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State of Enzymatic Antioxidant Defense System in Children Born after Assisted Reproductive Technologies

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Abstract

The study included examination of 45 children born after ARTs and 20 controls, which involved assessment of health status, presence of congenital developmental abnormalities, as well as data of neonatal history, to look for association with genetic developmental abnormalities of enzymes of antioxidant defense superoxide dismutase (SOD), catalase (CAT), as well as hypoxia-inducible factor (HIF). The study data showed relationship between incidence of some diseases with presence of mutation in these genes. Occurrence of heterozygous forms of these enzymes was associated with features of diseases found in subjects' histories: for SOD functional disturbances of digestive system (2.2 ± 2.2 %) r = 0.42, minor heart development abnormalities (11.1 ± 4.7 %) r = 0.34, atopic dermatitis (4.4 ± 3.1 %); for CAT urinary system diseases (42.1 ± 11.3 %), functional disorders of biliary tract (21.1 ± 9.4 %), minor heart abnormalities (52.6 ± 11.5 %), recurrent respiratory diseases (63.2 ± 11.1 %), retardation of physical (10.5 ± 7.0 %) and neural and mental development (21.1 ± 9.4 %), diseases of auditory and visual systems (21.1 ± 9.4 %); for HIF-1 minor heart abnormality (72.7 ± 13.4 %), signs of undifferentiated dysplasia of connective tissue (18.2 ± 13.4 %), manifestations of atopy (27.2 ± 13.4 %).

Keywords: Children Born after Assisted Reproductive Technologies; ART; Antioxidant Defense System; Superoxide Dismutase SOD2 (T58C); Catalase CAT (C-262T); Hypoxia Induced Factor HIF1A (Pro582Ser)

Abbreviations: SOD: Superoxide Dismutase; CAT: Catalase; HIF: Hypoxia-Inducible Factor; ROS: Reactive Oxygen Species; LPO: Lipid Peroxidation; ART: Assisted Reproductive Technologies; AOS: Antioxidant System; PCR: Polymerase Chain Reaction.

Introduction

Comprehensive assessment of health status takes into account the level of body functional capabilities in the setting of homeostatic self-regulation, as well as the range of body compensatory and adaptive reactions. One of important components of body homeostasis is the balance between pro-oxidant and antioxidant resources. The equilibrium between free-radical oxidation and functions of antioxidant system has substantial impact on cytochemical reactions [1-3]. Under physiologic conditions, the free-radical reactions involving reactive oxygen species induce the processes of lipid peroxidation necessary to regulate cell membrane permeability. Through activation of immunoglobulin synthesis, metabolites of oxygen participate in reactions of cell-mediated and humoral immunity, stimulate microbicide, cytotoxic and immunoregulatory effects, as well as affect processes of proliferation, differentiation, and apoptosis. However, excessive formation of reactive oxygen species (ROS) and activation of lipid peroxidation (LPO) may lead to development of oxidative stress. Excessive formation of ROS facilitates destruction of lysosomes, damages

cytoplasmic, mitochondrial and nuclear membranes, affects nuclear and mitochondrial genome, leads to impairment of microcirculation, disruption of permeability of blood-tissue barriers [4-6].

Oxidative stress initiates adaptive defensive mechanisms which restore redox status and regulate oxygen delivery to the cells. Antioxidant system (AOS) is responsible for depression of free-radical oxidative pathways and includes a number of enzymes, such as superoxide dismutase, catalase, glutathione-dependent peroxidases and transferases [7,8,3]. Activity of antioxidant defense pathways plays a major role in the status of adaptive and compensatory potential that body has at cellular level.

Imbalance of the antioxidant system that occurs due to various unfavorable factors may significantly affect occurrence of multiple morphological and functional shifts. Particularly, with the use of assisted reproductive technologies (ARTs) to address infertility, child develops in conditions that drastically differ from natural. Older reproductive age of parents, somatic and reproductive illness, compromised obstetric or gynecologic history, ovarian stimulation, use of medications during pregnancy, morbid conditions during neonatal period may cause discordance of antioxidant reactions and are serious predisposing factors to disruption of adaptive capabilities in a growing body [9-11]. Together with the environmental factors, genetic predisposition plays an important role in the pathogenesis of defects of regulation for key biochemical pathways. In view of the above, it is feasible to study the role of genes coding enzymes of antioxidant system (AOS) in children born after ARTs.

Purpose of the study: To study the state of enzymatic antioxidant defense system in children born after assisted reproductive technologies.

Materials and Methods

Forty-five children, 1 to 7 years old, born after ARTs, were examined. Gender structure had higher proportion of boys, $p > 0.05$. The control arm included 20 children, 1 to 7 years old, born naturally.

