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Research Article

Serum $VEGF_{165}$ and HGF in Egyptian Patients with Lung and Pleural Cancers

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Abstract

Background: Lung cancer is one of the most aggressive human malignancies with an increasing incidence worldwide. Mostly diagnosed in late stages, hence prognosis is very poor. Early diagnosis is important to improve its prognosis and treatment.

Aim: Our aim explore the circulating levels of $VEGF_{165}$ and HGF in lung and pleural cancers. Pointing out their clinical significance in the early detection and differential diagnosis of lung cancer subtypes compared to well-established markers; NSE and TPA. In addition, we investigated effect of some cancer predisposing factors (Age, gender and smoking) on the studied population as well as investigated parameters.

Patients and methods: Study included 64 lung and pleural cancer patients and 23 controls. Serum levels of TPA, NSE, HGF and $VEGF_{165}$ were determined quantitatively using ELISA technique. In addition, qualitative determination of $VEGF_{165}$ was done by Western blotting.

Results: Serum levels of TPA, HGF and $VEGF_{165}$ were significantly elevated in all patients, with no discriminative ability between different histological subtypes. NSE was significantly elevated in SCLC patients only. Accuracy was in descending order of TPA, HGF, $VEGF_{165}$ and NSE. Sensitivity of TPA, HGF, $VEGF_{165}$ and NSE was 100, 95.24, 95.24 and 8.1% respectively, while specificity was 85, 88.24, 100 and 100% respectively.

Conclusion: Our study was the first to discuss the clinical significance of HGF and $VEGF_{165}$ in Egyptian patients with lung and pleural cancers. We recommend the use of combination of markers for diagnosis of lung and pleural cancers. Moreover, HGF and $VEGF_{165}$ could be useful markers for lung and pleural cancers after standardizing their circulating levels and validating them in large-scale prospective clinical trials.

Key words: $VEGF_{165}$, HGF, NSE, TPA, mesothelioma, lung cancers.

1. INTRODUCTION

Lung cancer is currently the most frequently diagnosed solid tumor and the most common cause of cancer mortality worldwide, and non-small cell lung cancer (NSCLC) makes up about 80% [1]. An estimated 1.2 million people are diagnosed annually with lung cancer and 1.1 million of them die from their disease [2]. Accurate epidemiological data on lung cancer in Egypt is not available since a comprehensive national population-based cancer registry is lacking. However, official statistics as well as institution and hospital-based studies show that it is the 7th most common cancer in Egypt [3]. In spite of aggressive therapy available today, the prognosis of lung cancer patients is generally very poor. Therefore, the development of novel diagnostic techniques to identify lung cancer is important to facilitate earlier diagnosis of primary or recurring cancers leading to more effective treatment and improved prognosis [4].

In 1971, Folkman proposed a hypothesis that tumor growth is angiogenesis dependent. This hypothesis suggested that tumor cells and vascular endothelial cells within a neoplasm might be switched from a resting state to a rapid growth phase by a "Diffusible" chemical signal from tumor cells [5].

Tumor growth and metastasis have been considered to be the consequence of a series of biological events that are controlled by growth factors receptors and growth factors expression [6]. At least 20 molecules have been identified that are involved in initiation and regulation of angiogenesis; among which, vascular endothelial growth factor "VEGF" and hepatocyte growth factor "HGF".

VEGF is a homodimeric glycoprotein [7]. A cytokine [7] with 34-42 kDa molecular weight [8,9]. The VEGF gene is located on human chromosome 6 [10]. Alternative splicing of VEGF mRNA accounts for at least 6 different isoforms from a single gene until now): 121, 145, 165, 189, 206 [11,12], and 183 amino acids [13,14].

VEGF is expressed mainly by cells in close proximity to endothelial cells, but also reported to be expressed by many other cells [6], and many malignant tumor cells over express it [8]. VEGF is expressed by normal bronchiolar and differentiated columnar epithelial cells and by alveolar macrophages [15]. $VEGF_{121}$ and $VEGF_{165}$ are the only freely soluble isoforms. Others are mostly bound to heparin in the extracellular matrix [16]. $VEGF_{165}$ is the most abundant homodimer, which is produced by numerous cell types that include a variety of tumors [17].

