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Effect of Altitude on Vascular Endothelial Growth Factor Levels with Validation of its Prognostic Significance in Patients with Non-Small Cell Lung Carcinoma

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ABSTRACT

Background and aim of the work: Recent studies revealed that hypobaric hypoxia stimulates release of vascular endothelial growth factor (VEGF) and other studies found that high levels of this angiogenic factor are correlated with poor prognosis in patients with non-small cell lung cancer (NSCLC). In this study we will measure the serum levels of VEGF in both healthy individuals and in patients with operable non small cell lung carcinoma living in hypobaric oxygen environment (Taif) and validate the prognostic significance of its pretreatment level in those patients.

Patients and methods: Thirty one patients with operable (stage I, II and III A) non-small cell lung cancer (the patient group) and 15 healthy volunteers with matched gender and age (control group) were enrolled in this study from January 2010 to March 2015. The pretreatment level of VEGF was measured in patients in addition of its level in controls. All patients had the same diagnostic and therapeutic protocols. Mean follow up of patients was 30.4±7.8 months.

Results: The mean level of VEGF was high in control group, however, it was significantly lower than that in patient group (P value 0.041). The median survival of stage I patients was 13 months, stage II was 9 months, and of stage III A was 6 months. Univariate analysis showed a significant correlation between survival and pretreatment level of VEGF in patients with small lung cancer.

Conclusions: Our results revealed that hypobaric hypoxia significantly increases the circulating levels of VEGF in healthy individuals without remarkable effect on its level in patients with NSCLC. Our study verified also that the pretreatment mean serum level of VEGF showed a highly significant increase in NSCLC patients than that in control group and it was significantly correlated with patient survival in levels above 618 pg/ml.

Keywords: vascular endothelial growth factor – hypobaric hypoxia – non small cell lung cancer

INTRODUCTION

Taif is located in the western region of Saudi Arabia at moderate altitude and high altitude level with mean elevation of 1,879 ± 456 meters above average sea level (range: 1630- 2650 meters).[1]

As the altitude increases, atmospheric pressure decreases, with reduction of the partial pressure of oxygen and starts to affect humans at altitudes above 1,500 meters.[2] As a consequence of the hypobaric hypoxic environments, human would develop numerous physiologic responses, and recent studies verified that hypoxia induces the release of VEGF and other angiogenic factors.[2-4] The relation between these high levels of angiogenic factors and pathological disorders in hypobaric oxygen environment are still under investigations.[2, 3]

However, many investigators verified the role of angiogenesis in tumor growth and development of metastases and they found that it depends mainly on the balance between pro-angiogenic factors as vascular endothelial growth factor (VEGF) and .antiangiogenic factors.[3] Lung cancer has a high cancer specific mortality and non-small cell lung cancer (NSCLC) represent more than 80%, however, there is a variation in prognosis of patients even in the same stage, accordingly other factors contribute in prognosis other than the tumor stage, and a variety of factors have been reported as predictors for favorable or unfavorable prognosis of the disease.[5-7, 9]Several studies found a significant correlation between survival and the pretreatment levels of VEGF, but a widely accepted cut-off level is still lacking.[9]

In this study we will measure the serum levels of VEGF in both healthy individuals and in patients with operable non small cell lung carcinoma living in hypobaric oxygen environment (Taif) and validate the prognostic

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significance of its pretreatment level in those patients.

PATIENTS AND METHODS

Thirty one patients with operable (stage I, II and III A) non-small cell lung cancer were enrolled in this study. The patients presented to King Abdul Aziz specialist hospital and Al Hada Armed Force Hospital, Taif, Saudi Arabia from January 2010 to March 2015. Fifteen healthy volunteers with matched gender and age served as control group. The study was conducted after approval of the ethical boards of the hospitals and written informed consents were taken from all participants. The pretreatment level of VEGF was measured in patients in addition of its level in controls. Blood samples: (5 ml) of venous blood were obtained from each subject participating in the study, and centrifuged for the separation of serum, and stored at -20°c until used for analysis. Serum VEGF was measured by Enzyme-linked immune-sorbent assay (ELISA) with monoclonal antibodies specific for VEGF (Med Systems Diagnostics GmbH), according to the manufacturer's instructions.

