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The Efficacy of Propranolol in Retinopathy of Prematurity and its Correlation with the Platelet Mass Index

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ABSTRACT

Purpose: Retinopathy of Prematurity (ROP) is a proliferative vitreoretinopathy which is one of the most frequent causes of blindness in children. In an attempt to find a solution to this important problem in preterm children, the search for new, effective treatment modalities with fewer side effects is underway. In our study, which was planned for this reason, we aimed to investigate the effects of propranolol treatment applied to cases of ROP in various stages during the second phase (known as the neovascularization-hypoxia phase) and to determine the correlation of these effects with the platelet mass index (PMI).

Method: A total of 171 preterm infants at risk of ROP were selected randomly for inclusion in the study. All of the patients were classified according to their stage of ROP and were divided into control and treatment groups. While the cases in the control group were administered physiological saline solution, those in the treatment group were administered propranolol in the period that corresponded to the second stage of the disease. The thrombocyte and PMI values in the first and second stages of each study group were recorded.

Results: A significant difference was found between the control and treatment groups of the stage 2 ROP study subjects. In the stage 2 ROP study group, no significant difference was detected between the control and treatment cases in terms of platelet counts in phase 1 or in the PMI values and the thrombolytic counts in phase 2. On the other hand, in phase 2 of the stage 2 ROP study subjects significant differences were detected between the control and treatment group in terms of PMI values.

Conclusion: In the study, it was found in the stage 2 ROP study group that propranolol reduced the need for laser photocoagulation significantly. Also, in parallel to the efficacy of propranolol in this study group, a decrease was observed in PMI values.

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Platelet mass index; propranolol; retinopathy of prematurity; thrombocyte; vascular endothelial growth factor

Introduction

Retinopathy of prematurity (ROP) is a major retinal disease which is seen in preterm neonates.[1] It is an important problem in premature infants characterized by serious retinal detachments resulting from the discontinuation of normal retinal vascularization, and eventually lead to blindness.[2] This disease consists of two phases, each of which is the mirror image of the other. The first of these phases is that of retinal vascular inhibition which results from hyperoxia encountered by premature newborns postnatally (phase 1, vasoobliteration), while the second is that of abnormal vascular proliferation (phase 2, neovascularization) which develops as a response to the inhibition in phase 1. However, in phase 1, the lack of such mediators as vascular endothelial growth factors (VEGF), and, in phase 2, the superfluity of this mediator is in question.[2–5]

Regarding the solution to the problem of ROP, the aim of this study was to search for novel and effective medical treatment modalities with fewer side effects. We hypothesized that VEGF overexpression in ROP could be induced by β_2-Adrenergic Receptor (AR) stimulation, and that beta-blockers (such as propranolol) could be useful in the treatment of ROP. Our hypothesis is that in human preterm newborns with ROP, VEGF overexpression could be induced by β_2-AR stimulation, and that propranolol could reduce the progression of the disease. We aimed to investigate the effects of propranolol treatment when applied to cases of ROP in various stages during the second phase and to determine the correlation of these effects with the platelet mass index (PMI).

For this purpose, the characteristics of the above-mentioned phases were made use of, which are extremely important for the pathophysiology of the disease. It has been demonstrated in previous studies (mouse model of oxygen-induced retinopathy-OIR) that the blockage of adrenergic receptors in the second phase of ROP could reduce neovascularization and prevent the progression of the disease.[6–8]

With this in mind, we decided that propranolol, which acts by lowering VEGF levels in the treatment of vascular tumors in

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pediatric patients, could be effective in the same way, and thus could also be used in the treatment of ROP in phase 2.[9–11]

There are clinical and experimental studies in the literature demonstrating that platelets have important roles in angiogenesis.[12–14] Platelets exercise their effects through other mediators besides VEGF. However, the most important of these angiogenic proteins in platelets is VEGF.[12]

In the second stage of ROP, in addition to VEGF which is released from hypoxic retinal tissue, the VEGF released from thrombocytes can contribute to neovascularization. The conclusions drawn from these studies corroborate the hypothesis that there may be a correlation between VEGF, which is increased in the second phase, and PMI. Since propranolol exercises its effect by reducing VEGF levels, it is probable that a correlation also exists between its efficacy and PMI, and this possible relationship may be important in terms of monitoring its therapeutic effectiveness.[9]

With the proliferation of medical possibilities and knowledge in our time, there has been an extraordinary increase in the rate of preterm births, and, consequently, the visual problems associated with preterm births, particularly in developing countries, have also increased markedly. Unless managed properly, the visual problems in question can result in devastating consequences which can lead to blindness. The current techniques used to prevent the progression of the visual problems associated with prematurity (such as laser photocoagulation therapy and bevacizumab) have rather grave side effects, such as loss of visual field and poor visual acuity.[15–17] In addition, these techniques are not only rather grueling for physicians but are also limited in use, for they can only be used in some stages of ROP (cases of stage 3 or ROP cases in which zone 1 is involved).[18,19]

For all these reasons, our study aimed to find a solution to meet the need for a new, practical treatment method with fewer side effects to reduce the use of laser photocoagulation, which has far more side effects, in preterm cases at risk in terms of ROP. The agent considered for use in response to our search is propranolol, which has also been used with success in the treatment of benign vascular tumors in pediatric patients.[20] In our study, the effect of propranolol, which is the most ambitious agent in this respect, was studied in light of the current literature. For this purpose, the effect of propranolol was examined in all stages of ROP (except for cases at stage 3, which require laser photocoagulation therapy). In this context, our study has addressed a subject which, until now, has not been examined in the literature, and aimed to shed some light on it.

