

The Influence of the Lowered Natural Cellular Cytotoxic Activity and Tocopherol Concentration in the Umbilical Blood on the Health of the Babies in the First Three Years of Life

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Abstract

The activity of the NK cells, tocopherol concentration, tocopherol lipid saturation index in the umbilical blood of the 33 healthy newborns was examined. Subpopulations of early and late rosettes with rosette E test were identified. Blood for the study was drawn immediately after the delivery by the puncture of the umbilical vein. Mononuclear cells were isolated by centrifugation in the gradient of Ficoll and Uropolin. Rosette E test was performed.

The activity of the NK cells was assessed using Cr⁵¹ labelled leukemia line K-562 as the target cells.

Tocopherol concentration was examined spectrophotometrically according to Hasim.

The mean activity of the NK cells was 8.6 ± 1.5 (SE), and the mean tocopherol concentration was 0.22 ± 0.02 (SE).

Three years after the initial study mothers of the examined children got the questionnaire on the well being of their babies. The

statistical analysis has shown the negative correlation between the number of infections in the first year of life and the activity of NK cells with $P < 0.05$. It shows the possible prognostic value of NK cells test. The protective anti infective action of the breastfeeding was confirmed.

Zusammenfassung

Es wurde die Aktivität von NK Zellen, die Konzentration von Tocopherol und die Sättigung von Tocopherolfetten im Nabelblut von 33 gesunden Neugeborenen untersucht. Subpopulationen von aktiven und kalten Rosetten wurden identifiziert. Die durchschnittliche Aktivität von NK Zellen war mit 8.6 ± 1.5 (SE) tiefer als in den Studien anderer Autoren. Drei Jahre nach der ursprünglichen Studie wurde den Müttern der untersuchten Kinder ein Fragebogen über das Wohlbefinden ihrer Kinder vorgelegt. Die statistische Analyse hat eine negative Korrelation zwischen Infektionen und der Aktivität von NK Zellen gezeigt ($P < 0.05$). Der schützende antiinfektiöse Wert des Stillens und die immunomodulierende Eigenschaft von Tocopherol wurde gezeigt.

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The reactivity of the immunologic system during the neonatal period is different than in the adult. That is due to the slow maturation of this system. The role of the lymphatic system of the newborn during the higher susceptibility to the contagious factors is still very interesting to the perinatologist.

Natural killer (NK) cells play an important role in the immunologic surveillance, antiviral response and antitumor response. Natural cellular cytotoxicity phenomenon of these cells is killing target cells without the necessity of earlier immunization by antigen and is not dependent on the compatibility between effector and target cells in the MHC.

Vitamin E is important factor in the antioxidative complex^{2,16}, which protects newborns and older children against free radicals destruction such as retinopathy and bronchopulmonary dysplasia¹⁴. Lately a reports were published on immunomodulatory action of tocopherol^{19,22,28}. The action of this vitamin on the course of the infection in adults and children and on the percentage of active lymphocytes in the peripheral blood was noted earlier. Tocopherol is also an important protective, anti-oxidative factor for cellular membrane phospholipides. This is a immunocompetitive action, because the degree of the receptor expression is dependent on the functional state of the cellular membrane.

Aim of the Study

The aim of the study is to:

1. Evaluate the natural cytotoxic activity and tocopherol concentration in the umbilical blood of healthy newborns, and correlation of the results with the health of the children in the first few years of life.
2. Evaluate the tocopherol concentration and tocopherol lipid saturation index in the umbilical blood and examination of the influence of vitamin E on the formation of E rosettes by neonatal T lymphocytes.

Material and Method

33 healthy newborns after normal pregnancy and delivery were examined. All mothers were healthy, mean age 27.

The characteristics of the group is shown in the Table I.

Table I.

NUMBER OF BABIES	33
FEMALE	14
MALE	19
AGE OF MOTHERS	26.7±0.8 *
range	18-34
LENGTH OF PREGNANCY (weeks)	39.5±0.2 *
range	36-42
WEIGHT AT BIRTH (grams)	3321±81 *
range	2350-4400
APGAR IN THE FIRST MINUTE	9.6±0.15 *
range	6-10

* mean ± SE

Blood for the study was drawn from the umbilical vein immediately after the delivery and then centrifuged on the gradient of Ficoll and Uropolin. Mononuclear cells were obtained.¹³ After 20 minutes centrifugation at 450 g cells were washed twice with 0.9 % NaCl and brought up to the concentration of 10×10^6 cells in 1 ml of the rosette medium (10 % human absorbed inactivated serum AB group in PBS). The rosette test was performed: 0.1 ml of 5×10^6 /ml lymphocyte solution was added to 0.1 ml of SRBC (sheep red blood cell) at the concentration of 250×10^6 /ml. ARFC essays were fixed immediately, total rosettes (TRFC) were incubated at 4 °C for 18 hours.

