Volume-3, Issue-4, April-2019: 203-209
International Journal of Recent Innovations in Academic Research **ISSN: 2635-3040**

Expression of Vascular Endothelial Growth Factor in Juvenile Nasopharyngeal Angiofibroma in Haji Adam Malik General Hospital Medan

Yuli Tetriana Sari[1], Rizalina A. Asnir[1], Ashri Yudhistira[1], Sutoyo Elliandy[2] and Taufik Ashar[3]

[1]Department of Otorhinolaryngology Head and Neck Surgery, Faculty of Medicine Universitas Sumatera Utara/H. Adam Malik General Hospital, Medan Indonesia
[2]Unit Installation of Pathological Anatomy H. Adam Malik Medan General Hospital, Medan Indonesia
[3]Department of Environmental Health Faculty of Public Health Universitas Sumatera Utara, Medan, Indonesia
Corresponding Author E-mail: ulie8783@gmail.com

Received: April 16, 2019; **Accepted:** April 23, 2019; **Published:** April 27, 2019

Abstract

Background: Juvenile Nasopharyngeal Angiofibroma (JNA) is a benign blood vessel tumor that is locally aggressive in young adults. Vascular Endothelial Growth Factor (VEGF) is an important growth factor in tumor biology. VEGF overexpression has been associated with tumor progression and poor prognosis in various types of tumors. VEGF expression in angiofibroma has been previously noted and associated with proliferation and increasing blood vessel density.

Objective: To determine the expression of Vascular Endothelial Growth Factor (VEGF) in Juvenile Nasopharyngeal Angiofibroma (JNA).

Methods: A descriptive research with cross-sectional study design, the data was taken from medical records at the H. Adam Malik general hospital Medan from Januari 2011 to December 2017 with a total sample 24. Inclusion criteria included a sticky medical record and clear identify and had paraffin blocks with a juvenile nasopharyngeal angiofibroma diagnosis.

Results: VEGF overexpression was found in 14 male patients (60.9%), and 11 people (61.1%) out of 18 people under the age of 20 years. In stage III, VEGF overexpression was found to be 90% and at stage IV reached 100%.

Conclusion: VEGF overexpression in JNA was found especially at advanced stages.

Keywords: Juvenile Nasopharyngeal Angiofibroma (JNA), Vascular Endothelial Growth Factor (VEGF), Immunohistochemistry.

Citation: Yuli Tetriana Sari, Rizalina A. Asnir, Ashri Yudhistira, Sutoyo Elliandy and Taufik Ashar. 2019. Expression of Vascular Endothelial Growth Factor in Juvenile Nasopharyngeal Angiofibroma in Haji Adam Malik General Hospital Medan. International Journal of Recent Innovations in Academic Research, 3(4): 203-209.

1. Introduction

Juvenile Nasopharyngeal Angiofibroma (JNA) is one of the benign tumors in the nasopharynx which ranges around 0.5% of all neck head tumors that occur in 1 in 150.000 people. This disease usually attacks adolescents and young adults between the ages of 14-25 years, and tend to attack males. The incidence in Southeast Asian countries is greater than in western countries (Makhasana *et al.*, 2016). Diagnosis of JNA is based on patient history, physical examination, endoscopic evaluation and radiological examination such as Computed Tomography (CT), Magnetic Resonance Imaging (MRI) and angiography (Gidea *et al.*, 2012). Biopsy is a contraindication of JNA to avoid bleeding (Shamim *et al.*, 2013).

VEGF (Vascular Endothelial Growth Factor) expssion in tumor cells is stimulated by hypoxia, oncogenes (race) and inactivation of tumor suppressor genes (p53) and by various cytokines. Activation of the VEGF / VEGF receptor axis (VEGFR) triggers multiple signal networks that produce endothelial cell survival, mitogenesis, migration, differentiation, vascular permeability and mobilization of endothelial progenitor cells from the bone marrow to the peripheral circulation (Hickin and Ellis, 2005; Kawamura *et al.*, 2008; Farhat, 2009).