In line with these considerations, propranolol was applied to cases in various stages of ROP during the second phase – the neovascularization period of the disease when VEGF levels are on the rise. With the purpose of monitoring the efficacy of the treatment, the PMI values were also closely monitored, since they were reported to be crucial in storing, transporting and secreting VEGF.[12]

Methods

Randomly included in the study, which was conducted from April 2011 to April 2014 were newborns who were admitted to our neonatal service, with a gestational age (GA) under 32 weeks, birth weights (BW) under 1500 g, and with stage 0, 1, 2 ROP. The patients were divided into three study groups depending on their stages of ROP (0, 1, 2). Each study group was divided into sub-groups comprising control or treatment cases.

Our study was planned with ROP pathophysiology in view. Consequently, it is essential to understand the concepts of "phase" and "stage", which are the most important elements in comprehending both ROP and our study.

"Phase" is a term used to refer to the steps of postnatal vascular development observed on the walls of the retina. The first person to suggest the concept of "phase" and pioneer an understanding of ROP pathophysiology was Ashton.[3] Then, the concept of oxygen toxicity (phase-1) was established followed by hypoxia-mediated vasoproliferation (phase-2) through work in cats.[21–23]

The most important determining point in the pathology of ROP is considered to be the occurrence of phase-2. It is an important sign which indicates the onset of serious disease. Phase-1 (hyperoxia and loss of the maternal–fetal interaction resulting in an arrest of retinal vascularization) develops, as a result of the hyperoxy encountered postnatally by the preterm infant, and results in the suppression of retinal vascularization. As a result of this occurrence in phase-1, phase-2 (hypoxic, stimulating growth factor-induced vasoproliferation) is triggered. If precautions are not taken after the onset of phase-2 (such as ensuring that only the required amount of supplementary oxygen is administered) the disease can progress.Phase-2 is characterized by the proliferation of blood vessels largely in response to hypoxia-driven increases in VEGF and erythropoietin.[24,25] The new vessels poorly perfuse the retina and are leaky, which leads to fibrous scar formation and retinal detachment.[26] Owing to all these pathophysiological features of the concept of stage which we have tried to describe, we found it appropriate to initiate propranolol treatment in the second phase of ROP in the cases included in our study.

The concept of stage, however, is defined by retinal vascularization based on an imaginary picture formed by dividing the retina into three zones with the optic nerve at the center. Such a definition is quite helpful to clinicians in monitoring the progression of the disease to blindness. Classification of the stages of retinopathy of prematurity is necessary for the standardization of treatment practices, and so that interventions can be assessed at a defined stage when progression to blindness is likely.[27]

ROP cases at stage 1 and 2 can spontaneously revert and normalize. Stage-2 is characterized by a thin demarcation line between the vascularized and non-vascularized retina in addition to a ridge. In stages after stage-2, extraretinal neovascularization can develop, causing total retinal detachment and thus blindness. Owing to these pathophysiological reasons, stage-2 is crucial in the treatment of ROP and is a critical period in the monitoring of ROP.[21] For these reasons, throughout our study, we monitored the cases in stage-2 more closely, with physicians specialized in the field, than those in other stages. While treating the cases at stages 0,1, 2 with propranolol, we assessed the cases at stage 3 in terms of laser coagulation treatment.

Careful attention was exercised to ensure that propranolol was administered to stage 0 and 1 ROP patients at the end of phase 1 (i.e. the obliteration phase when VEGF levels were low) and at the beginning of phase 2 (i.e. neovascularization phase when VEGF levels were elevated). In cases of stage 0–1, we decided when propranolol treatment should be initiated after the first examination in risky cases. As for the stage 2 ROP cases, their propranolol treatment was started as soon as stage 2 ROP was detected in their retinal examination. Hypoxia is the driving force for blood vessel growth, through the reflex increase in VEGF gene expression, as observed during the second phase of ROP, which is a vasoproliferative phase (the neovascularization-hypoxia phase).[28–30] Thus, an effort was made to make use of the inhibitory effects of propranolol on vessels during the neovascularization period when VEGF levels rose (phase 2). The control cases received physiological saline solution only.

Propranolol was administered to the patients in the treatment group in physiological saline solution in doses of 0.5 mg/kg/6 hours at 30 minutes before feeding time. Any leftover solution, which was orally administered, was not used again. In other words, a fresh solution was prepared on each occasion. The data regarding all the preterms were plotted and recorded throughout their follow-up in lists prepared previously.

Post-menstrual age was taken into account for the period of transition from phase 1 to phase 2. No patient received propranolol before post-menstrual week 31. The cases born before gestational week 27 received propranolol at post-menstrual week 32 at the earliest; those born at gestational week 27–29 received propranolol at post-menstrual week 33 at the earliest; and finally, the cases born at gestational week 30 or later received propranolol at post-menstrual week 34, at the earliest. Those born with a birth weight below 1250 g did not receive propranolol before post-menstrual week 32, regardless of their gestational age.

One hour before their being examined, the infants' pupils were enlarged by means of 1% tropicamide and 2.5% phenylephrine drop applied 3 times, each at an interval of 15 minutes. Topical anesthesia was achieved with 0.5% proparacaine hydrochloride before examination. After the attachment of a cover speculum, the front segment and then the fundus were examined through a binocular indirect ophthalmoscope with 20–28 diopter lenses. The cases were assessed simultaneously by two experienced ophthalmologists blinded as to which patients were receiving propranolol, but the decision was made jointly. Propranolol administration and retinal examinations were continued in the study groups until retinal vascularization was completed.

