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Research into the bilirubin levels in the blood serum of newborns with retinopathy of prematurity

Retinopathy of prematurity was described for the first time in 1942 by Terry, an oculist from Boston (19) who observed the fibro-vascular proliferative masses behind the lens causing blindness in infants born before their due time. The above changes have been called retro-lental fibroplasia. At present it is known that the above term is appropriate for the description of one of the stages of the advanced form of the condition which nowadays is referred to as retinopathy of prematurity (ROP). This condition is now generally considered to be the main cause of sight loss in the newborns and is due in no small measure to the increase in keeping very immature, newly born babies alive (3, 10, 11, 12, 13).

Factors such as low birth weight, the short gestation period and the extended period of oxygen-therapy are main risk factors in the retinopathy of prematurity (5, 8, 10, 12). It is a commonly accepted belief today that the damage to immature retina vessels caused by free oxygen radicals is chiefly responsible for retinopathy of prematurity in newly born, premature infants (10, 12, 13).

In 66%–80% of newly born infants, physiological jaundice lasting until the 7th or the 10th day of life is observed. Its peak occurs in the 3rd to the 5th day, the bilirubin concentration in the serum not exceeding 12.5 mg%. In premature babies, jaundice can be prolonged in character and peak values can reach 15.0–16.0 mg%, its peak being observed in the 6th to the 7th day of life. This is caused by the insufficient adaptation of the newly born infant to bilirubin transformation and excretion. In full-term infants, jaundice usually disappears without the necessity for treatment (4, 9, 14).

In recent years, some researchers have drawn our attention to the antioxidant function of bilirubin (2, 18). In their experimental research, Stocker et al. proved that bilirubin at micro-molar concentration levels *in vitro*, effectively deactivates the free oxygen radicals chemically produced and its antioxidative activity increases with the decrease in experimental oxygen levels from 20% to 2%. The above studies proved that in liposomes at 2% oxygen concentration, bilirubin has a stronger antioxidative action than vitamin E (alpha-tocopherol) which was previously considered to be the strongest of antioxidants (15, 16, 17). Studies by Hayman et al., carried out on a group of 12 newborns, suggest that bilirubin can play a protective role in the retinopathy of prematurity (7). A paper by Hegyi et al. based on an analysis of 25 cases, stated that the higher bilirubin concentration in the blood serum of newborns is connected with the lower frequency of the occurrence of diseases with etiology relating to free radicals such as necrotic enteritis, bronchial-pulmonary dysplasis, brain ventricle hemorrhages and retinopathy of prematurity (6).

The aim of this study was to evaluate the relation between the total bilirubin level in the peripheral blood of newly born infants and the occurrence of retinopathy of prematurity in these babies and its clinical picture. The above studies are important when estimating procedures for the treatment of physiological jaundice in premature babies.

MATERIAL AND METHODS

Studies were carried out on a group of 110 premature, newly born infants under examination in the ROP Unit of the 2nd Ophthalmologic Clinic at the Medical University of Lublin.

Four groups relating to the advancement of retinopathy of prematurity were distinguished in the study material. The generally accepted acronym ROP was used in the description. The ROP-0 group consisted of 67 premature, newborns in which no symptoms of retinopathy were found during three routine examination rounds. The ROP-1 group consisted of 12 premature, newborns with symptoms of retinopathy of the 1st degree. The ROP-2 group consisted of 10 premature, newlborns with symptoms of retinopathy of the 2nd degree. The ROP-3 consisted of 21 premature, newborns with symptoms of retinopathy of the 3rd degree. Blood serum bilirubin levels were determined in all the infants subjected to this study. The mean values of the parameters obtained during this study for all groups are presented in Table 1 below.

Table 1. Mean bilirubin levels in the groups

Name of the group	No. of infants	Mean bilirubin level in mg%
ROP-0	67	12.46±2.38
ROP-1	12	6.41±4.11
ROP-2	10	7.34±4.57
ROP-3	21	6.48±2.53

All the premature, newly born infants studied were divided into the following two groups depending on their bilirubin levels. Premature babies with bilirubin blood serum levels up to 10 mg% were assigned to Group A, while premature babies with bilirubin blood serum levels of above 10 mg% were assigned to Group B.

