MÆDICA - a Journal of Clinical Medicine

ORIGINAL PAPER

MAEDICA – a Journal of Clinical Medicine
2021; 16(2): 170-178
https://doi.org/10.26574/maedica.2021.16.2.170

Impact of Surgery on the Hemangiogenic Profile, Especially VEGF Levels, in Lung Cancer Patients

Shabir Hussain RATHER[a], Showkat Ali BHAT[a], Arshad BASHIR[a], Abdul Majeed DAR[b], Muzzafar ZAMAN[a], Naveed NABI[a], Aliya SHAH[c], Akif Mutahar SHAH[d]

[a]Department of Surgery, SKIMS Medical College, Srinagar, Jandk, India

[b]Department of Cardiovascular Surgery, SKIMS Institute of Medical Scineces Soura, Srinagar, Jandk, India

[c]Department of Microbiology, GMC Srinagar, India

[d]Department of Anaesthesia, GMC Srinagar, India

ABSTRACT

Introduction: *Lung cancer was considered to be rare at the beginning of the 20th century, but it has now reached almost epidemic proportions. It is the leading cause of cancer deaths in developed countries and is also rising at alarming rates in developing countries.*

Aim: *The aim of our study is to establish an effect on serum levels of vascular endothelial growth factor (VEGF) after surgery in lung cancer patients.*

Methods: *This was a prospective study. For the estimation of VEGF, 50 lung cancer patients were studied. Both preoperative and postoperative levels of VEGF were estimated for all subjects. Blood samples were obtained from all cases both preoperatively and postoperatively (four weeks after surgery). Blood samples of 100 age and sex matched healthy controls were collected from the Outpatient Departments of SKIMS to establish normal serum VEGF levels.*

Conclusion: *Our findings show that serum VEGF levels are higher as the tumor stage progresses and tumor size increases, which explains the lower serum VEGF levels observed by us in the operable patient group.*

Keywords: lung, VEGF, cancer, angiogenesis.

Address for correspondence:
Muzzafar Zaman
Department of Surgery, SKIMS Medical College, Srinagar, India
Contact No.: 9816635960
Email: muzzafarzaman@yahoo.com

Article received on the 25th of March 2021 and accepted for publication on the 24th of June 2021

INTRODUCTION

Lung cancer was considered to be rare at the beginning of the 20th century, but it has now reached almost epidemic proportions. It is the leading cause of cancer deaths in developed countries and is also rising at alarming rates in developing countries (1). It has a worldwide incidence of 14%, whereas it accounts for 6.8% of all cancers in India (2). It is the leading cancer of both sexes in three of the Urban Cancer Registries (Bhopal, Delhi and Mumbai) in India (3).

In NSCLC, angiogenesis has been evaluated by intratumoral microvessel count or expression of angiogenic markers. Angiogenesis is controlled by stimulatory factors, e.g., vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), platelet-derived growth factor (PDGF), platelet derived endothelial cell growth factor (PDEC-GF)/ thymidine phosphorylase (TP) and inhibitory factors (e.g., angiostatin, endostatin whose balance determines the degree of angiogenesis (4). Tumors can up- or down-regulate these factors to produce an environment in which angiogenesis occurs. The VEGF/VEGFR signalling pathway is frequently up-regulated in lung cancer, and VEGF overexpression is associated with tumor progression (5). High VEGF levels have been identified as an independent prognostic factor correlated with poor prognosis in patients with lung cancer (6). VEGF family consists of seven members, including VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E, VEGF-F, and placental growth factor. Among angiogenic stimulators, VEGF-A plays an essential role in both vasculogenesis and angiogenesis. Placental growth factor (PlGF) shares eight cysteine residues in a VEGF homology domain (7, 8).

Although the importance of VEGF expression in tumor tissues is known, the clinical and prognostic significance of serum VEGF in patients with cancer is not defined. The clinical utility of serum VEGF in predicting clinical course and prognosis in NSCSL is under investigation as well as in other types of cancers. There is a possible implication that serum VEGF level may be an independent prognostic factor among the known clinical factors if they are consistently elevated in tumors as opposed to other conditions, including normal conditions. Therefore, the aim of this study was to investigate the relationship between preoperative and postoperative serum VEGF levels. ❑

MATERIALS AND METHODOLOGY

Chemicals and reagents

The various kits and reagents were procured from standard sources.

