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Total antioxidant status as marker of oxidative stress in infants with intrauterine growth restriction

Șadiye-Ioana Scripcariu[1,2], Andreea Avasiloaiei[1,2*], Demetra Socolov[1,2], Elena Mihălceanu[1,2], Daniela-Cristina Dimitriu[2,3], Mihaela Moscalu[4], Maria Stamatin[1,2]

1. Department of Mother and Child Health, "Grigore T. Popa" University of Medicine and Pharmacy, Iași, România

2. "Cuza-Vodă" Clinical Hospital of Obstetrics and Gynecology, Iași, România

3. Department of Preclinical Sciences, "Grigore T. Popa" University of Medicine and Pharmacy, Iași, România

4. Department of Interdisciplinary Sciences, "Grigore T. Popa" University of Medicine and Pharmacy, Iași, România

Abstract

***Aim**: The aim of this study is to identify correlations between total antioxidant status values of mothers and their infants and compare these values in accordance to the presence or absence of intrauterine growth restriction.* ***Material and methods**: This is a prospective, comparative study performed over a period of 3 years on a number of 52 infants and their mothers. Thirty-six of them had intrauterine growth restriction and 16 were appropriate for their gestational age and were used for comparative purposes. General information regarding the mother, infant and pregnancy were recorded. In addition, total antioxidant status was assessed from blood samples, taken right before delivery from mothers and from the cord blood in infants.* ***Results**: We found significant differences between total antioxidant status both between mothers and neonates with IUGR (intrauterine growth restriction) versus without IUGR ($p=0.018$, and $p<0.001$, respectively). In addition, in both groups, there was a significant direct correlation between maternal and neonatal values of serum total antioxidant status (TAS) ($p<0.001$). In* ***conclusion**, we can say that TAS values, as an important marker of the oxidative status of patients, are correlated with the presence of IUGR and values recorded from blood samples of the mother may be predictive for the oxidative status of the infant, thus of IUGR.*

***Keywords:** neonatal, total oxidative status, intrauterine growth restriction, oxidative status*

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Introduction

Intrauterine growth restriction (IUGR) is an important health issue, affecting approximately 5-10% of pregnancies worldwide. It is defined as "the impaired growth and development of the embryo and/or its organs during gestation" (1). Knowledge of the mechanisms implicated in

***Corresponding author**: Andreea Avasiloaiei, Department of Mother and Child Health, "Grigore T. Popa" University of Medicine and Pharmacy, Iași, Romania. E-mail: andreea.avasiloaiei@umfiasi.ro

IUGR is important for preventing the appearance of this pathological entity, mainly because fetal growth is influenced by both genetic, epigenetic and environmental factors that impact, among other mechanisms, uteroplacental blood flow and, thus, transfer of nutrients and oxygen from mother to fetus. We can see that oxygen plays an important role in fetal development.

In the normal organism, there is a balance between oxidant and antioxidant factors, so that the former does not harm cells (2). The break of this balance caused either by the increase in reactive oxygen species (ROS), or by the decrease in antioxidants, will determine molecules in the organism, such as lipids, proteins, and DNA to be damaged (3).

In the prenatal period, abnormal placentation leads to a reduction of perfusion and, subsequently, it leads to ischemia reperfusion injury to the placenta; this, in turn, results in the generation of reactive oxygen species (4). However, there are limited published data on oxidative stress in IUGR. Markers of oxidative stress have been found both in newborns with IUGR and in their mothers – in blood samples, placenta and amniotic fluid (5, 7). Moreover, studies conducted by Biri et al. have shown that maternal plasma levels of superoxide dismutase were higher in comparison with the control group, in contrast with placental levels that showed no significant differences between the two groups (7). Regarding the values of total antioxidant status (TAS) and total oxidant status (TOS), different studies in the literature reach contradictory conclusions. Thus, whereas Takagi et al. (5) identify lower serum TAS and higher TOS levels in the IUGR group, Mert et al. (4) show increased TAS and TOS in IUGR as well as in preeclampsia groups. Total oxidant and antioxidant capacity are means of exploring the relationship between oxidative stress and antioxidant factors, thus they can express the risk of development of IUGR as a result of oxidative stress during pregnancy.

The aim of this study is to identify correlations between TAS values of mothers and their infants and compare these values in accordance to the presence or absence of IUGR.

Material and Methods

We performed a prospective study over three years (2016-2018) in our maternity hospital on a group of 52 infants. Thirty-six infants had IUGR, diagnosed over the second or third trimester of pregnancy and were also small-for-gestational-age (SGA) – below the 10th percentile on the intrauterine growth curves (study group). Sixteen appropriate-for-gestational-age (AGA) infants, from healthy pregnancies were used as controls. We excluded from our study infants with congenital malformations of any kind.