Collectively, VEGF plays a crucial role in tumor expansion by initiating blood vessels permeability, extravasation of plasma proteins, invasion of stromal cells, and by causing the sprouting of new blood vessels that supply the tumor with nutrients [14].

Mature HGF is a Cytokine [18,19], an 82 kDa., 674 amino acid glycoprotein, that is part of a small family of factors that also includes an HGF-like factor known as macrophage stimulating protein, that lack significant homology with other known growth factors [20].

HGF is a key switch for turning on angiogenesis: A mechanism by which HGF induces tumor angiogenesis, with two distinct components. First, by acting directly on endothelial cells. Second, by up-regulating the expression of VEGF and down-regulating the expression of other angiogenesic inhibitor; Thrombospondin-1 [21].

Our aim explore the circulating levels of $VEGF_{165}$ and HGF in lung and pleural cancers. Pointing out their clinical significance in the early detection and differential diagnosis of lung cancer subtypes compared to well-established markers; NSE and TPA. In addition, we investigated effect of some cancer predisposing factors (Age, gender and smoking) on the studied population as well as investigated parameters.

2. SUBJECTS AND METHODS

2.1. Subjects

The study included patients who presented to out-patient clinics of NCI, Cairo University, for the evaluation of respiratory diseases or complaining from respiratory malfunction not attributed to other causes. Diagnosis was based on patient history, clinical examination, biopsy, and imaging studies. Abdomen, brain, and bone scanning were carried out when recommended. Performance status was estimated by ECOG scale. Histopathology was carried out according to the WHO classification [4], and modified TNM system was used for staging [22]. Patients who had no previous lung manipulation or treatment that could affect serum levels of the investigated parameters were included. All data were recorded for each patient.

2.2. Specimenes collection, handling and storage

Blood samples were collected from all patients prior to performing any clinical manipulation. blood was freshly withdrawn by venipuncture, collected in vacutainers, incubated in decline tubes at room temperature (25-37 ˚C) for 30 minutes, centrifuged twice at 3000 rpm for 10 minutes. Serum obtained was processed within one hour and immediately frozen at ≤ –20 ºC until time of analysis. These storage conditions were proven to be sufficient to prevent deterioration of the investigated proteins (According to the manufacturer instructions). After one cycle of slowly thawing, the serum was left to reach room temperature, thoroughly mixed then used for analysis.

2.3. Investigated parameters in serum

1. Quantitative determination of NSE (Prod. No. 420-10, Lot. 15347:1 CanAg Diagnostics AB, SE-41455, Gothenburg, Sweden.) [23].
2. Quantitative determination of TPA (IDeaL™ Monoclonal TPA_{cyk} ELISA, IDL Biotech AB; Bromma, Sweden, Cat. No. 10-023) [24].
3. Quantitative determination of HGF (Quantikine hHGF EIA, R&D Systems,Inc., Cat. No. DHG00, lot. No. 223614).
4. Quantitative determination of $VEGF_{165}$ (BioSource International, USA. hVEGF EIA Cat. KHG0112/KHG0111, lot.No. P102001).
5. Western blotting of $VEGF_{165}$ was used as a confirmatory test for the detection and identification of $VEGF_{165}$ (Positive control R&D systems) [25].

2.4. Statistical analysis

Statistics were calculated for the entire study cohort, using GraphPad Instat tm V2.04. Appropriate graphs were plotted when needed using Prism V4.03. Determination of the optimum cut-off value for VEGF and HGF among the studied groups was estimated using ROC curve using SPSS V10.0. Diagnostic accuracy was calculated [26].

3. RESULTS

3.1. Clinical and demographic profile of the studied groups

As shown in *table-1;* control group comprised 23 healthy (non-malignant) subjects. Cases included in the study were 64 lung and pleural cancer patients (26 malignant mesothelioma and 38 bronchogenic carcinoma). Group of bronchogenic carcinoma was further subdivided according to histopathological classification into: small cell lung cancer "SCLC", Non- small cell lung cancer "NSCLC". The NSCLC group according to histopathological classification comprised large cell lung cancer "LCLC', squamous cell carcinoma "SCC', adenocarcinoma"AC', and undifferentiated large cell lung cancer.