VEGF overexpression has been associated with tumor progression and poor prognosis in various types of tumors, including colorectal carcinoma, gastric carcinoma, pancreatic carcinoma, breast cancer, lung cancer and melanoma, acute myeloid leukemia, liver carcinoma, and ovarian cancer (Hickin and Ellis, 2005). Hota *et al.*, (2015) have reported that there are VEGF receptor tumor cells in 36 JNA patients, receptor expression is present in endothelial cells and in the stroma (Hota *et al.*, 2015).

This study aims to determine the expression of Vascular Endothelial Growth Factor (VEGF) in Juvenile Nasopharyngeal Angiofibroma (JNA).

2. Method

This research is descriptive with a cross-sectional study, which was conducted in the Division of Oncology-Head Neck Surgery Department of T.H.T.K.L FK USU and Pathology Anatomy Section of H. Adam Malik Hospital Medan during in the period of January 2011 to October 2018.

The sample in this study are all patients with a sticky medical record data and clear identify of juvenile nasopharyngeal angiofibroma in their results of the postoperative histopathological examination, which was then adjusted to paraffin blocks in the anatomical pathology section of H. Adam Malik General Hospital, Medan.

Preparation after cutting tissue (preparations/slides): preparations are heated in microwave high level for 5 minutes. Furthermore, the preparations were deparaffinated with xylol I-II-III respectively for 5 minutes, wash in running water for 5 minutes. Blocking with endogenous peroxidase (0.5% H2O2 in methanol) for 30 minutes. Next wash with running water for 5 minutes. Give tris EDTA pre-treatment in microwave: Cook I: high power level for 5 minutes. Cook II: medium power level for 5 minutes. Then it is cooled for about 45 minutes. Wash with PBS pH 7.4, then limit tissue with Pap-Pen. Blocking non-specific activity with normal serum for 20 '. Incubate preparations with VEGF primary antibodies for one night at 4°C (in the refrigerator). Wash with PBS pH7.4. Then incubate with Envision for 30 minutes. Wash with PBS pH 7.4-Twin 20 then PBS for 5 minutes each. Furthermore, the preparation was given chromogen to be colored with DAB (Diamino Benzidine) for approximately 5 minutes. Wash with running water. Counterstain with Hematoxylin Lilie Mayers. Wash with

running water. Saturated Lithium Carbonat (5% in aquadest for 1-2 minutes). Wash with running water. Next do dehydration with multilevel alcohol (80% alcohol, 96% alcohol, absolute alcohol I and II each for 5 minutes). Clearing with xylol I, II, III for 5 minutes each. Cover with Entellan and cover glass. The slides can be read directly and examined by two specialists in anatomical pathology.

VEGF expression is an assessment of VEGF protein from the results of brown color on the cytoplasm of tumor cells by summing the results of a broad score with an intensity score, so that the immunoreactive VEGF score is obtained using a light microscope and immunohistochemical methods. No VEGF overexpression is indicated with an immunoreactive score of 0-3 and VEGF overexpression is indicated with a score of 4-6 immunoreactive.

3. Results

The subjects of this study are 24 patients who met the inclusion criteria. The majority of the subjects were 23 males (95.8%), with the highest number in the age group <20 years old are 18 patients (75%). The average age of the subjects was 17.83 years old, with the youngest age is 13 and the oldest is 24. This is shown in table 1.

Table 1. Demographic Characteristics of Research Subjects

Demographic characteristics	Frequency	%
Sex		
Male	23	95.8
Female	1	4.2
Age		
< 20	18	75
20 – 29	6	25
Average (SD)	17.83 (2.70)	
Min-max	13-24	

Based on the results of the histopathological examination, all are images of angiofibroma. Most subjects (41.7%) were in stage III followed by stage II with 9 people (37.5%). Only 1 subject is in stage I. This is shown in table 2.

