Study of correlation between vascular endothelial growth factor receptor and some prognostic factors in papillary carcinoma of thyroid gland

Mohammadreza Jalali Nadoushan[1*], Sepideh Siadati[2], Amir Arsalan Amin[3], Ali Davati[4], Mohaddese Mirzapour[5]

1.Department of Pathology, Shahed University, Tehran, Iran
2.Department of Pathology, Babol University of Medical Sciences, Babol, Iran
3.Department of Social Medicine, Shahed University, Tehran, Iran
4. Clinical Research Development Center, Shahid Beheshti Hospital, Babol, Iran

Article info:
Received : 8 May 2012
First Revision: 28 June 2012
Accepted: 12 July 2012

Key Words:
Papillary Carcinoma of Thyroid Gland
VEGFR
Tumors Size
Lymph Node Involvement

A B S T R A C T

Background and Objective: Thyroid cancer is the most frequent endocrine malignancy in the world and papillary carcinoma is the most frequent thyroid carcinoma. Different markers used for determination of prognosis and VEGFR is one of them. The aim of present study was to determine the expression of Vascular Endothelial Growth Factor Receptor (VEGFR) as a prognostic marker in papillary carcinoma.

Materials and Methods: This study was conducted on 92 tissue blocks of papillary carcinoma during the period of 2009-2011 in Mostafa Khomeini Hospital, Tehran, Iran. Two tissue sections were prepared. One of them stained with Hematoxylin and Eosin and the other by immunohistochemistry to determine VEGFR expression. Also tumor size and lymph node status was determined. The data analyzed and the p-value of ≤o.o5 was considered statistically significant.

Results: The mean age of patients was 39.32± 16.93 years. 40.2% of tumors showed VEGFR expression. 23.9% of patients had lymph node involvement. The average tumor size was 3.6 ± 2.2 cm. No meaningful relationship was observed between VEGFR expression and sex and age of patients and the size of tumor. However, there was a direct significant relationship between the absence of VEGFR expression and uninvolvement of lymph nodes.

Conclusion: It seems that reduction in VEGFR expression in papillary carcinoma resulting in reduced numbers of involved lymph node and better prognosis. More complete studies in this field with patient follow up is recommended.

1. Introduction

According to latest reports of the Ministry of Health of Iran, thyroid cancer is the seventh most common cancer in women. Age-specific incidence rates in men and women are 2.80 and 1.60, respectively.Also the incidence of thyroid cancer in women and men is 1.19% and 4.36% of all cancers, respectively. The most common age of thyroid cancer in women is 45-48 years and in men 80-84 years (1). In United States the incidence of thyroid cancer is lower and includes less than 1% of all cancers (2% in women and 0/5% in men). The annual mortality from thyroid cancer is 6 per million. Papillary thyroid carcinoma (PTC) is the most common type of thyroid cancer which includes 80% of all thyroid malignancies (female to male ratio 2/1) (2, 3). PTC appears as a solitary nodule then

[*]***Corresponding Author:***
Mohammadreza Jalali Nadoushan
Department of Phathology, School of Medicine, Shahed University, Tehran, Iran
Email: jalalinadooshan@yahoo.com