Compared to control group, cancer patients showed: extremely significant difference with respect to age ($p<0.0001$ using Mann-Whitney test), male predominance among cancer patients with ratio 2.1:1, however with statistical significant at $p> 0.5$ (Fisher exact test). Also there was higher prevalence of cigarette smoking (51.56 %) among cancer (17.39 %) at $p<0.01$, with bronchogenic carcinoma showing higher prevalence than mesothelioma patients using Fisher exact test.

3.2. Descriptive analyses

3.2.1. Investigated serum markers:

As shown in table-2: TPA, HGF, $VEGF_{165}$ were significantly elevated in all patients (Median=13.8 ng/ml, 1920 pg/ml, 804 pg/ml respectively) compared to control at $p< 0.0001$. Bronchogenic carcinoma group showed significant higher values, while mesothelioma group didn't show any significant variation from that of control ($p< 0.0001$, > 0.05 respectively). All markers had no discriminative ability between mesothelioma and bronchogenic carcinoma groups. Serum levels of NSE showed no significant variation neither between cancer (Median=5 ug/L) and control nor between mesothelioma and bronchogenic groups. (using Kruskal-Wallis non-parametric ANOVA followed by post-hoc Dunn's multiple comparison test).

As shown in table-3: Median serum levels of TPA, HGF, $VEGF_{165}$ were significantly elevated in both SCLC and NSCLC compared to control ($p< 0.01$ and < 0.0001, $p<0.001$ and <0.0001, $p< 0.001$ and < 0.0001 respectively). All markers had no discriminative ability between SCLC and NSCLC groups. Only NSE showed significant variation between SCLC and NSCLC groups ($p< 0.0001$). Due to low sample size of SCLC group, ANOVA test wasn't carried out, and Mann-Whitney test was used for analysis of pairs.

3.2.2. Western blot for human Vascular Endothelial Growth Factor$_{165}$:

Figure-1 illustrates western blot for $VEGF_{165}$. All serum samples for randomly selected cancer patients showed a single band at 32-34 kDa., that corresponded to the band of $VEGF_{165}$ positive control standard. No bands appeared for the randomly selected control.

3.2.3. Effect of gender on the investigated markers:

Only TPA and VEGF showed significant difference among cancer patients, median ($p < 0.05$, using Mann-Whitney test was used for analysis of pairs). Males were demonstrated to have slightly higher values than females with respect to TPA (14.55, 9.6 ng/ml respectively), but the opposite was in case of VEGF (666, 1056 pg/ml respectively).

3.2.4. Effect of cigarette smoking on investigated markers:

There was no significant variation between smokers and non-smokers, in serum level of any of the investigated markers among cancer patients ($p < 0.05$, using Mann-Whitney test was used for analysis of pairs). Although there was ahigher percent smokers in cancer group (51.56%) compared to control group (17.39%) at $p<0.01$.

3.3. Correlation studies

3.3.1. Correlations between Age and investigated markers:

Only NSE showed a weak significant correlation with age (Pearson correlation coefficient "r" = 0.1664, $p < 0.05$).

3.3.2. Correlations between investigated markers:

HGF showed weak correlation with NSE and moderate correlation with TPA (as shown in table-4).

3.4. Diagnostic accuracy

A comparison of the effectiveness of TPA, VEGF, NSE and HGF as tumor markers in lung and pleural cancers was carried out by calculating the five diagnostic accuracy indices: Sensitivity, specificity, positive predictive value, negative predictive value,

and accuracy. In addition, false positive and false negative was estimated as shown in table-5.

3.4.1. ROC Curve:

TPA was the closest to the top left-hand corner (AUC ≈ 1). VEGF, HGF then NSE came in descending order of A and AUC (Figure-2).

4. DISCUSSION

Malignant tumors are ranked the third in developing countries after infectious-parasitic and cardiovascular diseases. Although lung cancer is not one of the leading cancers in Egypt, it is one of the highest mortality rates; a leading cause of cancer deaths in both men and women [27]. In the last statistical surveys made by the National Cancer Institute of Egypt 2002-2010, Lung and bronchus were the 7th among the most common cancers in both sexes, and the 4th with respect to men [3]. Overall, lung cancer has a very poor prognosis, with nearly 65% of patients dyeing within a year of diagnosis [28].

