RETINOPATHY OF PREMATURITY AND SERUM LEVEL OF INSULIN-LIKE GROWTH FACTOR-1

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SUMMARY – The aim of our study was to measure and compare serum insulin-like growth factor-1 (IGF-1) levels at postmenstrual age of 33 weeks between preterm infants with and without retinopathy of prematurity (ROP). ROP occurs in two phases. Low serum levels of IGF-1 during ROP phase 1 have been found to correlate with the severity of ROP. ROP phase 2 begins around postmenstrual week 33. We conducted a prospective cohort study to measure serum IGF-1 levels in premature infants at postmenstrual age of 33 weeks. The study included all premature infants (N=74), gestational age ≤33 weeks, hospitalized at Department of Neonatology, Clinical Center of Montenegro, from April 2008 to July 2009. The incidence of ROP in the study cohort was 50.7%. Infants with ROP had a significantly lower birth weight and significantly shorter gestational age. The mean level of IGF-1 at postmenstrual age of 33 weeks was 23.7 mcg/L. Study results showed that there was no significant difference in serum IGF-1 level between newborns with and without ROP at postmenstrual age of 33 weeks (in newborns with ROP, it was the beginning of ROP phase 2). A large controlled study with repeated measurement of IGF-1 level in the neonatal period is needed to confirm that restoration of IGF-I level occurs in ROP phase 2, i.e. that the low level of IGF-1 is only a feature of ROP phase 1.

Key words: *Retinopathy of prematurity; Insulin-like growth factor-1; Preterm infants*

Introduction

Retinopathy of prematurity (ROP) is a disease of preterm infants. It is a serious vasoproliferative disorder of immature retinal blood vessels. ROP is the most common cause of blindness in childhood in developed countries[1].

The incidence of ROP in the total population of preterm infants is 4.2%. In Montenegro, ROP incidence was 3.9% in 2005, 7.9% in 2006, 9.2% in 2007, and 12.1% in 2008[2]. The incidence of ROP is significantly higher, up to 50%, in preterm infants with extremely small birth weight (BW).

ROP is a pathologic process that occurs only in immature retinal tissue and can progress to tractional retinal detachment, which can result in functional or complete blindness. In infants born preterm, the retina is not fully vascularized. The more premature the child, the larger is the avascular area.

During the third trimester, normal retinal vascularization is stimulated by "physiological hypoxia"[3]. In response to hypoxia, vascular endothelial growth factor (VEGF) is secreted. For appropriate VEGF induced vessel growth, sufficient levels of insulin-like growth factor-1 (IGF-1) in serum are necessary[4].

IGF-1 is a peptide which is essential for both prenatal and postnatal growth of retinal vasculature. During fetal life, IGF-1 is secreted in most tissues, mostly in placenta. In the eye, IGF-1 mRNA was found in the sclera and cornea of fetuses aborted at 16-20 weeks of gestation[5]. After birth, IGF-1 is produced in most organs, mainly in the liver[6]. Serum levels are closely related to gestational age and are lower in more prematurely born infants[7]. Low serum levels of IGF-

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1 during the early postnatal days have been found to correlate with the severity of ROP[8].

ROP occurs in two phases. The ischemic phase 1 begins after premature birth with delayed retinal vascular growth. The reason for this are low values of IGF-1 after preterm birth because of the loss of IGF-1 sources from the placenta [7]. The proliferative phase 2 is characterized by pathologic neovascularization. This leads to ROP phase 2, occurring around postmenstrual week 33. When ROP phase 2 is initiated, IGF-1 level reaches the threshold and if VEGF is high, neovascularization proliferation proceeds[9]. If the process continues, proliferation advances, blood vessels grow in corpus vitreum, which precedes partial or total retinal detachment[8]. This condition is known as retrolental fibroplasia (RLF).

The aim of our study was to measure and compare serum IGF-1 levels between preterm infants with and without ROP at postmenstrual age of 33 weeks. In preterm infants with ROP, postmenstrual age of 33 weeks is the time when ROP phase 2 begins.

