковского, 26

### REFERENCES

1. Annual report on the state of health of the population, the sanitary-epidemic situation and the results of activity of the health care system of Ukraine. 2017 Ministry of Health of Ukraine, State Institution «WISD Ministry of Health of the country». – Kyiv: Medinform IEC, 2018. – 458 p.
2. Antioxidants: a clinical and pharmacological aspects / Chekman I.S., Belenichev I.F., Gorchakova N.A. et al. // Ukrainian Medical Journal. – 2014. – N 1 (99). – P. 22–28.
3. Batrak N.V., Malyshkina A.I. Risk factors for habitual miscarriage // Bulletin of the Ivanovo Medical Academy. – 2016. – Vol. 21, N 4. – P. 37–41.
4. Biochemical indicators of endotoxemia / Peshev S.L., Chudaykin A.N., Peshev L.P., Fomina O.A., Pesheva O.N. // Bulletin of the Peoples' Friendship University of Russia. Series «Medicine». – 2010. – N 3. – P. 88–93.
5. Chevari S., Chaba I., Szekey J. The role of superoxide dismutase in the oxidative processes of a cell and a method for determining it in biological materials // Laboratornoe delo. – 1985. – N 11. – P. 678–681.
6. Danilova L.A. Handbook of laboratory research methods. SPb.: Peter, 2003. – 736 p.
7. Miscarriage of pregnancy: current views on the problem (literature review) / I.I. Vorobyova, A.A. Zhivetskaya-Denisova, V.B. Tkachenko, N.V. Rudakova, S.M. Tolkach // Health of woman. – 2017. – N 3 (119). – P. 113–117.
8. Nosenko E.N., Zhuk S.I., Rutinskaya A.V. Problematic issues of large obstetric syndromes // Zhinochiy Likar. – 2017. – N 2. – P. 28–25.
9. Pathophysiological role of free radical processes in pregnancies end in miscarriage / Sosnova E.A., Bolevich S.B., Pokaleneva M.Sh., V.F. Snegirev // Archives of Obstetrics and Gynecology. – 2016. – N 3 (3). – P. 136–140.
10. Preclinical study of the specific activity of potential drugs of primary and secondary neuroprotection: a method. recommendations / I.S. Chekman, I.F. Belenichev, O.O. Nagornaya et al. Kiev: TOV «Vidavnicтво «Juston», 2016. – 80 p.
11. Pristrom A.M., Benhamed M. Oxidative stress and cardiovascular disease // Lechebnoe business. – 2012. – N 1 (23). – P. 21–28.
12. Sidorova I.S., Unanyan A.L. Miscarriage: impaired antioxidant defense and its correction // Russian Bulletin of the Obstetrician-Gynecologist. – 2009. – N 1. – P. 14–16.
13. The method of estimation of catalase activity / Korolyuk M.A., Ivanova L.I., Mayorova N.T., Tokarev V.E. // Laboratornoe delo. – 1988. – N 1. – P. 16–18.
14. von Linsingen R., Bicalho MDG., de Carvalho NS. Baby born too soon: an overview and the impact beyond the infection // J Matern Fetal Neonatal Med. – 2017. – N 30 (10). – P. 1238–1242.

Статья поступила в редакцию 20.11.2019