

COMPARING ANTIOXIDANT CAPACITY AND VITAMIN C VALUES BETWEEN UMBILICAL CORD AND MATERNAL BLOOD PLASMA AND THOSE IN NEWBORNS WITH HYPOXIA

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Abstract:

Objective

Oxygen intoxication plays an important role in the pathogenesis of some fetal diseases such as encephalopathy, ischemia & hypoxia. Antioxidants can reduce oxidative damage in newborns. The object of this research was measuring total value of antioxidant and vitamin C in blood sample of pregnant women before delivery, and also in umbilical cord blood of their newborn, and repeating these measurements 48 hours after birth in newborns with hypoxia and controlling group.

Materials & Methods

The sample included 32 healthy pregnant women presented for delivery & also their newborns. The total values of antioxidant & vitamin C in plasma were measured by staining methods.

Results

There was no statistical meaningful difference in total values of antioxidants & vitamin C in women's blood & their newborn's cord blood. Also total values of antioxidant & vitamin C meaningfully increased in newborns with hypoxia after 48 hours.

Conclusion

total values of antioxidant & vitamin C in blood plasma of newborn, increase in oxidative damage as a defense mechanism.

Key words: Antioxidants , Ascorbic Acid , Hypoxia , Umbilical cord blood.

Introduction

Oxygen intoxication has a key role in the pathogenesis of many newborn's diseases including hypoxic ischemic encephalopathy, bronchopulmonary dysplasia & necrotizing enterocolitis (1, 2, 3,4,5), antioxidant enzymes, such as superoxide dismutase, catalase, glutathione peroxidase, and reductase, are the main defense mechanisms against oxidative damage (6, 7, 8, 9, 10, 11). But other antioxidants including preventive antioxidants such as transferrin and ceruloplasmin and chain breaking antioxidants such as vitamin E, C, uric acid and Bilirubin can help this defense too (9, 12, 13).

The fetus is exposed with partial hypoxia before birth & it would be exposed with oxidative stress, immediately after birth, because of increased oxygen pressure. These effects can lead to the production of active oxidative compounds & therefore the balance between their production & elimination by oxidative materials & enzymes is maintained (13, 14). The serum levels of antioxidants (excluding Bilirubin) decrease or remain unchanged during the first days.

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Newborns, if needed, can reinforce their antioxidant defense system which includes extracellular & preventive antioxidants against oxidative stress, that is increasing the capacity at the first 48 hours after birth (14, 15). In this study at first antioxidant values in women's blood sample & in their newborns' umbilical blood cord were compared, then antioxidant values in the blood of newborns with hypoxemic ischemic encephalopathy, 48 hours after birth, compared with controlling group. By this comparison the antioxidants ability of the defense system of the newborns & the probable need to external antioxidants were evaluated.

Materials & Methods

The study population included 32 pregnant women presented to Bou Ali Hospital in Hamadan, in 2005 for delivery & also their newborns.

The blood samples of women were obtained before delivery for measuring antioxidant & vitamin C values, then cord blood samples of their full term newborns were obtained & antioxidant values of blood samples were compared. In newborns with hypoxic ischemic encephalopathy, the measurement of antioxidant values was repeated 48 hours later and compared with immediately after birth and controlling group values to evaluate the antioxidant defense system & its ability in reducing oxidative stress.

Total value of antioxidant: There are specific systems in the body to having encounter roles against free radicals damages, called Total value of antioxidant, including a) Catalytic factors b) Proteins as Transferrine c) compounds of low molecular weight with scavenging roles.

Measurement & Tools

Introducers for measuring total antioxidant capacity:

- 1) Saline phosphate buffer with pH = 7.4, NaCl 8 g, KCl 0.2 g, KH₂PO₄ 0.2 g and Na₂HPO₄ 1.12 g, in sterile water 1 liter.
- 2) RBC 10% suspension with saline phosphate buffer.
- 3) 0.2 Molar AAPH (a free radicals producer in aquatic phase) solution

Aquatic phase scavenger of red blood cells hemolysis in AAPH environments prepared for measuring total antioxidant capacity of plasma for different concentrations of ascorbic acid.