Patients and controls

Patients attending the Department of Cardiovascular and Thoracic Surgery, Medical Oncology and Internal Medicine of Sher-I-Kashmir Institute of Medical Sciences (SKIMS), India, were screened for lung cancer. A total of 50 histologically confirmed cases were included in the study. Blood samples from all cases were obtained both preoperatively and postoperatively (four weeks after surgery). Blood samples of 100 age and sex matched healthy controls were collected from the Outpatient Departments of SKIMS to establish the normal serum VEGF levels. Patients and controls had a mean age of 62.5 and 59 years, respectively. A written informed consent was obtained from all cases and controls. All patients who have received chemotherapy or had any other type of cancer were excluded from the study.

The demographic and clinic-pathological characteristics of each patient were recorded in a questionnaire to collect information about gender, age, dwelling, smoking status, histopathology, grade of differentiation, tumor stage, family history, etc. The collection and use of blood samples for this study has been approved by the Institute Ethical Committee.

Sample collection/storage

Peripheral blood (~ 5 mL) was obtained from each subject in EDTA containing vials (200 µL of 0.5 M, pH 8.0) and centrifuged at 3,000 rpms for 10 minutes in a refrigerated centrifuge. The resulting plasma was stored in small aliquots at -70°C until use.

Enzyme linked immunosorbent assay (ELISA)

Principle and application

The enzyme-linked immunosorbent assay (ELISA) is a test that uses antibodies and color change to identify a substance.

Procedure for ELISA

All precautions were taken to ensure contamination free quantification of VEGF. All samples were run in duplicate to ensure statistically accurate results.

Equipments and reagents

1. ELISA kit
    a. VEGF Microplate (Item A): 96 wells (12 strips x 8 wells) coated with anti-human VEGF
    b. Wash buffer concentrate (20x) (Item B): 25 mL of 20x concentrated solution.
    c. Standards (Item C): two vials, recombinant human VEGF
    d. Assay diluent A (Item D): 30 mL, 0.09% sodium azide as preservative; for standard/sample (serum/plasma) diluents
    e. Assay diluent B (Item E): 15 mL of 5x concentrated buffer; for standard/sample (cell culture medium/urine) diluents
    f. Detection antibody VEGF (Item F): two vials of biotinylated anti-human VEGF (each vial is enough to assay half microplate)
    g. HRP-Streptavidin conentrate (Item G): 200 µL 300x concentrated HRP conjugated streptavidin
    h. TMB one-step substrate reagent (Item H): 12 mL of 3,3',5,5'- tetramethylbenzidine (TMB) in buffered solution
    i. Stop solution (Item I): 8 mL of 0.2 M sulfuric acid
2. Microplate reader capable of measuring absorbance at 450 nm
3. Precision pipettes to deliver 2 µL to 1 mL volumes
4. Adjustable 1-25 mL pipettes for reagent preparation
5. 100 mL and 1 liter graduated cylinders
6. Absorbent paper
7. Distilled or deionized water
8. Log-log graph paper or computer and software for ELISA data analysis
9. Tubes to prepare standard or sample dilutions

