Mechanisms Of Oxidative Stress and Maternal-Fetal Involvement

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Abstract

Oxidative stress is manifested at the maternal-fetal interface from the beginning of pregnancy. It plays a role both in the normal development of the placenta and in the pathophysiology of complications: miscarriage, preeclampsia, intrauterine growth restriction (IUGR) and premature rupture of membranes. We studied from an immunohistochemical point of view nine placentas from pregnant women between the ages of 35 and 40 who had miscarriages in the absence of an obvious medical cause with a control group consisting of 9 normal, up to term placentas. We studied the anti-Gluthatione antibody and METH1 patterns of staining. The results showed an obvious association of low values of GLUTH and METH1 with miscarriages.

Keywords: oxidative stress, miscarriages, superoxide radicals, preeclampsia

INTRODUCTION

Oxidative stress is a process generated by the imbalance between the production and accumulation of reactive oxygen species (by-products of oxygen metabolism) in cells and tissues and the ability of a biological system to detoxify these reactive products. Even though oxidative stress is a pathological condition, harmful to the human body, is sometimes used as a treatment for some conditions, such as cancer, with a certain degree of clinical success. [1]

Free radicals are molecules that contain oxygen with an unequal number of electrons which allows them to easily interact with other molecules, sometimes causing chain reactions, oxidation reactions. They can be beneficial or, on the contrary, harmful. Superoxide radicals (O2-), hydrogen peroxide (H2O2), hydroxyl radicals (OH-) and singlet oxygen ($^1O2$) are reactive oxygen species (ROS). ROS results from metabolic processes mainly in mitochondria, both physiologically and pathologically. [2,3,4] The production of reactive oxygen species is mainly based on enzymatic and non-enzymatic reactions. The enzymatic reactions capable of generating ROS are those involved in the respiratory chain, prostaglandin synthesis, phagocytosis and the cytochrome P450 system. [5-15] Superoxide can also be generated by the leakage of electrons from the shorter electron transport chain within the endoplasmic reticulum (ER). [16] Other sources of superoxide under physiological conditions are: the enzyme nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, which generates substantial amounts throughout pregnancy, but especially at the beginning of gestation, cytochrome P450 and other redoxases. Various growth factors, drugs and toxins cause an increase in the amount of ROS. [17,18] Superoxide is converted by superoxide dismutase enzymes to hydrogen peroxide. Hydrogen peroxide is not a free radical, so it is less reactive than superoxide. However, it is included in the term ROS because it is closely involved in the generation and detoxification of free radicals. Because it is nonpolar, it is able to diffuse across cell membranes and therefore acts as a second-order messenger in signal transduction pathways. [5-15]

Antioxidants are molecules that can donate an electron to a free radical without losing its stability. Following such a reaction the free radical stabilizes and becomes less reactive. Cells initiate an antioxidant defense system based primarily on enzymatic components, such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), to protect against cell damage induced by reactive oxygen species. [19] Superoxide is converted to hydrogen peroxide by two isoforms of superoxide dismutase (SOD): manganese SOD which is limited to mitochondria and copper-zinc SOD which is in the cytosol. Catalase or glutathione peroxidase (tetrameric selenoprotein) breaks down hydrogen peroxide into water. The activity of glutathione peroxidase depends on the presence of reduced glutathione (GSH) as a hydrogen donor.[20]

Oxidative stress is manifested at the maternal-fetal interface from the beginning of pregnancy. It plays a role both in the normal development of the placenta and in the pathophysiology of complications: miscarriage, preeclampsia, intrauterine growth restriction (IUGR) and premature rupture of membranes. [21,22] It is accepted that placental development takes place at a relatively low concentration of oxygen, being supported by the secretions of the endometrial glands rather than by the maternal circulation. [23,24] Other researchers suggest that this environment protects the developing embryo from oxygen free radical-mediated teratogenesis. [25] The onset of circulation is associated with a tripling of the oxygen concentration in the placenta. [22] This will stimulate higher rates of generation of reactive oxygen species, especially in the critical syncytiotrophoblastic layer, at which the concentrations of antioxidant enzymes, copper-zinc superoxide dismutase and catalase are low. Consequently, the villi taken from the peripheral region of the placenta have high levels

of chaperone HSP70, nitrotyrosine residues - which indicate the formation of peroxynitrite - and reveal degenerative morphological changes in the syncytiotrophoblast, compared to specimens taken from the central region. Molecular evidence confirms that this apoptotic cascade is activated in peripheral villi and that this would be sufficient to explain their regression. [34-36]

Aim and objectives

It is known that the Glutathione (GLUTH) acts to minimize the oxidative stress in the tissue and it's protective mechanism is corelated with it's high expression. We aimed to quantify and prove this expression in case of miscarriages of unknown causes and also to analyze methionine synthase reductase (METH1) behavior from an immunohistochemical point of view.

MATERIAL AND METHODS

Our study consisted of a pilot group of nine placentas from pregnant women between the ages of 35 and 40 who had miscarriages in the absence of an obvious medical cause. The second group was represented by the control group consisting of 9 placentas from patients aged between 35 and 40 who had normal up to term gestation periods and gave birth to healthy children.