Material and Methods

The study was designed as a cohort, prospective, longitudinal type, based on other similar studies. The study included all premature infants (N=74), gestational age ≤33 weeks, hospitalized at Department of Neonatology, Clinical Center of Montenegro, from April 2008 to July 2009. This study did not include infants with conspicuous congenital anomalies.

A database was formed with demographic data, data from pregnancy and delivery medical history (the course of delivery, evaluation of vitality at birth, anthropometric measures at birth), and data from clinical monitoring of the newborn.

In every newborn included in the study, venous blood sample (0.5 mL) was obtained in 33rd postmenstrual week. Extracted serum was frozen and stored in a freezer (at -20 up to -80 °C) until completion of a series of samples. In all samples, quantitative determination of the requested biomarkers (IGF-1) was performed simultaneously, under the same conditions, using immunochemical enzyme-linked immunosorbent assay (ELISA), a method widely used on this hormone measurement[10].

The diagnosis of ROP was made by ophthalmic screening. The infants were examined according to a routine protocol, which consisted of dilated eye fundus examinations. After pupillary dilatation, the eyes were examined by indirect ophthalmoscopy. ROP was classified according to the international classification (ICROP) and subdivided into 5 stages. Proliferative retinopathy was defined as stage 3, stage 4 or stage 5 and moderate ROP as stage 1 or stage 2. After getting information about the existence of the active form of proliferative ROP, the primary cohort (74 newborns) was divided into two subgroups: newborns with ROP and newborns without ROP.

Ethics Committee of the Clinical Center of Montenegro in Podgorica approved the study protocol (consent number 03/01-3813/4), in accordance with standard operating procedures of the Ethics Committee of the Clinical Center of Montenegro and international guidelines for performance of biomedical research on humans.

Statistical data processing included calculation of descriptive measures, use of statistical tests and statistical software programs (NCSS, SPSS), for comparisons of parameters between the subgroups. The following statistical tests were used: t-test, χ^2-test, and Pearson's correlation test.

Results

Primary cohort included 74 preterm infants of gestational age (GA) ≤33 weeks. There were 42 (56.8%) male and 32 (43.2%) female newborns. Table 1 shows the structure of primary cohort according to the history of pregnancy (singleton or multiple) and type of delivery (vaginal or cesarean section).

Of the total number of newborns in primary cohort, 95.9% (n=71) were tested for ROP (ophthalmic

Table 1. Distribution of newborns according to pregnancy and delivery history data

	Without ROP	With ROP
Pregnancy		
Singleton	23 (47.9%)	25 (52.1%)
Multiple	12 (52.2%)	11 (47.8%)
Type of delivery		
Vaginal	18 (45.0%)	22 (55.0%)
Cesarean section	17 (54.8%)	14 (45.2%)

ROP = retinopathy of prematurity

Table 2. Distribution of newborns according to birth weight (BW), gestational age (GA) and levels of insulin-like growth factor-1 (IGF-1) in relation to the presence of retinopathy of prematurity (ROP)

Finding	Parameter					
	BW (g)		GA (weeks)		IGF (mcg/L)	
	χ	SD	χ	SD	χ	SD
Without ROP	1821.71	369.59	31.8	1.43	23.79	4.85
With ROP	1586.94	417.99	30.61	2.12	23.88	6.74
Significance	t=-2.50	p<0.05	t=-2.76	p<0.01	t=0.05	p>0.05

screening). Three (4.1%) newborns were transferred to other institutions for further diagnostic work-up and therapy, which was the reason for excluding them from the process of diagnosing ROP. Through ophthalmic screening, 36 (48.7%) newborns were diagnosed with proliferative form of ROP, whereas 35 newborns had normal ophthalmologic findings.

The mean birth weight (BW) of newborns in primary cohort was 1698.24±402.79g (range 990-2860 g). Table 2 shows mean BW of newborns with and without ROP. Student's t-test revealed the difference in the mean BW between newborns with and without ROP to be statistically significant, with negative direction (t=-2.50; p<0.05). Newborns with ROP had a significantly lower BW.

