Original Article

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The Effect of Antenatal L-Arginine and Antioxidant Supplementation on Oxidative Stress Marker Levels in Newborns

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ABSTRACT

Introduction:The present study was conducted in the Department of Obstetrics, Paediatrics & Biophysics IMS, BHU, Varanasi with an aim to study the role of L-arginine and oral antioxidants as a part of therapy in patients diagnosed with IUGR (Intra uterine growth restriction) and cord serum NO and oxidative stress markers GSH and SOD in newborns following delivery of such patients.

Materials and Methods: The study included 40 pregnant patient between 30-32 weeks of gestation diagnosed with IUGR who were divided into 3 groups: Group I (treated with L-arginine N=10), Group II (treated with antioxidants N=10), Group III (without nutritional supplementation N=10) and Group IV (healthy control pregnant patients of same gestational age range N=10) were taken. Cord serum NO & oxidative stress markers (GSH & SOD) were measured following delivery of patients from those four groups.

Results: The cord serum NO levels (µmol/lt) showed a significant increase & SOD (U/ml) & GSH (U/lt) values were increased in newborns to mothers diagnosed with IUGR after treatment with L-arginine. Similar results were obtained for treatment with antioxidants.

Conclusion: The reduced NO & reduced cord serum circulating levels of oxidative stress markers (GSH & SOD) activity may play an important role in the occurrence of IUGR.

Keywords: Antioxidants, L- arginine

INTRODUCTION

The most widely used definition of IUGR is a fetus whose estimated weight is below the 10th percentile for its gestational age and whose abdominal circumference is below the 2.5th percentile. It is estimated that 3 to 10% of infants are growth restricted. Approximately 6-30% infants in developing countries are classified as growth restricted [1]. The body contains a series of systems that form defense against free radicals. Several vitamins (A, C and E) have antioxidant properties. Anti-oxyenzymes as superoxide dismutase (SOD), catalase and glutathione peroxidase constitute important part of its defense. SOD, protects against superoxide radicals and catalyzes the transformation from superoxide to hydrogen peroxide [2-4].

Glutathione peroxidase is selenium dependent and selenium therefore, forms an important part of natural defense against free radicals [2]. Nitric oxide (NO) is an important regulator of placental perfusion, as it plays a role in placental vascular endothelial function [5]. Nitric oxide is synthesised from the physiologic precursor L-Arginine by the stereo specific enzyme, NO synthase in what is called the L-Arginine/NO pathway, and L-arginine is the only substrate for the production of NO.

The present study analyses cord serum levels of NO_2^-/NO_3^- and determines whether L-Arginine is effective in treating IUGR. The present study was also conducted to assess the status of oxidative stress in newborns born to the mothers diagnosed with IUGR by estimating the blood levels of free radical scavenger enzymes SOD and Reduced glutathione peroxidase)

MATERIALS AND METHODS

The present study was conducted in the Department of Obstetrics and Gynaecology, Pediatrics , NICU and Department of Biophysics Sir Sunder Lal hospital, Banaras Hindu University, Varanasi with 30 patients between age Group 20-32yrs after 30 -32wks gestation with fetuses diagnosed with growth restriction :

Group I (L-arginine supplementation, n=10)

Group II (those treated with antioxidants, n=10)

Group III (without nutritional supplementation, n=10)

and 10 patients after 30-32 weeks gestation with normal growth of fetuses which were considered as controls that is Group IV (Healthy controls, n=10) and cord serum levels were taken from neonates from all four groups following delivery of such mothers . Pregnancies with diagnosed growth restricted fetuses due to following were included in the study: Idiopathic IUGR, Pregnancy induced hypertension, Anaemia, Diabetes mellitus, Previous history of IUGR | Intrauterine death, APLAS (antiphospholipid antibody syndrome), Cyanotic heart disease, Pulmonary disease. Exclusion criteria: Antenatal cases with congenital anomalies and Intra uterine fetal death (IUFD) were excluded.

Study data was presented as percentage and Mean ± SD. various tests used to look for statistical significance was Chi-Square (2) & student t-test.

COLLECTION OF SAMPLE

Cord blood were collected, plasma separated by centrifugation and subjected to biochemical tests immediately stored at _20^{0}C in brown bottle. The quantitative determination of nitric oxide was done using the Assay Designs' nitric oxide assay kit (Assay Designs Inc., USA). Randox detection kit was used for the quantitative in vitro determination of SOD and glutathione Peroxidase in whole blood.