Ophthalmological examinations according to the scheme advised in the directive of the Minister of Heath and Social Care of July 25th, 1991, i.e. in the 4th, 8th, and 12th week of life, were carried out on all babies. Where symptoms of retinopathy were found, the examinations were carried out at weekly intervals. An indirect ophthalmoscope and aspheric lenses with a power of 20 and 29 dioptres were used for the above examinations. In order to obtain a better picture of the peripheral regions of the retina, the wall of the eye-ball was invaginated by squint hook. The degree of retinopathy advancement was determined in accordance with the instructions published by the "International Classification of Retinopathy of Prematurity' of 1984 (3). Blood serum levels of bilirubin were determined using the 'Jendrassik' colorimetric method (acc. to 1). The examinations were carried out on the 2nd, 4th, 6th, and 8th day of life.

Numerical data given in the statistical analysis characterises the numerical data of the bilirubin levels in the blood serum by means of arithmetical means X, standard deviation SD and variability coefficient V. The frequency of the occurrence of individual retinopathy degrees was expressed in absolute numbers and percentages. The significance of the differences between the mean values was tested using the T-Student's test. The hypothesis on the lack of relations between classifying features was verified for the data classed according to the degree of retinopathy and ranges of bilirubin levels established by means of the Chi-square test. The significance level assumed was $\alpha=0.05$. Calculations were carried out by means of the 'Statgraph 5.0' statistical software package.

RESULTS

The mean bilirubin level in the blood serum in the ROP-0 group was 12.46 ± 2.38 mg% and was statistically significantly higher than in the blood serum of the ROP-1 group where it was at a level of 6.41 ± 4.11 mg% ($p<0.001$), and was, in turn, statistically significantly higher than in the

blood serum of the ROP-2 group in which it was 7.34±4.57 mg% ($p<0.001$), and statistically significantly higher than in the blood serum of the ROP-3 group where it was at a level of 6.48±2.53 mg% ($p<0.001$). A graphic comparison between the mean bilirubin levels in the individual groups has been presented in Figure 1.

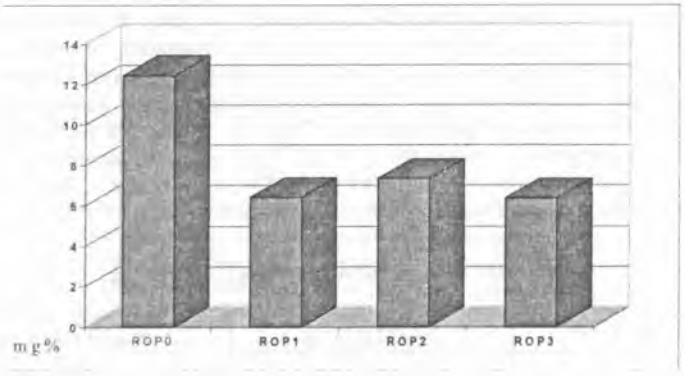

Fig. 1. Graphic comparison of the mean serum bilirubin levels in groups: ROP-0, ROP-1, ROP-2 and ROP-3

Table 2 presents the frequency of the occurrence of individual stages of ROP related to the classification of the study group of babies into group A with bilirubin levels up to 10 mg% and group B with bilirubin levels above 10 mg%. Highly significant statistical differences in the occurrence of the individual stages of ROP were found in relation to the bilirubin levels in the group up to 10 mg% and above 10 mg% ($p<0.001$).

Table 2. Comparison of the frequency of occurrence of the individual ROP stages in groups of infants with bilirubin levels up to 10 mg% (group A) and in groups of infants with bilirubin levels exceeding 10 mg% (group B)

Groups	No. of infants with ROP-0	No. of infants with ROP-1	No. of infants with ROP-2	No. of infants with ROP-3	Value of the Chi^2 function (Chi^2)	Level of significance
(A) 10 mg%	6 (9.8%)	10 (83.3%)	7 (70%)	21 (100%)	65.52	$p<0.001$
(B) (above) 10 mg%	61 (90.2%)	2 (16.7%)	3 (30%)	0 (0%)		

In the group of immature babies studied, ROP damage, of the 1st and 2nd stage types, occurs in the group with bilirubin levels up to 10 mg% and ROP damage, of the 3rd stage type, occurs in this group only, while to have no symptoms of ROP in this group is very rare.