Although lung cancer is the number one cause of cancer deaths; however, no specific serum biomarker is available till date for early detection. Currently available tumor markers are unsuitable for the screening of asymptomatic individuals [29].

In this study we investigated the circulating levels of VEGF165 and HGF in lung and pleural cancers. Pointing out their clinical significance in differential diagnosis of lung cancer subtypes compared to well-established markers; NSE and TPA. In addition, we investigated effect of some cancer predisposing factors (Age, gender and smoking) on the studied population as well as investigated parameters.

4.1. Age, gender, smoking effect

Age is one of the risk factors for cancer [28]. In Egypt, the mean age of cancer patients was 48 years [27], and increased to 53 years in 2004 "Cancer Statistics, Biostatistics and Epidemiology, NCI of Egypt, December 2005" [30]. In the present study mean age of cancer patients was 54.9 compared to 43.4 years for control group. Age correlated with extreme significance to incidence of cancer ($p<0.0001$), which came in accordance with Ferrigno et al. [31].

In Egypt there is a male predominance in cancer incidence with the ratio 1.4:1, Thus although males constitute 51.1% of the Egyptian population, they contribute by 58.3% of the cancer population; this denotes that males in general are at a higher risk, than females to develop cancer. Conversely, in developed countries as in USA, this male predominance is less striking with the ratio 1.1:1 [32]. Results of the present study showed a male predominance among cancer group with a more striking ratio 2.1:1, but with statistically non-significant difference from control group ($p>0.5$).

Smoking is one of the chief risk factor for the premature mortality of lung cancer [33], which is demonstrated here by the significantly higher percent smokers in cancer group.

4.2. Investigated markers

TPA was reported, as useful marker for lung cancer, even more than the Carcino-embryonic antigen "CEA" [34], which was contradicted by Rasmuson et al. [35]. In the present study, serum TPA was significantly elevated in all cancer patients in accordance with Plebani et al. [36] who reported that TPA increased in patients irrespective to histological type, and only extensive SCLC showed high levels of TPA. Lung cancer studies of large and non-selected populations showed that TPA had no clear preference for a specific cell type [37]. TPA serum determination can suggest a diagnosis of malignancy, but its evaluation, as a single test, is not useful to differentiate between malignant or benign disease [38].

Due to the different biology, prognosis and sensitivity to therapy of SCLC and NSCLC, their differentiation is very important. In SCLC, Neuron specific-enolase "NSE" is the best accurate among all other neuroendocrine markers [39], and that its measurement in serum is more useful than in pleural effusion [40]. Therefore, TPA and NSE are considered for routine clinical use with CEA. In the present study, NSE wasn't significantly elevated in cancer patients except for SCLC. In support to our results, Plebani et al. [36] reported that NSE levels in SCLC patients showed significantly higher levels than other histological types. The same was for Kasprzak et al. and Pujol et al. [41,38]. Serum NSE level might allow simple and cost-effective differentiation of SCLC and NSCLC [42], using an appropriate cut-off [43].

In cancer as well as many other serious diseases, the body loses control over apoptosis and angiogenesis where apoptosis is hindered and excessive angiogenesis occurs. It has become clear that the growth of solid tumors is dependent on the process of angiogenesis and that VEGF is a central positive regulator of this process. Collectively VEGF plays a crucial role in tumor expansion by initiating blood vessels permeability, extravasation of plasma proteins, invasion of stromal cells, and by causing the sprouting of new blood vessels that supply the tumor with oxygen and nutrients. VEGF increased expression has been demonstrated in lung cancer [44]. $VEGF_{165}$ has also been demonstrated to play an important role in tumorigenesis, and the most prominent isoform that can fully rescue expansion of the angiogenesis-deficient tumor *in vitro* [14].

Zhang et al. [21] studies identified HGF as a key switch for turning on angiogenesis: A mechanism by which HGF induces tumor angiogenesis, with two distinct components. First by acting directly on endothelial cells, inducing proliferation and migration. Second, by acting on tumor cells, up-regulating the expression of the proangiogenic factor VEGF, and down regulating the expression of an angiogenesis inhibitor "TSP-1" [45,10]. In lung cancer, HGF may exert its biological effects on tumor cells by stimulating their proliferation, inhibiting their apoptotic death, and especially through its mitogenic and scattering properties, favoring tumor cell migration along the alveolar basal membrane.