At birth, we recorded the following information: infant gestational age, weight, length, Roehr's ponderal index and we took blood samples from both mother and infant in order to analyze serum TAS. The samples, which were taken immediately prior to delivery from peripheral blood in mothers and from the cord blood in infants, were preserved up to 36 hours in the refrigerator (+2-+8°C) or in the freezer up to 14 days at -20°C. The analysis was performed on an RX Imola® automated wet chemistry analyser (Randox Laboratories Ltd., Crumlin, County Antrim, Northern Ireland), at a wavelength of 600 nm, using the ABTS technique, with Randox reagents. As reference for TAS, we used values between 1.23 and 1.77 mmol/L. Hemolyzed samples were considered inadequate and discarded.

The study was approved by the Hospital's ethics committee by decision number 1124/01.02.2017. Informed consent was taken from the infant's parents prior to any harvest of biologic material being done.

The data were analyzed using SPSS 24. (SPSS, Chicago, IL, USA). Numerical variables of continuous type were expressed as means and stan-

dard deviations, and for comparison, the t-student test or Mann-Whitney U test were applied. To evaluate the correlation among variables, the correlation coefficient was calculated, and statistical significance was assessed through the Pearson test. Statistical significance was defined as $p<0.05$.

Results

The infants we included had a mean gestational age of 36.6 weeks, a mean birth weight of 2456 grams, a mean length of 47.1 cm and a mean ponderal index of 2.25, with significant statistical differences between the two groups (Table 1). Serum TAS values in the mothers were between 0.89 and 1.98 mmol/L, with statistically significant differences ($p=0.018$) between the mean values in the IUGR group (1.32 mmol/L) and the non-IUGR group (1.46 mmol/L) (Table 2, Figure 1A).

TAS values determined in the serum of neonates were between 0.9 and 2.08 mmol/L and also showed significant differences ($p<0.001$) between the means we obtained in the IUGR group (1.34 mmol/L) and the ones in the non-IUGR group (1.61 mmol/L) (Table 2, Figure 1B).

In both groups, we found a significant direct correlation between maternal and neonatal values of serum TAS ($p<0.001$ for both groups) (Figure 2).

Table 1. Characteristics of the studied groups

Characteristic (†)	Total group	Study group	Control group	P-value (‡)
Gestational age (weeks)	36.6±2.8	35.8±2.9	38.3±1.4	<0.001*
Birth weight (g)	2456.1±830.9	2033.8±620.7	3406.9±252.7	<0.001*
Birth length (cm)	47.1±4.8	45.2±4.7	51.2±1.2	<0.001*
Ponderal index (kg/cm^3)	2.25±0.3	2.12±0.3	2.48±0.13	<0.001*

† mean± standard deviation; ‡ t-student test or Mann-Whitney U Test; (*) Marked effects are significant at $p<0.05$

Table 2. TAS values in studied groups

TAS values (†)	Total group	Study group	Control group	P-value (‡)
Mothers (mmol/L)	1.36±0.23	1.32±0.24	1.46±0.19	0.018*
Infants (mmol/L)	1.42±0.26	1.34±0.25	1.61±0.18	<0.001*

mean± standard deviation; † t-student test; ‡ Mann-Whitney U Test; (*) Marked effects are significant at $p<0.05$

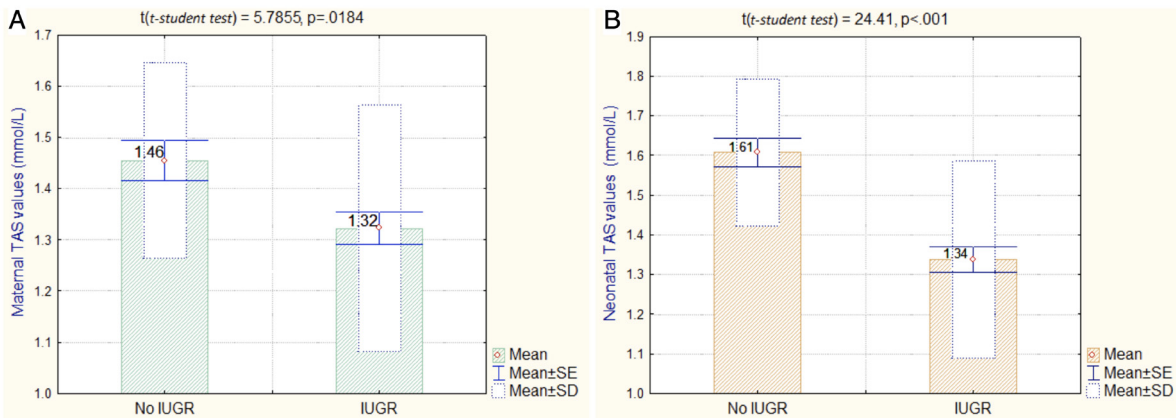

Fig 1. Mean values of (A)maternal and (B)neonatal serum TAS (mmol/L)