In the group with a bilirubin level above 10 mg%, no ROP symptoms was the most frequent occurrence, damage of the ROP of the 1st and 2nd stage was relatively rarely observed and there was no damage of the ROP of the 3rd stage type. Figure 2 presents a graphic comparison of the frequencies of the occurrence of individual ROP stages in relation to bilirubin levels up to 10 mg% (group A) and above 10 mg % (group B).

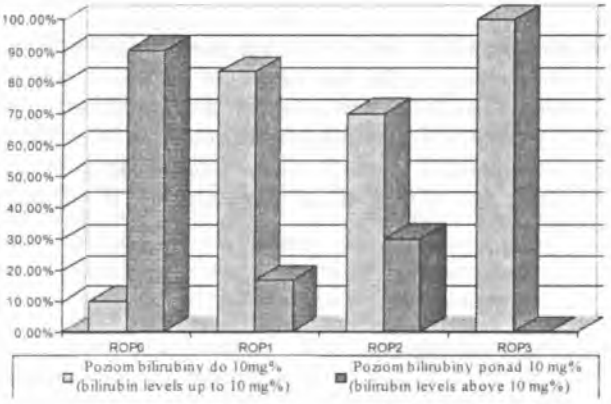

Fig. 2. Comparison of the frequency of occurrence of the individual ROP stages in groups of infants with bilirubin levels up to 10 mg% (group A) and in groups of infants with bilirubin levels above 10 mg% (group B)

DISCUSSION

The first reports on the anti-oxidative role of bilirubin come from 1987 (15, 16) and 1988 (18). Based on *in vitro* studies, Stocker et al. (16) stated that bilirubin, together with human albumin, deactivated free oxygen radicals in the ratio of 1 mol of bilirubin complex with albumin to 2 mols of free oxygen radicals. The above data point to the role of the bilirubin-albumin complex as a natural antioxidant in the blood serum.

Sullivan et al. (18) carried out studies on the antioxidant activity of blood serum in 25 newly born infants with a weight at birth from 830 g to 3700 g and found that the insufficient antioxidant activity of the blood serum could cause the development of retinopathy of prematurity. In their *in vitro* studies, Stocker and Peterhans (17) found that bilirubin and biliverdine can act synergically in the prevention of the perioxidation of lysosomal lipids. Benaron and Bowen (2) carried out studies on a group of 44 premature, newly born infants with symptoms of free radical disease and found statistically significant lower bilirubin levels in the blood serum of this group of babies as compared to the group of premature, newly born infants without the symptoms of diseases with free radical etiology.

The above results agree with the results obtained in the present research based on an examination of 110 infants where it was found that in the group of premature, newly born infants without symptoms of retinopathy, the mean bilirubin levels in the blood serum were statistically significantly higher than in the group of premature, newly born infants with symptoms of retinopathy of the 1st degree. This was also the case in the group of premature, newly born infants with retinopathy of the 2nd degree and also the case in the group of premature, newly born infants with retinopathy of the 3rd degree.

The data presented above do not explain all the factors influencing the development and clinical course of retinopathy of prematurity. However, they permit the assumption that the careful monitoring of the physiological hyperbilirubinemy of premature, newly born infants with low birth weight who are subjected to oxygen therapy in incubators can prevent the development of retinopathy of prematurity in these babies.

CONCLUSIONS

1. Factors such as a high bilirubin level in the blood, a low birth weight and a short gestation period along with an extended period of oxygen therapy, are parameters closely identified with immaturity in newly born infants. Of these, only a high bilirubin level in the blood can protect against retinopathy or condition its milder course.

2. The above results indicate that a low total bilirubin level in the blood of those newly born infants, threatened by retinopathy of prematurity, should be considered a risk factor for this disease.