Table 2. Results of Tumor Stadium Examination

Tumor Stadium	Frequency	%
I	1	4.2
II	9	37.5
III	10	41.7
IV	4	16.7

In 23 male subjects, 14 people (60.9%) showed VEGF overexpression while one female subject showed overexpression. This is shown in table 3.

Table 3. VEGF Expression Based On Sex

Sex	VEGF Expression	
	Overexpression	*No Overexpression*
Male	14 (60.9)	9 (39.1)
Female	1 (100)	0

Among the 18 subjects under the age of 20, there were 11 people (61.1%) showed VEGF overexpression. In subjects aged 20 to 29 years, which are 6 people, there were 4 of them (66.7%) with VEGF overexpression. This is shown in table 4.

Table 4. VEGF Expression Based On Age

Age	VEGF Expression	
	Overexpression	*No Overexpression*
< 20	11 (61.1)	7 (38.9)
20-29	4 (66.7)	2 (33.3)

There were no subjects in stage I who showed VEGF overexpression. In stage II there were 2 subjects (22.2%) who showed VEGF overexpression. Among the 10 subjects in stage III, 90% showed VEGF overexpression and all subjects (100%) in stage IV also showed VEGF overexpression. This is shown in table 5.

Table 5. VEGF Expression Based On Stadium

Stadium	VEGF Expression	
	Overexpression	*No Overexpression*
I	0	1 (100)
II	2 (22.2)	7 (77.8)
III	9 (90)	1 (10)
IV	4 (100)	0

4. Discussion

In this study, table 1 shows the demographic characteristics of the study subjects, out of 24 samples, 23 samples (95.8%) were male and one female (4.2%), with the highest age in the age group <20 years are 18 people (75%) and the average age is 17.83 with the youngest is 13 and the oldest is 24. This is consistent with research conducted by Saniasiaya *et al.*, (2016) that there were 11 male patients suffering from JNA in Kelantan, Malaysia from January 2000 to December 31, 2015, with ages ranging from 11 to 21 years (Saniasiaya *et al.*, 2016).

Simbolon (2016) reported that from October 2010 to October 2015 at the H. Adam Malik General Hospital in Medan there were 20 JNA patients with the number of male patients are more than women (Simbolon, 2016). The same was reported by Renkonen et al in the THT-KL Department of the Helsinki University General Hospital (Finland) that there were 27 JNA patients, all of whom were men with a mean age of 17 ranging from 11 to 33 years (Renkonen *et al.*, 2011). Anggreani *et al.*, (2011) at the RSCM Jakarta, also reported that there were 27 patients with a age range of 9-23 years and a mean age of 15.7 years ± 3.23 years (Anggreani, 2011).

JNA is a rare, hypervascular, locally invasive disease and noncapsulated tumor with different origins in the nasopharynx in young adult men. Genetic research has found a link between these blood vessel tumors and the expression of the androgen receptor, indicating that these tumors are related to androgens. This explains why JNA is more common in young adult men. Boghani (2013) in the United States found four female patients out of a total of 305 JNA patients. Hormonal theory as one of the causes of JNA, where high estrogen levels can protect women. This explains why only few cases of JNA have been reported in some literature. Possible causes of JNA in women are due to other etiologies such as congenital or inflammatory (Mishra, 2013).

Table 2 shows the results of histopathological examination of the tumor stage in this study, all of which are the most angiofibroma images; 10 people in stage III (41.7%), followed by stage II with 9 people (37.5%) and only one sample in stadium I. Itoo, AF, Iqbal, I. and Chiesti, AL, (2014) reported that most sufferers of JNA were found in stage III (14 patients).

Early stages are very rare and also too late to make a diagnosis. Out of the 24 patients, 6 patients (25%) were obtained with intracranial involvement (Itoo *et al.,* 2014). This is in accordance with the research that we reported, out of 24 patients, there were 4 patients (16.7%) who had involved intracranials. While the research conducted by Naz *et al.,* (2009) on 40 young adult male patients using the Chandler category and found 27 cases in stage III (67.5%), 10 cases in stage II (25%) and 2 cases in stage one. Only 1 case in stage IV (Naz *et al.,* 2009).