Figure 1 shows distribution of newborns from primary cohort according to GA. The mean GA in primary cohort was 31.18±1.87 (range 26-33) gestational weeks. Table 2 shows mean GA of newborns with and without ROP. Student's t-test showed the difference in the mean GA between newborns with and without ROP to be statistically significant, with a negative direction (t=-2.76; p<0.01). Newborns with ROP had a significantly shorter GA at birth.
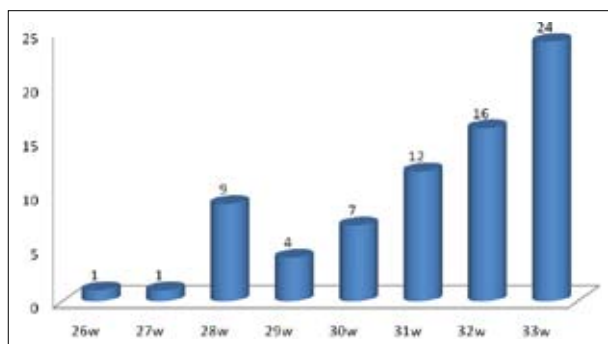
Out of 36 newborns with ROP, 20 (55.6%) were male and 16 (44.4%) female. Figure 2 shows distribution of male and female newborns according to morbidity from ROP. Pearson χ^2-test showed that there was no statistically significant difference in the frequency of ROP between male and female newborns (χ^2=0.12; p>0.05).

The mean level of IGF-1 in primary cohort was 23.74±5.79 mcg/L (range 15.44-46.33). In 19 (25.7%) newborns, the level of IGF-1 was lower than 20 mcg/L, in 33 (44.6%) newborns it was 20-25 mcg/L, and 21 (28.4%) newborns had IGF-1 above 25 mcg/L. The mean postnatal age at sampling was 12.56±11.97 days (range 1-56).

On average, male newborns had a lower level of IGF-1 (23.16±4.98 mcg/L) than female newborns (24.49±6.73 mcg/L). Pearson's correlation test yielded no significant correlation between sex and level of IGF-1 in 33rd postmenstrual week (p=0.07; p>0.05).

Table 2 shows mean levels of IGF-1 in newborns with and without ROP. Student's t-test showed no statistically significant difference in the mean levels
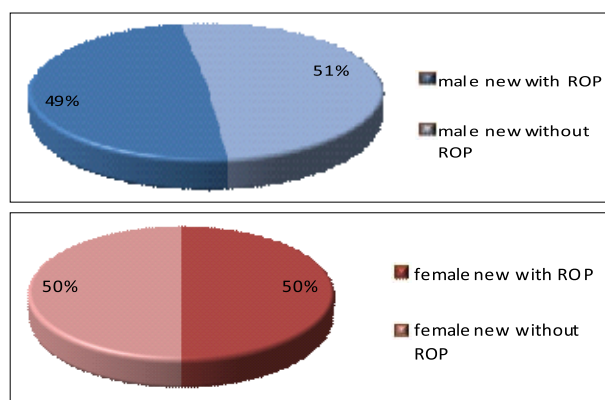

Fig. 1. Distribution of newborns from the primary cohort according to gestational age (weeks).

Fig. 2. Distribution of male and female newborns according to morbidity from retinopathy of prematurity (ROP).

of serum IGF-1 between newborns with and without ROP (t=0.05; p>0.05).

Pearson χ^2-test yielded no significant difference in the frequency of ROP between the cohorts of newborns with different levels of serum IGF-1 (χ^2=1.77; p>0.05).

Discussion and Conclusion

Premature birth is the main risk factor for ROP. Improved neonatal care has resulted in increased survival of extremely immature infants at a high risk to develop ROP. The increase in the incidence of ROP follows the increase in the incidence of blindness. Data from neighboring Croatia show that on every 26th day, one child gets blind because of ROP, adding that in the population of blind children up to 16 years of age ROP is the main cause of blindness. This tells us a lot about the importance of prevention and therapy for ROP for the overall health system of every country[11]. Better understanding of the pathophysiology of ROP contributes to achieving this goal.

The present study was performed at Department of Neonatology, the only center for medical treatment of the endangered preterm and term infants in Montenegro. Usually, 65% of the total number of preterm newborns in Montenegro are hospitalized at this Department. When we started the study in 2008, there were 8258 children born in Montenegro, of which 495 (6%) premature infants. In the same year, 860 newborns were hospitalized, of which 255 (29.6%) preterm, while 60 newborns were diagnosed with proliferative ROP and had the indication for laser treatment. In 2008, the incidence of proliferative ROP in Montenegro was 12.1%[2].