An immunohistochemical analysis was performed on 4 µm-thick sections prepared from formalin-fixed paraffin- embedded tissue by using an automated immunostainer (Bechmark XT, Ventana Medical Systems Inc., Tucson, AZ, USA). Immunohistochemical assays were performed on a Ventana Benchmark XT automated staining instrument according to the manufacturer's instructions. Slides were deparaffinized using EZprep solution (Ventana Medical Systems, Inc.) at 900C, and all reagents and incubation times were chosen as directed on antibody package inserts. Slides were developed using the OmniMap DAB (3,3'- diaminobenzidine) detection kit (Ventana Medical Systems, Inc.) and counterstained with Hematoxylin. [26-28] Sections were incubated with anti-Gluthatione primary polycloclonal antibody (ab9443, rabbit, IgG, cytoplasmatic, Abcam, Cambridge, CB2 0AX, UK) in accordance with the manufacturer's protocol.[29] The second antibody was METH1 (methionine synthase reductase) for sections incubation (primary polycloclonal antibody, rabbit, IgG, cytoplasmatic, Abmart,219 McMane Avenue, Berkeley Heights, NJ 07922, US) in accordance with the manufacturer's protocol. [30] Negative control was undertaken by omitting the primary antibody on the same section type. The specimens were analyzed by two skilled pathologists and were double blinded according with H score. The H score is determined by multiplying the percentage of cells demonstrating each intensity (scored from 0 to 3) and adding the results. There are 300 possible values. In this system, <1% positive cells is considered to be a negative result.[31] We used Leica DM3000 led microscope with intelligent automation and LAS EZ software (provided by Leica Biosystem) for capture of images and measurements.

RESULTS

The following table shows the H scores for each patient in both the control group and the study group. Glutathione expression is very high in the control group compared to the study group. In the latter there are values ranging from 0 to 205. (Table 1)

Table 1. Dispersion of H score values in the two studied groups

Nr crt	0	1+	2+	3+	**H-score**	**STUDY GROUP H Score**
1	90	10	0	0	**10**	290
2	0	0	0	0	**0**	290
3	0	0	0	0	**0**	295
4	0	20	55	25	**205**	285
5	80	20	0	0	**20**	280
6	0	80	20	0	**120**	290
7	0	0	0	0	**0**	280
8	0	35	35	30	**195**	295
9	0	0	0	0	**0**	295
Average					**61.11**	288.88

The comparative evaluation of the average values from the two groups shows us that the value in the study group is almost 5 times lower, which is associated with an increased oxidative stress.

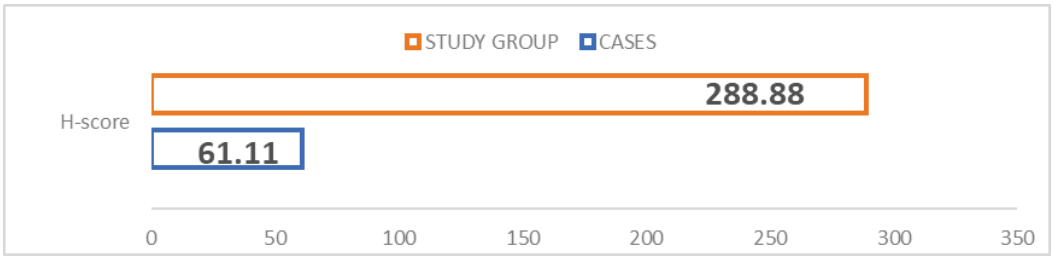

Figure 1. The average values of the H score in the groups included in the study

Analysis of mean values for METH1 revealed a smaller difference in the group of patients with comparative miscarriage compared to patients in the control group. (Fig. 2) The dispersion of the absolute values of the H score in the studied group is much smaller compared to the dispersion of the values obtained in the case of GLUTH. (Fig.3) The minimum value was 10 and the maximum was 280. (Table 2)

It's easily noticeble that in a case of miscarriage the value obtained was higher than the average value of the H score obtained in the control group.

Table 2. Dispersion of H score (METH1) values in the two studied groups

Nr crt	0	1+	2+	3+	**H-score**	**STUDY GROUP H score**
1	0	0	20	80	**280**	285
2	30	40	25	5	**105**	270
3	10	35	30	25	**170**	275
4	0	10	40	50	**240**	295
5	40	50	10	0	**70**	285
6	0	25	25	50	**225**	235
7	90	10	0	0	**10**	280
8	10	30	30	30	**180**	210
9	10	45	25	20	**155**	295
Average					**159.44**	270