Table 3 shows the expression of VEGF based on sex, there are 23 males in total; 14 males showed overexpression (60.9%) and only one woman who had overexpression. Table 4 shows VEGF expression based on age, out of 18 people under the age of 20, there were 11 people who were overexpressed (61.1%); and there are 4 people who showed overexpression in ranging age 20-29 years old. While table 5 shows VEGF expression based on the stage; there were 22.2% of stage II which showed overexpression; 90% overexpression in stage 3; and 100% overexpression at stage IV.

The study conducted by Liu *et al.,* (2015) Out of 70 male patients with age ranging from 9-41 years old, VEGF expression was seen in 67 patients. The VEGF overexpression was associated with a further tumor stage, it is suspected that VEGF is related to JNA growth (Liu *et al.,* 2015). This is also in accordance with the research conducted by Brieger *et al.,* (2004) with 10 JNA patients and VEGF expression was found in cell and blood vessel stroma (8 of 10). This study proves that VEGF is probably associated with proliferation and increasing blood vessel density (Brieger *et al.,* 2004). Saylam *et al.,* (2005) also conducted research using immunohistochemical methods and obtained VEGF expression in 24 out of 27 JNA sufferers (Saylam *et al.,* 2005).

In a previous study, Hota *et al.,* (2015) conducted VEGF expression studies on 36 samples of JNA in India, that were proven histopathologically. Within 36 samples, they obtained 36 samples of positive VEGF expression in endothelial cells and 34 samples of positive VEGF expression on stromal cells (Hota *et al.,* 2015). VEGF expression in JNA has previously been reported and is associated with proliferation and increasing blood vessel density (Brieger *et al.,* 2004; Saylam *et al.,* 2005).

In situ hybridization studies show that VEGF expressed in many tumors and anti-VEGF antibodies shows to have inhibitory effects on tumor cell growth, including small inhibitors of VEGFR signaling molecules, antisense oligonucleotides and anti VEGFR-2 antibodies. Survival is significantly increased in patients with chemotherapy (irinotecan, 5-fluorouracil, leucovorin) plus rhu MSB VEGF (an anti VEGF monoclonal antibody), providing information that VEGF plays an important role in the process of angiogenesis in solid tumors and hematological malignancies (Shibuya, 2013).

5. Conclusion

In this study, the number of JNA patients with VEGF overexpression was 14 males (60.9%); out of 18 subjects with JNA who were under 20, 11 people was found with VEGF overexpression (61.1%); whereas out of 6 JNA subjects aged 20-29, there were 4 of them

showed VEGF overexpressions (66.7%); 10 subjects in stage III (90%) and all subjects (100%) in stage IV showed VEGF overexpression. Thus, it can be seen that VEGF is increasingly overexpressed at the advanced stage so further research is needed to observe the relationship of VEGF overexpression toward the stage at JNA to improve the basic therapeutic effects of Nasopharyngeal Angiofibroma (JNA) in the future.

References

1. Anggreani, L., Adham, M., Musa, Z., Lisnawati, L. and Bardosono, S. 2011. Gambaran ekspresi reseptor estrogen β pada angiofibroma nasofaring belia dengan menggunakan pemeriksaan imunohistokimia. Oto Rhino Laryngologica Indonesiana, 41(1): 8-16.

2. Brieger, J., Wierzbicka, M., Sokolov, M., Roth, Y., Szyfter, W. and Mann, W.J. 2004. Vessel density, proliferation, and immunolocalization of vascular endothelial growth factor in juvenile nasopharyngeal angiofibromas. Archives of Otolaryngology–Head and Neck Surgery, 130(6): 727-731.

3. Farhat. 2009. Vascular Endothelial Growth Factor pada Karsinoma Nasofaring. Majalah Kedokteran Nusantara, 42(1): 59-65.