Reagent preparation

i. Bring all reagents and samples to room temperature (18 - 25°C) before use.
ii. Sample dilution: assay diluent A (Item D) should be used for dilution of serum/plasma samples. 1x assay diluent B (Item E) should be used for dilution of culture supernatants and urine. Suggested dilution for normal serum/plasma: 2-5 fold.
iii. Assay diluent B should be diluted 5-fold with deionized or distilled water.
iv. Preparation of standard: Briefly spin the vial of Item C and then add 640 µL of assay diluent A (for serum/plasma samples) or 1x assay diluent B (for cell culture medium and urine) into Item C vial to prepare a 50 ng/mL standard. Dissolve the powder thoroughly by a gentle mix. Add 60 µL of 50 ng/mL VEGF standard from the vial of Item C, into a tube with 440 µl of assay diluent A or 1x assay diluent B to prepare a 6,000 pg/mL standard solution. Pipette 400 µL of assay diluent A or 1x assay diluent B into each tube. Use the stock standard solution to produce a dilution series (shown below). Mix each tube thoroughly before the next transfer. Assay diluent A or 1x assay diluent B serves as the zero standard (0 pg/mL).
v. If the wash concentrate (20x) (Item B) contains visible crystals, warm to room temperature and mix gently until dissolved. Dilute 20 mL of wash buffer concentrate into deionized or distilled water to yield 400 mL of 1x wash buffer.
vi. Briefly spin the detection antibody vial (Item F) before use. Add 100 µL of 1x assay diluent B into the vial to prepare a detection antibody concentrate. Pipette up and down to mix gently (the concentrate can be stored at 4°C for five days). The detection antibody concentrate should be diluted 100-fold with 1x assay diluent B and used in step 4 of Part VI Assay Procedure.
vii. Briefly spin the HRP-Streptavidin concentrate vial (Item G) and pipette up and down to mix gently before use. HRP-Streptavidin concentrate should be diluted 300-fold with 1x assay diluent B.

ELISA protocol

ELISA for VEGF estimation was done as *per* manufacturer's protocol. Briefly, the following steps were followed:

1. All reagents and samples were brought to room temperature (18-25°C) before use. All standards and samples were in duplicate.

2. 100 µL of each standard (see Reagent Preparation step 2) and sample was added into appropriate wells. Then wells were covered and incubated for 2.5 hours at room temperature or overnight at 4°C with gentle shaking.
3. Solution was discarded and washed four times with 1x wash solution (300 µL). After the last wash, remaining wash buffer was removed by aspirating or decanting by inverting the plate and blotting it against clean paper towels.
4. 100 µL of 1x prepared biotinylated antibody (Reagent preparation step 6) was added to each well and incubated for 1 hour at room temperature with gentle shaking.
5. Discarded the solution. Repeated the wash as in step 3.
6. 100 µL of prepared Streptavidin solution (see Reagent Preparation step 7) was added to each well and incubated for 45 minutes at room temperature with gentle shaking.
7. Discarded the solution. Repeated the wash as in step 3.
8. 100 µL of TMB one-step substrate reagent (Item H) was added to each well and incubated for 30 minutes at room temperature in the dark with gentle shaking.
9. Added 50 µL of stop solution (Item I) to each well and was read at 450 nm immediately.

Statistical analysis

The continuous variables of the study have been in terms of descriptive statics and categorical variables in terms of frequency & percentage. The standard statical tests like chi-square test, Fisher`s Exact test, McNemar test, Wilcoxon ranksum test, and also repeated measurement analysis have been used to analyse the data. All the results so obtained have been discussed on 5% level of significance that is P valve less than 0.05 considered significant.

RESULTS

A total of 50 newly diagnosed lung cancer patients who underwent surgical resection at the Sher-I-Kashmir Institute of Medical Sciences (SKIMS) were selected for the study. This study was conducted in the department of cardiovascular and thoracic surgery from August 2012 to September 2014.

Characteristics of the study subjects

Fifty lung cancer patients were studied to estimate both preoperative and postoperative VEGF levels.

Patients' various clinicopathological features are summarized in Table 1.

Preoperative expression of VEGF in cases and controls

One hundred healthy age and sex matched volunteers were selected for the control group. The VEGF expression was determined by ELISA in all the 50 lung cancer cases and 100 controls in trip-