A Siemens Advia 2120i was used for reading thrombocyte values. This study was done using the PMI, which enables thrombocyte counts and mean platelet volume (MPV) values to be assessed together. The PMI was recorded as the value obtained from the multiplication of the thrombolytic count by MPV. [PMI = Platelet counts x MPV /1000].

Use was made of the National Institutes of Health (NIH) system for scoring bronchopulmonary dysplasia (BPD). Premature rupture of membrane (PROM) was considered to be positive if membranes ruptured more than 18 hours before delivery. Intraventricular hemorrhage (IVH) in patients with bleeding of at least phase 2 according to Volpe scoring was recorded as positive. In patent ductus arteriosus (PDA), those patients with a right atrium aorta root ratio of 1.4 were considered to be positive. Those on the borderline underwent dynamic echocardiography. Cases with necrotizing enterocolitis (NEC) and sepsis were assessed together and details were recorded in their follow-up charts. The Bell and Töllner scoring methods were employed for NEC and sepsis.

For the sake of objectivity in ROP follow-up, the retina was divided into 3 zones. **Zone 1**: the area with the optic disc in its center formed by a circle with a radius twice as long as the distance from the optic disc to the central macula. **Zone 2**: the area within a circle with a radius equal to the distance from the nasal ora serrata and with the optic disc in its center. **Zone 3**: the area outside zone 2 shaped like a half moon in the retina.

Grading ROP cases was done according to the International Classification of Retinopathy of Prematurity (ICROP) system. **Stage 0**: no sign of ROP located in the retina. **Stage I**: sighting of the thin whitish demarcation line which separates the avascular and vascular retinal areas from each other. **Stage 2**: sighting of the ridge characterized by the demarcation line gaining width and volume, **Stage 3**: defined as irregularities in the ridge and the sighting within it the formation of new vessels. **Plus Disease** was defined as tortuosity and dilatation in the posterior retinal vessels. Finally, **Pre-Threshold Disease** was defined as plus disease or ROP at stage 3 in zone I, while it was defined in zone 2 as plus disease accompanied by ROP at stage 2/3 ROP.

Excluded from the study were those with cardiovascular anomaly, renal failure, apnea, hypoglycemia, bradycardia, those who did not take their medicine throughout one day, those whose families wanted to remove their children from the study, and finally those who failed to gain sufficient weight. None of the cases received blood transfusion in the first 10 days.

Bradycardia was defined as a cardiac rate below 100 beats per minute, the presence of bradycardia in addition to the interruption of apnea respiration for longer than 20 minutes, and saturation falling below 88. Hypotension, however, was defined as mean arterial blood pressure less than the 10th percentile for gestation/birth weight and postnatal age. Occurrence of any one of these in any of the cases, even if on only one occasion, was enough to justify the exclusion of that case from the study.

Propranolol is generally well tolerated in newborns and infants. However, premature infants have a high risk of associated complications (severe bradycardia, hypotension, renal failure, persistent apnea, severe hypoglycemia) that could make this drug unsafe.[10] When these side effects were seen in newborns, the administration of propranolol was permanently discontinued and these newborns were excluded from the study. These side effects were usually observed within the first three days after administration of propranolol. Apnea was observed in four newborns, hypotension in one newborn and hypoglycemia in three patients, all of whom were removed from the study. In

Table 1. Stage 0 ROP study groups.

n: 56	Control group *n*: 30 (%) median ± SD	Treatment group *n*: 26 (%) median ± SD	*p* value
Gestational age (week)	29.0 ± 1.5	29.0 ± 2.1	0.83
Birth weight (g)	1054 ± 233	1099 ± 319	0.58
Mechanical ventilation (hour)	24.0 ± 409.5	168.0 ± 499.3	0.27
Oxygen using (day)	26.52 ± 32.9	22.05 ± 21.0	0.40
Platelet count in phase 1 (mm^3)	211000 ± 110500	184000 ± 87500	0.96
PMI value in phase 1	2059 ± 1039.5	1793 ± 877.5	0.67
Platelet count in phase 2 (mm^3)	264000 ± 194000	304 ± 128200	0.45
PMI value in phase 2	3048 ± 1618.2	3029 ± 1293.9	0.77
BPD	2 (6.7)	4 (15.4)	0.28
RDS	13 (43.3)	16 (61.5)	0.25
İVH	15 (50.0)	10 (38.4)	0.32
PDA	12 (40.0)	8 (30.8)	0.48
Sepsis-NEC	5 (16.7)	7 (26.9)	0.32
PROM	10 (33.3)	2 (7.7)	**0.02**
Preeclampsia	7 (23.3)	9 (34.6)	0.42
Prenatal steroid	25 (83.3)	22 (84.6)	0.88
Laser photocoagulation	0	1 (3.9)	0.28

PMI: platelet mass index, BPD: bronchopulmonary dysplasia, RDS: respiratory distress syndrome, IVH: intraventricular hemorrhage, PDA: patent ductus arteriosus, NEC: necrotizing enterocolitis, PROM: premature rupture of membranes.

addition, when propranolol was administered to unstable newborns, these side effects were more common. Therefore, only stable newborns were included in the study.

The absence or presence of a significant difference between the cases in the control and treatment groups in terms of diseases thought to affect BW, GA, and ROP was researched; this enabled us to determine whether or not a statistical significance existed between the control and treatment groups which would affect the study (Tables 1, 2 and 3).