through thyroid lymphatic vessels spreads to subcapsular and pricapsular area. It presents with a palpable mass in 80% of children and 29% of adult and can metastases to lung or bone (4). After diagnosis of cancer for determining of the prognosis and to choose the type of treatment, multiple clinical parameters such as TNM staging, pathological andmolecular factors are used. Vascular endothelial growth factor (VEGF) and its receptors (VEGFR) are considered as factors in determining the prognosis. VEGF is the strongest known protein that leads to specific angiogenic and mitogenic change in endothelial cell and increases the vascular permeability. This factor induces significant angiogenic response in vivo (5-9). The new vessels endothelial cell growth is dependent on VEGF (10). Three endothelial receptors with high affinity to VEGF are known. These receptors are VEGFR-1/ Flt-1 ، VEGFR-2/ Flk-1/ KDR, VEGFR-3/ Flt-4 that transmit signals during vessel formation (11). VEGFR-1 and VEGFR-2 are cell surface tyrosine kinase receptors which localized endothelial cells during embryonic development. Association of VEGF gene expression patterns and its receptors show that these proteins play an important role in vessels development in fetal period (12, 13). VEGFR-Tks is a member of tyrosine kinase large family and play an important role in messaging pathway leads to proliferation, differentiation, migration and anabolism of cells. Recently, Bernatches et al. used oligomers against FLK-2 and FLt-2 and show that proliferation, differentiation and migration of endothelial cells and platelet-activating factor are dependent on Flk-2, whereas inhibition of Flt-1 has no effect on VEGF activates. These studies showed that targeting VEGFR-2 messaging process could be effective in prevention of angiogenesis in tumors (14). VEGF and its receptors have a role in angiogenesis in many solid tumor like cancers of breast, colon, urinary bladder, gastric, liver, and prostate. Since solid tumors growth are dependent on angiogenesis, several therapeutic strategies based on targeting the VEGF pathway as anti-cancer therapy is being formed. Main approaches are prevention of the secretion of VEGF, neutralization of VEGF in capillary blood circulation and prevention of VEGF binding to its receptors (15-20). The purpose of this study was to determine the VEGF receptor expression as a prognostic marker in the prognosis of papillary carcinoma of thyroid gland.

2. Materials and Methods

Data of 70 diagnosed cases of papillary carcinoma of thyroid gland during the period of 2009 to 2011 were retrieved from the files of Department of Pathology of Mostafa Khomeini hospital, Tehran, Iran.

The data were analyzed according to age, gender, tumor size and lymph node status.Tissue blocks were cut at 5 micron, stained by conventional Hematoxylin and Eosin method to identify tumor and lymph node metastasis.

Another slide with 3 micron thick sections was prepared. These slides were deparaffinized in xylene and rehydrated through graded concentrations of ethanol. After washing with 10% phosphate buffered saline (PBS), the slides were placed in H2O2- methanol solution (1/9) for 10 minutes, washing with 10% PBS. Then they were placed in EDTA at 120 ºc for 15 minutes. The slides were chilled at room temperature, and washed with 10% PBS.

At next step, the slides were immunohistochemically stained according to kit instruction (Novacastra, UK).

At the end of immunostaining, we used Mayers Hematoxylin for counterstaining, then rinising slides with distilled water, rehydrated them through graded concentrations of ethanol, mounted with entellane and coverslipped. The expression or absence of VEGFR was examined by light microscopy.

Statistical analysis was performed using SPSS statistical software, Mann-Whitney and Chi-Square. We used " percent" for descriptive purpose. A p-value ≤ 0.05 was considered statistically significant.

3. Results

This study was done on 92 cases of papillary carcinoma of thyroid gland. The mean age of patients was 39.32± 16.93 years, ranging from 13 to 80 years. Meanwhile, 40.2% (37 cases) were positive for VEGFR expression and 59.8% (55 cases) were negative.

"Figure 1" details these data. 14.1% (13) cases were male and 85.9% (79 cases) were female (Figure 2). In this study, 23.9% (22 cases) had

lymph node metastasis and 76.1% (70 cases) were negative for lymph node metastasis (Figure 3).