TABLE 1. Patients' clinicopathological features

Parameter	Number (percentage) N=50	Total number
Age <60 >60 Mean age	 27 (54%) 23 (46%)	50
Sex Female Male	 8 (16%) 42 (84%)	50
Lymph node N0 N+	 34 (68%) 16 (32%)	50
Stage I II-III	 34 (68%) 16 (32%)	50
Grade Moderately & poorly differentiated Well differentiated	 12 (24%) 36 (72%)	50
Histology ADC & Others SCC	 19 (38%) 31 (62%)	50
Smoking status Non-smoker Smoker	 8 (16%) 42 (84%)	50
Chest pain Absent Present	 32 (64%) 18 (36%)	50
Breathlessness Absent Present	 35 (70%) 15 (30%)	50
Hemoptysis Present Absent	20 (40%) 30 (60%)	50
Pallor Absent Present	26 (52%) 24 (48%)	50
Anorexia Absent Present	 25 (50%) 25 (50%)	50
Cough Absent Present	 26 (52%) 24 (48%)	50
Lung involved Right Left	 31 (62%) 19 (38%)	50
Dwelling Rural Urban	 32 (64%) 18 (36%)	50

Parameter	Preoperative VEGF expression		Total	P-value
	Normal	Elevated		
Age			50	
<60	19	8		0.055
>60	10	13		
Sex				
Female	5	3	50	0.778
Male	24	18		
Lymph node				
N0	24	10	50	0.009*
N+	5	11		
Stage				
I	24	10	50	0.009*
II-III	5	11		
Grade				
Moderately & poorly differentiated	1	11	50	<0.001*
Well differentiated	28	10		
Histology				
SCC	20	11	50	0.233
ADC & Others	9	10		
Smoking status				
Non-smoker	4	4	50	0.617
Smoker	25	17		
Chest pain				
Absent	18	14	50	0.738
Present	11	7		
Breathlessness				
Absent	21	14	50	0.662
Present	8	7		
Hemoptysis				
Present	15	5	50	0.880
Absent	20	10		
Pallor			50	0.598
Absent	16	10		
Present	13	11		
Anorexia				
Absent	14	11	50	0.774
Present	15	10		
Cough				
Absent	14	12	50	0.255
Present	17	7		
Lung involved				
left	12	7	50	0.563
Right	17	14		
Dwelling				
Rural	20	12	50	1.00
Urban	11	7		

TABLE 2. Preoperative VEGF expression and clinicopathological features

licates. The normal value was determined by establishing a normal distribution curve. The value of 150 ng/mL was taken as normal and other concentrations above it were considered elevated. The average value of triplicates was considered a true value.

The VEGF expression was elevated in 42% of cases. Among the different clinicopathological parameters, VEGF expression was found to be statistically significant for lymph node positive cases (P-value 0.009), higher pathological stage (P-value 0.009) and higher grade (P-value 0.000). The p value was calculated by using the chi-square test. Statistical information for preoperative VEGF expression and various clinicopathological features is shown in Table 2.

Postoperative VEGF expression

To observe the impact of curative surgical resection of tumours on VEGF levels, we estimated the VEGF expression at four weeks after surgery. Again, the VEGF levels were estimated by ELISA technique using standard kit. All sample were run in triplicates to assure quality results. All results were subjected to a statistical analysis. Chi-square test was performed to get the level of significance. We found that all those factors for which VEGF expression was statistically significant preopera-

Parameter	Postoperative VEGF expression Normal	Elevated	Total	P value
Age			50	
<60	25	2		0.247
>60	17	6		
Sex				
Female	5	3	50	0.105
Male	37	5		
Lymph node				
N0	30	4	50	0.249
N+	12	4		
Stage				
I	30	4	50	0.249
II-III	12	4		
Grade				
Moderately & poorly differentiated	10	2	50	1.000
Well differentiated	32	6		
Histology				
SCC	27	4	50	0.459
ADC & Others	15	4		
Smoking status				
Non-smoker	6	2	50	0.598
Smoker	36	6		
Chest pain				
Absent	28	4	50	0.436
Present	14	4		
Breathlessness				
Absent	29	6	50	1.000
Present	13	2		
Pallor				
Absent	24	2	50	0.132
Present	18	6		
Anorexia				
Absent	21	4	50	1.000
Present	21	4		
Cough				
Absent	22	4	50	1.000
Present	20	4		
Lung involved				
Right	25	6	50	0.693
Left	17	2		
Dwelling				
Rural	28	4	50	1.00
Urban	14	4		

TABLE 3. Postoperative VEGF expression and clinicopathological features

TABLE 4. McNemar test

Postoperative VEGF levels					
Preoperative VEGF levels		Normal	Increased	Total	P-value
	Normal	29	0	29	<0.001
	Increased	13	8	21	
Total		42	8	50	

tively (lymph node positive, clinical stage and grade) became non-significant (p value >0.05).