The activity of NK cells was evaluated using leukemia K-562 cells as target cells.⁷ Target cells were cultured in the RPMI 1640 (Sigma) medium and labelled with ^{51}Cr by the incubation with 100 $\mu\text{Ci/ml}$ $\text{Na}_2^{51}\text{CrO}_4$ (Swierk). Target cells (2×10^4) were then mixed with effector cells (1×10^6 in RPMI) and incubated for four hours at 37 °C. After the incubation cells were centrifuged and radioactivity

of ^{51}Cr in the supernatant was evaluated (gamma counter, Amnes Miles). The percentage of the cytotoxicity was calculated from the formula:

$$\% \text{ of cytotoxicity} = \frac{\text{experimental liberation} - \text{spontaneous liberation}}{\text{maximal liberation} - \text{spontaneous liberation}} \times 100$$

The spontaneous liberation was derived from the target cells incubated in the control medium, maximal liberation from the cells incubated with Triton X-100. Assays were repeated three times.

Tocopherol concentration was measured spectrophotometrically according to Hasim, S.A. et al.¹⁰, and lipid concentration according to Zollner, N. et al.²⁹. Tocopherol was presented as tocopherol lipid saturation index (mg/dl/g of lipids), and calculated from the following formula:

$$\text{INDEX} = \frac{\text{tocopherol}}{\text{lipids}} \times 1000$$

Tocopherol lipid saturation index of the blood more accurately informs us about the state of the nutrition of the organism with the vitamin E, than the tocopherol concentration itself⁶.

Three years after the initial study mothers of the examined children got the questionnaire about the well being of their babies. Number of infections in babies, number of infections treated with antibiotics, and the length of the breast-feeding was emphasized. The results were analyzed statistically using correlation coefficient according to Spearman and the descriptive statistics. All the calculations were done on the personal computer using Goode Software QUASAR.

Results

The results of the study are shown below.

Table II shows mean natural cellular cytotoxicity in the whole group of the examined newborns, and according to sex.

Table II.

NEWBORNS	Nº OF CASES	% CYTOTOXICITY
		E:D 50:1
EXAMINED GROUP	33	8.6 ± 1.5 *
FEMALE	14	4.8 ± 0.8 *
MALE	19	11.7 ± 2.3 *

* mean value ± SE

The mean activity of the NK cells in the umbilical blood was 8.6 ± 1.5 (SE), and was higher in male newborns than in female.

Table III shows tocopherol concentration and tocopherol lipid saturation index of the serum.

Table III.

	CONC. OF TOCOPHEROL mg/dl	TOCOPHEROL/LIPIDS mg/dl/g lipids
NEWBORNS n=30	0.22±0.02 *	0.45±0.04 *

* mean ± SE

In the group of the examined newborns the concentration of the tocopherol in the serum was 0.22 mg/dl. and tocopherol lipid saturation index 0.45 mg/dl/g of lipids.

Table IV shows the subpopulation of T lymphocytes forming active (ARFC), cold (CRFC) and total (TRFC) rosettes.

Table IV.

	ARFC	CRFC	TRFC
NEWBORNS n=30	16±3.1 *	16±2.2 *	32.1±4.2 *

* mean ± SE

The mean percentage of active rosettes was 16.1 and cold rosettes 16.

Table V presents the existing correlations.

Table V.

	LENGTH OF BREASTFEEDING	ACTIVITY OF NK CELLS
NUMBER OF INFECTIONS IN THE FIRST YEAR OF LIFE	NEGATIVE CORRELATION P<0.01	NEGATIVE CORRELATION P<0.05
TOTAL NUMBER OF INFECTIONS IS THE FIRST THREE YEARS OF LIFE	NEGATIVE CORRELATION P<0.01	NO CORRELATION
NUMBER OF INFECTIONS TREATED WITH ANTIBIOTICS IN THE FIRST THREE YEARS OF LIFE	NEGATIVE CORRELATION P<0.05	NEGATIVE CORRELATION P<0.05

The number of infections in the first year of life is negatively correlated with the length of breastfeeding with $P < 0.01$ and activity of the NK cells of the umbilical blood $P < 0.05$. The number of the infections treated with the antibiotics in the first three years of life is negatively correlated with the length of breastfeeding and activity of the NK cells ($P < 0.05$).

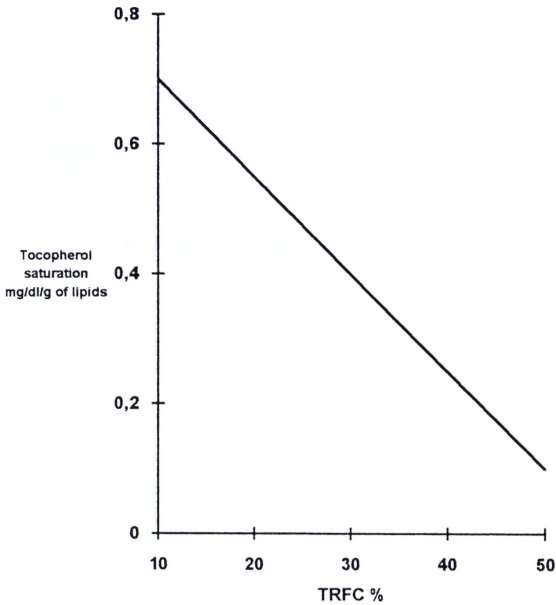


Fig. 1. $P < 0.01$ $Y = 0.87 - 0.014x$

Figure 1 shows the correlation between the TRFC and tocopherol lipid saturation index.

