Research Article

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Detection of Serum Associated Proteins as Diagnostic Biomarkers in Patients suffering from Lung Cancer

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Abstract

Objective:
Lung cancer (LC) is one of the leading causes of cancer related deaths worldwide. Among other reasons, one cause of LC deaths is the lack of early diagnosis. Serum biomarkers might be helpful in early detection of this cancer but there is no up to date specific biomarker reported to diagnose LC. Aim of the present study was to predict the tumor associated proteins as potential biomarkers for the early detection of Lung Cancer (LC).

Material and Methods:
A total of 50 serum samples from LC patients were tested and SDS-PAGE profiling was performed. The visualization of detected protein bands in the gels were stained by Coomassie blue staining method.

Results:
Two bands were differentially expressed by LC patients comparing control sera. A 41 kDa band was stained in 21% testing sera while 110 kDa band was stained with 17% testing sera of patients.

Conclusion:
These protein bands might serve as candidate potential biomarkers for early LC diagnosis after mass spectrometry confirmation with large scale testing sera.

Key Words: *Sera, Diagnostic biomarker, Lung cancer*

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INTRODUCTION

Lung cancer is furthest the common origin of cancer related death in both men and women worldwide. Which is responsible for almost 1.4 million deaths per year (Jemal et al., 2011). In the United State lung cancer is defined as the main reason of cancer related deaths, with more than 190,000 death cases reported per year. The 60% patients diagnosed as suffering from lung cancer die within a year of their diagnosis, and near 75% patients die within 2 years, and the survival rate of 5 years is fewer than 16%. Less survival rate is mainly because of the late stage to which the LC is presently been detected. The death rate of LC is high than that of prostate cancer, breast cancer and colon cancer (Jemal et al., 2011). LC are of two main subtypes, small-cell lung cancer(SCLC), and the non-small cell lung cancer(NSCLC), which is accounted for about 85% of diagnosed with lung malignancies (Hassanein et al., 2012). However, the survival rate of 5 years in the initial stage, treatable, (NSCL) non-small cell LC is about 50-70%. The patients with 5 year survival with cancer diagnosis, their ratio drops from 2-5%, when their tumor is already been spread (Goldstraw et al., 2007). However, the initial recognition helps in survival, tests performed for screening of high risk person by the chest X-rays or by the sputum cytology does not shows progress in specific disease survival, the existent, the LC diagnosis is mainly depends on the symptoms (e.g., cough, chest pain, breath shortness, and hemoptysis), and the recognition is frequently happened by the therapeutic interference (i.e., surgery) is not conceivable (Melamed & Flehinger, 1984).

Tumor markers might be noticed by the tissues, sera, and urine from the malignant tumors patients, which can further be helpful for the particular diagnosis, perception of the malignant tumor, continuation of treatments, and for the estimation by patient's result (Patel, Erickson, Roberts, & Grenache, 2010). For the further identification an effective biomarkers is used which is proved to be helpful in determining that the identified screening, suspicious nodule is malignant or is something else, thus decreasing the sum of incorrect positives by operation or clinical biopsy(Veronesi, Bianchi, Infante, & Alloisio, 2016).

Current blood diagnostic tests focuses on tumor associated antigen (TAA) detection, several markers are used, such as the (CEA) carcinoembryonic antigen, (NSE) neuron-specific enolase, chromogranin, (CA) carbohydrate antigen CA19-9 and CA125 that display an improved positivity at progressive phases (Tarro, Perna, & Esposito, 2005). These are infrequently used as the early biomarkers because of short specificity and sensitivity. Nevertheless, serum tumor-associated autoantibodies (TAAbs) blood tests against the misfolded, mutated, overexpressed, and abnormal cellular autologous antigens formed by the cells of cancer (Heo, Bahk, & Cho, 2012; Veronesi et al., 2016), might identify entities with early LC and differentiate

between great threats of smokers with nonthreatening nodules from those with LC. The autoantibodies stays longer in the circulating blood than that of the antigens themselves, these autoantibodies are potentially said to be highly helpful diagnostic markers of cancers, and they can be easily identified from the patient's plasma that develops the LC, the autoantibodies circulation has initiated up to five-years earlier than the CT will be able to identify the tumor (Zhong et al., 2006).