Statistical information for the postoperative VEGF expression and various clinicopathological features is shown in Table 3.

Table 4 is showing that VGEF levels were elevated in 21 (42%) patients before surgery, decreased to statically significant levels in 13 (62%) patients after surgery, and remained elevated in eight (38%) patients (p-value <0.001).

TABLE 5. Wilcoxon-rank sum test

Group	(mean±SD) Median	Mean difference	P-value
Preoperative	365.08±497.13 (130)	223.5	<0.001
Postoperative	132.58±113.86 (104)		

Table 5 shows that the mean VGEF level was 365.08 pg/mL preoperatively and 132 pg/mL postoperatively, with a mean difference of 223.5 pg/mL, and it was statically significant (p-value <0.001).

Table 6 is showing the effect of decrease in postoperative VEGF levels by controlling the tumor stage, nodal status and grade through repeated measurement analysis.

The table shows there is no interaction on decrease in postoperative VEGF levels with involvement of nodes, higher stage and grade of tumor; means patients who were having no node in-

Effect on VEGF levels	Value Pillars trace (0.3450)	p-value 0.001
VEGF* node	0.00	1.00
VEGF* stage	0.00	1.00
VEGF* grade	0.064	0.080

TABLE 6. Effect of decrease in postoperative VEGF levels

volvement, lower stage and grade of tumor, VEGF levels decreased in them also. ❑

DISCUSSION

As the field of angiogenesis research is undergoing an explosive growth and the development of highly sensitive ELISA tests made it possible to analyse circulating angiogenic factors in clinical blood samples, we set out to investigate the diagnostic and prognostic potential of circulating VEGF in lung cancer patients undergoing surgery. The serum VEGF levels were useful for diagnostic in a study by Tamura M *et al* (9) or prognostic (Matsuyama W *et al*, Chol-JH *et al*, Brattstrom D *et al*) (10-12). Their predictive value with regard to surgery (13) and treatment for advanced lung cancer (14) has been previously explored and represented the topic of this study. Although our study is the first of its kind in estimating the VEGF levels both preoperatively and postoperatively, comparison will be made with studies in which VEGF values have been estimated prior to surgery.

In the present study, the total number of selected patients was 50. Serum VEGF levels were higher in our patient group than healthy controls and they were shown to be statistically significant.

Patient sex and VEGF

Out of all 50 cases, 84% were males and 16% females, which was not comparable to the male:female ratio observed by Qiang *et al* (7) but in agreement with that reported by Akin *et al* (8). The VEGF values were not statistically significant for gender (p-value 0.778) and were consistent with the findings of Qiang *et al* (15). Thus, age has no effect on VEGF expression in lung cancer.

Smoking and VEGF

In our study, we found no statistical association between VEGF values and smoking (p-value 0.617), although 84% of our patients were smokers and smoking was an independent risk factor for lung cancer, as observed by Hecht *et al* (17).

Lymph node status and VEGF

For patients with elevated VEGF values and lymph node positive status, a strong statistical significance (p-value 0.009) was noted, which is in agreement with studies carried out by Tamura *et al* (9), but in contradiction with findings reported by Ahmet *et al* (16). In our study, the VEGF values were elevated in only 22% of lymph node positive cases, while lymph node positivity was seen in 32% of patients and in around 20% of cases had elevated serum VEGF concentrations but negative lymph node status. However, VEGF levels decreased postoperatively and became statistically non-significant. It appeared that serum VEGF increased with lymph node involvement and also may reflect tumor burden. Our results are promising, as VEGF reduction postoperatively gives a clue regarding the use of anti-VEGF where VEGF remains or increases postoperatively, suggesting either a tumor relapse or residual tumor growth or disease aggressiveness.