In addition, with respect to external interventions likely to impact ROP (O_2 treatment day, mechanical ventilation in hours, prenatal steroid use etc.), the absence or presence of statistical differences between the control and treatment groups which might affect the study was also researched (Tables 1, 2 and 3).

Laser photocoagulation therapy and the statistical significance of its distribution between the control and treatment groups are specified at the end of each table (Tables 1, 2 and 3).

With a view to researching the efficacy of propranolol, the platelet counts and PMI values in each of the treatment and control cases were recorded. The distribution of these values were researched together with their statistical significance between the cases in the control group, to which propranolol was denied, and the cases in treatment groups who were administered propranolol, and between the first (vasooblitera-tion) and second (neovasularization) phases of the disease (Tables 1, 2 and 3) (Figures 1 – 4).

This study was approved by the Ethics Committee of Erciyes University, Kayseri, Turkey. All the participating families were informed during the study and their written informed consents were obtained.

Table 2. Stage 1 ROP study groups.

n: 62	Control group *n*: 32 (%) median ± SD	Treatment group *n*: 3 (%) median ± SD	*p* value
Gestational age (week)	28 ± 1.5	28 ± 1.2	0.94
Birth weight (g)	1000 ± 260	1055 ± 205	0.73
Mechanical ventilation (hour)	144 ± 620	216 ± 404	0.18
Oxygen using (day)	34.55 ± 25.37	25.20 ± 29.0	0.29
Platelet count in phase 1 (mm^3)	225000 ± 113500	215000 ± 73000	0.18
PMI value in phase 1	2106 ± 1087.6	1943 ± 805.2	0.65
Platelet count in phase 2 (mm^3)	344000 ± 190800	293000 ± 149300	0.08
PMI value in phase 2	3490 ± 1475.7	2769 ± 1523.1	0.35
BPD	11 (34.3)	8 (26.7)	0.51
RDS	23 (71.8)	19 (63.3)	0.45
İVH	15 (46.8)	15 (50.0)	0.88
PDA	11 (34.3)	10 (33.3)	0.94
Sepsis-NEC	4 (12.5)	5 (16.7)	0.25
PROM	5 (15.6)	2 (6.7)	0.22
Preeclampsia	4 (12.5)	7 (23.3)	0.31
Prenatal steroid	20 (62.5)	20 (66.7)	0.69
Laser photocoagulation		1 (3.1)	2 (6.7) 0.51

PMI: platelet mass index, BPD: bronchopulmonary dysplasia, RDS: respiratory distress syndrome, IVH: intraventricular hemorrhage, PDA: patent ductus arteriosus, NEC: necrotizing enterocolitis, PROM: premature rupture of membranes.

Table 3. Stage 2 ROP study groups.

n: 53	Control group *n*: 26 (%) median ± SD	Treatment group *n*: 27 (%) median ± SD	*p* value
Gestational age (week)	28.7 ± 2.31	28.0 ± 2.53	0.47
Birth weight (g)	1070 ± 329.1	885 ± 317.5	0.65
Mechanical ventilation (hour)	84 ± 392.1	300 ± 517.0	0.07
Oxygen using (day)	24.42 ± 42.9	22.05 ± 31.0	0.40
Platelet count in phase 1 (mm^3)	206000 ± 104100	163000 ± 58800	0.44
PMI value in phase 1	2005 ± 994.9	1834 ± 632.1	0.37
Platelet count in phase 2 (mm^3)	284000 ± 164900	286000 ± 81800	0.10
PMI value in phase 2	3059 ± 1353.2	2814 ± 198.5	**0.014**
BPD	5 (19.2)	9 (33.3)	0.08
RDS	11 (42.3)	16 (59.2)	0.15
İVH	16 (61.5)	12 (44.4)	0.42
PDA	11 (42.3)	10 (37.0)	0.93
Sepsis-NEC	3 (11.5)	5 (18.5)	0.23
PROM	7 (26.9)	5 (18.5)	0.56
Preeclampsia	7 (26.9)	2 (7.4)	0.07
Prenatal steroid	22 (84.6)	20 (74.0)	0.66
Laser photocoagulation		7 (26.9)	1 (3.7) **0.024**

PMI: platelet mass index, BPD: bronchopulmonary dysplasia, RDS: respiratory distress syndrome, IVH: intraventricular hemorrhage, PDA: patent ductus arteriosus, NEC: necrotizing enterocolitis, PROM: premature rupture of membranes.

Statistical analyses

The data were analyzed by using the SPSS 16.0 (SPSS Inc. Chicago, Illinois) statistical package program. Distribution of the data was controlled via the Shapiro–Wilk normality test. Between groups, normally distributed variables were compared by using the independent sample t-test; variables without normal distribution were compared by using the Mann–Whitney U test. The chi-square test was utilized to analyze rational data. A p value of <0.05 was accepted as statistically significant.

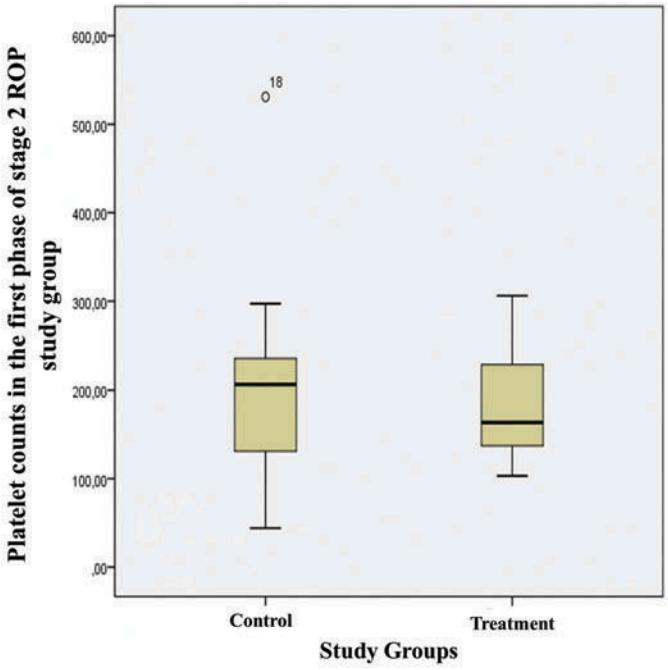

Figure 1. Platelet count distribution between the control and treatment cases in the first phase of the stage 2 ROP study group.

