

# THE ANALYSIS OF SELECTED MICROELEMENTS IN NEONATAL UMBILICAL CORD BLOOD

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## Abstract

Microelements and trace elements play a vital role in the body. Low quantities of these elements are essential to ensure proper metabolic processes.

The aim of the thesis was to determine levels of selected microelements (zinc, copper, iron, manganese, chromium and aluminium) in cord blood plasma, and to find out if the gestational age, gender and birth weight of a newborn affect these concentrations.

The research was conducted on the cord blood of 71 newborns. Regarding the gestational age, three groups were distinguished: I – neonates born after 37 week of pregnancy ( $n=7$ ), II – neonates born between 38 and 41 week ( $n=59$ ), and III – neonates born after 42 week of pregnancy ( $n=5$ ). Subsequently, another division concerned the gender: male ( $n=35$ ) and female ( $n=36$ ) neonates, as well as their weight: neonates with regular birth weight – 2.500-3.500 g ( $n=61$ ), and neonates with high birth weight – over 3.500 g ( $n=10$ ). The concentrations of zinc, copper, iron, manganese, chromium, and aluminium in cord blood plasma were determined on the basis of inductively coupled plasma atomic emission spectroscopy method. The results were subjected to statistical analysis using Statistica 10.0 software, with the assumption that the level of significance was  $p<0.05$ .

The average concentrations of analysed elements in umbilical cord blood were the following:  $18.67\pm 3.05 \mu\text{mol Zn dm}^{-3}$ ,  $16.60\pm 2.64 \mu\text{mol Cu dm}^{-3}$ ,  $23.32\pm 3.29 \mu\text{mol Fe dm}^{-3}$ ,  $0.96\pm 0.21 \mu\text{mol Mn dm}^{-3}$ ,  $2.81\pm 0.14 \mu\text{mol Cr dm}^{-3}$  and  $0.041\pm 0.028 \mu\text{mol Al dm}^{-3}$ , which is within the standard ranges, suggesting efficient regulating mechanisms of the developing foetuses. The research proved that the neonate's gestational age, gender and birth weight

had no substantial impact on concentrations of these elements in the cord blood, except the concentration of iron, which was statistically significantly correlated with the gender of neonates.

Key words: microelements, neonate, cord blood.

## ANALIZA STĘŻEŃ WYBRANYCH MIKROPIERWIĄSKÓW W KRWI PEPOWINOWEJ NOWORODKA

### Abstrakt

Dużą grupę pierwiastków wchodzących w skład organizmu ludzkiego stanowią pierwiastki śladowe. Wśród pierwiastków niezbędnych do prawidłowego funkcjonowania organizmu znajdują się również te, które określa się mianem toksycznych (mangan, chrom i glin). Niewielkie ich ilości zapewniają prawidłowe funkcjonowanie enzymów, syntezę hormonów oraz budowę związków o ważnej roli biologicznej, budulcowej i regulacyjnej organizmu ludzkiego.

Celem pracy było oznaczenie stężenia wybranych mikropierwiastków (cynku, miedzi, żelaza, manganu, chromu i glinu) w osoczu krwi pępowinowej noworodków oraz ustalenie, czy termin porodu, płeć i urodzeniowa masa ciała noworodka mają wpływ na stężenie tych mikropierwiastków.

Badano krew pępowinową 71 noworodków urodzonych w Szpitalu Wojewódzkim Nr 2 w Rzeszowie. Noworodki pochodziły od zdrowych matek w wieku od 16 do 44 lat. Porody odbywały się siłami natury lub przez cesarskie cięcie. Ze względu na tydzień ukończenia ciąży wyodrębniono 3 grupy: pierwszą stanowiły noworodki urodzone do 37. tyg. ciąży ( $n=7$ ), drugą – noworodki urodzone między 38. a 41. tyg. ciąży ( $n=59$ ), trzecią – noworodki urodzone w 42. i późniejszych tygodniach ciąży ( $n=5$ ). Ponadto dokonano podziału noworodków ze względu na płeć: noworodki płci męskiej ( $n=35$ ) i noworodki płci żeńskiej ( $n=36$ ), a także ze względu na urodzeniową masę ciała: noworodki z normalną masą urodzeniową: 2500-3500 g ( $n=61$ ) i noworodki z dużą masą urodzeniową: powyżej 3500 g ( $n=10$ ). W osoczu oznaczono stężenia cynku, miedzi, żelaza, manganu, chromu i glinu metodą emisyjnej spektrometrii atomowej z plazmą indukcyjnie wzbudzoną (ICP-AES – Inductively Coupled Plasma – Atomic Emission Spectrometry) z użyciem spektrometru Liberty 2 AX firmy Varian. Wyniki poddano analizie statystycznej, stosując oprogramowanie Statistica 10.0 (test Anowa Kruskala-Wallisa, test Manna-Whitneya), przyjmując za istotne statystycznie różnice przy  $p<0,05$ .