4. Gidea, D.F., Iovanescu, G., Cotulbea, S., Maria, G.R., Horhat, D. 2012. Juvenile nasopharyngeal angiofibroma–case report. Jurnalul Pediatrului, 15(59-60): 54-8.

5. Hicklin, D.J. and Ellis, L.M. 2005. Role of the vascular endothelial growth factor pathway in tumor growth and angiogenesis. Journal of Clinical Oncology, 23(5): 1011-1027.

6. Hota, A., Sarkar, C., Gupta, S.D., Bhalla, A.S. and Thakar, A. 2015. Expression of vascular endothelial growth factor in juvenile angiofibroma. International Journal of Pediatric Otorhinolaryngology, 79(6): 900-902.

7. Itoo, F.A., Iqbal. I. and Chiesti, L.A. 2014. Juvenille Nasopharyngeal Angiofibroma: A Tertiary Hospital-Based Experience. Indian Journal of Clinical Practices, 25(4): 352-355.

8. Kawamura, H., Li, X., Welsh, M. and Claesson-Welsh, L. 2008. VEGF Signal Tranduction in Angiogenesis. In: Figg, W.D., Folkman, J. (Eds.), Angiogenesis, Springer, Boston, MA.

9. Liu, Z., Wang, J., Wang, H., Wang, D., Hu, L., Liu, Q. and Sun, X. 2015. Hormonal receptors and vascular endothelial growth factor in juvenile nasopharyngeal angiofibroma: immunohistochemical and tissue microarray analysis. Acta oto-laryngologica, 135(1): 51-57.

10. Makhasana, J.A.S., Kulkarni, M.A., Vaze, S., Shroff, A.S. 2016. Juvenile nasopharyngeal angiofibroma. Journla of Oral Maxillofacial Pathology, 20(2): 330.

11. Mishra, S., Praveena, N.M., Panigrahi, R.G. and Gupta, Y.M. 2013. Imaging in the diagnosis of juvenile nasopharyngeal angiofibroma. Journal of Clinical Imaging Science, 3(Suppl 1): 1.

12. Naz, N., Ahmed, Z., Shaik, S.M. and Marfani, M.S. 2009. Juvenille Nasopharyngeal Angiofibroma Role of Imaging in Diagnosis, Staging and Recurrence. Pakistan Journal of Surgery, 25(3): 185-189.

13. Renkonen, S., Hagström, J., Vuola, J., Niemelä, M., Porras, M., Kivivuori, S. M., ... & Mäkitie, A.A. 2011. The changing surgical management of juvenile nasopharyngeal angiofibroma. European Archives of Oto-Rhino-Laryngology, 268(4): 599-607.

14. Saniasiaya, J., Abdullah, B. and Ramli, R.R. 2017. Surgical management and outcome of juvenile nasopharyngeal angiofibroma in a single centre: A fifteen year experience. Egyptian Journal of Ear, Nose, Throat and Allied Sciences, 18(1): 39-41.

15. Saylam, G., Yucel, O.T., Sungur, A., *et al.*, 2005. Proliferation, Angiogenesis and Hormonal Marker in Juvenille Nasopharyngeal Angiofibroma. International Journal of Pediatric Otorhinolaryngology, 70(2): 227-34.

16. Shamim, A.A., Ghias, K. and Khan, M.J. 2013. Juvenile nasopharyngeal angiofibroma: experience at a tertiary care centre in Pakistan. Journal of Pakistan Medical Association, 63(1): 134-138.

17. Shibuya, M. 2013. Vascular endothelial growth factor and its receptor system: physiological functions in angiogenesis and pathological roles in various diseases. The Journal of Biochemistry, 153(1): 13-19.

18. Simbolon, R. 2016. Profil Penderita Angiofibroma Nasopharyngeal Juvenille di RSUP H. Adam Malik Medan. Tesis Magister, Medan, FK USU.