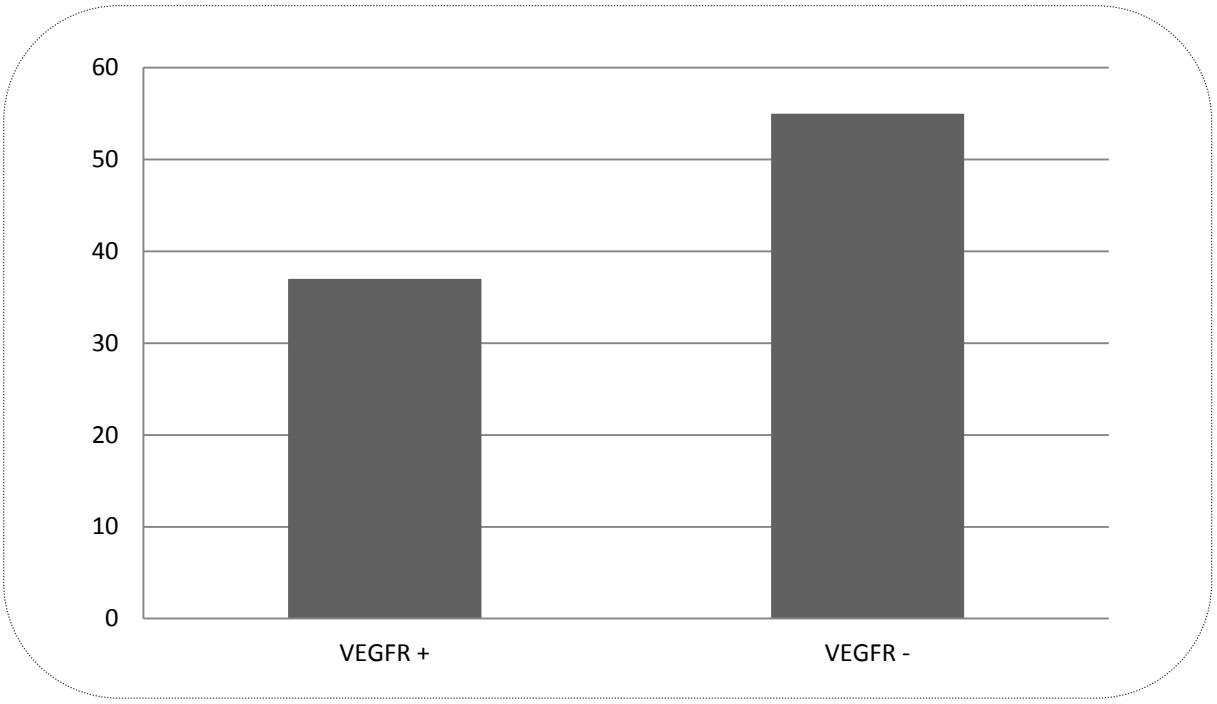

Figure.1: The frequency of VEGFR expression in papillary carcinoma of thyroid gland

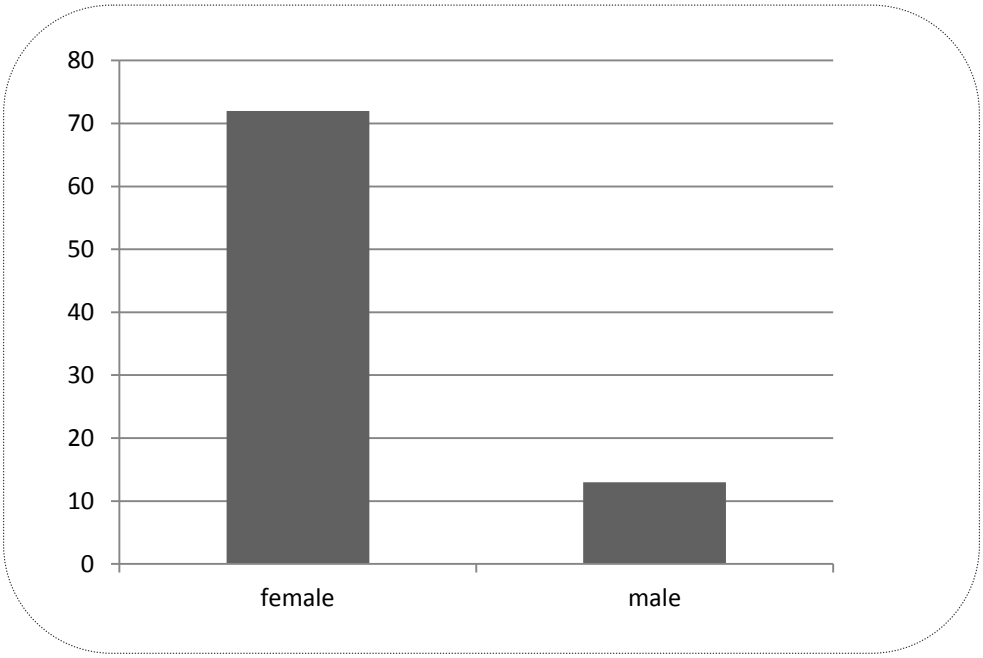

Figure.2: The frequency of gender of patients with papillary carcinoma of thyroid