Wykazano, że średnie stężenia cynku, miedzi, żelaza, manganu, chromu i glinu we krwi pępowinowej noworodków mieściły się w zakresie wartości uznawanych za prawidłowe (Cu:  $16,60\pm 2,64$   $\mu\text{mol dm}^{-3}$ ; Zn:  $18,67\pm 3,05$   $\mu\text{mol dm}^{-3}$ ; Fe:  $23,32\pm 3,29$   $\mu\text{mol dm}^{-3}$ ; Mn:  $0,96\pm 0,21$   $\mu\text{mol dm}^{-3}$ ; Cr:  $2,81\pm 0,14$   $\mu\text{mol dm}^{-3}$ ; Al:  $0,041\pm 0,028$   $\mu\text{mol dm}^{-3}$ ), co może świadczyć o sprawnie funkcjonujących mechanizmach regulacyjnych w organizmie rozwijającego się płodu. Zarówno termin porodu, jak i płeć oraz masa urodzeniowa noworodka nie wpłynęły w istotny sposób na stężenia badanych pierwiastków we krwi pępowinowej noworodków. Jedynie znamienne statystycznie różnice w stężeniach żelaza zanotowano w zależności od płci noworodków.

Słowa kluczowe: mikropierwiastki, noworodek, krew pępowinowa.

## INTRODUCTION

Microelements and trace elements play an important role in the body. Low quantities of these elements are essential for the proper functioning of numerous compounds which play some crucial biological, building and regulating roles, e.g. enzymes or hormones.

One of such essential microelements is zinc. It is a cofactor of over 200 enzymes; it participates in the metabolism of hormones and in the synthesis of proteins; it also acts as a regulator of transcription and metabolism of vitamin A. As a component of copper-zinc superoxide dismutase (CuZnSOD), a vital antioxidative enzyme, zinc is involved in the antyoxidative defence of the body (MERIALDI et al. 2004, CANTIN et al. 2007, OZDEMIR et al. 2007). Zinc deficiency in the foetal period may lead to an innate immune system failure, neural tube defects, and even teratogenic disorders (MERIALDI et al. 2004, CANTIN et al. 2007). Zinc is also involved in interactions with other elements, e.g. copper and iron (OZDEMIR et al. 2007, SHIRVANI et al. 2010).

Copper occurs in serum in combination with the ceruloplasmin, albumins and histidine, while inside a cell it is bound with metallothioneins, which prevents its cytotoxicity and plays an important part in protecting the body against free radicals (KOMASZYŃSKA, MAĆKIEWICZ 2007). Copper is indispensable in the synthesis of haemoglobin in a developing organism, and, as a cofactor of many enzymes, it forms copper-zinc (CuZnSOD) and extracellular superoxide dismutase (ecSOD), eliminating reactive oxygen species (ROS) (YIEN-MING et al. 2006, FORMIGARI et al. 2007). Copper deficiency interferes with the synthesis of catalase and manganese-dependent superoxide dismutase (MnSOD), reduces the concentration of ceruloplasmin in serum, and, in the prenatal period, can contribute to foetal growth disorders, cause anaemia, neutropenia and bone demineralization (SCHULPIS et al. 2004). Due to a high copper concentration in the body, lipids, proteins and DNA are prone to oxidative damage, which leads to disorders of the nervous system, the development of neurodegenerative diseases and cancer (YIEN-MING et al. 2006, FORMIGARI et al. 2007, ZEYREK et al. 2009).