Devices:

- Spectrophotometer JENWAY
- Retsch mixer
- Shaker
- Digital scale

Preparation of Introducer solutions for measuring vitamin C in plasma:

1) Metaphosphoric acid solution (6g/h): 3 g of Metaphosphoric acid (HPO₃) dissolved in sterile water up to 500 ml total volume prepared.

2) Sulfuric acid solution (4.5 mol/h): 250 ml of concentrated sulfuric acid mixed with sterile water up to 500 ml total volume.

3) Sulfuric acid solution (12 mol/h). 300 ml of sterile water added to 650 ml of concentrated sulfuric acid.

4) 2,4 Dinitrophenyl hydrazine (DFH) 4.5 mol/h sulfuric acid in 2 g/h: 10 g of DFM powder dissolved in 4.5 mol/h sulfuric acid solution (solution number 2)

5) Thiourea solution (5 g/h): 5 g urea dissolved in sterile water with 100 ml total volume.

6) Copper sulfur solution (0.6 g/h): 0.6 g of copper sulfur (Aqua free) added to sterile water with 100 ml total volume.

7) dinitrophenyl hydrazine- Thiourea-copper solution (DTCS): 5 ml of thiourea solution + 5 ml of copper sulfate solution + 100 ml of DFM solution = DTCS solution

Measurement of vitamin C in plasma:

Calibration solutions:

a) 50 mg/dL ascorbic acid: 50 mg of vitamin C metaphosphoric acid solution (solution number 1), with 100 ml total volume.

b) 10 ml of solution (a) added to sterile water with 100 ml total volume.

c) Working solution: 0.5, 2, 4, 6, 10, 15 & 20 ml of solution (b) added to 7 lab tubes, then number 1 solution (6 g/h metaphosphoric acid) added to 25 ml total volume. The concentrations of 7 tubes was 0.1, 0.4, 0.8, 1.2, 2, 3, & 4 mg/dL, respectively.

d) 1.2 ml of metaphosphoric acid solution added to all tubes.

e) 0.4 ml of DTCS solution added to each tube & all tube were then incubated at 37 C temperature for 3 hours.

COMPARING ANTIOXIDANT CAPACITY AND VITAMIN C VALUES BETWEEN ...

f) 2 ml of 12 mol/h sulfuric acid solution (solution number 3) added to each tube after 10 minutes, and mixed with vortex.

g) The absorption of samples read in spectrophotometer device with 250 nm wave length.

Results

The average of antioxidant capacity in mothers' blood sample plasma was 5.42 mmol/L at the time of delivery & the total antioxidant capacity of umbilical cord blood of their newborns was 5.32 mmol/L, which didn't show statistical significant difference using Paired t test (table 1).

The average value of vitamin C in mothers' blood plasma was 168.0 mg/dL at the time of delivery & the average value of vitamin C in their newborns' blood plasma was 167.0 mg/dL. Paired t test didn't show significant difference.

The average value of antioxidant in umbilical cord blood of hypoxic newborns was 5 mmol/L before hypoxia & 6.2 mmol/L at 48 hours after hypoxia (Tables 1 and 2). This increase in antioxidant value was significant using

paired t test ($p < 0.05$). In against of controlling group, correlation coefficients of antioxidant values in hypoxemic newborn's blood sample showed significant relation before hypoxia & after it ($p < 0.05$).

The average value of vitamin C in umbilical cord blood of hypoxic newborns was 184.0 mg/dL before hypoxia & 280.0 mg/dL 48 hours after hypoxia. In against of controlling group, Paired t test didn't show significant difference. The average value of total antioxidant capacity in blood sample of mothers with hypoxic newborns & blood sample of mothers with non-hypoxic newborns compared & paired t test didn't show statistical significant difference (table 3).

The average value of vitamin C in blood samples of mothers with hypoxic newborns 48 hours after birth & blood samples of mothers with non-hypoxic newborns was compared by using t test & there wasn't statistical significant difference.

Total capacity values of antioxidants in hypoxic & non-hypoxic newborns' umbilical cord blood were compared by using t test which didn't show statistical significant difference.