Staging and VEGF

Since the sample size was low, we clubbed the staging into two groups, one with stage I and the other one as stage II/III. Again, a strong statistical association was observed for elevated VEGF values with higher stage (p-value 0.009). Among our patients, 68% had a stage I tumor, with elevated VEGF levels in only 20% of them, and 32% of patients had stage II/III, with elevated VEGF levels in 22% of them. However, our findings are in contradiction with those provided by earlier studies (7, 8, 10, 12), but in agreement with those conducted by Ohta *et al* and Imoto *et al*. Postoperatively, serum VEGF concentrations decreased to significant levels, showing serum VEGF levels increase with tumor growth, and might also reflect the tumor burden. A serum assay can be easily and frequently performed due to its minimal invasiveness.

Tumor histopathology and VEGF

Out of 50 patients, 31 (62%) had histology squamous cell carcinoma, with only 11 (22%) of them having increased serum VEGF levels, and 19 (38%) patients had histology adenocarcinoma and other types, with 10 (20%) of them having elevated VEGF levels. Although adenocarcinoma is the

most frequent histological type, squamous cell carcinoma was the most common histological type in our study; this may be attributed to the fact that 84% of our patients were smokers, which is strongly associated with smoking. No statistical significance was observed for histopathology (p-value 0.617). In this case, our study is in agreement with earlier studies carried out by other authors (16). Again, no statistical significance was observed for decrease in VEGF levels postoperatively with regard to histopathology.

Tumor grade and VEGF

Since tumors with different grading show different biological phenomenon, we compared the VEGF levels with different tumor gradings. We clubbed well to moderately differentiated tumors as low grade and poorly differentiated tumors as high grade; 78% of patients were low grade, with increased VEGF levels in only 20% of them, while 24% of patients were high grade, with elevated VEGF levels in 22% of them. A strong statistical significance was observed between patients with higher grade and elevated VEGF levels (p-value 0.000). Soon after surgery, the VEGF levels became non-significant as was the case with lymph node status and tumor stage. Our results are in total disagreement with earlier studies carried out by Akin *et al* and Ahmet *et al* (16), which may be attributed to the low sample size in our study.

Other factors and VEGF

For all other factors such as anorexia, chest-pain, hemoptysis, breathlessness, cough and lung involved, no statistical significance was observed both pre- and postoperatively with serum VEGF levels. Common presenting symptoms, in order of decreasing frequency, were as follows: anorexia in 25 (50%) patients, cough [24 (48%)], hemoptysis [20 (40%)], chest pain [18(36%)] and breathlessness [15 (30%)]. On clinical examination pallor was seen in 24 (48%) patients.

As expected, the curative surgery had a great impact in normalizing the circulating VEGF levels. Postoperatively, we found a drastic change in serum VEGF levels. Preoperatively, the VEGF levels were statistically significant for lymph node metastasis, higher pathological stage and higher tumour grade. The mean preoperative and postoperative VEGF levels were 365.08 pg/mL and 152.5 pg/mL, respectively, with a mean difference of 223.5 pg/mL. This was statically significant change (p-value <0.001). This change is a direct indication of circulating VEGF levels as having a prognostic significance, as suggested by studies carried out by Akin *et al*. There was an overall decreased trend in postoperative VEGF levels, and no statically significant interaction was seen on decrease in postoperative VEGF levels by controlling the tumor stage, grade and nodal status, meaning that VEGF levels decreased in other patients who also had raised levels, but were node negative and lower tumor stage and grade.

Our data is mostly in agreement with previous studies which showed a role of VEGF as a prognostic factor. Since surgery is the best treatment for early stage lung cancer, the role of VEGF estimation postoperatively will be of tremendous help in managing patients suffering from this disease and will help in regulating the kind of therapy required for individual patients. Any kind of recurrence will not only raise the VEGF but will also give a good information regarding the underlying residual tumour. For those with higher stage lung cancers, for whom surgery is not viable option, the VEGF levels will help in diagnosing the aggressiveness of the disease. Cancers with higher VEGF levels will not only give information regarding its invasive potential but will also help in determining which is the suitable therapy for each patient.

