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Original Article

The Short-Term Effects of Intravitreal Injection of Bevacizumab on the Plasma Levels of Vascular Endothelial Growth Factor, Insulin-Like Growth Factor-1, and Growth Parameters in Infants with Retinopathy of Prematurity

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Abstract

Purpose: To determine the changes in serum levels of free vascular endothelial growth factor (VEGF), insulin-like growth factor-1 (IGF-1), and growth parameters in infants with retinopathy of prematurity (ROP) who received intravitreal injection of the bevacizumab (IVB).

Methods: A prospective interventional case series study, including 10 infants with Type 1 ROP was conducted. Using the enzyme-linked immunosorbent assay, serum levels of VEGF and IGF-1 were measured before, 1 month and 2 months after treatment with IVB in both eyes. Growth parameters, including weight, length, and head circumference and their Fenton's z-score, were also measured.

Results: Serum VEGF levels were suppressed 1 month after IVB ($P = 0.007$) and then increased between 1 and 2 months ($P = 0.064$). Z-scores of all growth parameters except weight z-score decreased in the 1st and 2nd months.

Conclusion: Serum VEGF levels showed a transient reduction after IVB which lasted at least 2 months. Growth velocity of premature infants may be affected by anti-VEGF therapy and should be followed with particular attention.

Keywords: Bevacizumab, Growth parameters, Insulin-like growth factor-1, Retinopathy of prematurity, Vascular endothelial growth factor

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Introduction

Vascular endothelial growth factor (VEGF) is the main growth factor responsible for angiogenesis which also mediates retinal neovascularization in retinopathy of prematurity (ROP).[1] Insulin-like growth factor-1 (IGF-1) is also critical for normal vascular formation, and its low serum level is strongly associated with the development of ROP.[2]

Bevacizumab (Avastin®; Genentech, Inc., South San Francisco, CA, USA) is a recombinant humanized monoclonal antibody that is directed against VEGF-A. Intravitreal injection of the bevacizumab (IVB) has been used as a treatment modality for Type 1 ROP.[3] A major concern regarding anti-VEGF therapy in ROP is the risk of systemic absorption. There are few studies that evaluated serum concentration of VEGF after IVB in infants with ROP, and all of them have consensus in a significant reduction of serum VEGF level following IVB.[4-7]

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Concerns have been raised about systemic absorption of bevacizumab and its effect on developing tissues, including the brain. A decrease in serum levels of VEGF and dependent factors, especially IGF-1, has potential side effects such as impaired neurogenesis and neuroprotection.[8,9] Head circumference growth has special relevance to neurodevelopment in preterm infants, which is used as an indicator of brain size.[10] On the other hand, the role of suboptimal postnatal growth of head circumference[11] and weight[12] has been established in the development of ROP. Therefore, anti-VEGF drugs can change infantile growth parameters such as weight, length, and head circumference by alleviating ROP stage or slowing growth speed. To the best of our knowledge, there is only one study which evaluated the body weight gain in infants with ROP who had undergone IVB and laser therapy, and found no change after both treatments.[4] Recruitment of other growth indices such as body length and head circumference is less addressed.

The aims of this study were to: (1) evaluate serum concentrations of VEGF and IGF-1 in infants with ROP who received IVB up to 2 months and (2) evaluate the changes in three main growth parameters after IVB using Fenton's z-scores (it is a measure of deviation from the mean adjusted for gestational age [GA]).

Methods

This study was a prospective interventional case series on infants with Type 1 ROP referred to a university hospital (Rassoul Akram Hospital, Tehran, Iran) from July 2016 to December 2017. Informed consents from the parents of all patients and Ethics Committee approval of Iran University of Medical Sciences (IUMS) (IUMS-9311257003) were obtained. This study adhered to the tenets of the Declaration of Helsinki. ROP screening was performed with dilated fundus examination. The stage of the ROP determined according to the International Classification of Prematurity System.[13]

Included were infants with ROP Type 1, Stage 3+ ROP in zone I or zone II posterior in both eyes. Based on the BEAT-ROP study,[3] infants underwent intravitreal injection of bevacizumab (0.625 mg/0.025 ml) per eye per dose by topical anesthesia with a 30G needle, 1.5 mm posterior to the corneal limbus under sterile conditions in the operating room. After the injection procedure, topical antibiotics and steroid eye drops were prescribed. Both eyes were treated in all patients. Exclusion criteria were history of laser therapy or laser therapy after IVB. The infants who underwent pre- or post-IVB whole blood transfusion were also excluded due to the possible changes of the anti-VEGF systemic level as well as poor health status of patients.

The following data were gathered: stage of ROP at enrollment, gender, GA, postmenstrual age (PMA) at the time of treatment, and birth weight. Body weight, body length, and head circumference were also measured before, 1 month, and 2 months after treatment.