All patients had the same diagnostic and therapeutic protocols. After full clinical assessment of all patients, the initial work up to diagnose lung cancer included; chest x-rays, sputum cytology, fiber-optic bronchoscopy and biopsy which was used primarily to diagnose central lung lesions while CT-guided biopsy was performed mainly to diagnose peripheral tumors. Video assisted thoracoscopic biopsy was performed if bronchoscopy and/or CT guided biopsy had failed to obtain a diagnostic tissues. Metastatic workup included total body bone scan and CT scan of the brain and abdomen. Tumors removed during surgery were examined and staged based on TNM descriptions (International Union Against Cancer) and the stage grouping system (International Association for the Study of Lung Cancer). Exclusion criteria included; patients who were unable to cooperate with the requirements of the study, those participating in another clinical study, those with a major comorbidity as other malignancy, hepatic, renal, or cardiac dysfunction, patients who were found irresectable during surgery, and those who died during surgery or in early postoperative period. Mean follow up of patients was 30.4±7.8.

Statistical analysis:

SPSS 18.0 (SPSS, Chicago Illinois) was used for carrying out statistical analysis. Group differences were further analyzed by χ 2 and difference between means of continuous variables was tested by Student's t test. Serum VEGF was first examined as continuous variable in univariate Cox regression survival analysis and due lack of a widely accepted cut-off level, the first, second, and third quartiles were used as categorical variables. Level of significance was determined at $P < 0.05$.

RESULTS

Table 1 shows that there was no significant difference between control and patient groups regarding demographic data, but the mean preoperative level of VEGF was significantly higher in patient group than its level in control group. Table 2 shows distribution of the staging, pathological findings, and mortality in patient group. The median survival of stage I patients was 13 months, stage II was 9 months, and of stage IIIA was 6 months. The median of preoperative level of VEGF was 488 pg/ml with interquartile range of 251. Univariate analysis of the data of the patient group showed a significant correlation between preoperative serum VEGF levels and survival (Table 3).

DISCUSSION

VEGF is a potent mitogen in endothelial cells therefore, it plays a critical role in the induction and regulation of angiogenesis in physiological processes such as embryonic development and the menstrual cycle as well as in pathological conditions which include tumor growth and atherosclerosis.[5]Vascular endothelial growth factor release is induced by hypoxia either in subjects exposed to experimental hypoxic conditions or in residents of hypobaric hypoxic environment (>1500 m above sea level).[2]

In the present study the mean level of VEGF in the control group was 248.2±55.1 pg/ml which is significantly higher than that recorded in other studies on healthy individuals living in low altitude environment where its mean level ranged between 15-49

pg/ml.[2, 3, 4] However, investigators found no correlation between high levels of VEGF induced by hypobaric hypoxia and respiratory disorders, explaining that elevated VEGF induced by chronic hypoxia is associated with blockade of its receptors in pulmonary endothelium.[6] San *et al.* and Voelkel and Tuder reported in their studies that hypobaric hypoxia increases the level of VEGF, but, its concentration in the pulmonary capillary remained unchanged.[2, 6]

Direct evidence that hypoxia can promote tumor angiogenesis has not been approved, however, several angiogenic factors can be involved in tumor angiogenesis, Voelkel and .Tuder [6] and Rofstad *et al.*[7]reported in their studies that hypoxia can increase VEGF synthesis without increase the angiogenic potential of the cells of all tumors and they concluded that exposure to hypoxia just led to secretion of redundant VEGF.