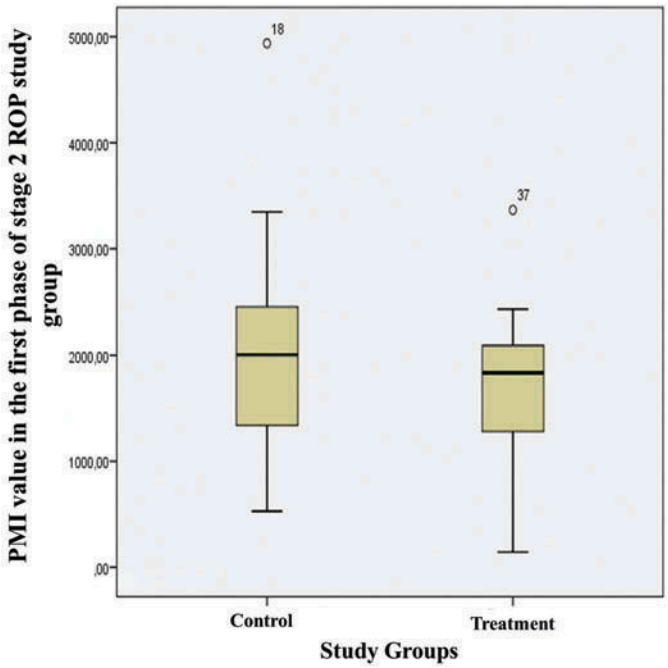

Figure 2. PMI value distribution between the control and treatment cases in the first phase of the stage 2 ROP study group.

Results

Throughout the study, three cases in the stage 0 ROP group, two cases in the stage 1 ROP group, and one case in the stage 2 ROP group were excluded from the study at their parents' request. Five cases in the stage 0 group, six cases in the stage 1 group, and two cases in the stage 2 group were excluded from the study because they had not taken their medication throughout the day.

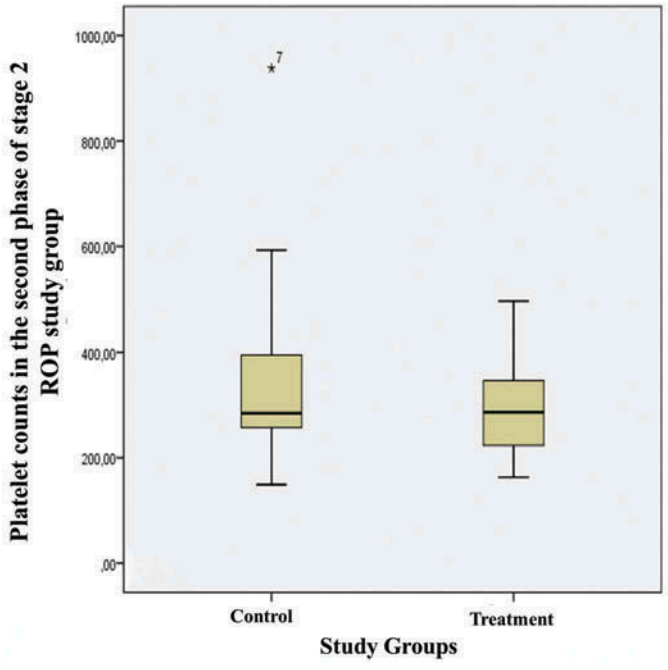

Figure 3. Platelet count distribution between the control and treatment cases in the second phase of the stage 2 ROP study group.

Three cases in the stage 0 study group, two cases in the stage 1 study group, and four cases in the stage 2 study group had to be excluded from the study for reasons of apnea development in the stage 0 study group, increasing ventilator need in the stage 1 study group, and hypoglycemia and increasing ventilator need in the stage 2 study group, respectively.

Three cases in the stage 0 and 1 ROP study groups underwent laser photocoagulation therapy upon the detection of an avascular area at their retinal examinations. Patients found to have pre-threshold ROP in their follow-up received laser photocoagulation therapy. Three patients in the stage 2 ROP group were administered intravitreal bevacizumab, and they were excluded from the study.

The distribution of the diseases affecting ROP within the study groups, their thrombolytic counts in ROP phases, PMI values, and the need of the cases for laser photocoagulation are presented in Tables 1, 2, 3 and Figures 1, 2, 3, 4.

A total of 56 cases were included in the stage 0 study group. The control and study groups did not differ significantly in terms of GA and BW ($p > 0.05$). No difference statistically significant enough to affect the results of the study was found between the control and treatment groups in terms of conditions such as birth weights, gestational age, diseases likely to affect ROP (BPD, respiratory distress syndrome [RDS], IVH, PDA sepsis-NEC, preeclampsia), prenatal steroid use, duration of mechanical ventilation, or duration of O_2 use ($p > 0.05$) (Table 1). In this study group a significant difference was found in favor of the control group in terms of PROM ($p = 0.02$) (Table 1).