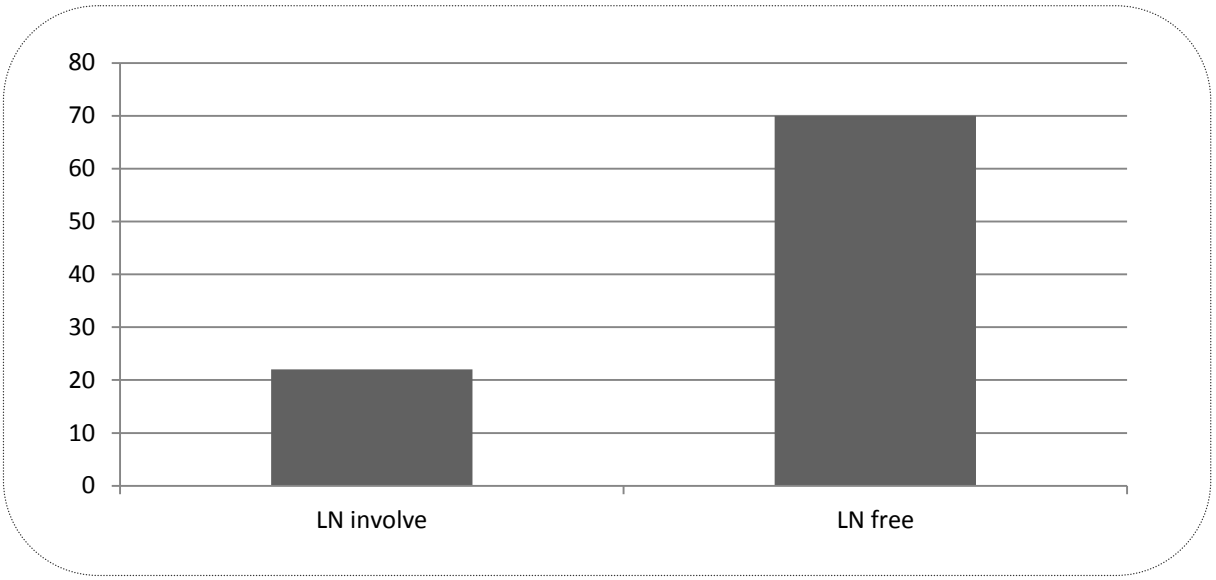

Figure.3: The frequency of Lymph nodes involvement

Tumor size ranged from 0.5 to 14 cm (mean 3.6± 22 cm).

Using Chi-Square, no significant relationship between the expression of VEGFR and the patients age was found (p=0.633).

Table 1 details the numbers of VEGFR positive and negative cases in both sexes. From 37 cases

with VEGFR expression, 14 cases had lymph node metastasis and from 55 cases with no expression of VEGFR, 47 cases found with no lymph node metastasis.

According to Chi-Square, meaningful relationship was observed between the absence of VEGFR expression and un-ivolvement of lymph nodes(p=0.01). These data are shown in Table 2.

Using Mann-Whithney test, no significant relationship was found between VEGFR expression and tumor size (p=0.458) (Table3).

Table 1: Frequency of VEGFR

Sex	Positive	Negative
Male	6	7
Female	31	48

Table 2: The correlation between lymph node involvement and VEGR expression

LN	Positive	Negative
Positive	14	8
Negative	23	47

Table 3: The correlation between tumor size and VEGR expression

VEGFR	N	Mean Rank
Positive	55	48.19
Negative	37	43.99

4. Discussion

The present study was performed on 99 tissue blocks of papillary carcinoma of thyroid gland. Our findings showed that there was no meaningful relationship between VEGFR expression and tumor size and gender of patients, but this relationship was seen between the expression of VEGFR and lymph nodes involvement.

Jiang et al. studied 115 cases of PTC and 20 cases of nodular goiter. They showed that the expression of VEGF was strongly related to growth of PTC and so VEGF-D and VEGF-C have a close relation to lymphatic metastases of PTC, and could be predictive factors for lymphatic metastases of this tumor (21). On the other hand, regarding our study, expression of this factor is not related to tumor size but there was a relation between expression of VEGFR and lymph node metastases. It appears that our study complete Jiang study because we studied the relation of patient sex with VEGFR and this factor was not evaluated in their study.