Negative correlation between the percentage of TRFC and tocopherol lipid saturation index was shown with $P < 0.01$.

Figure 2 shows the correlation between ARFC's and tocopherol lipid saturation index.

The graph shows positive correlation between the active rosettes and tocopherol lipid saturation index.

Discussion

The activity of the NK cells in the umbilical blood is lower than in adults^{23,25,26}. Higher natural cellular cytotoxic activity in male newborns was proven.

The questionnaire on the well being on the babies shows that the number of the infections in the first year of life, and number of the infections treated with antibiotics in the first three years of life is negatively correlated with the activity of NK cells examined in the umbilical blood. It determines the possible prognostic value of the test of the NK cells activity. Additionally, the analysis of the material obtained from the questionnaire proves the protective value of the breastfeeding against various infections.

Maternal milk is important for the newborn not only because of its chemical composition but also because of the components protecting the newborn against various infective factors. It was thought that the influence of the maternal milk on the immunologic system of the newborns is mainly due to the IgA class anti-

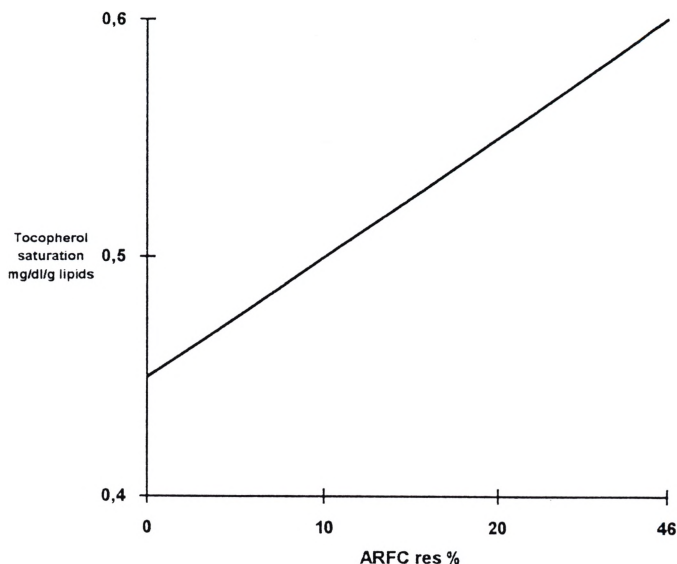


Fig. 2.

$$P < 0.05 \quad y = 0.44 + 0.002x$$

bodies. Now we know that the immunologic system of the newborn can be modulated by the numerous peptides and immunocompetent cells in the maternal milk.

The negative correlation between tocopherol lipid saturation index and TRFC is very interesting. It could point to the role of tocopherol in the activity of the lipoprotein factors leading to the inhibition of the expression of the receptors on the surface of the T lymphocytes. In the LDL fraction many factors inhibiting various functions of lymphocytes among them inhibiting the expression of the receptor to SRBC were found^{1,27}.

Vitamin E is transported into the cells by the receptor with high affinity to the LDL lipoprotein²⁴. Suppressor Fcγ⁺ are most sensitive to the inhibitors in the LDL fraction³. It is possible that the immunostimulatory function of tocopherol is partly due to the inhibition of suppressor T lymphocytes by the lipoprotein bound with it.

A positive correlation between rosette forming T lymphocytes (young and activated cells) and tocopherol lipid saturation index was found. It confirms the suggestions of the other authors on the role of the vitamin E in the activation of the lymphocytes.

The concentration of tocopherol in the umbilical blood of the examined group is much lower than the results by other authors – Ehrenkranz, R.A.⁵. A linear correlation between the concentration of tocopherol in the mothers and child's blood serum was found¹⁵. According to the nutritional requirements, the concentration of tocopherol in the blood serum should not be lower than 0.7 mg %; a concentration below 0.5 mg % is considered to be a vitamin E deficit. It seems that this threshold should be lower in children and should be 0.3 mg/dl.⁶ Toco-

pherol lipid saturation index should also be lower in children and its threshold should be 0.6 mg/dl/g of lipids.

Gutcher, G.R. et al.⁸ found the deficiency of vitamin E in the premature and SGA newborns, suggesting the supplementation of this vitamin. Ehrenkranz, R.A.⁴ proved that the newborns given tocopherol are in less risk of bronchopulmonary dysplasia due to oxygen therapy. The same positive effect should be seen in the prophylaxis of the hemolytic anemia caused by the deficiency of vitamin E⁵.

Conclusions

1. The low tocopherol lipid saturation in the umbilical blood is due to its deficiency in the diet during the pregnancy and may have an influence on the formation of cellular immunity in the newborns.
2. Finding of lowered activity of NK cells in the umbilical blood directly after the delivery may suggest higher infectious diseases morbidity in children in the first years of life, and might be a prognostic factor.

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