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Apolipoprotein-A1 as lung cancer serum protein biomarker detected by proteomic strategies

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Abstract: Lung cancer is emerging as a prime cause of cancer deaths in under developed countries. Extremely high mortality rates are due to non-awareness of health regarding issues like smoking, radiation exposure and air pollution along with specific occupational toxicity leading to great incidence of lung malignancy. Biomarker is defined as any type of specific parameter which can assess any kind of undergoing abnormality and unnatural happening in living beings. Recent proteomic methodologies have been providing worthwhile advancements in indication, identification and control of disease. In this study, comprehensive set of modern proteomic methodologies have been implied to identify the specific role of lung cancer serum proteins which may prove as a standard criteria for evaluation of disease compared to other types of diseases. 100 lung cancer patients along with 50 age matched healthy persons participated in study fulfilling all ethical guidelines. SDS-PAGE, two dimensional gel electrophoresis coupled with in-gel digestion, Maldi-Tof-Tof technique, electro-elution of identified proteins and immune-detection by western blotting have been used in present study to identify and elaborate the lung cancer biomarker proteins. 100 lung cancer patients and 50 age matched healthy controls were included in this study following exclusion and inclusion criteria and differentially expressed protein spots resulting from two dimensional serum profiling were excised, in-gel digested and analyzed by Maldi-Tof-Tof technique coupled with Mascot database search. A 28.061 kDa protein Apolipoprotein- A1 with isoelectric point of 5.27, identified at peptide mass tolerance of 149ppm, was investigated to be up regulated in squamous cell lung carcinoma cases compared to other types of lung cancer and healthy controls. Significant score of 117 and peptide sequence coverage of 56% authenticated the results of peptide mass fingerprinting and electro-elusion was performed to raise primary antisera in response to human Apolipoprotein- A1 to validate the proposed findings. Western blotting outcomes further validated the suggestion that Apolipoprotein –A1 is overexpressed in squamous lung carcinoma, leading to the useful diagnostic pathway establishment on clinical basis.

Keywords: Apo-A1, lung cancer serum biomarker, two dimensional gel electrophoresis, Maldi-Tof analysis, electroelusion.
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INTRODUCTION

Lung cancer is the topmost cause of cancer mortality for both males and females in the United States, with frequency of 160,000 new cases per anum. Poor prognosis can be seen by low survival figures i.e. 5 to 10 year total survival rates for subjects taking therapy were found to be only 14% and 8%, respectively[1]. Lung cancer is broadly classified into two main divisions i.e., Non-small-cell lung cancer NSCLC (having three types based on tissue study types, like adenocarcinoma, large-cell carcinoma, and squamous-cell carcinoma) with 85% prevalence of total statistics and small-cell lung cancer (SCLC), almost 15% of figures[2].

Biomarkers are stated as the entities serving as a best source for computable analysis of biological homeostasis, which discriminate what is unnatural from what is natural[3]. Cancer biomarkers provide valuable direction in vast field of cancer discipline. They are supportive in early detection of malignancies and offer an indispensable tool for corroboration of cancer phases, to assess the treatment feedback[4].

Levels of carcinoembryonic antigen (CEA) are found to be raised in lung cancer patients especially in adenocarcinoma and large cell lung cancer. CYFRA 21-1 is a signal of elevated levels of cytokeratin 19 fragments that suggests the presence of lung cancer and is a good indicator for recognizing therapy outputs of late stage non-small cell lung cancer subjects[5]. In comparison to CYFRA21-1, tissue polypeptide antigen (TPA) estimates cytokeratin fragments (8, 18 and 19) identifying non-small cell lung cancer[6]. Progastrin-releasing peptide (ProGRP) released by the neuroendocrine tissues of the gastrointestinal and respiratory tracts and is listed as a recommended biomarker for SCLC[7]. Elevated levels of NSE in SCLC present an important source for post treatment of the disease. All these above mentioned biomarker proteins generally exhibit too less specificity to the lung cancer to be consigned as disease related molecules[3].