Yasuoka et al. studied 49 cases of PTC and showed that expression of VEGF-D and Flt-4 was associated with increasing of lymphatic vessels density, through which probably plays an important role in tumor metastasis to lymph nodes (22).

This study was similar to our study, although we evaluated this relation with gender and tumor size and the numbers of our cases (92) was greater than their study.

Scarpino et al. evaluated 61 cases of PTC, including 14 cases with lymphatic metastasis and showed low expression of NCAM (CP56) leading to negative regulation of VEGF-D production, therefore could be effective on reduction of lymphangiogenesis and lymphatic metastasis (23). We found similar results, but we didn't evaluate the relationship of NCAM with VEGFR.

Also the determination of relationship between VEGFR with gender and tumor size in our study could be complementary of Scarpino and his colleagues.

Jebreel et al. performed a study on 66 cases including multinodular goiter (17), Graves disease (14), follicular adenoma (10), ashimoto's thyroiditis (8), PTC (7) and 10 cases of normal thyroid tissue. They concluded that VEGF-1

expression and small vessels density in different pathologic entities had no significant difference. Expression of VEGF and its receptors in autoimmune conditions were less than neoplastic lesions. In the other model, expression of VEGF-1 and VEGF-2 were highly increased in neoplastic diseases of thyroid gland (24).

In contrast to our study, they evaluated the expression of these factors in different diseases of thyroid gland with no assessment of relationship between these conditions and prognostic factors.

We studied PTC, as a most frequent thyroid carcinoma in the world, and evaluated the relation between VEGFR expression and prognostic factors in this carcinoma, like tumor size and lymph nodes involvement. Considering these factors our study is complementary to Jebreel study.

Fenton et al. performed a study on papillary carcinoma (42), follicular carcinoma (8) and benign lesions (15) of thyroid gland in children and young adults. They found VEGFR expression in papillary and follicular carcinoma were more than benign lesions. Expression of FIT-1 in PTC was more than follicular carcinoma and benign lesions. Also they found that there was meaningful relationship between VEGF type 1 receptor (FIT-1) and tumor size in children and young adult (25).

In contrast to Fenton et al., we studied only PTC and found no meaningful relationship between VEGPR expression and tumor size. However, this difference may attributable to age group (children and young adult in Fenton study). We considered the relationship between VEGPR expression with patient sex and lymph nodes status. Thus, our study was complementary to Fento et al. study.

Considering our findings and the previous data, it seems that VEGFR is an important prognostic factor to determine the outcome of patients with papillary carcinoma of thyroid gland. Further studies with more cases and long-time follow up for evaluation of recurrence and metastasis is recommended.

4.1. Acknowledgment

This paper is the result of medical student thesis and has been financially supported by esearch council of Shahed University.

References

1. Ministry of Health, Treatment, and Medical Eduacation. Cancer Registry Report Tehran: Tandis; 2009.

2. Billar T, Dunn D, Hunter J, Pollock R. Schwarts Textbook of Surgery. New York: Mc Graw Hill; 2005.

3. Andreoli T, Carpenter C, Griggs R. Cecil Essentials of Medicine. 23rd ed. New York:Mosby; 2004.

4. Kasper DL, Braunwald E, Fauci AS, Hauser SL, Longo DL, Jameson JL. Harrison's principles of internal medicine. 17thed. New York: McGraw Hill; 2008.

5. Connolly DT, Heuvelman DM, Nelson R. Tumor vascular permeability factor stimulates endothelial cell growth and angiogenesis. J Clin Invest 1989; 84: 1470-8.

6. Plate KH, Breier G, Weich HA. Vascular endothelial growth factor is a potential tumor angiogenesis factor in human gliomas in vivo. Nature 1992; 359: 845-8.

7. Phillips GD, Stone AM, Jones BD. Vascular endothelial growth factor (rhVEGF165) stimulates direct angiogenesis in the rabbit cornea. In Vivo 1994; 8: 961-5.