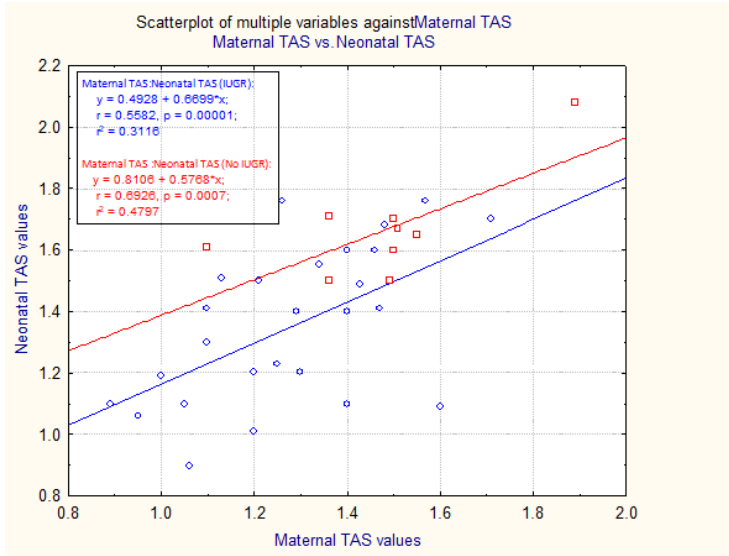

Fig 2. Regression analysis of maternal versus neonatal serum TAS in IUGR group and non-IUGR group

Discussions

Under physiological circumstances, reactive oxygen species, as byproducts of oxidative stress, are cleared from the cell environment by antioxidant molecules – both enzymes and vitamins. Antioxidants are responsible for the protection of cells from peroxidation reactions, the limitation of cellular damage, as well as the maintenance of cellular membrane integrity (8).

TAS represents an overall measure of the antioxidant activity of biological fluids and it provides an integrated parameter, rather than the simple sum of measurable antioxidants, by taking into account their synergistic interaction (9) thus providing an integrated parameter rather than the simple sum of measurable antioxidants. The capacity of known and unknown antioxidants and their synergistic interaction is therefore assessed, thus giving an insight into the delicate balance in vivo between oxidants and antioxidants. Measuring plasma AC may help in the evaluation of physiological, environmental, and nutritional factors of the redox status in humans. Determining plasma AC may help to identify conditions affecting oxidative status in vivo (e.g., exposure to reactive oxygen species and antioxidant supplementation.

Although it is known that IUGR is a condition marked by limited antioxidant defense, some studies investigating antioxidant activity in IUGR show conflicting results. Toy et al. showed that serum TAS levels were significantly lower in women with IUGR pregnancies, compared to the control group, whereas TOS levels and the oxidative stress index were significantly higher in the IUGR group compared to controls (10).

Biri et al. showed that plasmatic levels of malondialdehyde and xanthine oxidase, as main products of lipid peroxidation and also glutathione peroxidase and superoxide dismutase, were all significantly higher in women with IUGR than in the control group (7).

Hracsko et al. showed that infants with IUGR have lower values of antioxidant enzymes and lower ferric reducing ability of plasma, as a measure of plasmatic antioxidant capacity, and also higher malondialdehyde values (11).

Our study showed, in a similar fashion, that both mothers and infants from IUGR pregnancies

have significantly lower values of serum TAS, compared to those from normal pregnancies. Moreover, there is a significant direct correlation of maternal and neonatal TAS values, both in IUGR and non-IUGR pregnancies.

By contrast, the research performed by Mert et al. on women whose pregnancies were complicated with preeclampsia and IUGR, showed markedly elevated levels of both total oxidant status and TAS when compared with healthy pregnant women (4). That would imply that although oxidative stress is markedly high in IUGR pregnancies, there are enough resources to counteract its deleterious effects, as high levels of TAS in the cord blood of infants from pre-eclamptic mothers are presumably protective against oxidative stress (12). Although we also opted to study TAS in the cord blood of IUGR infants, we could not assume the same protective effect.

In the extensive study by Saker et al., birth weight has been proved to be an important factor in the alteration of oxidant-antioxidant balance: both SGA infants and their mothers had low plasma total antioxidant activity and low values for antioxidant vitamins (C and E), when compared to their AGA peers (13). Interestingly enough, although mothers of LGA infants had antioxidant values comparable with mothers of AGA infants, LGA infants themselves had low antioxidant activity, similar to that of SGA infants.