The examination routine included analysis of subject clinical and history data, laboratory and instrumental findings. The state of antioxidant defense was studied using molecular genetic analysis with determination of polymorphism features for the following enzyme genes: superoxide dismutase (SOD-2), catalase (CAT), as well as hypoxia-inducible factor (HIF-1).

Assessment of allele polymorphisms was performed using polymerase chain reaction (PCR) followed by restriction fragment analysis. Analysis involved amplification of regions in the specified genes using specific primers: SOD2 (T58C), CAT (C-262T), HIF1A (Pro582Ser).

Methods

Molecular genetic methods of investigation Isolation of DNA from cells of buccal epithelium was performed using modified Chelex method (2013). 200 μL of 5 % Chelex 100 solution in sterile distilled water (Chelex in the sodium form, 100–200 mesh, Bio-Rad) were added into an Eppendorf tube containing an applicator with a swab of epithelial cells. The tube was incubated at 56 °C for 30 minutes. After that, the tube was incubated at 96 °C for 8 minutes. After the incubation the tube was centrifuged (using Eppendorf Centrifuge 5424). Concentration and purity of the DNA preparation were determined with spectrophotometer (NanoPhotometer, Implen); the sample for this assay was taken directly from the tube with DNA solution (5 μL aliquot). For PCR, 5 μL of supernatant was taken.

Genotyping

Allele variants of genes SOD2 T58C, CAT C-262T were assessed using allele-specific polymerase chain reaction (PCR). The amplification of the investigated gene sites was performed concurrently in two Eppendorf tubes for wild-type and mutant gene variants in 20 μL of buffer solution and with 100 nmol of each oligonucleotide primer (Lytech, Russia), 100–150 ng DNA.

Allele variants of HIF1A Pro582Ser gene were detected using PCR-RFLP method by treating the amplification products with restriction enzyme NmuC1. The amplification was performed using thermal cycler CFX96 (Bio-Rad). Fractionation of amplification products was performed in horizontal 2 % agarose gel, prepared in 1x tris-borate buffer (1xTBE) with voltage of 100 V for 45 minutes. Molecular weight marker—DNA pUC19: Msp1.

A total of 246 PCR runs were performed in this study.
Statistical analysis of the acquired data was performed using Statistica 7 software package. Relationships of nominal variables were determined using Pearson's test. Non-parametric correlation analysis was used to evaluate significance and direction of correlation between parameters. Differences were considered statistically significant at $p < 0.05$.

Results and Discussion

Taking into account that development and growth of children born from induced pregnancy occurs in changed conditions, which may substantially differ from natural, and is often associated with exposure to a number of unfavorable factors,

it is feasible to study the functional reserve of a child's body and its adaptive capabilities. Exploration of the state of antioxidant defense systems is of special interest, because the imbalance of pro-oxidant and antioxidant resources facilitates loss of adaptation, worsening of tissue hypoxia, development of energy deficiency, and secondary damage to cell membranes.

To a certain degree, features of adaptation pathways at cellular level can be studied by analyzing individual components of the antioxidant system. Antioxidant enzymes superoxide dismutase (SOD) and catalase (CAT) are biochemical indicators of body's general resistance.

SOD is considered to be the key enzyme of antioxidant defense, with MnSOD being one of its isoenzymes. This isoenzyme is the one that is capable not only to neutralize the oxygen radical, but also to support balance of O_2-H_2O_2 in the intracellular space by regulating main signal channels, as well as block release of cytochrome C and proteins of Bcl-2 family into the cell cytoplasm from mitochondria, thus preventing development of apoptosis.

Assessment of SOD2 (T58C) polymorphism findings in the main arm of children demonstrated the presence of T/T genotype in 32 (71.1 ± 6.8 %), and T/C in 13 (28.9 ± 6.8 %) of subjects (see table). Analysis of occurrence of the allele variants showed domination of homozygotes (T/T genotype). Children with T/C genotype had history of functional disturbances of digestive system (2.2 ± 2.2 %) $r = 0.42$, minor heart development abnormalities (11.1 ± 4.7 %) $r = 0.34$, atopic dermatitis (4.4 ± 3.1 %). Among subjects with minor allele, girls were more prevalent ($p < 0.05$).

It should be noted that (38.4 ± 13.5 %) children with heterozygous variant of the gene had history consistent with complications in antenatal period (premature placental abruption, placental circulation insufficiency, oligohydramnios). In the group of homozygotes for the main allele (T/T), there were 25.0 ± 7.7 % premature births, while in the heterozygote (T/C) group there were 69.2 ± 12.8 %; $r = 0.51$, $p < 0.05$. Comparison of anthropometric data (body mass and length at birth) showed that impairment of physical development was reported more frequently in children with T/C genotype (69.2 ± 12.8 %), $p < 0.05$.