No significant difference was found between the control and treatment groups in the stage 0 study group in terms of the thrombolytic count in phase 1 and phase 2 ($p = 0.96$, $p = 0.45$) and PMI ($p = 0.67$, $p = 0.77$) (Table 1).

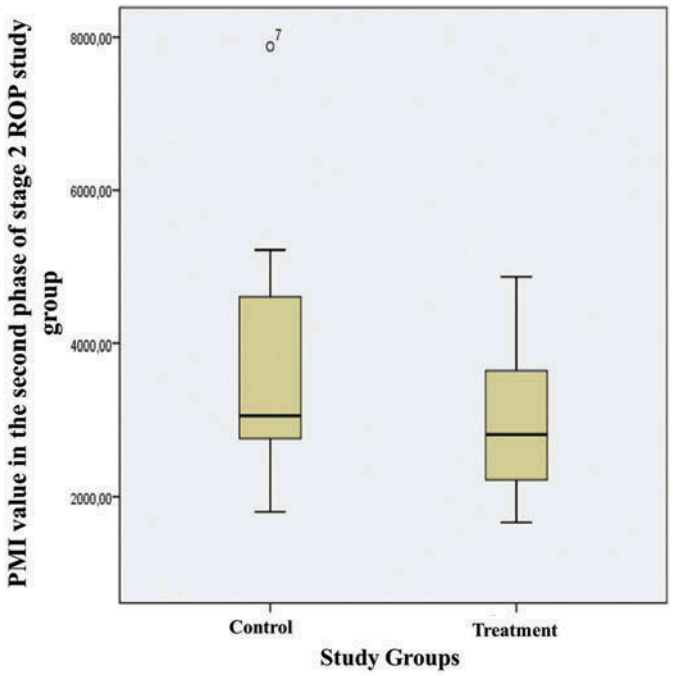

Figure 4. PMI value distribution between the control and treatment cases in the second phase of the stage 2 ROP study group.

An examination of the study results of the stage 0 ROP group revealed that while none of the 30 cases in the control group needed laser photocoagulation, one (3.84%) of the 26 cases in the treatment group did require it. In this study group, there was not a statistically significant difference in the need for laser photocoagulation therapy between the control and treatment cases ($p = 0.28$) (Table 1).

No difference significant enough to affect the results of the study was detected between the control and treatment groups of 62 cases in the stage 1 ROP study group in terms of GA, BW, and diseases that might affect ROP [BPD, RDS, PROM, IVH, PDA sepsis-NEC, preeclampsia, duration of mechanical ventilation, duration of O_2 use] ($p > 0.05$) (Table 2).

One (3.12%) of the 32 cases in the control group of the stage 1 ROP study group needed laser photocoagulation compared to 2 (6.66%) of the 30 cases in the treatment group. In this study group there was no statistically significant difference in terms of the need for laser photocoagulation ($p = 0.51$). In this group, in control and treatment cases, no statistically significant difference was detected in platelet count in phase 1 and 2 ($p = 0.18$, $p = 0.08$) and PMI values ($p = 0.65$, $p = 0.35$) (Table 2).

No significant difference that might impact the stage 2 study group's results was detected between the control and treatment groups in terms of diseases likely to affect GA, BW, and ROP (BPD, RDS, PROM, IVH, PDA sepsis-NEC, and preeclampsia), prenatal steroid use, duration of mechanical ventilation, or duration of O_2 use ($p > 0.05$) (Table 3).

While 7 (26.9%) of the patients in the control group needed laser photocoagulation, in only one (3.7%) of the 27 cases on propranolol did the need for laser photocoagulation arise. In this study group, a statistically significant difference was found in favor of the control group in terms of the need for laser photocoagulation ($p = 0.024$ (Table 3).

In the stage 2 ROP study group, no difference was observed between the control and treatment groups in terms of thrombolytic count ($p = 0.44$) and PMI values ($p = 0.37$) in phase 1 and the platelet count in phase 2 ($p = 0.10$) (Table 3) (Figures 1, 2 and 3). However, significant differences were found between the control and treatment cases in this study group in terms of PMI values in phase 2 ($p = 0.014$) (Table 3 and Figure 4).

Discussion

Despite all the therapeutic modalities, retinopathy of prematurity continues to be among the leading causes of childhood blindness in developed and developing countries.[4]

At present, other than laser photocoagulation, which reduces visual acuity and visual field,[31] and bevacizumab,[16] which can be effective in ROP involving only zone 1, there is no effective therapeutic application. For this reason, the search for novel, effective treatment modalities with fewer side effects continues.

Previously conducted animal studies have clearly demonstrated the effects of a mouse model of the OIR adrenergic system on ROP. The data obtained from this study demonstrated that neovascularization, which constitutes the basis of the disease, could be reduced by blocking AR in the second phase (known as the hypoxic or neovascularization phase) of ROP. The mechanism occurs through the blocking of β_2-AR, which is predominantly localized in the retinal Müller cells. In addition, hypoxia was shown to up-regulate VEGF, VEGF receptor-1, VEGF receptor-2, IGF-1, and IGF-1 receptor messengers. Treatment with propranolol partially restored the hypoxia-induced increase in IGF-1-mRNA and VEGF-mRNA.[6,7,10,32]

Increased VEGF expression is seen in the retina during the development of neovascularization, and an increase in VEGF receptors is observed in the vicinity of target endothelial cells.[33–35] Therefore, propranolol was administered during the second phase (the neovascularization or hypoxia phase) of ROP in this study.

