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Patterns of Angiogenic Factors (VEGF, Angiopoietin-1) in Patients with Benign Thyroid Lesions Before and After Surgery

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ABSTRACT

Background: Angiogenesis plays an important role in goiter development with endothelial cell proliferation occurring before increased proliferation of the thyroid follicular cells and increase in both vascular endothelial growth factor (VEGF) and Angiopoietin-1 (Ang-1) in the serum and intrathyroid tissue.

Objective: The aim of the current work was to measure the levels of VEGF and Ang-1 in patients with benign thyroid disorders before and after thyroidectomy and compare it to the normal control subjects.

Patients and Methods: This prospective study included a total of 80 patients provisionally diagnosed as benign thyroid disorders and 50 apparently healthy subjects served as a control group, attending at Outpatient Clinics of Endocrine Surgery Unit, Mansoura University Hospital. This study was conducted between February 2017 to February 2020. Of the 80 patients, 12 (15%) were male and 68 (85%) were female and their age ranged from 19 to 60 years (median = 40.41 years). On admission, careful history taking, clinical examinations and different investigations were done.

Results: Thirty patients had benign multinodular goiter, 20 had solitary follicular adenoma, 10 had diffuse toxic, 10 had nodular toxic goiter, and 10 had Hashimoto thyroiditis. In all patients, pre-operative circulating VEGF and Ang-1 levels were increased compared to controls (P<0.001), and a decrease after thyroidectomy was observed in the levels of VEGF (P<0.001) in all of them. This decrease was significant after total thyroidectomy compared to near total and hemi-thyroidectomy. Circulating Ang-1 were significantly increased (P<0.001) after thyroidectomy in all patients.

Conclusion: It could be concluded that this study raise the potential for VEGF and Ang-1 factor to be used as biomarkers of the effectiveness of thyroidectomy in benign thyroid lesions. These results needs further investigations and may have potential prognostic implications.

Keywords: Angiogenesis – Thyroidectomy – VEGF – Angiopoietin-1.

INTRODUCTION

Angiogenesis is the formation of new capillaries from the existing vasculature. Thyroid follicular cells secrete several angiogenic factors, e.g., vascular endothelial growth factors (VEGFs), angiopoietins, fibroblast growth factors (FGFs), and anti-angiogenic factors, e.g., thrombospondin, angiostatin, and proteases, and their production is regulated by TSH through cyclic AMP or by other follicular cell growth factors [(1)]. Rise in TSH activates the follicular cells to produce angiogenic factors. Thyroid follicular cells also express the angiopoietin receptor, Tie-2 and possibly also the VEGF receptor, VEGFR1 [(2,3)].

The vascular endothelial growth factors are perhaps the best known of the angiogenic factors in the thyroid and elsewhere, and they act in cooperation with FGFs to stimulate angiogenesis [(4)]. VEGF expression is increased by hypoxia, estrogen, explaining the male: female incidence (1:4) of thyroid disease, and increased by hepatocyte growth factor [(5,6)]. VEGF expression is suppressed by dexamethasone and somatostatin analogues, which may suggest potential areas for therapy [(7)]. The ligands for angiopoietins 1, 2, and 3 and their receptor Tie2 are synthesized by thyroid follicular cells and other cell types. Estrogen and thyroid hormone increase tissue production of Ang-2 and decrease that of Ang-1 [(8,9)].

The thyroid is a highly vascular organ, and the thyroid vasculature is increased in goiter, induced either by thyrotropin (TSH) receptor stimulation or by tumorigenic transformation. This makes the thyroid an attractive model in which to study physiologic and pathologic angiogenesis and its regulation during organ growth [(1)].

Angiogenesis occurs in the thyroid during disease processes including goiter, Graves' disease, thyroiditis and cancer. The molecular mechanisms controlling angiogenesis are becoming clearer, and therapy targeting these processes is coming closer to clinical fruition. Both promoters and inhibitors of angiogenesis have been identified in the thyroid, including vascular endothelial growth factor (VEGF), angiopoietins, fibroblast growth factor, and thrombospondin [(10)].