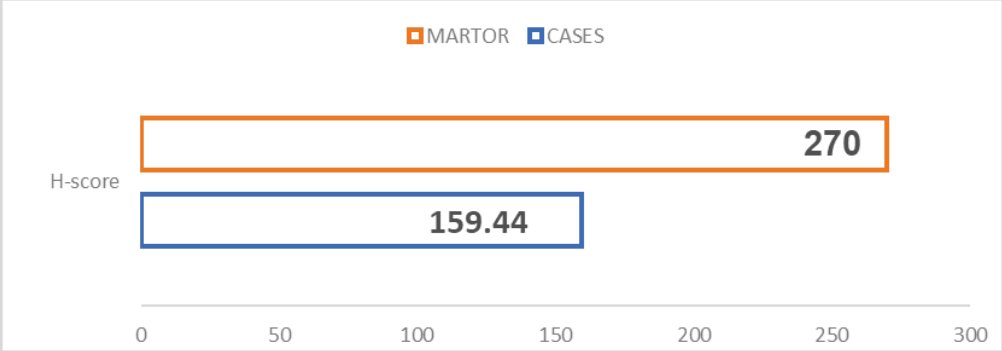

Figure 2. The average values of the H score (METH1) in the groups included in the study

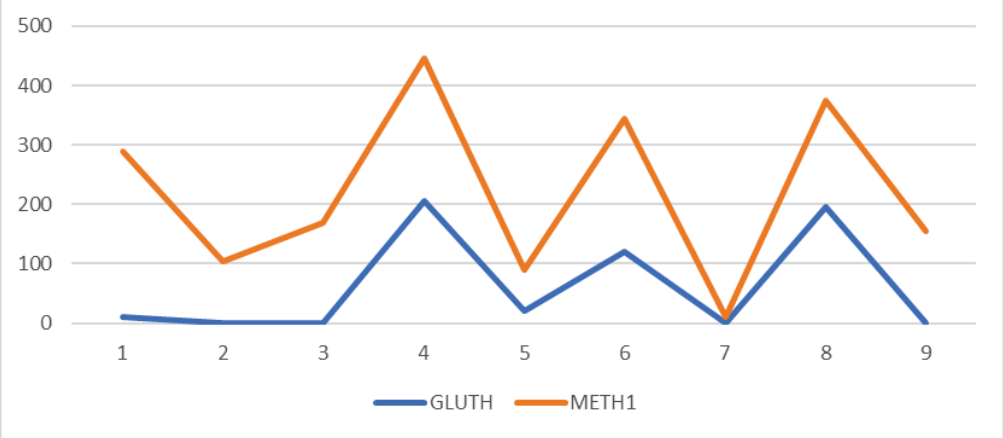

Figure 3. Dispersion of H score (METH1/GLUTH) values in the two studied groups

DISCUSSIONS

The concept of pro-oxidant-antioxidant balance is essential for understanding oxidative stress for several reasons. First, this concept emphasizes that the disturbance can be caused by changes that can occur in either part of the balance: like abnormally high generation of ROS, or deficiencies in antioxidant defense, or structural changes occurred in proteins or potentially conformational isomers that could cause their enzymatic activity to be lost or to be impaired. [15.32] Uncontrolled, oxidative stress and/or decreased protective mechanisms, and therefore glutathione levels (as our results have also sugested) can be responsible for inducing several conditions, both chronic and degenerative, can accelerate the aging process, are associated with cancer, Parkinson's disease, Alzheimer's disease and can also cause acute conditions (e.g. stroke). [37,1] The concept of equilibrium draws attention to the fact that there will be a gradual response to oxidative stress. Therefore, minor balance disturbances can lead to homeostatic adjustments in response to changes in the immediate environment, while major disturbances can lead to irreparable damage and cell death. The line between physiological and pathological changes is difficult to specify. It is currently estimated that complex interactions occur between oxidative stress and other forms of cellular stress, such as endoplasmic reticulum (ER) stress. [33]

It is known that the glutathione acts to minimize the oxidative stress in the tissue. The results obtained by us reveal a very high H score of GLUTH expression for patients who gave birth at term, almost 5 times fold (4,71x) then for patients with miscarriages. The high expression of glutathione is associated with a very high antioxidant protection mechanism. The analysis of the results obtained in the group of patients with miscarriages reveals that in 6

cases included in the study the values of the H score is 0 or close to 0. It is obvious that in these cases there was no antioxidant protection or it was very low being below the sensitivity limit of the method used. All this leads us to believe that the values of oxidative stress are high in miscarriages. In several studies, the decrease or enzyme deficiency of methionine synthase reductase is associated with an increase in homocysteine values, associated with blindness, neurological symptoms, atherosclerosis, congestive heart failure, age-related disease and birth defects.[38].

The results obtained by us are consistent with studies conducted so far. For patients with miscarriages, there is a low value of methionine synthase reductase compared to the results obtained in the control group. The analysis of the mean values of the H score in the case of METH1 in the groups included in our study reveals a decrease of 41% in the values in the case of spontaneous abortions compared to the control group. The comparative distribution of curves for GLUTH and METH1 reveals a parallel between the mechanisms of antioxidant protection. Low glutathione levels have been associated with low methionine synthase reductase levels which validates our results. Unfortunately for our pilot study, the results may be obscured by the small number of cases studied. For this reason, we believe that further research needs to be done in the future.

CONCLUSIONS

The results we obtained highlights, as expected, that low values of Glutathione (GLUTH) are associated with miscarriages but also the values of Methionine Synthase Reductase (METH1) in such conditions are low.

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