Iron is involved in many biochemical processes, hence it is necessary for the proper growth and functioning of the body. It is an essential component of haemoglobin, myoglobin and other proteins. Cooperating with low molecular weight antioxidants, iron ions stabilize the structure of cell membranes, which protects the body from oxidative processes (CANTIN et al. 2007, FORMIGARI et al. 2007). Iron plays an important role in the process of erythropoiesis, being simultaneously a substantial component of the DNA and RNA synthesis, and an element required in the process of building cellular immunity and in the synthesis of myelin during the foetal brain development (LIEU et al. 2001, GURZAU et al. 2003, SHIRVANI et al. 2010). Health consequences of iron deficiency in pregnant women are associated

with miscarriages, premature births with low birth weight, increased risk of neonatal deaths, and psychomotor disorders and hearing impairment later in children's life (O'BRIEN et al. 2003, ANDERSEN et al. 2006, YOUNG et al. 2010).

The proper functioning of the human body is also dependent on trace amounts of manganese, chromium and aluminium (TAKSER et al. 2004, ASCHNER et al. 2005, BRONISZ, PASTERNAK 2008). A major function of manganese is related to antioxidant processes because, as a constituent of superoxide dismutase (MnSOD), it helps to scavenge harmful free radicals generated in metabolic transformations of a cell. Manganese is essential in the processes of cellular respiration, prevents the formation of atherosclerosis and participates in the process of hematopoiesis (ASCHNER et al. 2005, SOLDIN, ASCHNER 2007, VIGEH et al. 2008, RUIZ-SANZ et al. 2011). Low levels of manganese result in an impaired psychomotor development of the organism, growth inhibition and anaemia. On the other hand, prolonged exposure to high doses of manganese during the foetal period causes nervous system disorders, diseases of the skeletal system and intrauterine growth restriction (IUGR) (TAKSER et al. 2004, ASCHNER et al. 2005, SOLDIN, ASCHNER 2007, VIGEH et al. 2008).

Chromium appears to be an essential component of a diet owing to its effects on the glucose metabolism (ANDERSON et al. 2006, BRONISZ et al. 2007). Chromium deficiency among pregnant women is a factor in the development of metabolic syndrome. On the other hand, excess of chromium, apart from the prooxidative influence, can cause iron metabolism disorders facilitating the development of anaemia (GOMES et al. 2005, ANDERSEN et al. 2006, RUIZ-SANZ et al. 2011).

Aluminium in the human body is an inhibitor of enzymes participating in the carbohydrate metabolism, processes of phosphorylation, dephosphorylation, and proteolytic enzymes connected with the cell membrane (SANZ-MEDEL et al. 2002, BRONISZ, PASTERNAK 2008). High levels of aluminium help to reduce the activity of antioxidant enzymes (superoxide dismutase and glutathione peroxidase), disturb iron and zinc distribution and increase lipid peroxidation (SANZ-MEDEL et al. 2002, GURZAU et al. 2003, ALEXANDROV et al. 2005). Exposure to high levels of aluminium in the intrauterine life leads to progressive neurodegeneration and death (ALEXANDROV et al. 2005, KAWAHARA 2005).

The aim of the thesis was to determine the levels of selected microelements (zinc, copper, iron, manganese, chromium and aluminium) in cord blood plasma, and to find out if the gestational age, gender and birth weight of a newborn affect these concentrations.

## MATERIAL AND METHODS

The research was conducted on the cord blood of 71 neonates born in the Provincial Hospital No 2 in Rzeszów. The children were born by healthy mothers aged 16 to 44. These were both natural labours and caesarian sections.

Regarding the gestational age, three groups were distinguished: the first group comprised neonates born up to the 37<sup>th</sup> week of pregnancy ( $n=7$ ), the second one consisted of neonates born between the 38<sup>th</sup> and 41<sup>st</sup> week of pregnancy ( $n=59$ ), and the third one was composed of neonates born in the 42<sup>nd</sup> week and later ( $n=5$ ). Two other divisions distinguished between the genders: male ( $n=35$ ) and female ( $n=36$ ) neonates, newborns' weight: neonates with regular birth weight – 2500-3500 g ( $n=61$ ), and neonates with high birth weight – over 3500 g ( $n=10$ ).