The aim of the current work was to measure the levels of VEGF and Ang-1 in patients with benign thyroid disorders before and after thyroidectomy and compare it to the normal control subjects.

PATIENTS AND METHODS

This prospective study included a total of 80 patients provisionally diagnosed as benign thyroid disorders and 50 apparently healthy subjects served as a control group, attending at Outpatient Clinics of Endocrine Surgery Unit, Mansoura University

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Of the 80 patients, 12 (15%) were male and 68 (85%) were female and their age ranged from 19 to 60 years (median = 40.41 years). On admission, careful history taking, clinical examinations and different investigations were done.

These investigations included:

- Measurement of thyroid functions (T3, T4, TSH), auto antibodies, serum calcium (total & ionized), platelet count.
- Neck ultrasonographic, color Doppler imaging.
- Neck ultrasound elastography for cases of simple multinodular goiter (MNG) and solitary thyroid nodule (STN).
- Fine-needle aspiration cytology (FNAC) was done for patients with MNG and STN.
- Radioactive thyroid scan or computed tomography was done if needed.
- Levels of serum VEGF and Ang-1 were estimated for all patients with thyroid disorders by ELISA technique. Blood samples were taken preoperatively and two months after thyroidectomy for all patients. Serum VEGF and Ang-1 were also estimated for control cases.

The included subjects were divided into five groups: **Group (I)** included 30 patients who subdivided into Group (I a): simple multinodular goiter (20 Patients), where near total thyroidectomy was done and Group (I b): simple multinodular goiter (10 patients), where total thyroidectomy was done, **Group (II)** included 20 patients with solitary thyroid nodule, where hemithyroidectomy was done, **Group (III)** included 20 patients who subdivided into Group (III a): Diffuse toxic goiter (10 Patients) and Group (III b): Toxic nodular goiter (10 patients), where near total thyroidectomy was done, **Group (IV)** included 10 patients with chronic thyroiditis where near total thyroidectomy was done, and **Group V (control)** included 50 healthy subjects admitted from the general surgery clinic with benign lesions other than thyroid disorders proved by different methods of investigations (38 females and 12 males).

Different surgical procedures were performed including:

1. **Hemithyroidectomy:** One lobe of the thyroid is removed with the isthmus and pyramidal lobe. The isthmus is divided close to the contralateral lobe.
2. **Near total thyroidectomy:** Same as total thyroidectomy, but after the inferior artery branches are ligated, the lobes are then transected leaving a cuff of posterior thyroid tissue which lies on either sides of the trachea.
3. **Total thyroidectomy:** We did a bilateral hemithyroidectomy.

Principle of the Assay (VEGF & Ang-1):

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human VEGF has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any VEGF present is bound by the immobilized antibody.

Ethical consent:

An approval of the study was obtained from Mansoura University Academic and Ethical Committee. Patients were interviewed and written informed consents were obtained.

Statistical Analysis

Data were fed to the computer and analyzed using IBM SPSS Corp. Released 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp. Qualitative data were described using number and percent. Quantitative data were described using median (minimum and maximum) for non-parametric data and mean, standard deviation for parametric data after testing normality using Kolmogrov-Smirnov test. Significance of the obtained results was judged at the (0.05) level.

Chi-Square test for comparison of 2 or more groups. Parametric tests: Student t-test was used to compare 2 independent groups. One Way ANOVA test was used to compare more than 2 independent groups with Post Hoc Tukey test to detect pair-wise comparison. Non Parametric tests: Mann-Whitney U test was used to compare 2 independent groups. Kruskal Wallis test was used to compare more than 2 independent groups with Mann Whitney U test to detect pair-wise comparison. Wilcoxon signed Rank test to compare between 2 studied periods. Receiver Operating Characteristic (ROC) curve analysis: The diagnostic performance of a test or the accuracy of a test to discriminate diseased cases from non-diseased cases is evaluated using Receiver Operating Characteristic (ROC) curve analysis. Sensitivity and Specificity were detected from the curve and PPV, NPV and accuracy were calculated through cross tabulation.