In the present study, serum HGF and $VEGF_{165}$ were significantly elevated in all cancer patients with no discriminative ability among different histological subtypes. In line, Cressey et al. [14] investigation on 18 NSCLC (9 AC and 9 SCC), revealed extremely significant higher level of serum VEGF in lung cancer (1251 ± 568 pg/ml) than a healthy volunteer group (543 ± 344 pg/ml). Ilhan et al. [15] concluded that increased serum $VEGFR_1$ and VEGF levels are important parameters in lung cancer detection, since VEGF levels of patients M±SD and Median; 449.48±175.54 and 428.9 pg/ml respectively) were extremely significant higher in patients than in healthy subjects (77.06±47.26 pg/ml) ($p<0.0001$). This study was compatible with the results of other studies [46]. High standard deviation of VEGF levels had been demonstrated in most studies, which may present a problem in the use of serum VEGF as a biological marker.

In line, Bharti et al. [47] findings suggest that serum levels of HGF may serve as useful serum marker in SCLC (6 limited disease, 7 extensive disease, 4 relapsed disease), and Siegfried et al. [18] results suggested that elevated HGF might predict a more aggressive biology in NSCLC.

4.2.1. Western blot analysis of VEGF:

In our study, western blotting of VEGF confirmed the results obtained by Eliza, where randomly selected serum samples of cancer patients showed a clear single band which corresponds to that of the VEGF protein standard at 32-34 kDa. No bands appeared for the selected control sample. Our data was previously suggested by Brown et al. and Al-Eryani [8,9], who stated that molecular weight of VEGF is 34-42 kDa., and 34-46 kDa. [45]. However, it was 40-45 kDa. according to Folkman and Kalluri report [48].

4.3. Correlation studies

In our study, NSE showed a weak significant correlation with age, in accordance with Iwasaki et al. [49], that there is no significant association between both of VEGF and HGF levels in tissue extracts and age. In the contrary, Van Zandwijk et al. [50] reported that high NSE level does not correlate with age in NSCLC.

In this study, Males were demonstrated to have slightly higher values than females with respect to TPA, but the opposite was in case of VEGF. On the contrary, Cressey et al. [14] reported that gender didn't show any impact on circulating level of VEGF. Iwasaki et al. [49] reported that there were no significant associations between both of VEGF and HGF levels (in tissue extracts) and gender. Van Zandwijk et al. [50], reported that high NSE level does not correlate with sex or histology in NSCLC.

In the present study, HGF showed weak correlation with NSE and moderate correlation with TPA. Our results are in the contrary to previous studies: Bivariate correlation analyses showed that the serum level of NSE was significantly related to the levels of TPA [51,30]. In line, Hasegawa et al. [52] reported that serum VEGF level did not correlate with serum NSE. Fuhrmann-Benzakein et al. [53] reported that plasma levels of HGF correlated with high plasma VEGF. On the contrary Iwasaki et al. [49] reported no relation, which was in agreement with our results.

4.4. Diagnostic accuracy of investigated markers

On studying the diagnostic accuracy of TPA and NSE at their reference cut-off values (1 ng/ml and 13 ug/L respectively), it was 96.3% and 32.94% respectively. While Plebani et al. [36] reported that TPA had an accuracy of 78% when using cut-off values of 1 ng/ml. The sensitivity and specificity of a tumor marker are important in establishing its potential clinical utility for a specific type of neoplasm. In the current study, TPA and NSE had a sensitivity of 100% and 8.1% at their reference cut-off values. However, specificity was 85% and 100% respectively. So TPA was more sensitive but less specific than NSE. In lung and pleural cancers, studies of large and non-selected populations showed that, TPA sensitivity rates was 51-85% [54], and 46-85% [36]. In Cioffi et al. [55] TPA sensitivity (NSCLC+SCLC) was 58.7% and more than that of NSE (35.8%). In Molina et al. [56], NSE sensitivity was 22%. TPA has been determined in 271 mesothelioma patients, 131 pulmonary neoplastic diseases, and 140 benign lung diseases, where TPA had a sensitivity and specificity of 65% [37]. From the previous reports we can conclude that TPA is a very sensitive marker but not tumor specific, which came in accordance with our results.