Because angiogenesis is controlled between angiogenic and antiangiogenic factors, it is reasonable to use serum or plasma VEGF levels to correlate with the clinical outcome. Antiangiogenic therapy against VEGF and its receptors has been widely applied in the management of lung cancer patients. Serum VEGF levels should be measured and might be helpful to lead an efficient individualized antiangiogenic therapy for patients with lung cancer in the future. ❑

CONCLUSION

In conclusion, we have demonstrated that serum VEGF levels are higher as the tumor stage progresses and tumor size increases, so naturally we had lower serum VEGF levels in the operable patient group. ❑

Conflicts of interest: none declared.

Financial support: none declared.

Acknowledgements: Thanks to all coauthors, hospital staff and patients who participated in this study.

References

1. **Khuri FR, Herbst RS, Fossella FV.** Emerging therapies in non-small cell lung cancer. *Ann Oncol* 2001;12:739-744.
2. **Nanda Kumar A.** Consolidated report of the population based cancer registries, incidence and distribution of cancer, 1990-1996. National Cancer Registry Programme. New Delhi: Indian Council of Medical Research. 2001.
3. **Greenlee RT, Murray T, Bolden S, Wingo PA.** Cancer statistics. *CA Cancer J Clin* 2000;1:7-33.
4. **Gridelli C, Maione P, Rossi A, De Marinis F.** Role of bevacizumab in the treatment of nonsmall cell lung cancer: current indications and future developments. *The Oncologist* 2007;10:1183-1193.
5. **Sun S, Schiller JH, Spinola M, Minna JD.** New molecularly targeted therapies for lung cancer. *J Clin Invest* 2007;10:2740-2750.
6. **Herbst RS, Onn A, Sandler A.** Angiogenesis and lung cancer: prognostic and therapeutic implications. *J Clin Oncol* 2005;14:3243-3256.
7. **Brock CS, Lee SM.** Anti-angiogenic strategies and vascular targeting in the treatment of lung cancer. *Eur Respir J* 2002;3:557-570.
8. **Pang RWC, Poon RTP.** Clinical implications of angiogenesis in cancers. *Vasc Health Risk Manag* 2006;2:97-108.
9. **Tamura M, Ohta Y, Kajita T, et al.** Plasma VEGF concentration can predict the tumor angiogenic capacity in non-small cell lung cancer. *Oncol Rep* 2001;8:1097-102.
10. **Matsuyama W, Hashiguchi T, Mizoguchi A, et al.** Serum levels of vascular endothelial growth factor dependent on the stage progression of lung cancer. *Chest* 2000;118:948-951.
11. **Choi JH, Kim HC, Lim HY, et al.** Vascular endothelial growth factor in the serum of patients with non-small cell lung cancer correlation with platelet and leukocyte counts. *Lung cancer* 2001;33:171-179.
12. **Battstrom D, Bergqvist M, Larsson A, et al.** Basic fibroblast growth factor and vascular endothelial growth in sera from non-small cell lung cancer patients. *Anticancer Res* 1998;18:1123-1127.
13. **Battstrom D, Bergqvist M, Hesselius P, et al.** Elevated preoperative serum levels of angiogenic cytokines correlate to larger primary tumors and poorer survival in non-small cell lung cancer patients. *Lung cancer* 2002;37:57-63.
14. **Kido Y.** Vascular endothelial growth factor (VEGF) serum concentration changes during chemotherapy in patients with lung cancer. *Kureme Med J* 2001;48:43-47.
15. **Qiang Lin, Maoquan Li, Zhen-Ya Shen, et al.** Prognostic Impact of Vascular Endothelial Growth Factor-A and E-Cadherin Expression in Completely Resected Pathologic Stage I Non-Small Cell Lung Cancer. *Jpn J Clin Oncol* 2010;7:670-676.
16. **Ahmet Demirkazik, Hakan Akbulut, A Fusun Ulger, et al.** Serum vascular endothelial growth factor levels in patients with nonsmall cell lung cancer. *Ann Saudi Med* 2004;5.
17. **Hecht SS.** Cigarette smoking and lung cancer chemical mechanism and approach to prevention. *Lancet Oncol* 2002;J3:461-469.