RESULTS

This study included 12 males and 68 females, the age of the studied patients ranged from 19 to 60 years with a mean age of (40.41 ± 9.02) with a slight higher mean age in females than males, but with no statistical significance (P value = 0.198).

Table (1): Demographic characteristics of the studied groups.

	Control n=50	**Cases** n=80	**Test of significance**
Age/years Zean±SD	36.94±7.53	40.41±9.02	t=2.27 p=0.025*
Sex			
Male	12 (24.0 %)	12 (15.0 %)	χ^2=1.66
Female	38 (76.0 %)	68 (85.0 %)	p=0.198

t: Student t test , χ^2=Chi-Square test * statistically significant if p<0.05

All patients proved to be benign thyroid disorders, including colloid nodular (30 patients, 37.5 %), diffuse toxic (10 patients, 12.5 %), toxic nodular (10 patients, 12.5 %), follicular adenoma (20 patients, 25%), Hashimoto thyroiditis (10 patients, 12.5 %).

Table (2): Diagnosis of the studied patients (80).

Diagnosis	**n=80**	**%**
MNG	30	37.5
STN	20	25.0
Diffuse toxic	10	12.5
Toxic nodular	10	12.5
Hashimoto	10	12.5

The control group included 50 individuals with benign non thyroid lesions, 12 were males (24%) and 38 females (76%) ranging in age from (21years) to (51 years), the mean age was (36.94 ± 7.53).

Table (3): Comparison of **VEGF** and **Ang-1** among the control group and all patients (pg\mL).

	Control	**Cases**	**Test of significance**
VEGF (pg\ml) Median (min-max)	77 (25-446)	351.5 (42-918)	Z=8.16 P<0.001*
Ang-1 (pg\ml) Median (min-max)	118 (34-632)	596 (71-3819)	Z=7.49 P<0.001*

Z: Mann Whitney U test *statistically significant if p<0.05

This table shows a significantly (p<0.001) high levels of VEGF 351.5 (42- 918) pg\mL in all patients, compared to the healthy control group 77 (25-446) pg\mL. It shows also a significantly high (p<0.001) Ang-1 of all patients 596 (71 – 3819) pg\mL, compared to the healthy control group 118 (34-632).

Table (4): Pre- and post-operative distribution of VEGF according to the pathology.

Pathology	**VEGF**		**Test of significance (Wilcoxon signed rank test)**
	Pre-operative	**Post-operative**	
Colloid nodular (30)	353(111-918)^{A}	110.5(20.9-380)	*P*<0.001*
Follicular adenoma (20)	216(42-739)^{ABC}	95(28.4-441)	*P*<0.001*
Diffuse toxic (10)	365(195-899)^{B}	121(57-352)	*P*=0.005*
Nodular toxic (10)	365(195-899)^{C}	121(57-352)	*P*=0.005*
Hashimoto (10)	382.5(197-890)	93.5(53-354)	*P*=0.005*
Test of significance	KW *P*=0.026*	KW *P*=0.816	

VEGF described as median (min-max), KW: Kruskal Wallis test *statistically significant if p<0.05, similar superscripted letters denote significant difference between studied groups within same column by Post Hic Mann Whitney U test.

Table 4 shows significant lower levels of pre-operative serum VEGF of the follicular adenoma group, compared to the other pathological types, but at the same times all the pathological groups shows a significant decrease of VEGF after thyroidectomy.

Table (5): Pre- and post-operative distribution of Ang-1 according to the pathology.