Immediately after the delivery, a fragment of the umbilical cord was clamped and the blood was taken out from the placental vein to heparinized test tubes. The plasma was obtained by spinning a tube of fresh blood for 15 minutes at 3000 rpm and was stored until the analysis at the temperature of  $-20^{\circ}\text{C}$ .

Trace elements such as zinc, copper, iron, manganese, chromium and aluminium were determined in the blood plasma by the inductively coupled plasma atomic emission spectroscopy (ICP-AES), using a Varian Liberty 2 AX spectrometer.

The results underwent statistical analysis using Statistica 10.0 software (Anova Kruskal-Wallis test, Mann-Whitney test), with the level of significance set at  $p<0.05$ .

## RESULTS AND DISCUSSION

The concentrations of zinc, copper, iron, manganese, chromium and aluminium in the umbilical cord blood and the standard values for these microelements are presented in Table 1.

Average concentrations of the analysed microelements in particular subdivisions relating to the gestational age, gender and birth weight of a newborn are presented in Table 2.

According to the research, the average zinc concentration in the cord blood of the examined neonates was  $18.67\pm 3.05 \mu\text{mol dm}^{-3}$  and stayed within the standard limits (Table 1). The level of this element was on the increase in relation to the gestational age. A little higher zinc concentration was observed in female neonates, but the results were similar in all the sub-groups distinguished according to the newborns' birth weight (Table 2).

Table 1

Concentrations of zinc, copper, iron, manganese, chromium, aluminum in neonatal umbilical cord blood

Micro-element	Microelement concentration ( $\mu\text{mol dm}^{-3}$ ) in umbilical cord blood						Range of norm ( $\mu\text{mol dm}^{-3}$ )
	$\bar{x}$	SD	range of variation	bottom quartile	median	upper quartile	
Zn	18.67	3.05	11.84-24.48	16.30	19.16	21.23	12.2-21.3
Cu	16.60	2.64	12.99-26.02	14.59	15.96	17.87	12.6-25.2
Fe	23.32	3.29	14.28-27.52	20.38	23.94	26.42	12.5-26.9
Mn	0.96	0.21	0.14-1.54	0.86	0.93	1.05	0.72-3.64
Cr	2.81	0.14	2.48-3.16	2.69	2.79	2.90	2.80-2.88
Al	0.041	0.028	0.014-0.142	0.021	0.035	0.051	> 0.37

$\bar{x}$  – mean, SD – standard deviation

Similar observations were made by ELIZABETH et al. (2008), who examined relationships between the zinc level, gestational age and birth weight. They proved differences in the concentration of zinc depending on the gestational age and birth weight. OZDEMIR et al. (2007) analysed the zinc level in neonates in respect of the birth weight. Their research showed that the zinc concentration in neonates was related to the birth weight.

Despite differences in levels of the examined elements, the research of ELIZABETH et al. (2008), OZDEMIR et al. (2007), as well as authors' own study revealed that the zinc concentration in the cord blood stayed within the range of reference values.

The copper concentration in the cord blood in the examined neonates equalled  $16.60 \pm 2.64 \mu\text{mol dm}^{-3}$ , which falls into the standard range (Table 1). Regarding the date of delivery, the copper level results showed a downward trend depending on the gestational age. The highest concentration of that microelement was observed for neonates born before 37<sup>th</sup> week of pregnancy, while the lowest one was determined for those born after the 42<sup>nd</sup> week of pregnancy. Yet, the differences between these groups were not statistically significant. Similarly, the gender and birth weight had no substantial impact on the copper concentration, although slightly higher results were observed for male neonates and newborns with regular birth weight (Table 2).

OZDEMIR et al. (2007) proved that the copper level in the cord blood of neonates born between 38<sup>th</sup> and 42<sup>nd</sup> week of pregnancy varied depending on the birth weight of these newborns. They noted that the highest concentration of Cu was characteristic for newborns with low birth weight (up to 2,500 g), and the lowest one – for those with birth weight over 4,000 g. The copper level in the cord blood of neonates with high birth weight

Table 2

Concentrations of zinc, copper, iron, manganese, chromium and aluminum in the subgroups in dependence of the week of delivery, gender and birth weight