Apolipoproteins are designated as an assemblage of proteins linked with lipoproteins in variable extents and show noticeable part in multiple disease arrays. A variety of Apolipoproteins, e.g. Apolipoprotein A, B, C, D, E, H, and J in addition to their sub-types have been reported along with few novel discoveries. These proteins have diverse but positive role in natural body functioning. Blood levels of apolipoproteins intensely correlate with medical environments in course of enormous diseases, and their implementation as a biomarker in diagnostic and prognostic fields and risk assessment of few severe ailments has been made effective[8].

Human Apolipoprotein A1 (Apo-A1) is acknowledged as the chief protein constituent of high-density lipoprotein (HDL) of plasma[9]. It is produced in liver and intestine as a zymogen pre pro-protein. Levels of Apolipoproteins reveal the alterations in concentration of HDL detected in normal subjects but

are also affected by other likely factors e.g. age, alcohol consumption, hormonal therapy, gender, race, BMI, and coronary arterial disorders. Apo-Al is a well-known as a negative indicator of inflammation, as, greater than 25% reduction in its level has been observed during inflammation. Apo-A1 has been found to be a particular inhibitor of cytokine synthesis by monocytes and macrophages as stimulation of T cells is compromised. It also functions as constitutive anti-inflammatory element, and fall in HDL-related Apo-A1 level may be a sign of chronic spread of inflammation. It was represented that concentration of Apo-A1 in blood is declined in various cancer forms[10].

In present study, effective implementation of recent age proteomic approaches like SDS-PAGE, two dimensional gel electrophoresis ,in-gel digestion, Maldi-Tof-Tof, electro-elusion and western blotting have been provided for sorting out differentially expressed lung cancer serum proteins compared to controls and to elaborate the vital role of disease specific biomarker proteins with strong emphasis to arrest the malignancy at primary phase.

MATERIALS AND METHODS

Sample collection, processing and estimation of serum protein contents

Blood samples (n=100) of diagnosed lung cancer cases were collected from Gulab Devi Chest Hospital, Lahore and a performa was filled up with the help of relevant medical specialist with complete record of gender, age, smoking history, occupational exposure, type and stage of cancer and relevant details of medication given. 50 age-matched healthy control subjects (with no smoking history) were also included in the study group and 5ml of blood was drawn from each participant. Serum was isolated by centrifuging whole blood at 4000rpm for 15min after incubating the whole blood sample for 2 hours at room temperature without shaking. Total protein contents of each patient and control serum were measured by sensitive Bradford assay as recommended in standard protocol[11]. Protein amount of each sample was computed as µg/µl by plotting the standard curve of BSA with a broad range of ultra-pure dilutions.

One dimensional SDS PAGE analysis

In order to note the variation pattern of protein resolution in patients and control samples, 5µg of serum samples of lung cancer patients and control subjects were electrophoresed onto 12% SDS gel according to the protocol described by Laemmli[12]. One dimensional characterization of serum also gave valuable information for optimum concentration of each sample to be run on two dimensional gel electrophoresis.