The measurement of serum level of vascular endothelial growth factor and insulin-like growth factor-1

Blood samples were collected just before IVB injection as well as at 1 month and at 2 months after treatment. The blood samples were collected in sterile tubes with ethylenediaminetetraacetic acid and centrifuged at 5000 revolutions per minute for 10 min until a clear separation between plasma and cellular parts became visible. After centrifugation, blood plasma was kept frozen at − 80°C until analysis was performed. Enzyme-linked immunosorbent assay (ELISA) kits (Quantikine ELISA for human VEGF and human IGF-1; RandD Systems, Minneapolis, MN, USA) were used to measure the serum levels of free VEGF and IGF-1. Working standards were provided based on the manufacturer's recommendations. The results were applied to a microplate reader at wavelengths of 450 nm and 570 nm for all values. All assays were performed in duplicate to acquire an average.

Fenton and Kim[14] growth z-scores were calculated for growth parameters, including weight, head circumference, and body length. We chose to use z-scores rather than crude amount of weight, head circumference, and length measurements because this provides standardization to a recognized scale, widely used and validated.[14] The negative change in z-score represents the less than the predicted growth rate whereas a positive change indicates a growth rate greater than the expected rate.[14]

Statistical analysis

Data were analyzed using SPSS software (version 22.0, IBM Corporation, Armonk, NY, USA). Normal distribution of data was evaluated by Shapiro–Wilk test because of the ≤50 number of sample size. Repeated measures ANOVA with a Greenhouse–Geisser correction was used to evaluate the changes of variables at follow-up times. *Post hoc* tests using the Bonferroni correction was used to compare follow-up times. $P < 0.05$ was considered statistically significant except *post hoc* comparison that was set at a significant level of <0.017 based on a Bonferroni correction.

Results

We initially enrolled 14 patients in this study; however, four patients were excluded due to severe cardiopulmonary disease that could confound the relationship between serum level of VEGF and infantile growth. Finally, 10 infants (4 boys and 6 girls) with ROP Type 1, Stage 3+ ROP in zone I or zone II posterior in both eyes were included. The infants had a mean GA of 27.5 ± 2.8 weeks and a mean PMA of 34.0 ± 1.9 weeks (ranging from 31 to 37 weeks). The mean birth weight of infants was 1279.0 ± 364.7 (ranging from 820 to 3050 grams). Table 1 shows baseline growth parameters.

All data had normal distribution based on Shapiro–Wilk test ($0.075 \leq P \leq 0.899$).

Serum levels of free vascular endothelial growth factor

Serum-free VEGF levels had significant changes after IVB ($P = 0.002$). They decreased 774.00 ± 581.36 pg/ml at

Table 1: Growth parameters, serum levels of vascular endothelial growth factor, and insulin-like growth factor-1 in ten infants with type 1 retinopathy of prematurity who underwent intravitreal injection of the bevacizumab

Variable	Baseline	Month 1		Month 2			$P^{\ddagger}$
	Mean±SD, median (IQR)	Mean±SD, median (IQR)	$P^{\dagger}$ (preoperative)	Mean±SD, median (IQR)	$P^{\dagger}$ (month 1)	$P^{\dagger}$ (preoperative)	
Weight (g)	1599.0±422.4, 1510.00 (1196.25 to 2057.50)	2558.5±438.0, 2582.50 (2118.75 to 2890.00)	**<0.0001**	3410.5±463.3, 3430.00 (2922.50 to 3787.50)	**<0.0001**	**<0.0001**	**<0.0001**
Weight z-score	−1.47±0.89, −1.44 (−2.17 to −1.01)	−1.20±1.01, −1.13 (−1.83 to −0.73)	0.358	−1.01±0.99, −0.89 (−1.72 to −0.53)	0.115	0.225	0.079
Length (cm)	40.1±3.4, 39.00 (37.75 to 42.25)	43.1±3.3, 42.75 (41.00 to 45.13)	**<0.0001**	45.3±3.2, 45.25 (43.13 to 47.13)	**<0.0001**	**<0.0001**	**<0.0001**
Length z-score	−1.59±1.24, −1.63 (−2.84 to −0.67)	−2.38±1.32, −2.37 (−3.43 to −1.32)	**<0.0001**	−3.31±1.38, −3.37 (−4.19 to −2.35)	**<0.0001**	**<0.0001**	**<0.0001**
HC (cm)	28.6±2.4, 27.25 (26.88 to 31.00)	30.2±2.5, 29.50 (27.50 to 32.63)	**<0.0001**	31.6±2.5, 31.75 (28.88 to 33.75)	**<0.0001**	**<0.0001**	**<0.0001**
HC z-score	−1.43±1.51, −1.32 (−2.85 to −0.51)	−2.39±1.63, −2.26 (−4.27 to −0.98)	**0.001**	−3.11±1.67, −2.86 (−5.20 to −2.47)	**0.001**	**<0.0001**	**<0.0001**
VEGF (pg/ml)	1267.05±487.53, 1193.23 (968.42 to 1454.13)	493.05±377.03, 457.82 (206.76 to 677.79)	**0.007**	900.77±452.05, 897.64 (636.22 to 1186.89)	0.064	**0.001**	**0.002**
IGF-1 (ng/ml)	30.16±5.88, 28.04 (24.82 to 33.96)	28.87±5.24, 28.55 (23.67 to 32.59)	1.00	35.38±4.03, 34.52 (32.71 to 37.78)	0.157	0.063	0.062