One of the strong points of our study is that it demonstrates that the antioxidant status of the IUGR infant is closely linked to that of its mother and, thus, can be influenced by it. Oxidative stress in IUGR pregnancies can be reduced to a point by antioxidant supplementation, such as vitamins and trace elements. Not only is this difficult to quantify objectively but also, IUGR pregnancies are more often than not, high-risk pregnancies, due to possible malnourishment or other conditions of the mother, such as pre-eclampsia or hypertension.

Maternal malnourishment ultimately leads to intrauterine malnutrition, which is linked to a rise in oxidative stress. Gupta et al. ascertained this in their study, by the increase of malondialdehyde activity and decrease of antioxidant enzymes, such as superoxide dismutase, catalase and reduced glutathione (14) catalase, reduced glutathione, and serum malondialdehyde (MDA. Pre-eclampsia is an independent risk factor for oxidant-antioxidant imbalance (1). Infants from mothers with pre-eclampsia have high lipidic peroxidation markers and low TAS, as demonstrated by Namdev et al. Moreover, in their study, it was proved that the level of oxidative stress was linked to poor neonatal outcome, such as necrotizing enterocolitis, sepsis or respiratory distress (15).

Obesity and its link to oxidative stress may be another risk factor for the appearance of IUGR. Thus, the study of comorbidities that obesity can be associated with and the link between them through oxidative stress has been an interesting subject in literature (16). An interesting fact is that, while ischemia-modified albumin has not been demonstrated to be associated with obesity as a result of oxidative stress in this pathology (17), studies in literature link ischemia-modified albumin to oxidative stress in pathologies such as stroke (18) and chronic heart failure (19). So, oxidative stress has been demonstrated as a pathophysiologic factor in multiple diseases, but the mechanisms through which it functions are still incompletely investigated, making this subject an interesting and promising one.

The importance of personalized medicine (20) is an emerging concept that is sustained by the data presented in this article. Biomarkers, especially ones with predictive value may have benefits in lowering perinatal morbidity and mortality and are applicable in a wide array of pathologies, from sepsis to necrotizing enterocolitis

(21). In addition, in perinatology, the difficulty resides not only in the delicacy of the patient, but also in the ambiguous pathophysiology of certain diseases.

As complete blood counts are useful in the positive and differential diagnosis of neonatal sepsis (22), the evaluation of antioxidant capacity of the human body is important for the identification of oxidative stress and, subsequently, its adverse effects (23).

The small number of included patients derives, on the one hand, from the low number of IUGR not associated with other comorbidities or congenital malformations and, on the other hand, from the relatively low number of correctly followed pregnancies with IUGR and the low compliance of these patients (who, unfortunately, quite often come from more precarious living environments) to the investigations that need to be done in order to correctly diagnose IUGR and link it to oxidative stress (24) whereas intrauterine growth restriction (IUGR. Nonetheless, the problem at hand is of utmost importance and the number of patients has not been an issue from a statistical point of view, thus we do not consider this a weak point of this study.

The major limitations of our study are the small number of patients and the lack of biochemical determinations from placenta and amniotic fluid that could further support our claims.

Taking into consideration that smoking and drinking are important factors that influence oxidative stress parameters, we consider that this issue may be addressed in future studies, by excluding these influencing factors, with the downside of further reducing the number of enrolled patients.

One other limitation could be the statistically significant difference in gestational age. However, while oxidative stress may be different in preterm and term infants, TAS is not influenced by gestational age, as it was demonstrated by Ferencz et al in their study on IUGR infants (25).

Conclusions

Through this study we managed to demonstrate that TAS values are significantly lower in mothers and infants with IUGR, as compared to mothers with normal pregnancies, resulting neonates without IUGR. In addition, we have shown that there is a direct correlation between TAS values of infants and their mothers, regardless of the presence or absence of IUGR.

In conclusion, we can say that TAS values, as an important marker of the oxidative status of patients, are correlated with the presence of IUGR and values recorded from blood samples of the mother may be predictive for the oxidative status of the infant, thus of IUGR.

Abbreviations

AGA – appropriate-for-gestational-age
DNA – deoxyribonucleic acid
IUGR – intrauterine growth restriction
LGA – large for gestational age
ROS – reactive oxygen species
SGA – small-for-gestational-age
TAS – total antioxidant status
TOS – total oxidant status

Author's contribution

SIS – Concept, Data collection, Literature search, Writing the article, Critical review
AA – Interpretation of results, Writing the article, Literature search, Critical review
DS – Literature search, Data acquisition, Interpretation of results, Critical review
EM – Literature search, Data acquisition, Critical review
DCD – Data processing, Literature search, Critical review
MM – Formal data analysis, Interpretation of results, Critical review
MS – Literature search, Writing the article, Critical review, Supervision

Conflict-of-interest statement

Authors declare no conflicts of interest.

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