MATERIALS & METHODS

Work Place and Sampling

All experiments were performed in Biotechnology laboratory of CASVAB (University of Baluchistan Quetta). A total of 50 blood samples were collected from diagnosed lung cancer patients from Bolan Medical Complex Hospital (BMCH) and Sandeman Provincial Hospital Quetta. 3-5 ml venous blood was drawn in sterile vacutainer with informed written consent of patients. 20 blood samples were also collected from healthy subjects, as negative control.

Sample Processing

Sera were obtained from all samples individually by centrifuging at 8000 rpm for 10 minutes and collected serum samples were stored at -80°C in Biotech Laboratory till use.

Protein Estimation

Quantity of protein present in each serum sample was estimated by Lowry method (Lowry et al., 1951) using BCA Protein assay kit (Bio-RAD) that allows measuring the concentrations of proteins up to 0.025%.

Gel Electrophoresis (SDS- PAGE)

Proteins present in each serum sample of lung cancer patient were separated by SDS-PAGE that separates the proteins according to their molecular weight in a given matrix. Polyacrylamide gel (12%) was prepared as running gel (Tris-HCL pH=8.8, 10% SDS solution, 30% acrylamide, 0.8% bis-acrylamide, 10% ammonium persulfate and TEMED). 4% stacking gel was also prepared which contained (Tris-HCL pH=6.8, 10% SDS solution, 30% acrylamide, 0.8% bis-acrylamide, 10% ammunium persulfate and TEMED). Protein samples were loaded onto the top of stacking gel in the wells constructed by comb and allowed to migrate according to protocol as adopted by Mustafa MZ et al., 2016.

Gel Staining

For visualization of detected protein bands, the gels were stained by coomasie blue staining method.

RESULTS

Reactivity of serum samples

SDS-PAGE based reactivity of all 50 serum samples of lung cancer patients was performed and compared with a pool of 20 normal subject's sera (Figure 1 & 2).

A very prominent protein band of 41 kDa molecular weight was detected in 42% (n=21/50) of reacting sera with moderate to strong intensity. This 41 kDa band did not detect in pool of 20 normal sera.

Another protein band of 110 kDa was detected in 34% (n=17/50) with strong

intensity and did not observe in pool of control sera (Figure 1 & 2).
Few other bands of different molecular weights were also detected with patients' sera as well as stained with control sera too.

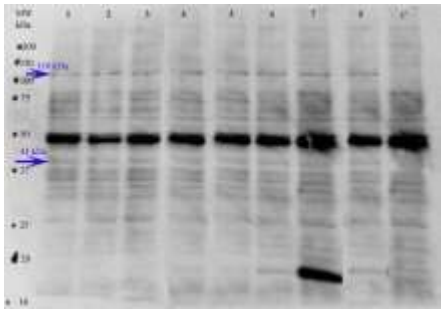

Figure 1. Representative SDS-PAGE migration pattern of proteins presents in sera of lung cancer patients (n=8), 41 kDa and 110 kDa protein bands are differentially stained with patients' sera and did not stain with pool of control (C) sera.

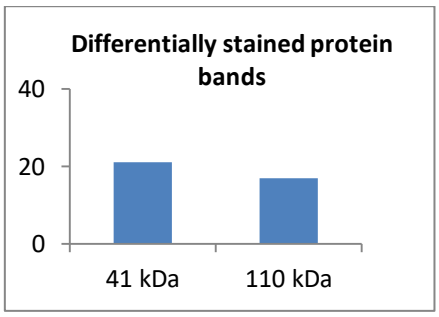

Figure 2. X-axis indicates the differentially stained protein bands of 41 kDa and 110 kDa molecular weights which were not detected in normal control sera, Y-axis indicates the number of patients' sera stained with these bands.

