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Original Article

Serum transforming growth factor-β levels and severity of retinoblastoma in children

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Abstract

Background Transforming growth factor-β (TGF-β) expression contributes to the growth of retinoblastoma. TGF-β is produced or released by infiltrating cells such as lymphocytes and monocytes/macrophages. TGF-β levels are a potential marker of disease severity.

Objective To assess the difference in serum TGF-β levels before chemotherapy in patients with retinoblastoma grades III and IV.

Methods This cross-sectional observational study was done at Dr. Wahidin Sudirohusodo Hospital, Makassar, Indonesia from January to November 2019. Subjects were pediatric patients with grade III and IV retinoblastoma who had not undergone chemotherapy. Patients who met the inclusion criteria provided blood specimens for TGF-β testing. We analyzed the difference in serum TGF-β level between grade III and grade IV patients.

Results We obtained 38 subjects, consisting of 13 grade III and 25 grade IV retinoblastoma patients. Mean TGF-β levels were in 1,061 ng/L in grade III and 988 ng/L in grade IV patients. The Mann-Whitney U test revealed no significant difference between the levels of TGF-β, retinoblastoma grade III and IV (P=0.655). However, TGF-β levels in both groups were markedly above the normal value (100 ng/L).

Conclusion TGF-β levels are markedly increased in grade III and IV retinoblastoma patients. There was no significant difference in TGF-β level between grade III and IV patients. Our findings suggest that TGF-β plays an important role in tumor cell development. Further research on differences in TGF-β levels between late stages (grades III and IV) and early stages (grades I and II) of retinoblastoma to elucidate the role of TGF-β as a marker of retinoblastoma severity. **[Paediatr Indones. 2023;63:169-72; DOI: https://doi.org/10.14238/pi63.3.2023.169-72**].

Keywords: *TGF-β; retinoblastoma; severity*

Retinoblastoma is the most common primary intraocular malignant tumor in children. Its incidence has been reported to be 1 in 15,000 to 18,000 live births, or approximately 12, 6, and 4 cases per 1 million children younger than 5, 10, and 15 years, respectively.[1] It is also the third most common intraocular tumor. Retinoblastoma can affect all ages but most commonly affects children under the age of 3 years.[2] Prior to 2002, Dr. Cipto Mangunkusumo Hospital in Jakarta had 15-20 new cases of retinoblastoma per year, which had risen to 40 cases per year in 2002-2003.[3] Dr. Soetomo Hospital in Surabaya reported 115 retinoblastoma cases from 1999 to 2003.[3] In Dr. Wahidin Sudirohusodo Hospital, Makassar, 95 new cases of retinoblastoma were treated in the Department of Child Health between 2013 and 2018.[4] From 2005 to 2010, the outpatient clinic of Dr. Wahidin Sudirohusodo Hospital had 67 cases of retinoblastoma involving 70 eyes, out of which 12.9% were in stage I, 22.9% in stage II, 44.3% in stage III,

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and 20% in stage IV.[4]

TGF-β is a cytokine that plays a role in the proliferation, differentiation, migration, and survival of cells. TGF-β is secreted in a dormant state, then activated by proteases or thrombospondin before binding to serine/threonine kinase receptors. TGF-β receptor types include TGF-β type II (TR-II) receptors and two different TGF-β type I receptors. The existing signal is transduced from the cell membrane to the cell nucleus via the Smad family of signal transducers. TGF-β acts as a tumor suppressor in normal cells and early-stage cancer cells, so it is believed that most tumor development is caused by mutations or deletions of genes that encode TGF-β signal formation. However, as the tumor progresses, the protective and cytostatic effects of TGF-β slowly disappear. TGF-β transforms into a tumor promoter, causing tumor progression, invasion, and metastasis.[5] In other words, the level of TGF-β influences the severity of the stage of cancer, such as retinoblastoma[6] The aim of this study is to assess serum levels of TGF-β before chemotherapy in patients with retinoblastoma grades III and IV.

Methods

This cross-sectional observational study included patients aged 1 month to 18 years diagnosed with grade III or grade IV retinoblastoma treated at Dr. Wahidin Sudirohusodo Hospital, Makassar, from January to November 2019 who had not received chemotherapy. Patients with second malignancies in addition to their retinoblastoma were excluded. Subjects underwent TGF-β measurements from peripheral venous blood specimens. We used a TGF-βnormal reference value of <100 ng/L.[7]

Data analysis was performed using *SPSS version 26.0* (*IBM*, Armonk, New York). The chi-square test was used to analyze associations between categorical variables. The independent t-test was performed to compare means between grade III and grade IV retinoblastoma patients. Data normality was assessed using the Kolmogoro-Smirnov test. A P value less than 0.05 was considered statistically significant. This study was approved by Universitas Hasanuddin Faculty of Medicine Ethics Committee, Indonesia. Subjects' parents provided written informed consent for participation in the study.

Results

During the study period, 43 patients aged 1 month to 18 years were diagnosed with retinoblastoma (grades III and IV). Thirty-eight patients, consisting of 13 grade III and 25 grade IV retinoblastoma patients, were included. Five patients did not complete the TGF-β level examination due to a lack of sample size.

Subject characteristics are shown in **Table 1**. There were 12 males (32%) and 26 females (68%). Most subjects (58%) had good nutritional status, but 11 (29%) children were severely malnourished. The majority of subjects (76%) subjects were aged 1-5 years.