Two dimensional gel electrophoresis

Two dimensional gel electrophoresis has the advantage of more profound separation of proteins and complex protein mixtures by fractionating the sample into two dimensions of isoelectric point and molecular weight, respectively. 18cm long IPG strips with pH range of 3-10, non-linear (Serva, Cat# 43012.01) were used and rehydration of serum sample was processed using rehydration buffer (8M Urea, 4% CHAPS 3-(3-Cholamidopropyl-dimethylammonio-propanesulfonic acid), 50mM DTT(dithreitol, Fermantas, Cat# R0861) 0.2% Servalyte and 0.0002% bromophenol blue, at 20°C overnight. First dimension was operated on (Amersham Scie plus, UK) flat-bed chamber , run at a total of 6000kW with sequential cycles of voltage and time as follows: 150v for 1 hour, 300 v for 1hour, 600volts for 1 hour, 1000volts for 12 hours, 2000v for 5 hours, 3000volts for 2 hours and a final 4000volts for 8 hours. Second dimension was carried out on 12 % SDS gel by the method as described by Lammeli[13]. In place of stacking gel, molten (0.5%) agarose was used to cast the well and to support the strip. Strip was given a 10 min incubation (with shaking on orbital shaker)in equilibration buffer 1 (6M urea, 30% glycerol, 2% SDS, 50mM Tris-HCl (pH 8.8), and 0.01% bromophenol blue , with 0.1g DTT fresh/10ml of buffer . After, same procedure was repeated with equal quantity of equilibration buffer 2 containing 0.5g of Iodoacetamide, in place of DTT. 0.5% molten agarose solution was prepared , in double autoclaved deionized water with few grains of bromophenol blue and poured onto pre-cast uniform surface of resolving SDS-gel. Strip was immediately aligned carefully with only 1mm edge of strip touching the gel. A vertical plastic well was properly inserted within the gel to be solidified and 5µl of unstained protein ladder (Fermantas, PageRular, SM0661) was loaded in well after polymerization of agarose and setting of well. The whole gel assembly was run at 60 volts till sample stayed in agarose portion, and later on at a constant voltage of 120volts, until dye come to the bottom of the gel. It was stained in G-250 colloidal coomassie stain overnight after protein fixation (30% ethanol, 10% acetic acid and 60 % deionized water) for 4 hours. Destaining was carried out with several repeated changes of deionized water. Image was scanned until a clear background of the gel was obtained.

In-gel digestion

Destaining of gel spots

The gel spots were excised with sterilized blade, washed with double autoclaved deionized water and

shifted to an autoclaved eppendorff tube holding sufficient quantity of deionized water to soak the gel pieces. It was left at room temperature for 15 min followed by addition of 100µl of mixture of acetonitrile and double autoclaved deionized water, prepared in equivalent amount and incubated at room temperature for 15 min again with subsequent addition of 100µl of acetonitrile and left at room temperature till gel spot texture turned sticky and white. Later on, 100µl of 100mM ammonium bicarbonate (Ambic) was added after removal of acetonitrile and mixture was incubated for 5 min at room temperature. Same amount of acetonitrile was added again with further incubation of 15 minutes. Gel pieces was dried in a freeze dryer (Christ Alpha1-4, Loc-Im) for approximately 15 minutes, after separation of added solution.

Table 1: Clinical data chart of patients history.

Age of lung Cancer subjects	Pathological stage	Type of cancer	Sample size
35-67	III&IV	Adenocarcinoma	48
32-56	III&IV	Squamous cell carcinoma	34
40-68	III&IV	Large cell lung carcinoma	06
48-62	III&IV	Small cell lung carcinoma	12

Reduction and alkylation

100µl of 10mM DTT was added in above dried gel pieces so that reduction of disulphide bonds may take place by incubating the vial at 56^{o}C for 45 min. 100µl of 55mM iodoacetamide (Fluka, Cat#57670) was also added instantly just after removing the DTT solution and incubated in dark for 30 minutes. Added solution was decanted and gel piece was rinsed with 100mM Ambic by 5 minutes incubation. Afterwards, same amount of acetonitrile was transferred in tube with incubation for 15 minutes. The gel piece was completely desiccated again as mentioned above in a freeze dryer for a time period of 15 minutes.

Tryptic digestion of protein

20-40µl (depending upon the spot size) of reconstituted trypsin (Promaga, Sequencing grade Cat# V5111) was added to the dried gel pieces and mixture was placed on ice for 45 min. 50mM of Ambic was added again after removing the trypsin ensuring that the gel spot was fully immersed sin buffer and left at 37^{o}C, overnight.