Our results proved that NSE has no sensitivity except for SCLC, that was in agreement with a study done

by Ebert et al. [57], who reported that NSE is the first choice marker for SCLC, with sensitivity 77- 85%. However in Hasegawa et al. study sensitivity decreased to 42% for SCLC [52]. The incidence of false positive for TPA tests in patients affected by benign diseases was between 2-10% [58], and 2-12% [36]. In accordance, our results showed 15% false positive for TPA, while NSE showed no false positive.

On studying the diagnostic accuracy of HGF and $VEGF_{165}$ at their reference cut-off values (955 and 123 pg/ml respectively), accuracy was 96.25% and 85.39% respectively. Both HGF and $VEGF_{165}$ had a 95.24% sensitivity which was less than TPA but much greater than NSE. VEGF specificity was 100% the same as NSE, for HGF it was 88.24%, i.e. all are more specific than TPA. HGF showed no false positive, while for $VEGF_{165}$ it was 11.8%. On the contrary, it was reported that in SCLC, at cut-off value 500pg/ml, VEGF was less sensitive as a tumor marker compared to NSE with sensitivity 31% and 42% respectively [52].

Angiogenic factors are poor prognostic indicators for tumor aggressiveness and survival [59]. Both HGF and VEGF are potent angiogenic factors, and from our results, it was clear that both have almost same diagnostic accuracy indices: Sensitivity for both was 95.24%, specificity was 88.24 and 100%, positive protective value was 100 and 96.77%, negative protective value was 83.33 and 84.1%, and accuracy was 96.25 and 85.39% respectively. Even false positive was 0 and 11.8% and both have false negative of 4.8%. This in part may be due to they are both angiogenic promoters even if they function through different mechanisms, or may be due to the fact that HGF indirectly, and transcriptionally induces VEGF expression in keratinocytes, in addition to VEGF-independent actions on angiogenesis [45,10].

4.4.1. ROC curve analysis:

In the present study, analysis of ROC revealed that the highest diagnostic accuracy was achieved by TPA (AUC≈ 1), and then comes $VEGF_{165}$, HGF and NSE in descending order. In agreement with our results, Plebani et al. [36] reported that by using the ROC method, TPA showed the highest diagnostic accuracy among other lung markers.

Until now there is no specific sole tumor marker for lung cancer detection [60], and for the differential diagnosis between NSCLC and SCLC, and both from benign diseases, and mesothelioma a combination of more than one marker is preferable [39].

5. CONCLUSION

From the present data we can conclude that:

1. HGF and $VEGF_{165}$ have almost same diagnostic accuracy indices and can be considered moderately informative tumor markers; they both may be useful in diagnosis of lung and pleural cancers. However, further studies are needed to confirm this suggestion.
2. TPA was the most accurate in lung cancer diagnosis, but lacked the needed specificity at the used cut-off (1 ng/ml).
3. NSE at the used cut-off (13 ug/L), is considered the best tumor marker for SCLC, the fact that our results agree with, but this is not the same for other lung cancer subtypes.
4. None of the investigated markers had the ability to discriminative between different histological subtypes, except for NSE.
5. In conclusion, combination of HGF with each of TPA and NSE is more valuable than the use of one of them alone

6. RECOMMENDATIONS

Circulating levels of VEGF and HGF may be valuable future tools for diagnosis. However, needs standardization by large-scale prospective clinical trials, to establish clear and definite sharp cut-off values that can be practically applicable. This study recommends the use of a combination of markers for the confirmed diagnosis of lung and pleural cancers.