Micro-element	Microelement concentration ( $\mu\text{mol dm}^{-3}$ ) in umbilical cord blood in dependence of:						Statistical analysis
	week of delivery						
	37 <sup>th</sup> week and below		38-41 <sup>th</sup> week		42 <sup>th</sup> week and above		
	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	
Zn	17.78	2.22	18.63	3.22	20.32	0.72	0.474
Cu	16.76	4.82	16.65	2.43	15.75	0.30	0.507
Fe	21.71	2.86	23.48	3.23	23.73	4.54	0.160
Mn	1.06	0.26	0.95	0.21	0.87	0.12	0.293
Cr	2.82	0.20	2.80	0.13	2.88	0.19	0.730
Al	0.045	0.043	0.041	0.027	0.035	0.008	0.938
	gender of a neonate						$p^{**}$
	male			female			
	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	
	Zn	18.50	3.10	18.83	3.03	16.49	2.65
Cu	16.70	2.66	16.49	2.65	16.49	2.65	0.683
Fe	22.49	3.55	24.13	2.83	24.13	2.83	0.046
Mn	0.98	0.25	0.94	0.17	0.94	0.17	0.447
Cr	2.81	0.15	2.81	0.14	2.81	0.14	0.861
Al	0.047	0.033	0.035	0.021	0.035	0.021	0.095
	birth weight of a newborn						$p^{**}$
	normal birth weight			high birth weight			
	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	
	Zn	18.67	3.07	18.68	3.10	18.68	3.10
Cu	16.60	2.70	16.57	2.33	16.57	2.33	0.849
Fe	23.31	3.22	23.40	3.87	23.40	3.87	0.791
Mn	0.96	0.22	0.92	0.15	0.92	0.15	0.524
Cr	2.80	0.15	2.83	0.12	2.83	0.12	0.336
Al	0.040	0.026	0.049	0.039	0.049	0.039	0.804

$\bar{x}$  – mean, SD – standard deviation,  $p$  – level of the probability,

\* ANOVA Kruskal-Wallis test, \*\* Mann-Whitney test

was significantly lower than of those with low or regular birth weight. JONES et al. (JONES et al. 2010) proved that the copper concentration in neonates of Afro-American origins born by mothers with pre-eclampsia was lower than in the case of those born by healthy mothers. Moreover, they noticed that neonates with low birth weight (<2,500 g) born before 37<sup>th</sup> week of pregnancy are characterized by a higher copper concentration in comparison to neonates with the birth weight over 2,500 g born on the expected date of delivery or later.

Contrary to the research by JONES et al. (2010) that substantiated the relation between the copper level and birth weight, the authors' own research did not prove any statistically significant differences between copper concentrations versus the gestational age, gender and birth weights.

The average concentration of iron in the cord blood of examined neonates reached to  $23.32 \pm 3.29 \mu\text{mol dm}^{-3}$ , which was within the standard range (Table 1). During the last four weeks before due date, most iron bound to maternal serum with transferrin passes to the placenta, and from there, by the placental transferrin receptor, goes to the foetus (O'BRIEN et al. 2003). This is confirmed by the authors' own research, which indicated that iron concentrations in the examined neonates showed an upward trend in relation to the gestational age. The level of this element in the cord blood depending on the gender was significantly higher in case of female neonates, and a little higher for the newborns with high birth weight. The research demonstrated that a statistically significant difference in the iron level was related to a neonate's gender (Table 2).

ELIZABETH et al. (2008) showed that neonates with low birth weight born prematurely had a lower iron level than the control group. Both this research and the authors' own investigations indicate that higher iron concentrations are positively correlated with the gestational age. YOUNG et al. (2010), however, showed different results, having observed as lower iron levels in neonates with birth weight ranging from 2,150 to 4,705 g and born around the due date ( $40.0 \pm 1.2$  week of pregnancy). The researchers claimed there was a relationship between the concentration of iron in neonates and their mothers, and the placental TfR protein. They stated that the demand for iron during the foetal period is related to an increase in the mother's needs and the developing pregnancy. Besides, the expression of the placental TfR protein seems to be a vital factor for the pregnancy.

Trace amounts of manganese are essential for the proper functioning of the body because this element participates in antioxidative processes. It is also required to keep a healthy bone structure in a developing organism (ASCHNER et al. 2005, SOLDIN, ASCHNER 2007).