DISCUSSION

Lung cancer is furthermost the 2nd common cancer, which is the principal cause of cancer related deaths for men as well as for women (Kanodra, Silvestri, & Tanner, 2015; Smittenaar, Petersen, Stewart, & Moitt, 2016). It is estimated that, the 60% patients, those are diagnosed by the lung cancer die within a year of their diagnosis. LC are of two main subtypes, small-cell lung cancer(SCLC), and the non-small cell lung cancer(NSCLC), which is accounted for about 85% of diagnosed with lung malignancies (Hassanein et al., 2012).
Several serum-based tumor markers are used for the LC, such as CEA (Carcino-Embryonic Antigen), which is one of the best-known biomarkers for lung cancer. Other markers are the chromogranin A, CYFR A 21–21, CA-125, neuron-specific enolase (NSE), retinol-binding protein (RBP), squamous cell carcinoma antigen (SQCC) and α1-antitrypsin. Nevertheless, there is no single blood test exists for LC diagnosis. CEA is said to be the most widely used tumor marker, the percentage estimated for the SCLC patients is raised by 0-38% for the patients with limited disease, whereas is about 40-65% with the extensive disease. The percentage for the NSCLC defined with the known biomarker CEA is 30-65% (Boffetta, 2006; Al-Shagahin et al., 2009; Chen et al., 2010; Chmura et al., 2009; Holdenrieder et al., 2008; Kosacka & Jankowska, 2009; Nisman et al., 2009).
Some other potential serum based biomarkers includes, squamous cell carcinoma antigen (SCC), GM-CSF, stem cell factor (SCF), and VEGF which is proven to be associated with NSCLC, conversely all of these have

been failed to establish the required sensitivity and specificity to be permitted by clinical developments to be used as diagnostic tools (Buccheri, Torchio, & Ferrigno, 2003; Greenberg & Lee, 2007; Schneider, 2006).

In our study we detected a 41 kDa protein band which may correspond to tissue polypeptide antigen (TPA), a 40 kDa protein. Currently, a sum of different supplementary factors exists in the serum have been estimated for their prospective effectiveness as the biomarkers for LC. These factors contain tissue polypeptide antigen (TPA) and Intercellular adhesion molecule 1 (ICAM-1). These are endothelial cell adhesion molecules which are members of the immunoglobulin super gene family (Springer, 1990; Carlos and Harlan, 1990; Montefort and Holgate, 1991). We also detected a 110 kDa protein band which was not observed in pool of control sera, it has been predicted that this protein band might corresponds to ICAM-1. The molecular weight of ICAM-1 ranges from 76–114 kDa; and the degree of glycosylation of the core protein (molecular weight of 55 kDa.) determines the variation is dependent on which has a, epithelial cadherin E-cadherin, Serum Amyloid A (SAA) (Cremona et al., 2010). In a recent study, it was found that the elevated levels of two proteins SAA1 and SAA2 in LC patients' tissue samples and blood with reference to healthy donors and patients detected with other cancers. This study suggests that SAA1 and SAA2 are the candidate for further investigations as specific diagnostic biomarkers in the future (Sung et al., 2011) with E-Selectin (Gogali et al., 2010), High mobility group box 1 HMGB1(Shang et al., 2009), urokinase-type plasminogen activator receptor uPAR (Almasi, Hoyer-Hansen, Christensen, & Pappot, 2009), and angiopoietin-1 (Park et al., 2009).

The unsatisfactory representation of individual biomarkers and the occurrence of further biomarker contenders have managed numerous agents to improve panel's multi-analyte in expectancies of succeeding the stages of specificity and sensitivity. The amount of these panels consists of circulating proteins (Molina et al., 2003; Patz et al., 2007) and of the tumor-associated autoantibodies together (Farlow et al., 2010; Nolen et al., 2011) and which is assessed in LC with the promising results.

CONCLUSION

In conclusion, further investigation on large scale with higher number of patients' sera should be done in order to propose an effective and potential diagnostic biomarker or a panel of diagnostic markers with increased sensitivity and specificity. Identification of all detected proteins is recommended by mass spectrometry for confirmation.

Conflict of interest

Authors declare that there is no conflict of interest.

Ethical approval

Ethical and other necessary approvals were taken from Ethical Review Board of the University of Balochistan, Quetta, Pakistan.

Consent for Publication

All authors approved manuscript for publication.

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