RESULTS

In our study, cord serum NO (µmol/L) levels in group I is significantly less than the control group ($p<0.001$). Similarly in cord serum, SOD (mg/ml) & GSH (U|ml) values were significantly smaller than control group with $p<0.01$ & $p< 0.001$ respectively [Table/Fig-1]. In this study, cord serum NO ,SOD & GSH levels

when compared between group I & II, NO & SOD values were smaller in group II than group I & GSH higher in group II than group I but without any significance [Table/Fig-2].

Cord serum NO levels were found to be higher in group II when compared to group III (p<0.01) . SOD levels were smaller in group III when compared with group II without any significance [Table/Fig-3]. Cord serum NO, SOD & GSH values were significantly smaller in group 3 when compared to controls with significance (p <0.001) thus showed that oxidative stress plays a role in IUGR [Table/Fig-4]. In our study, cord serum NO levels were significantly higher in group I when compared with group III. SOD & GSH values were lower in group III as compared to group I but without any significance [Table/Fig-5].

		Mean ± SD	t-value	p-value
Nitric Oxide (µmol/L	Group I	25.10±3.60	10.14	<0.001
	Group IV	41.00±3.39		
Superoxide dismutase (mg/ml)	Group I	7.27±0.54	3.18	<0.01
	Group IV	7.91±0.32		
Glutathione peroxide (U/ml)	Group I	30.60±2.45	7.14	<0.001
	Group IV	40.50±3.62		

[Table/Fig-1]: Comparison of cord serum NO2- / NO3-, SOD and GSH levels of Group I with controls

		Mean ± SD	t-value	p-value
Nitric Oxide (µmol/L)	Group I	25.10±3.60	1.938	NS
	Group II	21.70±4.21		
Superoxide dismutase (mg/ml)	Group I	7.27±0.54	1.778	NS
	Group II	6.89±0.39		
Glutathione peroxide (U/ml)	Group I	30.60±2.45	1.347	NS
	Group II	32.40±3.43		

[Table/Fig-2]: Comparison of cord serum NO2- / NO3-, SOD and GSH levels in Group I and Group II

		Mean ± SD	t-value	p-value
Nitric Oxide (µmol/L)	Group II	21.70±4.21	3.543	<0.01
	Group III	16.60±1.71		
Superoxide dismutase (mg/ml)	Group II	6.89±0.39	0.551	NS
	Group III	6.75±0.69		
Glutathione peroxide (U/ml)	Group II	32.40±3.43	2.250	<0.05
	Group III	29.20±2.89		

[Table/Fig-3]: Comparison of cord serum NO2- / NO3-, SOD and GSH levels in Group II and Group III

		Mean ± SD	t-value	p-value
Nitric Oxide (µmol/L)	Group III	16.60±1.71	20.27	<0.001
	Group IV	41.00±3.39		
Superoxide dismutase (mg/ml)	Group III	6.75±0.69	4.76	<0.001
	Group IV	7.91±0.32		
Glutathione peroxide (U/ml)	Group III	29.20±2.89	7.69	<0.001
	Group IV	40.50±3.62		

[Table/Fig-4]: Comparison of cord serum NO2- / NO3-, SOD and GSH levels of Group III with controls (Group IV)

		Mean ± SD	t-value	p-value
Nitric Oxide (µmol/L)	Group I	25.10±3.60	6.736	<0.001
	Group III	16.60±1.71		
Superoxide dismutase (mg/ml)	Group I	7.27±0.54	1.856	NS
	Group III	6.75±0.69		
Glutathione peroxide (U/ml)	Group I	30.60±2.45	65	NS
	Group III	29.20±2.89		

[Table/Fig-5]: Comparison of maternal serum NO2- / NO3-, SOD and GSH levels in Group I and Group III

DISCUSSION

Intrauterine growth restriction (IUGR) may be defined as pathological decrease in the rate of fetal growth. This ultimately results in a fetus who does not achieve its inherent growth potential, putting it at increased risk of perinatal mortality and morbidity. The most widely used definition of IUGR is a fetus whose estimated weight is below the 10th percentile for its gestational age and whose abdominal circumference are below the 2.5th percentile. Approximately 6-30% infants in developing countries are classified as growth restricted [1].

L-Arginine is the natural substrate of NOS and the natural precursor of NO. The availability of L-Arginine is critical to the regulation of NO production. L-Arginine (precursor of NO) has a more significant effect on the improvement of uteroplacental microcirculation, which, obviously, can improve the placental oxygen-supplying function in cases of IUGR. The human body contains a series of systems that form a defense against free radicals by strict control mechanism. Reduced levels of these defenses predispose an organism to greater injury due to free radicals. Superoxide dismutase is an enzyme which catalyses the conversion of superoxide radicals to hydrogen peroxide and molecular oxygen, and thus protects cells against the potential toxicity of reactive free radicals produced from oxygen. Gluthathione peroxidase catalyses the reduction of hydrogen peroxide, organic hydroperoxides or lipid peroxides by reduced glutathione and hence protect cell from oxidative damage.