Evaluation of SOD-2 features in the compared groups found that presence of T/C genotype is significantly higher in ART-children (χ^2 4.88, $p < 0.05$). Absence of minor allele subjects (C/C) in ART-children arm and its low occurrence in the control arm are consistent with available literature [1].

Taking into consideration contingency for functioning of antioxidant factors, the state of catalase enzyme was evaluated concurrently. This enzyme is one of the "fastest" enzymes due to its effect on dismutation reaction.

In the ART-children arm, assessment of CAT (C-262T) polymorphism showed C/C genotype in 48.9 ± 7.5 %, C/T — in 42.2 ± 7.4 %, and T/T — in 8.9 ± 4.2 %. Domination of C/T genotype was associated with history of urinary system diseases (42.1 ± 11.3 %), functional disorders of biliary tract (21.1 ± 9.4 %), minor heart abnormalities (52.6 ± 11.5 %), recurrent respiratory diseases (63.2 ± 11.1 %), retardation of physical (10.5 ± 7.0 %) and neural and mental development (21.1 ± 9.4 %), diseases of auditory and visual systems (21.1 ± 9.4 %).

Presence of T/T polymorphism was found in four children. In the first case, despite the presence of the mutation, and after a pregnancy complicated by placental insufficiency, a girl was born at 39 weeks, with body mass 3310 g, body length 52 cm, and Apgar score of 7–8 points. The second child, a girl, was born at 38 weeks, with body mass 2840 g and body length 49 cm. The pregnancy had no complications. The other two had retardation of physical development with preterm birth. It is characteristic that minor heart development abnormalities were found in these children.

The complications of pregnancy were documented in 13.3 ± 5.1 % of cases and were associated with T/T genotype. Among preterm births, C/T genotype was more prevalent (19.0 ± 8.6 %). Deviations in variables of physical development at birth in children were associated with C/T genotype (31.6 ± 10.7 %).

General morbidity among children correlated with presence of hetero- and homozygous alleles, $r = 0.75$.
Frequency of occurrence of the minor allele (C/T + T/T) was 23 (51.1 ± 7.5 %). No gender differences were found with regard to subjects with CAT minor allele.

When compared to results of control arm, the ART-children arm had significantly higher proportion of C/C genotype and minor alleles (χ^2 4.08, $p < 0.05$). Taking into account the unfavorable effect of hypoxia conditions on child development and their association with pregnancy disorders, this study also involved analysis of hypoxia-inducible factor (HIF-1) in children born after ARTs.

HIF-1 is a dimeric protein complex, and it is one of the primary genes taking part in the process of homeostasis. It plays crucial role in body's reaction to low oxygen concentrations and hypoxia. Moreover, HIF can induce expression of glycolytic genes and affect ATP synthesis, and it also participates in glucose metabolism and regulates lipid homeostasis. Dysfunction of this factor is already determined during prenatal period, when presence of hypoxia of various

scale may lead to gross morphological abnormalities or disruption of fetal maturation. Complete deficiency of HIF-1α was shown to cause embryo death due to neural tube defects, cardiovascular malformations and death of cerebral cells.

Analysis of HIF1A (Pro582Ser) polymorphism features allowed to find C/C genotype in 71.1 ± 6.8 %, C/T in 24.4 ± 6.4 %, and T/T in 4.4 ± 3.1 %. Heterozygous genotype variant (C/T) was associated with history of minor heart abnormality (72.7 ± 13.4 %), signs of undifferentiated dysplasia of connective tissue (18.2 ± 13.4 %), manifestations of atopy (27.2 ± 13.4 %).

Genotype T/T was documented in two children. One boy was born at gestational age of 39 weeks, with body mass 3000 g, body length 51 cm, and Apgar score of 7–8 points. According to child's chart, he had history of the following diagnoses: syndrome of undifferentiated dysplasia of connective tissue, recurrent obstructive bronchitis, attention deficit and hyperactivity syndrome.

Complicated pregnancy was documented in 1 child (2.2 ± 2.1 %) (C/C genotype). Preterm births occurred more frequently for children with C/C genotype (15.6 ± 7.6 %). Deviations in variables of physical development at birth in children were also associated with C/C genotype (31.6 ± 10.7 %). Frequency of occurrence of the minor allele (C/T + T/T) was 28.8 ± 6.8 %. The subgroup of subjects with minor allele had higher proportion of boys, $p > 0.05$.

Compared to control arm, ART-children arm had genotype C/T, but the difference was not significant (χ^2 2.75, $p = 0.09$).