Bold *P* values are statistically significant. [¥]Repeated measures ANOVA with a Greenhouse-Geisser correction, [†]*Post hoc* tests using the Bonferroni correction. HC: Head circumference, VEGF: Vascular endothelial growth factor. IGF-I: Insulin-like growth factor-1, SD: Standard deviation, IQR: Interquartile range

month 1, which was significant ($P = 0.007$) and remained lower than pretreatment levels after 2 months ($P = 0.001$) [Figure 1].

Serum levels of insulin-like growth factor-1

Serum IGF-1 levels did not have significant changes during 2-month follow-up ($P = 0.062$), including reduction of 1.28 ± 9.80 ng/ml at month 1 ($P = 1.00$) and then 6.51 ± 7.38 ng/ml increase at month 2 ($P = 0.063$) [Figure 2 and Table 1].

Growth pattern

All growth parameters showed a continuous increase over time. However, z-scores of all growth parameters except the z-score of weight decreased in the 1st and 2nd months [Figure 3 and Table 1]. Up to 2 months following treatment, a weak positive correlation was detected between the IGF-1 serum levels and the infant's weight ($r_s = 0.4$, $P = 0.033$), length ($r_s = 0.4$, $P = 0.029$), and head circumference ($r_s = 0.4$, $P = 0.036$) on the day that the serum was collected. However, the VEGF level did not have any correlation with growth parameters and IGF-1 levels ($0.086 < P < 0.983$). Furthermore, the Spearman correlation test was done between the declines of serum bevacizumab versus changes of the z-scores of the anthropometric indices. No significant correlation was found ($0.21 \leq P \leq 0.93$).

Discussion

Bevacizumab (Avastin) is an anti-VEGF agent which leaks from the vitreous cavity into the systemic circulation and suppresses the VEGF levels in infants with ROP.[4-7] The highest blood level of bevacizumab is attained around one to 2 weeks after the intravitreal injection.[15] Significant reduction of serum VEGF level 1 month after IVB ($P = 0.007$) and its rise up to month 2 in our study is consistent with the short-term systemic effect of bevacizumab in infants.[4,6] Furthermore, IVB was injected in both eyes in the current study, which may affect the systemic VEGF more than unilateral injections.

Dysregulation of other cytokines, such as IGF-1, may happen following the reduction of serum VEGF levels. Hong *et al*.[5]

Figure 1: Serum level of free vascular endothelial growth factor and its 95% confidence interval at baseline, 1 month and 2 months of follow-up

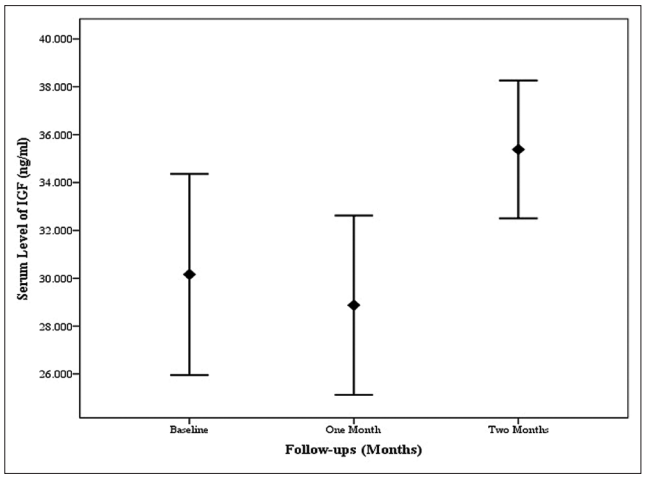

Figure 2: Serum level of insulin-like growth factor-1 and its 95% confidence interval at baseline, 1 month and 2 months of follow-up