Fisher's exact test revealed no significant difference in sex distribution between subjects with grade III vs. IV retinoblastoma (P=0.714), with females making up the majority of both groups. The Kolmogorov-Smirnov test revealed that there was no significant difference in nutritional status distribution between the two groups (P=0.806), with well-nourished subjects comprising more than half of both groups. There was no significant difference in age group distribution between grade III *vs.* IV retinoblastoma patients (P=0.243), with most subjects in both groups being 1-5 years of age (**Table 2**).

The mean TGF-β levels of subjects with grade III and IV retinoblastoma were 1,061 ng/L and 988 ng/L, respectively. On analysis using the Mann-Whitney U test, this difference was not statistically significant (P=0.655) (**Table 3**).

Table 1. Subjects ' characteristics

Characteristics	(N= 38)
Sex, n	
Male	12
Female	26
Nutritional status, n	
Well-nourished	22
Underweight	5
Malnourished	11
Age, n	
< 1 year	4
1-5 years	29
5-10 years	5

Table 2. Analysis of subject characteristics and severity of retinoblastoma

Characteristics	Retinoblastoma		P value
	Grade III (n = 13)	Grade IV (n = 25)	
Sex			
Male	5 (38.5)	7 (28.0)	0.714*
Female	8 (61.5)	18 (72.0)	
Nutritional status			
Well-nourished	8 (61.5)	14 (56.0)	0.806**
Underweight	3 (23.1)	2 (8.0)	
Malnutrition	2 (15.4)	9 (36.0)	
Age			
< 1 year	2 (15.4)	2 (8.0)	0.243**
1-5 years	10 (76.9)	19 (76.0)	
5-10 years	1 (7.7)	4 (16)	

*Fisher's exact test; **Kolmogorov-Smirnov

Table 3. Comparison of TGF-β levels in grades III and IV retinoblastoma patients

TGF-β levels	Retinoblastoma	
	Grade III (n = 13)	Grade IV (n = 25)
Mean (SD), ng/L	1,140 (319)	1,174 (562)
Median (range), ng/L	1,061 (728-1661)	988 (623-2723)

Mann-Whitney U test (P=0.655)

Discussion

We found no significant difference in TGF-β levels between patients with grades III and IV retinoblastoma. However, TGF-β levels were markedly increased in both groups, compared to the normal reference value of <100 ng/L.[7] Similarly, in a previous study reported by Shehata et al., serum TGF-β levels in advanced-stage retinoblastoma (groups D and E) did not differ significantly. However, serum TGF-β levels in both groups were increased well above the cut-off value of 370.76 pg/mL, with a mean (SD) of 421.71 (SD 64.37) in Group D and 447 (SD 50.44) in Group E.[8]

As previously explained, TGF-β acts as a tumor suppressor in normal and early-stage cancer cells, but due to mutations of genes that encode TGF-β signal formation, the protective and cytostatic effects of TGF-β slowly disappear as the malignancy advances in stage. TGF-β then transforms into a tumor promoter, causing tumor progression, invasion, and metastasis. Although a limitation of this study was the lack of comparison with early-stage (grades I and II) retinoblastoma, the presence of elevated TGF-β suggested that TGF-β plays an important role in the progression of tumor cells to an advanced stage.

In our study, there were no significant differences in sex, age, or nutritional status distribution between grade III and IV patients. We noted, however, that the majority of subjects were girls. In addition, most retinoblastoma patients were in the age range of 1-5 years (76%), similar to the findings of a previous study.[9] The majority of subjects in both groups were well-nourished, in line with findings reported by a study which reported good nutritional status in 67% of retinoblastoma patients.[10] Taba *et al.*[11] found no significant correlation between the severity of retinoblastoma and sex, nutritional status, or age. To the best of our knowledge, there have been few publications examining the relationship between TGF-β levels and retinoblastoma. Shehata *et al.*[8] found that serum TGF-β levels in retinoblastoma patients before therapy were significantly higher than controls (patients who would undergo eye surgery for congenital cataracts, secondary intraocular lens implantation, and congenital glaucoma). Tumor cell growth of greater than 2-3 mm in diameter necessitates the intake of nutrients from new blood vessels to ensure survival, a process known as angiogenesis, in which TGF-β is an important growth factor. TGF-β has direct and indirect effects on the mechanism of angiogenesis. TGF-β induces the expression of vascular endothelial growth factor (VEGF), which plays a direct role in the proliferation and migration of endothelial cells into the collagen matrix. TGF-β also acts as a strong chemoattractant for monocytes, causing them to release angiogenesis-promoting cytokines.[12,13]

Our study was conducted at the largest referral hospital in Eastern Indonesia, with the majority patients coming from this region. Hence, our results can be used as a reference for TGF-β levels in retinoblastoma patients in this area of the country.

Our study had several limitations. The cross-sectional design only provided pre-chemotherapy TGF-β data without follow-up evaluation of post-chemotherapy levels. Moreover, we did not include grade I and II patients due to the small number of such patients at our center. Finally, some serum specimens could not be analyzed because of insufficient volume, so the sample size was smaller than planned.

In conclusion, there are no significant differences in pre-chemotherapy serum TGF-β levels between retinoblastoma grade III and IV patients. However, serum TGF-β levels were markedly increased in both groups, suggesting that TGF-β plays an important role in the progression of tumor cells to an advanced stage. Follow-up studies are needed to compare TGF-β levels in advanced stage (grades III and IV) retinoblastoma to those in early stage (grades I and II) disease and to healthy controls.

Conflict of interest

None declared.

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