Pathology	Ang-1		Test of significance (Wilcoxon signed rank test)
	Pre-operative	Post-operative	
Colloid nodular (30)	596(83-3891)^{ABC}	3757.5(2304-4036)^{A}	*P*<0.001*
Follicular adenoma (20)	361(71-1044) ^{ADEF}	3538.5(766-4000)^{BC}	*P*<0.001*
Diffuse toxic (10)	912(445-1671)^{BD}	3885.5(3614-4000)^{BD}	*P*=0.005*
Nodular toxic (10)	912(445-1671)^{C}	3885.5(3614-4000)^{CE}	*P*=0.005*
Hashimoto (10)	713(510-943)^{EF}	3350(788-4000)^{ADE}	*P*=0.005*
Test of significance	KW *P*<0.001*	KW *P*=0.003	

Ang-1 described as median (min-max), KW: Kruskal Wallis test *statistically significant if *p*<0.05, similar superscripted letters denote significant difference between studied groups within same column by Post Hic Mann Whitney U test. Table 5 shows significant higher levels of pre-operative serum Ang-1 of the diffuse, nodular toxic goiter and Hashimoto thyroiditis groups compared to the colloid nodular and solitary follicular adenoma groups. All serum levels of Ang-1 are significantly increased after thyroidectomy.

Table (6): Comparison between pre- and post-operative levels of VEGF and Ang-1 according to type of operation done

		Hemi thyroidectomy (20)	Total thyroidectomy (10)	Near total thyroidectomy (50)	Mann Whitney U test
VEGF	Pre-operative	216 (42-739)	604.5 (111-918)	355 (153-899)	*P*1=0.012* *P*2=0.002* *P*3=0.293
	Post-operative	95 (28.4-441)	89 (20.9-380)	111.5 (39-354)	*P*1=0.965 *P*2=0.272 *P*3=0.552
Wilcoxon signed rank test		Z=3.51 *P*<0.001*	Z=2.67 *P*=0.008*	Z=6.14 *P*<0.001*	
Ang-1	Pre-operative	361(71-1044)	477.5(83-3819)	668.5(354-3733)	*P*1=0.301 *P*2<0.001* *P*3=0.007*
	Post-operative	3538.5(766-4000)	3805.5(2304-4000)	3800(788-4036)	*P*1=0.341 *P*2=0.082 *P*3=0.842
Wilcoxon signed rank test		Z=3.92 *P*<0.001*	Z=2.80 *P*=0.005*	Z=6.12 *P*<0.001*	

*p*1: between hemi-thyroidectomy, *p*2: difference between hemi-thyroidectomy and near total thyroidectomy, *P*3: difference between total & near total thyroidectomy.

Table 6 compares the pre- and postoperative serum levels of VEGF and Ang-1 after different types of thyroidectomy. It shows that serum levels of VEGF are significantly decreased after total thyroidectomy compared to either near-total or hemi-thyroidectomy, while the serum levels of Ang-1 shows no significant difference in the post-operative increase after different types of thyroidectomy.

Table (7): In the multinodular group only (30 patients): comparison between levels of VEGF and Ang-1 (pre- and post-operative) in patients who did total against near total thyroidectomy.

		Total thyroidectomy(10)	Near Total thyroidectomy(20)	Test of significance
VEGF	Pre-operative	604.5(111-918)	344.5 (153-850)	*P*=0.173
	Post-operative	89(20.9-380)	113.5(39-321)	*P*=0.509
		P=0.008*	*P*<0.001*	
Ang-1	Pre-operative	447.5(83-3819)	634(354-3733)	*P*=0.039*
	Post-operative	3805.5 (2304-4000)	3757.5 (3142-4036)	*P*=0.859
		P=0.005*	*P*<0.001*	

Table 7 shows that in the multinodular goiter group, total thyroidectomy is followed by a statistically significant decrease in the levels of serum VEGF compared to patients who underwent near total thyroidectomy. At the same time, levels of Ang-1 show no statistical difference in its increase after total or near total thyroidectomy.