In our study mean cord serum NO (µmol/lt.) values in Group I 25.10±3.60, Group II 21.70±4.21, Group III 16.60±1.71 when compared to mean cord serum NO levels in Group IV control 41.00±3.39, Values taken from [Table/Fig-1,2,4.] showed lower values in IUGR babies compared to control healthy neonates (p<0.001) and values in Group I (those being treated with L- arginine) tend to be higher than Group 2 (treated with antioxidants) & Group III (without nutritional supplementation) in mother during course of pregnancy. Thus in our study, L- arginine therapy increases the cord serum NO levels more than antioxidants treatment. Patients with IUGR tend to have lower serum NO levels [6].

The ongoing clinical trials indicate that the curative effect of L-Arginine combined with routine therapy that is the treatment of the cause of IUGR like antihypertensives in preeclampsia, haematinics in anemia is greater than that of routine therapy alone. L-Arginine has a more significant effect on the improvement of uteroplacental microcirculation, which, obviously, can improve the placental oxygen-supplying function in cases of IUGR [7].

In the present study the enzymatic activity of superoxide dismutase & glutathione `peroxidase were found to increase with increasing birth weight on comparison of other Group neonates with controls [Table/Fig-1,2 & 4]. In our study the mean SOD (mg|ml) & GSH (U/ml) in cord serum value in Group I & Group II, which was lower in IUGR babies compared to healthy control ones, with treatment these values tend to increase [Table/Fig-4].

Similar study by S.Basu et al., showed decreased oxidative stress markers in Small for gestational age when compared to Appropriate for gestational age infants [8].

The study by S.Basu et al., showed the activity of MDA (Malondialdehyde) was increased (5.33 ± 0.72 vs 2.55± 0.22 nmol/mL; $p < 0.0001$) while levels of superoxide dismutase (493.6±54.9 vs. 786.8 ± 79.1 U/g Hb; $p < 0.0001$), catalase (1.48 ± 0.24 vs. 2.31± 0.20 U/g Hb; $p < 0.0001$) and reduced glutathione (2.84 ± 0.37 vs 6.42 ± 0.23 Umol/g Hb, $p < 0.0001$) were decreased in term SGA(small for gestational age) born to undernourished mothers as compared to term AGA(Appropriate for gestational age) born to healthy mothers [8].

In our study the mean SOD (mg|ml) value in Group I neonates in cord serum was found to be 7.27±0.54, Group II 6.89±0.39, Group III 6.75±0.69 when compared with controls mean 7.91±0.32, showing this oxidative marker is significantly decreased in IUGR babies when compared to healthy control ones and with treatment these values tend to increase.

From the present study, it is difficult to infer whether the oxidative stress is a cause or effect of intrauterine growth retardation. If oxidative stress can be proven [8] to be the cause of intrauterine growth restriction, antioxidant therapy may be considered as a prophylactic/therapeutic modality for preventing intrauterine growth retardation secondary to maternal malnutrition. Micronutrient supplementation to pregnant women, who are at higher risk of having fetal growth retardation, may alleviate oxidative stress and promote anti-oxidant defense mechanisms in their offspring. This may act as a catalyst in increasing their size at birth and decreasing subsequent neonatal morbidity and mortality, especially in the first week of life [9-10].

In our study sample size was smaller for each Group as compared to other studies, study by S. Basu et al., 20 SGA & 20 AGA neonates were taken.

CONCLUSION

Patients with an IUGR fetus should be counselled that their neonates may have some immediate complications at birth but also some longterm complications including impaired cognitive function such as learning disabilities and spastic cerebral palsy.

This study shows that there is evidence of oxidative stress in the IUGR babies born to mothers as evidenced by reduced free oxygen radical scavenger system in neonate. Micronutrient supplementation to pregnant women, who are at higher risk of having fetal growth restriction, may alleviate oxidative stress and promote anti-oxidant defense mechanisms in their offspring. This may act as a catalyst in increasing their size at birth and decreasing subsequent neonatal morbidity and mortality, especially in the first week of life. Similarly, L-Arginine, potent precursor of NO, has a more significant effect on the improvement of uteroplacental microcirculation, which, obviously, can improve the placental oxygen-supplying function in cases of IUGR. L- arginine supplementation to mothers diagnosed with IUGR may prove beneficial.

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