In our clinical study we aimed to make a positive contribution to the search for novel therapeutic modalities in ROP. The efficacy of propranolol, a reliable beta-blocker agent which has been proven to act on vascular tumors in those of pediatric age by lowering VEGF levels, has been researched.[20] The data obtained from our study demonstrated that propranolol administered in the neovascularization phase does not have a significant effect on early stage ROP (stages 0,1), but it reduces the need for laser photocoagulation to a considerable extent in advanced stage ROP cases (stage 2) (Table 3).

In previous experimental studies, it was demonstrated not only that propranolol possessed a VEGF reducing effect but also that this effect was greater in hypoxic tissue where VEGF levels are elevated.[6,20] This observation was also supported recently by clinical and mouse models in OIR trials.[9]

The concern that propranolol could damage the development of the premature infant's brain as a result of its effects on VEGF is justified by experimental evidence showing that, in mice, VEGF participates in brain morphogenesis and that severe reductions in VEGF levels lead to degeneration of the cerebral cortex.[36,37] In experiments in animals, propranolol only reduced VEGF overproduction in the

hypoxic retina of mice with OIR without affecting the VEGF levels in the normoxic retina of control mice, suggesting different mechanisms of VEGF regulation in normoxic and hypoxic conditions. In addition, β_2-AR blockade did not influence VEGF levels in the brain, lungs, or heart, whereas the OIR model did not induce hypoxia or VEGF up-regulation.[6] Propranolol reduced the levels of VEGF in the hypoxic retina; however it did not affect the VEGF levels in the normoxic retina. This possibility is supported by the additional finding that β-AR blockade does not influence VEGF levels in normoxic tissue. These data also suggest that only the VEGF produced under hypoxia-ischemia can be affected by the inhibition of β-ARs.[10] Finally, these results support the view that propranolol is reliable in terms of its side effects and also demonstrate the absence of effects on neurodevelopmental outcome.[9,10]

In our clinical study, the differing effect of propranolol in low stage (stage 0, 1) and high stage (stage-2) ROP cases was ascribed to VEGF levels being low in preterms in the second phase of low stage ROP, while they were high in preterms in the second phase of high stage ROP.[6,20] Our study, when viewed from this perspective, also appears to corroborate experimental studies which propounded that the effect of propranolol occurs, not in normal tissues but mostly in hypoxic tissue, and thus, demonstrated that this agent is reliable in normal tissues in experimental studies.[6,20]

There are also clinical and experimental studies in the literature that have demonstrated that platelets are the major regulators of angiogenesis.[12,13] Platelets manifest their effects in question through VEGF, basic fibroblast growth factor, epidermal growth factor, platelet derived growth factor, and matrix metalloproteinases. The majority of these angiogenic proteins, the most important of which is VEGF, are stored in 200–500 nm-dimensioned α granules. In ROP, along with VEGF released from hypoxic retinal tissue (in the second or neovascularization phase), VEGF and other proangiogenic factors released from platelets may also contribute to neovascularization[12] The data from the studies done so far reinforce the hypothesis that there might be a close relationship between VEGF, which is transported, stored, and released by platelets, and PMI values.

In conclusion, in the control and treatment cases of the stage 2 ROP study group, in which the therapeutic effect of propranolol is seen, significant relationships were detected between the efficacy of propranolol and PMI values in the second phase of the disease (Table 3 and Figure 4). This significant relationship was ascribed to low PMI values, which reflected that the VEGF levels decreased as a result of propranolol administered in the second phase of the disease. This is because propranolol reduces VEGF levels, and this reduction manifests itself with falling PMI values. In this respect, the data from our study clinically corroborate experimental studies which demonstrated the efficacy of platelets in angiogenesis.[12,13]

Owing to VEGF levels being estimated to be low due to the nature of the pathophysiology of the disease, propranolol was not as effective in the stage 0 and 1 ROP study groups in the second phase (hypoxic or neovascularization phase). For this reason, propranolol did not have a sufficient reducing effect on VEGF in these cases, which means its healing effect could not be determined in these groups, either. Propranolol did not significantly affect PMI values in these cases, as mentioned above. In conclusion, the treatment and study groups did not significantly differ in PMI values.

It may be thought that the VEGF levels in the second phase are higher in the stage 2 ROP cases than in the stage 0–1 ROP study groups owing to the nature of the pathophysiology of the disease. For this reason, in the stage 2 ROP treatment cases, it is interesting that propranolol administered in the second phase of the disease also reduced PMI levels more markedly in the control group, as a result of its reducing VEGF levels more effectively (Table 3, Figure 4). In addition, significant differences were found in this study group between treatment cases and control cases in terms of PMI, which is important in monitoring the therapeutic effect of propranolol. The PMI levels in the stage 2 ROP group, were parallel to the therapeutic effect manifested in this group by propranolol due to its reducing VEGF levels (Table 3 and Figure 4).

This study demonstrated that ROP is a multifactorial disease which increases with lower birth weight, younger GA, higher rate of RDS, surfactant usage, PDA, septicemia, IVH, BPD, longer duration of oxygen use, mechanical ventilation, and continuous positive airway pressure (CPAP).[38–42]

In our study, there was no difference statistically significant enough to affect the study results between the control and treatment cases with respect to these predisposing factors. This is very important for a healthy interpretation of the data.