8. Tolentino MJ, Miller JW, Gragoudas ES. Vascular endothelial growth factor is sufficient to produce iris neovascularization and neovascular glaucoma in a nonhuman primate. Arch Ophthalmol 1996; 114: 964-70.

9. Cao Y, Linden P, Farnebo J. Vascular endothelial growth factor C induces angiogenesis in vivo. Proc Natl Acad Sci USA 1998; 95: 14389-94.

10. Alon T, Hemo I, Itin A. Vascular endothelial growth factor acts as a survival factor for newly formed retinal vessels and has implications for retinopathy of prematurity. Nat Med 1995; 1: 1024-8.

11. Mustonen T, Alitalo K. Endothelial receptor tyrosine kinases involved in angiogenesis. J Cell Biol 1995; 129: 895-8.

12. Breier G, Albrecht U, Sterrer S. Expression of vascular endothelial growth factor during embryonic angiogenesis and endothelial cell differentiation. Development 1992; 114: 521-32.

13. Jakeman LB, Armanini M, Phillips HS. Develop-

mental expression of binding sites and messenger ribonucleic acid for vascular endothelial growth factor suggests a role for this protein in vasculogenesis and angiogenesis. Endocrinology 1993; 133: 848-59.

14. Bernatchez PN, Soker S, Sirois MG. Vascular endothelial growth factor effect on endothelial cell proliferation, migration, and platelet-activating factor synthesis is Flk-1-dependent. J Biol Chem 1999; 274: 31047-54.

15. Kurebayashi J, Otsuki T, Kunisue H. Expression of vascular endothelial growth factor (VEGF) family members in breast cancer. Jpn J Cancer Res 1999; 90: 977-81.

16. Shaheen RM, Davis DW, Liu W. Antiangiogenic therapy targeting the tyrosine kinase receptor for vascular endothelial growth factor receptor inhibits the growth of colon cancer liver metastasis and induces tumor and endothelial cell apoptosis. Cancer Res 1999; 59: 5412-16.

17. Yoshiji H, Kuriyama S, Hicklin DJ. KDR/Flk-1 is a major regulator of vascular endothelial growth factor-induced tumor development and angiogenesis in murine hepatocellular carcinoma cells. Hepatology 1999; 30: 1179-86.

18. Droller MJ. Vascular endothelial growth factor is a predictor of relapse and stage progression in superficial bladder cancer. J Urol 1998; 160: 1932.

19. Kitamura M, Toi M, Arai K. Concentrations of vascular endothelial growth factor in the sera of gastric cancer patients. Oncol Rep 1998; 5: 1419-24.

20. Balbay MD, Pettaway CA, Kuniyasu H. Highly metastatic human prostate cancer growing within the prostate of athymic mice overexpresses vascular endothelial growth factor. Clin Cancer Res 1999; 5: 783-9.

21. Jiang HG, Gao M, Tang WP, Li FH, Cai QZ. Expression and significance of VEGF, VEGF-C, and VEGF-D in papillary thyroid carcinoma. Ai Zheng. 2005; 24(9): 1136-9.

22. Yasuoka H, Nakamura Y, Zuo H, Tang W, Takamura Y, Miyauchi A, et al. VEGF-D expression and lymph vessels play an important role for lymph node metastasis in papillary thyroid carcinoma. Mod Pathol. 2005; 18(8): 1127-33.

23. Scarpino S, Di Napoli A, Melotti F, Talerico C, Cancrini A, Ruco L. Papillary carcinoma of the thyroid: low expression of NCAM (CD56) is associated with downregulation of VEGF-D production by tumour cells. J Pathol. 2007; 212(4): 411-9.

24. Jebreel A, England J, Bedford K, Murphy J, Karsai L, Atkin S. Vascular endothelial growth factor (VEGF), VEGF receptors expression and microvascular density in benign and malignant thyroid diseases. Int J Exp Pathol. 2007; 88(4): 271-7.

25. Fenton C, Patel A, Dinauer C, Robie D,Tuttle M. The Expression of Vascular Endothelial Growth Factor and the Type 1 Vascular Endothelial Growth Factor Receptor Correlate with the Size of Papillary Thyroid Carcinoma in Children and Young Adults. Thyroid. 2000; 10(4): 349-57.