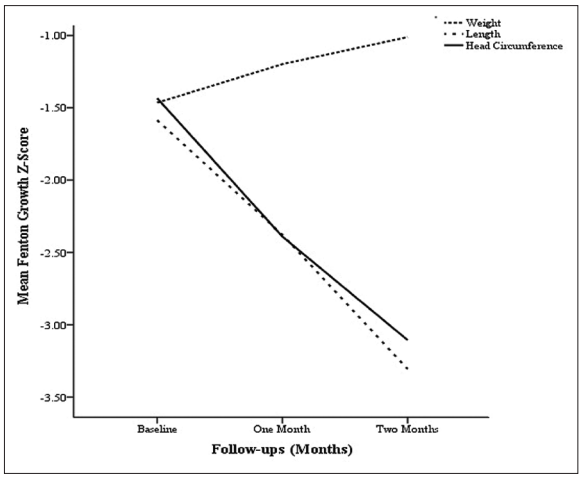

Figure 3: Changes of mean Fenton z-scores of weight, length, and head circumference at baseline, 1 month and 2 months of follow-up

and Wu *et al*.[6] found no significant effect of intravitreal bevacizumab on the IGF-1 levels up to 2 and 8 weeks after therapy, respectively. In contrast, Kong *et al*.[4] reported significant acute reduction at day 2 and then an increase up to day 42 after treatment. In the current study, in which all infants had PMA more than 30 weeks, IGF-1 did not show significant changes up to 2 months ($P = 0.062$) while concentration of IGF-1 in preterm infants rises after 30–32 weeks of PMA.[2] The insignificant decrease at month 1 might be a sign of adverse influence of anti-VEGF. However, we might have missed the acute reduction of IGF-1 levels within the 1st week due to either a limited number of follow-up visits or low power of the study with a small sample size.

Anthropometric measurements are routinely performed in the pediatric settings, being used around the world as health and development indices during infancy and childhood. In particular, weight, length, and head circumference are attributed to neurodevelopmental outcomes.[16] Suboptimal postnatal growths of head circumference[11] and weight[12] are associated with the development of Type 1 ROP. Baseline negative z-scores of the three main growth parameters in our study may be related to the study population who were premature infants with Type 1 ROP [Table 1].

Our results showed no systemic adverse effect up to 2 months after intravitreal bevacizumab, which compares favorably to previous studies regarding the effects of IVB in infants with ROP.[4-7] However, we observed a steady decrease in the z-scores of head circumference and body length [Table 1]. Although the current study revealed the transient systemic effect of IVB as well as insignificant reduction of IGF-1 after bevacizumab injection, the effect of IVB on the changes in growth parameters cannot be overlooked by considering the important role of these two growth factors in organ development, especially neurogenesis.[8,9] Moreover, weight z-scores showed a different behavior; they were positive and were not changed significantly at 1 and 2 months after treatment ($P = 0.358$ and $P = 0.225$, respectively), which was consistent with Kong's study[6]. However, the small sample size of our study can affect the insignificance of the results, which should be interpreted with caution. Therefore, we recommend that growth parameters, especially head circumference, should be meticulously examined in infants with ROP who underwent intravitreal bevacizumab injections. Additional studies are required to establish this connection.

There are several limitations in our study, including the small numbers of patients and few blood samplings. Future studies should recruit a large number of patients and have multiple subgroups undergo IVB injection with various doses and potencies to reach valid and powerful results about the possible "cause and effect" relationship between serum bevacizumab level and infantile growth parameters. Furthermore, we did not have a matched control group to compare. Since choosing a matched control group necessitates withholding therapy for some infants with ROP Type 1, it would raise ethical issues. Selecting controls from patients with less severe ROP would have also violated matching. In practice, we treat Stage 3+ ROP in zone I or zone II posterior with Avastin, and ROP Stage 2 and 3 with plus in zone II with laser. The inclusion of laser-treated patients in this study as a control group could create a big confounding effect, where the smallest and sickest infants were treated with anti-VEGF. In other words, there may be bias between the groups in that typically infants treated with bevacizumab are younger, smaller, and more fragile, and therefore may have different growth trajectories than those who received laser treatment. This can significantly affect their posttreatment growth rate parameters, too. Therefore, it would not be possible to conclude that any of the differences found in the various growth parameters were due to the treatment and/or VEGF/IGF profile. Moreover, this study has a short follow-up period, which is not enough for infant neurodevelopmental assessment. Long-term studies are required to determine whether IVB could cause neurodevelopmental impairment in infants with ROP and whether growth parameters such

as head circumference are reliable predictive factors for neurodevelopment.

In conclusion, the serum levels of free VEGF were suppressed transiently after IVB injection, which lasted up to 2 months while the serum levels of IGF-1 were not. Z-scores of growth parameters in premature infants who underwent anti-VEGF therapy should be measured and followed with more caution than individuals without anti-VEGF therapy.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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