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SUMMARY

The damage to immature retina blood vessels caused by free oxygen radicals plays a basic role in the pathogenesis of the retinopathy of prematurity (ROP). The main risk factors in ROP are low birth-weight, short gestation period and the extended period of oxygen therapy. In recent

years. some researchers have drawn our attention to the anti-oxidative role of bilirubin. The subject of this paper is an explanation of the relation between bilirubin levels in the blood of premature babies and the occurrence and clinical picture of the retinopathy of prematurity. The results obtained by the present author are important for the appropriate treatment of physiological jaundice in premature newborns in connection with ROP prophylaxis. The present study was carried out on a group of 110 newly born infants examined in the ROP Unit of the 2nd Department of Ophthalmology at the Medical University of Lublin. An indirect ophthalmoscope was used in the clinical examination and the bilirubin levels were determined using the 'Jendrassik' colorimetric method. The mean bilirubin level in the blood serum of the group without ROP symptoms was 12.46 ± 2.38 mg% and was statistically significantly higher than in the serum of the group exhibiting symptoms of retinopathy of prematurity where it was at a level of 6.41 ± 4.11 mg% ($p<0.001$); it was also statistically significantly higher than in the serum of the group of newborns exhibiting symptoms of retinopathy of prematurity of the second degree where it was at a level of 7.34±4.57 mg% ($p<0.001$) and was statistically significantly higher than in the serum of those prematurely newborns with symptoms of retinopathy of the third degree where it was 6.48±53 mg% ($p<0.001$). The results presented here prove the protective role of bilirubin in the course of the retinopathy of prematurity. A high blood bilirubin level, a low birth weight, a short gestation period and a long period of oxygen therapy are all parameters which are closely related to immaturity in the newly born. Of all these parameters, only a high blood bilirubin level can protect from the occurrence of retinopathy or influence its milder course, whereas a low bilirubin level could be a risk factor in the retinopathy of prematurity.

Badania poziomu bilirubiny w surowicy krwi noworodków z retinopatią wcześniaków

Podstawową rolę w patogenezie retinopatii wcześniaków (RW) odgrywa uszkodzenie niedojrzałych naczyń siatkówki przez wolne rodniki tlenowe. Głównymi czynnikami ryzyka RW są: niska waga urodzeniowa, krótki wiek ciążowy i długi czas tlenoterapii. W ostatnich latach niektórzy autorzy zwracają uwagę na antyoksydacyjną rolę bilirubiny. Tematem pracy jest wyjaśnienie związku między poziomem bilirubiny we krwi wcześniaków a występowaniem i obrazem klinicznym retinopatii wcześniaków. Wyniki pracy mogą mieć znaczenie dla właściwego prowadzenia fizjologicznej żółtaczki u wcześniaków w połączeniu z profilaktyką RW. Praca dotyczy 110 wcześniaków przebadanych w Poradni Retinopatii Wcześniaków II Kliniki Okulistyki AM w Lublinie. Do badania klinicznego użyto wziernika pośredniego obuocznego, a do określenia poziomu bilirubiny zastosowano metodę kolorymetryczną Jendrassika. Średnia poziomu bilirubiny w surowicy krwi w grupie bez RW wynosiła 12,46 ± 2,38 mg% i była statystycznie wysoce istotnie wyższa niż w surowicy krwi grupy z retinopatią wcześniaków pierwszego stopnia, gdzie wynosiła 6,41 ± 4,11 mg% ($p<0,001$), była statystycznie wysoce istotnie wyższa niż w surowicy krwi w grupie z retinopatią wcześniaków drugiego stopnia, gdzie wynosiła 7,34±4,57 mg% ($p<0,001$) oraz była statystycznie wysoce istotnie wyższa niż w surowicy krwi w grupie z retinopatią wcześniaków trzeciego stopnia, gdzie wynosiła 6,48±53 mg% ($p<0,001$). Przedstawione wyniki badań świadczą o ochronnej roli bilirubiny w przebiegu retinopatii wcześniaków. Wysoki poziom bilirubiny we krwi, niska waga urodzeniowa, krótki czas trwania ciąży i długi czas tlenoterapii są parametrami ściśle związanymi z niedojrzałością noworodka. Między nimi tylko wysoki poziom bilirubiny we krwi może ochronić od wystąpienia retinopatii lub spowodować łagodniejszy jej przebieg. Natomiast niski poziom bilirubiny we krwi może pełnić rolę czynnika ryzyka w retinopatii wcześniaków.