Amorín Kajatt**[8]** verified that there was no significant difference in pathological types, pretreatment level of angiogenic factors, and prognosis in his study on Peruvian patients with lung cancer living in high altitude environment compared with those living in lowland. Comparable results were obtained in the present study where the mean preoperative level of serum VEGF was significantly higher in patients than in healthy individuals, however, this level was not significantly different from that recorded in other studies on non small lung cancer patients living in normobaric environment.[8,9]Moreover**,** recent studies found that the likelihood of developing lung cancer was significantly lower for people living at higher altitudes, reporting that the risk of lung cancer decreased by 7.23 cases per 100,000 people for every 1000 meters of altitude, concluding that the correlation was evident both in the raw data, and when corrected for smoking rates and education.[10-15]

Rimmaudo *et al* [16]found in their study that high levels of circulating VEGF have been recorded in patients with non-small cell lung cancer and concluded that serum levels of angiogenic growth factors are thought to reflect the *in vivo* activity of tumor angiogenesis and their levels might be correlated with cancer metastasis and worse prognosis. Yoshimoto *et al.*[17] found in their study that circulating levels of VEGF increase significantly according to disease stage progression, which is in accordance with the findings of this study.

Dudek and Mahaseth[18] recorded in their study that patients with pretreatment serum VEGF levels more than 500 pg/ml had a significantly lower survival rate than patients with serum VEGF less than 500 pg/ml. other studies reported similar results.[19, 20]The results of this study verified a significant correlation between preoperative serum level of VEGF above 618 pg/ml and survival, which is higher than that recorded in Dudek and Mahaseth[18] study and this may be explained by difference in methodology, difference in patient stages in both studies, in addition to the smaller number of patients in our study.

Walker *et al* [19] found in their study that the pre-operational levels of serum VEGF, and endostatin (ES) were remarkably higher in patients with NSCLC than in healthy controls, but only an increased VEGF level was correlated with poor survival and they recommended more aggressive chemo and radiotherapy for those patient in addition to antiangiogenic therapy.

CONCLUSIONS

Our results revealed that hypobaric hypoxia significantly increases the circulating levels of VEGF in healthy individuals without remarkable effect on its level in patients with NSCLC. Our study verified also that the pretreatment mean serum level of VEGF showed a highly significant increase in NSCLC patients than that in control group and it was significantly correlated with patient survival in levels above 618 pg/ml.

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Conflicts of Interest: The author declares that he has no conflicts of interest.

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Table 1: Comparison between Patient group and control group

Parameter	Taif Group n = 31	Control Group n = 15	p value	Sig.
Mean age±SD (years)	58±14.1	55±13.4	0.734	NS
Sex Male Female	 23 (74%) 8 (26%)	 44 (69%) 20 (31%)	0.771	NS
Smoking +ve -ve	 17/31 (55%) 14/31 (45%)	 34 (53%) 30 (47%)	0.528	NS
VEGF level Mean±SD	677±164 pg/ml	248.2±55.1 pg/ml	0.041	S

NS: non-significant; S: significant

Table 2: Staging, pathological findings, and mortality in patient group

Stage I Number (%)	8/31 (26%)
Stage II Number (%)	13/31 (42%)
Stage III A Number (%)	10/31 (32%)
Adenocarcinoma Number (%)	14//31 (45%)
Squamous cell carcinoma Number (%)	11/31 (36%)
Adenosquamos carcinoma Number (%)	4/31 (13%)
Large cell carcinoma Number (%)	2/31 (6%)
Mortality	18/31 (58%)

Table 3: Univariate survival analysis of serum VEGF as continuous and categorical variables

Variables	**Preoperative serum VEGF**		
	HR	**(95% CI)**	***P value***
Continuous	1.008	(1.005–1.016)	0.001
Categorical			
The lower quartile (367 pg/ml)	1.704	(0.832–3.065)	0.148
The median (488 pg/ml)	1.458	(0.858–2.358)	0.163
The upper quartile (618 pg/ml)	1.911	(1.049–3.229)	0.034

CI, confidence interval, HR, hazard ratio, VEGF, vascular endothelial growth factor.