SOD2 T58C		C-262T CAT		HIF1A (Pro582Ser)	
Genotypes	n (%)	Genotypes	n (%)	Genotypes	n (%)
T/T	71.1 ± 6.8	C/C	48.9 ± 7.5	C/C	71.1 ± 6.8 %
C/T	28.9 ± 6.8	C/T	42.2 ± 7.4	C/T	24.4 ± 6.4 %
C/C	-	T/T	8.9 ± 4.2	T/T	4.4 ± 3.1 %
T-allele	0.7	C-allele	0.7	C-allele	0.7
C-allele	0.3	T-allele	0.3	T-allele	0.3
Deviation from genetic balance	$\chi^2 = 16.04$ $p < 0.0001$		$\chi^2 = 0.83$ $p < 0.04$		$\chi^2 = 16.04$, $p < 0.00006$

Table 1: Frequency of occurrence of genotypes and alleles studied in ART-children.

Thus, the obtained results allowed to find features of enzyme antioxidant system in children born after ARTs and to assess the significance of SOD2, CAT, and HIF-1 polymorphisms.

Conclusions

The significance of polymorphisms in the enzymes of antioxidant system in children born after ARTs was demonstrated: SOD2 T/C (χ^2 4.68, $p = 0.030$) and CAT (χ^2 3.85, $p = 0.049$).

It was shown that the state of antioxidant defense system in ART-children arm differed due to presence of predominantly homozygous variants of enzyme genotypes for SOD2 (T/T), CAT (C/C, T/T), HIF1 (C/C, T/T).

Occurrence of heterozygous forms of these genes was associated with features of diseases found in subjects' histories: for SOD, principally there were congenital heart abnormalities; for CAT, there were diseases of respiratory and urinary systems; for HIF-1, there were signs of connective tissue dysplasia and allergic reactions.

References

1. Ruiz-Sanz JI, Aurrekoetxea I, Matorras R, Ruiz-Larrea MB, (2011) Ala16Val SOD2 polymorphism is associated with higher pregnancy rates in in vitro fertilization cycles Fertil Steril 95(5): 1601-1605.

2. Emene CC, Kravchenko IE, Aibatova GI, Rizvanov AA (2017) Analysis of Serum Cytokines and Single-Nucleotide Polymorphisms of SOD1, SOD2, and CAT in Erysipelas Patients. Journal of Immunology Research pp: 14.

3. Vnukov VV, Panina SB, Milyutina NP, Krolevets IV, Zabrodin MA (2016) Analysis of the Relationship between Antioxidant Enzyme Gene Polymorphisms and Their Activity in Post-Traumatic Gonarthrosis. Bull Exp Biol Med 161(1): 75-78.

4. Scott RT, Treff NR, Stevens J, Forman EJ, Hong KH, et al. (2012) Delivery of a chromosomally normal child from an oocyte with reciprocal aneuploid polar bodies. J Assist Reprod Genet 29(6): 533-537.

5. Ricci G, Bogatti P, Fischer-Tamaro L, Giolo E, Luppi S, et al. (2011) Factor V Leiden and prothrombin gene G20210A mutation and in vitro fertilization: prospective cohort study. Hum Reprod 26(11): 3068-3077.

6. Kimura N, Tsunoda S, Iuchi Y, Abe H, Totsukawa K, et al. (2010) Intrinsic oxidative stress causes either 2-cell arrest or cell death depending on developmental stage of the embryos from SOD1-deficient mice. Mol Hum Reprod16(7): 441-451.

7. Komatsu K, Iwase A, Mawatari M, Wang J, Yamashita M, et al. (2014) Mitochondrial membrane potential in 2-cell stage embryos correlates with the success of preimplantation development Reproduction 147(5): 627-638.

8. Desmyttere S, Rycke MD, Staessen C, Liebaers I, Schrijver DF, et al. (2012) Neonatal follow-up of 995 consecutively born children after embryo biopsy for PGD Human Reproduction 27(1): 288-293.

9. Figueira RCS, Setti AS, Cortezzi SS, Martinhago CD, Braga DPAF, et al. (2012) Preimplantation diagnosis for β-thalassemia combined with HLA matching: first "savior sibling" is born after embryo selection in Brazil. J Assist Reprod Genet 29(11): 1305-1309.

10. Zheng HY, Shi XY, Wang LL, Wu YQ, Chen SL, et al. (2011) Study of DNA methylation patterns of imprinted genes in children born after assisted reproductive technologies reveals no imprinting errors: A pilot study Exp ther med 2(4): 751-755.

11. Kolesnikova LI, Bairova TA, Pervushkina OA (2013) Genes of Antioxidant Enzymes. Vestnik RAMN 68(12): 83-88.