The data showing the presence of an oxygen–ROP relationship were first propounded in the 1950s.[43] Hypoxia that arises during the first phase of the disease is the most important factor to cause first vasoconstriction then reflex vasoproliferation by lowering VEGF levels.[21] Laboratory studies on rats and mice have revealed that both hypoxia and changes in oxygen levels resulted in abnormal structuring in the development of retinal vascularization.[44,45] It has indisputably been demonstrated in studies on animals and humans that oxygen used in the first phase of the disease in particular is among the most important factors in the development of ROP.[23,46,47]

However, it has been reported that O_2 administered after post-menstrual week 32, in contrast to O_2 administered in the early stage, reduces neovascularization and has an ameliorative effect on ROP.[48] Given that O_2 administration was carried out in view of the phases of ROP in this study, it is important to note that O_2 administered in the second phase prevented the progress of the disease. In our study, with respect to O_2 consumption, no statistically significant difference was found between the control and treatment cases of the study groups which were classified according to the stages of ROP (Tables 1, 2, and 3). This was important for a healthy interpretation of the findings of the study.

ROP can be accompanied by neurological dysfunctions, abnormalities in the composition of the brain, NEC-sepsis, BPD, IVH, and other neonatal diseases.[49,50] Intraventricular hemorrhage was shown to be a significant factor related to the incidence of ROP in reported studies.[51] Some studies implicated the similarity in the circulation of the central nervous system (CNS) with the retina, which has a simultaneous effect on the retina and CNS when the circulation is impaired.[39] However, there are also studies which have demonstrated the

absence of a relationship between IVH and ROP.[52,53] Therefore, the effects of IVH on ROP should be discussed further.

The increased frequency of ROP in our times in parallel to BPD indicates the presence of an association between these two diseases, and it has been demonstrated in a multitude of studies that BPD is important in the development of ROP. In some studies, BPD has been linked to posterior ROP in particular.[41,42]

In our study there was no statistically significant difference between the control and treatment cases of any study group. The absence of difference in BPD in our study led us to believe that the interpretation of the findings from the study is ideal.

The increase of cytokines such as IL-6, IL-8, and TNF-α in infants with early sepsis has been found to be related with ROP.[52] There are also studies which have demonstrated that sepsis and BPD are linked to posterior ROP, and that the risk of ROP increases in fungal infections.[41,50,54]

While some studies argue the absence of a relationship between PROM and ROP,[41] others have reported an important relationship between clinical chorioamnionitis and elevated maternal white blood cell count.[55] In this respect, discrepant results have been reported regarding the relationship between ROP and PROM. Maternal preeclampsia was found to be associated with increased ROP development risk in premature infants.[42,56–58] However, there are studies in the literature which dispute this argument.[58]

The reduction in anti-oxidant defense and the increase in secondary O_2 pressure in mechanical ventilation aggravate the pathology, especially in preterm infants.[50] It has been reported that shortness of mechanical ventilation and CPAP in infants reduced the number of risk factors, and thus the prevalence of ROP.[42,59–61] The absence of significant differences between the control and treatment cases in the ROP groups is important in terms of an accurate interpretation of data.

Important studies have been conducted that show the relationship between ante-natal or post-natal steroid use and ROP. These studies reported decreased incidences of ROP, RDS, BPD, grade 3-4 IVH, NEC, sepsis, and mortality when ante-natal steroids were administered.[62,63] Apart from this, the comparison of cases receiving a high or low dose of post-natal steroid and their counterparts who did not receive any steroid revealed that the incidence of ROP was lower in cases without steroids,[64] which may suggest that post-natal use of steroids increases the incidence of ROP. Despite these studies, the effect of ante-natal and post-natal betamethasone on ROP is unclear.[42]

In our study no significant difference in propranolol efficacy was detected between the control and treatment cases in the early stage ROP study groups. For this reason, no significant difference in PMI values was found between the treatment and control cases in the stage 0–1 ROP study groups, either (Tables 1 and 2). Nevertheless, remarkably significant correlations were detected in the treatment cases of the stage 2 ROP study group compared to the control cases, with respect to PMI values depending on propranolol. It was concluded that these relations would provide physicians the opportunity to monitor the efficacy of propranolol treatment with great ease (Table 3 and Figure 4).

Limitations of the study

Our study, which planned to conduct research into the effects of propranolol on ROP, was limited with only 171 cases. Also, we failed to study VEGF levels for technical reasons. This is a major limitation of our study. Increasing the number of cases and close follow-up of blood VEGF levels would enable better results to be obtained and thus yield clearer results, corroborating, with greater force, the proposal we try to present based on the findings of our study. Notwithstanding such limitations, it is important that our study covered ROP cases not only in the stage-2 ROP but in all stages.

Conclusions

No significant difference was detected between the control and treatment groups in the stage 0 and 1 ROP study groups in terms of the therapeutic effect of propranolol owing to VEGF being already thought to be low. Consequently, no significant difference was found in PMI, either.

When viewed from the viewpoint of the therapeutic effect of propranolol, the control and treatment cases in the stage 2 ROP study group differed significantly from each other owing to VEGF levels being thought to be high in this group. In these cases significant differences were also found between the control and treatment groups in terms of PMI values in parallel to the effect of propranolol.

The data obtained from our study are consistent with those of experimental studies which demonstrated that propranolol could reduce excess production of VEGF in hypoxic retina (stage 2 ROP cases), in which levels are elevated, but is not effective in normoxic retina (stage 0, 1 cases), in which VEGF levels are normal.

In addition, it was concluded that follow-up of the response to propranolol in stage 2 ROP cases could only be achieved through PMI values and that this follow-up would facilitate clinicians' monitoring of the disease after propranolol administration.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the article.

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