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8. DECLARATION

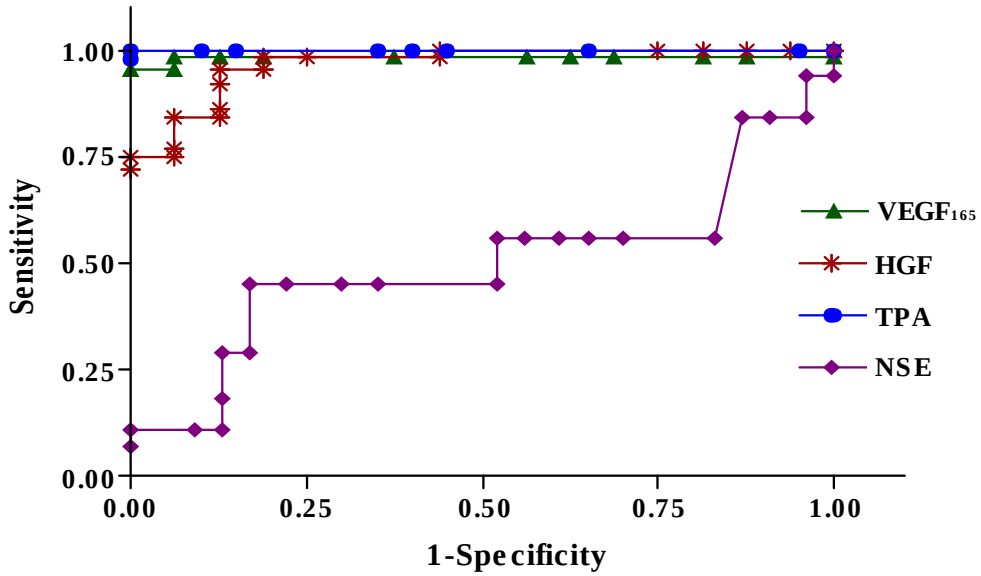
Work of this study was a part of PhD thesis of Dr. Amel Hashim, under the supervision of the rest of authors. The Egyptian Academy of Scientific Research and Technology had partially participated in financial costs of the study.

9. CONFLICT OF INTEREST AND FUNDING

Authors declare no conflicts of interest that might bias the study.

Lane 1: Molecular weight marker, lane 2: $VEGF_{165}$ positive control standard, lane 3: Control sample, lanes 4-10: Patients' samples. A single band of 32-34 kDa., was observed in lanes 4-10 that corresponded to the band in lane 2. No bands appeared for control sample in lane 3.

Fig 1
Western blot for $hVEGF_{165}$.



Qualitatively, the closer the curve, to the top left-hand corner, the higher the overall accuracy of the test is. Quantitatively, the area under the curve is an overall measurement of the accuracy. TPA was the closest to the top left-hand corner (Showed the biggest AUC ≈ 1). VEGF, HGF then NSE came in descending order of A and AUC.

Fig 2
ROC curve for all studied Markers.

Table 1
Clinical and Demographic Profile of the Studied Population

Parameters	Control	Lung and Pleural Cancers				
		Total	*Mesothelioma*	*Bronchogenic Carcinoma*		
				Total	SCLC	NSCLC
№	23	64	26	38	5	33
Age (years):						
Mean ± SE	43.4±1.53	54.9±1.53***	49.1±2.26	58.9±1.80	62.2±5.88	58.4±1.90
Median	45.0	53.5	45.5	60.5	68.0	59.0
Range	31 - 55	33 - 76	33 – 76	38 - 76	39 - 71	38 - 76
Gender :						
Male	65.2%	67.2%	42.3%	71.1%	80%	69.7%
Cigarette smoking:						
Smokers	17.4%	51.6%**	34.6%	63.2%	80%	60.6%
NSCLC Pathological Subtypes:						
AC	-	12	-	12	-	12
LCUC	-	14	-	14	-	14
SCC	-	7	-	7	-	7
Pathological Grade: %, (№)						
I	-	4.7% (3)	11.5% (3)	-	-	-
II	-	23.4% (15)	26.9% (7)	21.1% (8)	20% (1)	21.2% (7)
III	-	15.6% (10)	-	26.3% (10)	20% (1)	27.3% (9)

№: Total number in each group, SCLC: Small cell lung cancer, NCLC: Non-small cell lung cancer. AC: Adenocarcinoma, LCUC: Large cell undifferentiated carcinoma, SCC: Squamous cell carcinoma. ***: p< 0.0001 when compared to group control using nonparametric Mann-Whitney test. **: p< 0.01 when compared to group control using Fisher exact test.