Peptides recovery

10% TFA (trifluoroacetic acid) was added to the tube for lowering down the pH of the solution to approximately 3.0 and top fraction of the tube contents having peptides was carefully decanted, saved and storage in a sterile eppendorff vial. The left over gel piece was supplemented with 0.1%TFA and 60% acetonitrile blend and shifted to an ultrasonic water bath (Ultrasonic, LC30/H) for 30 min at 20^{o}C so as to collect any remaining fraction of peptides and resulting liquid was dehydrated in freeze dryer as earlier until 10µl volume was left. It was stored at 20^{o}C till peptide mass fingerprinting profiling.

Peptide mass fingerprinting and database searching

Extracted peptides were analyzed on Maldi-Tof-Tof MS (Ultraflex III, Bruker-Daltonics, Germany) with following analytical considerations: 337 nm nitrogen laser beam and 2GHz digitizer. Peaks of spectra were recorded in linear positive mode with value of ion acceleration 25kv and that of lens potential as 6kv. 100 Hz laser frequency was used with 60-70% intensity 7.5 gain of detector with 0.5 GS/s rate was adjusted. Matrix used was Alpha-cayano-4-hydroxy cinnamic acid (HCCA) and 1µl each of matrix and peptides fraction was applied on Maldi sample spotting plate and analysis was precede following instrument operation guidelines. Mascot database was used for protein identification with following optimized conditions: carbamidomethyl modification and methionine was adjusted to 1 missed cleavage, trypsin as digesting enzyme, database used was Swiss Prot and high significant score was obtained at peptide tolerance value of 149ppm. Mascot results were authenticated by obtaining prerequisite sequence coverage and high scoring of protein.

Immunization and validation of targeted proteins

15% SDS- polyacrylamide gel electrophoresis

In order to collect bulk amount of targeted protein to be eluted, several SDS gels were run (15%) to collect high quantity of same protein bands. Fixation, staining and destaining of the gels was done as above. Image was scanned and saved using gel documentation system.

Electro-elusion of protein from gel and immunization of mice

Serum samples containing about 100µg of protein were electrophoresed on 15% preparative gels masking wells of the comb to load maximum volume of sample and 5µl of standard protein ladder (Fermentas) was also run along the sample and gel was run at constant voltage of 120 volts followed by same procedures of staining and destaining. Required gel bands were marked, excised, minced with blade and shifted to pre-treated dialysis tube filled with protein elusion buffer (0.2 M Tris- acetate buffer (pH 7.4), 0.1% SDS and 100mM DTT ,added in each 1ml of elusion buffer). Horizontal electrophoresis assembly with running buffer (50mMTris-acetate

buffer (pH 7.4), 0.1% SDS, and 0.5mM sod-thioglycolate) was used and electro-elusion was continued at 50 volts till fully transparent gel bands were observed. Resulting elute was desiccated in freeze dryer completely and protein pellet was dissolved in (PBS, 137mM sodium chloride, 2.7mM potassium chloride, 4.3mM sodium hydrogen phosphate, 1.47mM potassium hydrogen phosphate, pH 7.4) and again 15% SDS-PAGE was carried out to review the quality of eluted protein. Intact distinct bands were reconstituted in PBS again after complete drying by 2 day incubation at 37°C (with open lid), centrifuged at 13000rpm for 10 min and top fraction containing a total of 50µg of required protein was injected in four weeks older Albino mice strains in triplicates for immunization purpose, after confirmation of the protein identity by peptide mass fingerprinting. Four periodic doses (after 10 day cycle) were given to the experimental animals and antisera was collected by cardiac puncture after complete round of the procedure.

Purification of anti-sera using protein A columns

Serum was isolated from animal clotted blood by spinning at 4000rpm for 15 minutes and immediately processed into IgG purification protein A columns (Pearl IgG purification kit Cat# 786-798, G-Biosciences) according to the recommended protocol.