The incidence of proliferative ROP in our primary cohort was 50.7%. This high incidence of ROP stems from the fact that the participants of our study were 'endangered' preterm infants who, after birth, had to be transferred to Department of Neonatology. This fact implies the higher presence of risk factors for the development of ROP (oxygen therapy, mechanical ventilation, anemia, transfusion).

Study results showed that there was no statistically significant difference in the incidence of ROP between male and female preterm infants. Newborns with ROP had a significantly lower BW and shorter GA at birth. The difference in GA was highly significant.

The mean level of serum IGF-1 in primary cohort was 23.7 mcg/L, which was also the mean level of IGF-1 at postmenstrual age of 33 weeks in endangered preterm infants in Montenegro. Serum IGF-1 level was slightly lower in male than in female newborns, but this difference was not statistically significant. In the cohorts formed on the basis of interval levels of IGF-1, there was no significant difference in the incidence of ROP.

There was no significant difference in serum IGF-1 level at postmenstrual age 33 weeks between newborns with and without ROP. The level of IGF-1 in ROP phase 2 did not differ significantly from the level of IGF-1 in newborns of the same postmenstrual age without ROP.

A large controlled study with repeated measurement of IGF-1 level in the neonatal period is needed to confirm that restoration of IGF-I level occurs in ROP phase 2, i.e. that the low level of IGF-1 is only a feature of ROP phase 1.

Several clinical studies have shown that persistent low serum concentrations of IGF-I after premature birth are associated with later development of ROP[9]. However, some studies have shown that there is no significant difference in serum IGF-1 level between the proliferative ROP group and non-proliferative group in any postnatal period[12].

The possible reason for different results should be looked for in rapid nutritional and metabolic changes during postnatal life, which have great effect on IGF-1 level. Future studies should take in consideration the effect of nutritional and metabolic status of the newborns on serum level of IGF-1.

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Sažetak

PREMATURNA RETINOPATIJA I SERUMSKA RAZINA INZULINU SLIČNOG FAKTORA RASTA–1

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Cilj rada je bio izmjeriti i usporediti serumsku razinu insulinu sličnog faktora rasta-1 (IGF-1) u 33. postmenstrualnom tjednu između prijeterminske novorođenčadi s prematurnom retinopatijom (PR) i bez PR. PR se javlja u dvije faze. Nađeno je da je niska serumska razina IGF-1 tijekom prve faze PR u korelaciji s težinom PR. Druga faza PR počinje oko 33. postmenstrualnog tjedna. Provedena je kohortna prospektivna studija u kojoj se mjerila serumska razina IGF-1 kod prijeterminske novorođenčadi u 33. postmenstrualnom tjednu. U studiju su uključena sva prijeterminska novorođenčad (N=74) gestacijske starosti ≤33 tjedna koja su bila hospitalizirana u Centru za neonatologiju Kliničkog centra Crne Gore od travnja 2008. do srpnja 2009. Incidencija PR u studijskoj kohorti iznosila je 50,7%. Novorođenčad s PR imala su značajno nižu porođajnu tjelesnu masu i značajno kraću gestacijsku dob. Prosječna razina IGF-1 u 33. postmenstrualnom tjednu iznosila je 23,7 mcg/L. Naši rezultati su pokazali da u 33. postmenstrualnom tjednu (za novorođenčad s PR to je početak druge faze PR) nema značajne razlike u serumskoj razini IGF-1 između novorođenčadi s PR i bez PR. Potrebno je provesti veću kontroliranu studiju s ponavljanim mjerenjem razine IGF-1 u neonatalnom razdoblju kako bi se potvrdilo da u drugoj fazi PR dolazi do restitucije razine IGF-1, odnosno da je niska razina IGF-1 odlika samo prve faze PR.

Ključne riječi: *Prematurna retinopatija; Inzulinu sličan faktor rasta-1; Prijeterminska novorođenčad*