Table (8): Surgical treatment and pathology of the studied patients.

	N=80	%
Thyroidectomy		
Hemi thyroidectomy	20	25.0
Total thyroidectomy	10	12.5
Near total	50	62.5
Pathology		
Colloid nodular	30	37.5
Follicular adenoma	20	25.0
Diffuse toxic	10	12.5
Nodular toxic	10	12.5
Hashimoto	10	12.5

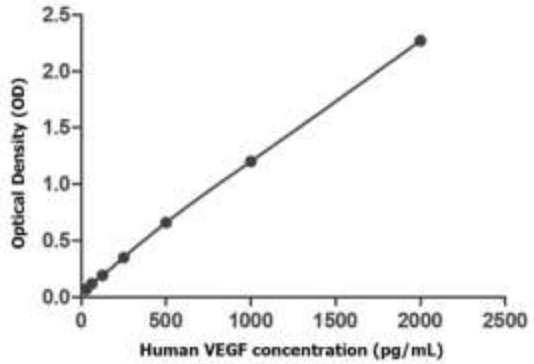
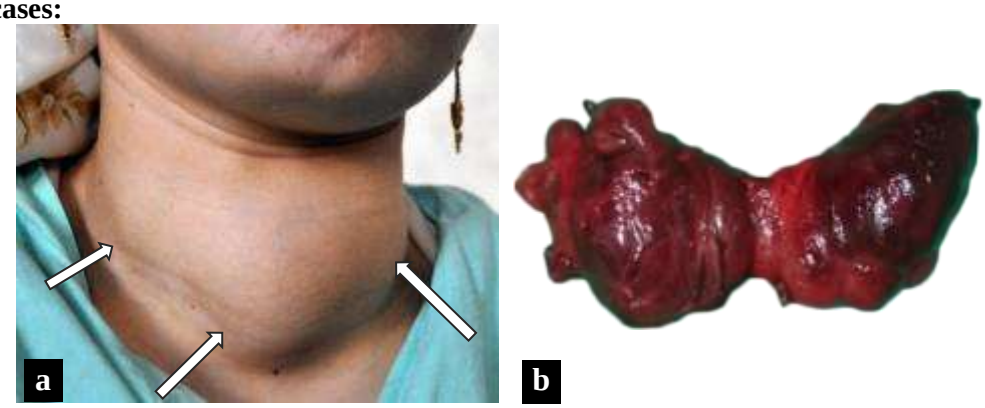
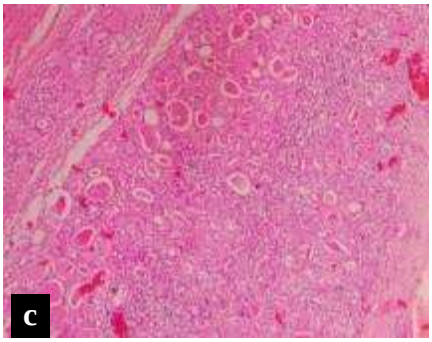


Figure (1): VEGF standard curve.

Figure (2): ELISA analysis set**.**

Sample of cases:



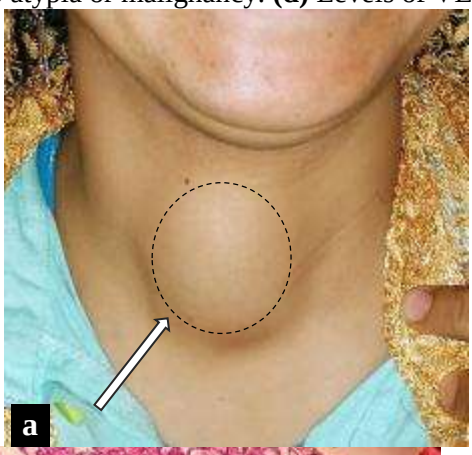


c

	Pre-operative	2 months post-operative
VEGF	841	20.9
Ang-1	364	3442

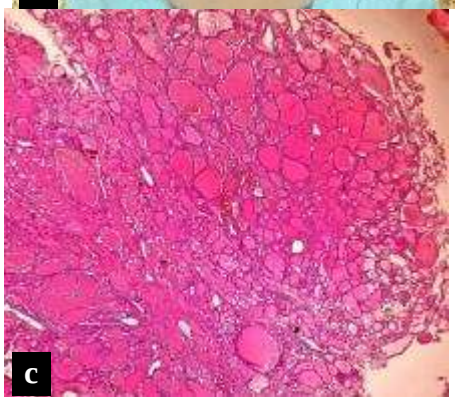
d

Figure (3): (a) Female patient 30 years old presented with simple multinodular goiter. **(b)** Gross appearance of the specimen removed. **(c)** Histopathological examination revealed colloid nodular goiter with degeneration features, no atypia or malignancy. **(d)** Levels of VEGF, Ang-1 by ELISA (pg/mL)



a

b



c

	Pre-operative	2 months post-operative
VEGF	580	180
Ang-1	83	3966

d

Figure (4): (a) Female patient 32 years old presented with right thyroid nodule. **(b)** Gross appearance of the specimen removed. **(c)** Histopathological examination revealed follicular nodule with hyperplastic changes. **(d)** Levels of VEGF, Ang-1 by ELISA (pg/mL)

DISCUSSION

Angiogenic activity is essential to the growth and progression of benign thyroid lesions [9], and in recent years there have been many efforts to investigate and present suitable biomarkers of thyroid angiogenesis because dysregulation of angiogenic cytokines could result in benign thyroid lesions and cancer initiation and progression. This is a study using ELISA to perform cumulative profiling of prominent serum angiogenic cytokines among patients with benign thyroid lesions before and after thyroidectomy and normal control subjects, respectively.

This study has found that before thyroidectomy, VEGF and Ang-1 were all increased in patients with benign thyroid diseases compared to control subjects, consistent with the known role of these cytokines in thyroid growth. HGF and VEGF are known to be potent mitogens for follicular thyroid cells, and the over expression of HGF, and VEGF has been described in nodular hyperplasia, in contrast to minimal production in normal thyroid tissue. Ang-1

shows high expression in benign thyroid lesions than in normal tissues.

The greater preoperative levels of all VEGF and Ang-1 in benign thyroid lesions with respect to healthy control subjects support the angiogenic and mitogenic role of them benign lesions, including thyroid inflamations. Greater preoperative serum VEGF and Ang-1 levels in benign lesions than the normal controls are consistent with previous studies done by **Handkiewicz-Junak *et al.*** [(11)].

Total thyroidectomy in both the main and subgroup analyses were associated with a postoperative decrease in the levels of VEGF and an increase in the levels of Ang-1 in benign thyroid lesions s, serum VEGF that were increased in other studies newly diagnosed benign lesions ,decreased progressively to levels comparable to those of control subjects with benign disease 2 months after operative treatment, suggesting that a subsequent increase of either of these markers during follow-up might be regarded as a sign of tumor recurrence.

In this study, the postoperative levels of all Angiogenic factors did not decrease to the respective levels of control subjects, probably owing to the timing of sampling that occurred 2 months postoperatively, demonstrated that the decreases in VEGF back to normal levels is likely a time-dependent process.

CONCLUSION

It could be concluded that the present study analyzed the expression and biologic importance of VEGF and Ang-1 in patients before and 2 months after thyroidectomy for benign thyroid disorders. The different profiles of the preoperative and post-thyroidectomy decreases in the levels of VEGF may suggest a different role of these 2 cytokines in the pathogenesis of benign thyroid disease. If validated, results from our study might have implications for the eventual use of serum VEGF or Ang-1 as biomarkers of the effectiveness of the operative treatment of benign thyroid disorders or to be used as building blocks for further research to improve detection and treatment of them.

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