The average manganese concentration in the examined sample was  $0.96 \pm 0.21 \mu\text{mol dm}^{-3}$ , falling into the range of values regarded as standard (Table 1). The concentrations of this element showed a downward trend in



relation to the gestational age, with the highest values for neonates born before 37<sup>th</sup> week of pregnancy and the lowest ones for those born after 42<sup>nd</sup> week of pregnancy. Additionally, higher manganese levels were observed in male neonates and newborns with regular birth weight (Table 2).

Similar research was conducted by JONES et al. (2010) who determined manganese concentrations in the cord blood depending on the gestational age and birth weight. These researchers claimed that the level of manganese was higher in neonates with low birth weight (<2,500 g) born before 37<sup>th</sup> week of pregnancy than in those with birth weight over 2,500 g and born on due date. However, the differences were not statistically significant, similarly to the results obtained by the authors. VIGEH et al. (2008) compared the manganese levels in the maternal whole blood and cord blood of healthy neonates and IUGR neonates, depending on a neonate's gestational age and gender. They proved that the manganese concentration in the maternal whole blood of mothers having IUGR newborns was significantly lower than in mothers with healthy newborns. At the same time, the concentration of manganese in the cord blood was higher for IUGR newborns than for healthy ones.

It seems that trace amounts of chromium in pregnant women's diets are essential since its deficiency is a factor of the metabolic syndrome. Pregnancy increases the excretion of and the demand for trivalent chromium, simultaneously boosting the insulin resistance and the concentrations of hormones acting antagonistically to insulin (ANDERSON 2007).

According to the authors' own research, the average chromium concentration in the cord blood of the examined neonates was  $2.81 \pm 0.14 \mu\text{mol dm}^{-3}$ , which is within the standard limits (Table 1). The level of this element changed in relation to the gestational age, as the highest values were observed in neonates born after 42<sup>nd</sup> week of pregnancy and the lowest ones - for those born between 38<sup>th</sup> and 41<sup>st</sup> week of pregnancy. The average chromium concentrations depending on the gender and birth weight were comparable in both groups. Similarly, the research by JONES et al. (2010) on chromium concentrations in relation to the gestational age and birth weight did not show any statistically significant differences. The chromium levels in the cord blood they detected were comparable to the results reported herein.

Aluminium is one of the most common metals in nature. According to the authors' own research, the average aluminium concentration in the cord blood reached  $0.041 \pm 0.028 \mu\text{mol dm}^{-3}$ , which was in the range of standard values (Table 1). During pregnancy, aluminium toxicity appears to be negatively correlated to the gestational age. The physiological immaturity of preterm infants' kidneys favours certain accumulation of aluminium as a result of the substitution of calcium ions, a process which weakens bone mineralization processes (REINKE 2003, GURA, PUDER 2006). However, the research did not show any statistically significant relationships between the

gestational age and aluminium concentrations. It was demonstrated that the level of this microelement shows a downward trend in relation to the gestational age. Also the gender and birth weight had no substantial impact on the aluminium concentration in the cord blood (Table 2).

The role of this element appears to be vital during pregnancy and lactation, as it induces an increase of newborns' body weight rate (YOKEL 1984). On the other hand, there is a wealth of research (REINKE et al. 2003, CHU et al. 2006, GURA et al. 2006, MIU et al. 2006) indicating that it is necessary to limit contact with this element due to its possible interference with the normal development of the organism. The aluminium is excreted from the body by kidneys, and preterm newborns are especially prone to the effect of this element. Aluminium toxicity seems to be negatively correlated with the gestational age because the physiological immaturity of preterm infants' kidneys favours some accumulation of aluminium as a result of the substitution of calcium ions, which weakens bone mineralization processes (REINKE et al. 2003, GURA et al. 2006).

## CONCLUSIONS

1. The average concentrations of zinc, copper, iron, manganese, chromium and aluminium in cord blood stayed within the ranges recognized as standard, which indicates efficient regulating mechanisms of the developing foetuses.

2. The neonate's gestational age, gender and birth weight had no substantial impact on concentrations of the examined elements in the cord blood, except iron, whose concentration was noticed to be statistically significantly correlated to the neonates' gender.

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