Table 2
Serum level of investigated markers in the studied population

Markers (cut-off)	Control	Malignant Mesothelioma	Bronchogenic Carcinoma
TPA: (1 ng/ml)			
№	20	26	34
Median	0.4	15.98	12.55***
Range	0.096 - 1.2	5.4 - 53.1	2.16 - 60.3
NS: (13 ug/L)			
№	23	26	36
Median	5.2	7.5	2.5
Range	1 - 12.4	0 - 12.5	0 - 38
HGF: (955 pg/ml)			
№	17	26	37
Median	620	2010	1860 **
Range	300 - 1390	900 - 6180	660 - 9450
$VEGF_{165}$: (123pg/ml)			
№	16	26	37
Median	52	960	720 ***
Range	10 - 120	3.6 - 2460	126 - 3060

№: Total number in each group, TPA: Tissue polypeptide antigen, NSE: Neuron specific enolase, HGF: Hepatocyte growth factor, $VEGF_{165}$: Vascular endothelial growth factor isoform 165. **: p< 0.001, ***: p< 0.0001 compared to control using Kruskal-Wallis nonparametric ANOVA followed by Dunn's multiple comparison test. Data were approximated to the second decimal. Determination of optimum cut-off value for $VEGF_{165}$ and HGF among studied groups was done using ROC curve.

Table3
Serum level of investigated markers in control, SCLC and NCLC groups

Markers (cut-off)	Control	SCLC	NSCLC
TPA : (1 ng/ml)			
№	20	4	30
Median	0.4	11.10 **	12.53 ***
Range	0.096 - 1.2	2.16-53.4	3.3-60.3
NSE: (13 ug/L)			
№	23	5	31
Median	5.2	19 ***	2.5 #
Range	1 - 12.4	15-38	0-12.5
HGF: (955 pg/ml)			
№	17	4	33
Median	620	2520 **	1500 ***
Range	300 - 1390	2070-3090	660 - 9450
$VEGF_{165}$: (123 pg/ml)			
№	16	4	33
Median	52	756 **	720 ***
Range	10 - 120	276-1320	126 - 3060

№: Total number in each group, TPA: Tissue polypeptide antigen, NSE: Neuron specific enolase, HGF: Hepatocyte growth factor, $VEGF_{165}$: Vascular endothelial growth factor isoform 165, SCLC: small cell lung cancer, NCLC: non-small cell lung cancer. **: $p< 0.001$, ***: $p< 0.0001$ when compared to group control using nonparametric Mann-Whitney test. #: $p< 0.0001$ when compared to SCLC group using nonparametric Mann-Whitney test. Determination of optimum cut-off value for $VEGF_{165}$ and HGF among studied groups was done using ROC curve. ANOVA test wasn't carried out for analysis of SCLC group, due to low sample size.

Table 4
Non-parametric correlations in-between the investigated markers.

Tested correlation	*№*	*r*	*p*
NSE vs. $VEGF_{165}$	62	0.0723	> 0.05
NSE vs. TPA	60	- 0.0048	> 0.05
NSE vs. HGF	62	0.3798	< 0.001
HGF vs. $VEGF_{165}$	63	0.0753	> 0.05
HGF vs. TPA	60	0.4658	< 0.0001
TPA vs. $VEGF_{165}$	60	0.0197	> 0.05

№: Total number of cancer patients, TPA: Tissue polypeptide antigen, NSE: Neuron specific enolase, HGF: Hepatocyte growth factor, $VEGF_{165}$: Vascular endothelial growth factor isoform 165. r: Spearman rank correlation coefficient.

Table 5
Diagnostic accuracy indices of the studied markers at their reference cut-off values in serum

Markers	Sn	Sp	PPV	NPV	A
TPA	100	85	95.24	100	96.3
$VEGF_{165}$	95.24	100	96.77	84.1	85.39
HGF	95.24	88.24	100	83.33	96.25
NSE	8.1	100	100	40.35	32.94

TPA: Tissue polypeptide antigen, NSE: Neuron specific enolase, HGF: Hepatocyte growth factor, VEGF165: Vascular endothelial growth factor isoform 165, Sn: Sensitivity, Sp: Specificity, PPV: Positive Predictive Value, NPV: Negative Predictive Value, A: Accuracy. Cut-off values: TPA= 1 ng/ml, NSE= 13ug/L, VEGF165 = 123 pg/ml, HGF= 955 pg/ml. All data are expressed in percentage.

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