Table 1: Distribution of frequency, average, standard deviation , maximum & minimum of total antioxidant capacity & vitamin C in blood plasma of women during delivery & their newborn's umbilical cord blood.

variable	frequency	min	max	mean	SD
Total antioxidant capacity in blood plasma of women during delivery (mmol/lil)	33	3.30	6.70	5.42	1.10
Total antioxidant capacity in newborns' umbilical cord blood (mmol/lil)	33	3.60	6.60	5.32	0.66
vitamin C in blood plasma of women during delivery (mg/dl)	31	0.010	0.600	0.168	0.173
vitamin C in newborns' umbilical cord blood(mg/dl)	33	0.040	0.380	0.167	0.073

Table 2: Distribution of frequency, average, standard deviation , maximum & minimum of total antioxidant capacity & vitamin C in plasma, 48 hours after birth.

variable	frequency	min	max	mean	SD
Total antioxidant capacity in health newborns' blood, 48 hours after birth (mmol/lil)	33	3.64	6.70	5.40	0.68
Total antioxidant capacity in hypoxic newborns' blood 48 hours after birth (mmol/lil)	4	5.80	6.70	6.20	0.37
vitamin C in health newborns' blood, 48 hours after birth.(mg/dl)	33	0.045	0.420	0.173	0.081
Vitamin C in hypoxic newborns' blood 48 hours after birth(mg/dl)	5	0.160	0.580	0.280	0.174

Table 3: Total antioxidant capacity averages & vitamin C average in women's blood during delivery, in women with hypoxic newborns & women with non-hypoxic newborns.

Variable	frequency	mean	SD
total antioxidant capacity averages in women with non-hypoxic newborns(mmol/lit)	28	5.407	1.130
total antioxidant capacity averages in women with hypoxic newborns(mmol/lit)	5	5.480	1.049
vitamin C averages in women with non- hypoxic newborns(mg/dl)	27	0.154	0.160
vitamin C averages in women with hypoxic newborns(mg/dl)	4	0.257	0.253

Table 4: Comparison of total antioxidant capacity average & vitamin C average in umbilical cord blood of newborns with hypoxia 48 hours after birth , and healthy newborns.

variable	frequency	mean	SD
total antioxidant capacity average in umbilical cord blood in healthy newborns(mmol/lit)	28	5.335	0.644
total antioxidant capacity average in umbilical cord blood of newborns with hypoxia 48 hours after birth (mmol/lit)	5	5.200	0.827
vitamin C average in umbilical cord blood in healthy newborns (mg/dl)	28	0.163	7.499
vitamin C average in umbilical cord blood of newborns with hypoxia 48 hours after birth (mg/dl)	5	0.184	7.022

Discussion

The results of present study show vitamin C & antioxidant values in blood samples of mothers at the time of delivery & in umbilical cord blood samples didn't have considerable differences. Also vitamin C & antioxidant capacity in blood plasma of newborns with hypoxia were compared which showed that both had significant increase 48 hours later (from 0.167 to 0.280 mg/100 ml for vitamin C & from 5 to 6.2 mmol/L for antioxidant capacity). Research of Szabo & et al showed that, the newborn's health depends on the antioxidant's concentration in body fluids & increasing of total antioxidant capacity can reduce incidence of oxygen toxicity related disorders (14).

Arikan & et al study showed that presence of antioxidants, such as glutathione & vitamin C during delivery can reduce oxidative damage in fetus (18).

Boonsiri & et al study showed that oxygen therapy in premature newborns increases vitamin C concentration in plasma, that is increasing the oxygen concentration can produce Reactive Oxygen Species (ROS) compounds & the probable reaction is increasing vitamin C concentration as an antioxidant (16).

Lindman & et al studies' findings showed that umbilical cord blood's ability in reducing free radicals depends on antioxidant concentrations, such as vitamin C & intracellular enzymes like catalases and peroxidases(14). Findings of present research are compatible with previous researches in this field.

Oxygen intoxications increases ROS compounds production, which leads to new conditions and the balance of oxidants & antioxidants is impaired .So one of the possible ways of modifying this condition is increasing the amount of antioxidant. In newborns who these balance changes led to hypoxic damage, the amount of antioxidants in plasma increased after 48 hours to restore this balance.

It is suggested that antioxidant capacity measuring test be used in similar studies with considering other effective factors such as the duration of the newborn's hospitalization in intensive care unit, numbers of mother's deliveries, mother's age, drugs consumption, using vitamin supplements, and etc (because they can affect the bodies' resistance against oxygen intoxication) to provide more accurate and definitive results.

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