Western blotting

Immunoblotting was performed by the method given by Towbin et al[13]. 10µg of Serum proteins were separated on 15% SDS-gel and electrophoresed to nitrocellulose membrane sheet (8.5×7.5, 0.22µm, G-Biosciences, Cat#786-018NC)) and allowed transfer of proteins for 2 hours, at constant voltage of 18 volts carried out in Semi Dry Transfer Cell (Bio-Rad Trans-Blot ,Sd). After protein transfer, 3% BSA (prepared in TBS-Tween-20) was applied to blot for blocking of non-specific protein substances and shaked for 2 hours. Purified primary antibody in 1:500 dilution made in TBS-Tween-20) was added to blot membrane after giving three washings with wash buffer (TBS, 25mM Tris, 150mM sodium chloride, 2mM potassium chloride, pH 7.4) and placed in refrigerator overnight followed by addition of conjugate (Goat anti-mouse IgG, AP-conjugated, Cat# 786-R43,G-Biosciences) diluted (1:5000) in same dilution buffer as for primary antibody after washing with the same wash buffer thrice and incubated for 2 hours. Blot was washed as stated above (thrice) and treated with alkaline phosphatase (AP) substrate buffer solution (100mM Tris-HCl, pH 9.5) 100mM sodium chloride, 5mM magnesium chloride, supplemented with 50mg/ml each of 5-bromo-4-chloro-3-indolyl-phosphate (BCIP) and nitro blue tetrazolium chloride (NBT) by gentle shaking and developing solution (substrate buffer) was removed as required density of bands was observed. Blot membrane was carefully dried by pressing between thick stack of clean filter paper and image was scanned and saved in gel documentation system.

RESULTS

Disease specific biomarker discovery is the urgent need of the time for effective eradication of a particular disorder. Present age proteomic layouts and associated ultra-sensitive high throughput screening tactics have made possible to identify, quantify and validate a particular agent to sort out an easy solutions to the complex problem of finding out disease-related biomarkers. In present study, 100 lung cancer sera along with 50 healthy control serum samples underwent proteomic characterization by the sequential steps of techniques, e.g. One dimensional SDS-PAGE, two dimensional gel electrophoresis, in-gel digestion, peptide mass fingerprinting, electro-elusion and western blotting.

One dimensional SDS PAGE

12% SDS PAGE was performed for patient and control serum samples with 5µg of total protein. Comparison of protein bands display in lung cancer patients versus healthy controls gave showed discrepancies at different molecular weights. Protein bands at about 28kDa were observed to be overexpressed in patient samples as compared to control one as shown in (Figure 1). For verification of one dimensional gel electrophoresis results, two dimensional gel electrophoresis was carried out to locate the variably present proteins on exact molecular weight position with accurate isoelectric point, so that the proposed observations may be certified.

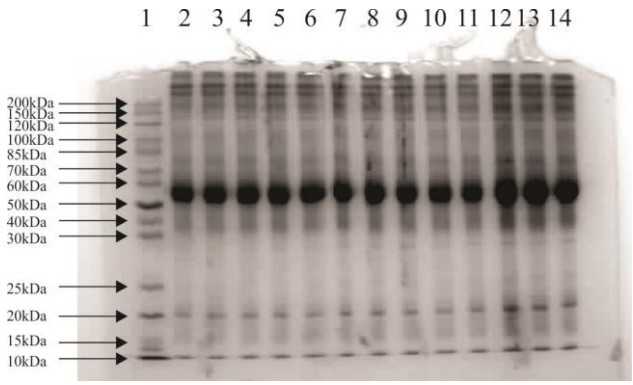

Figure 1: One dimensional protein resolution view of lung cancer serum samples compared to healthy control samples. Lane 1: protein marker (Fermentas, PageRular, SM0661), (10-200kDa) .Lane 2-7: control serum samples, Lane 8-14: patient serum samples (5µg) .

Two dimensional gel electrophoresis

Two dimensional gel electrophoresis was performed for both control and patient sera by loading 500µg total serum protein on 18cm long, non-linear IPG strips with pH range of 3-10 by successive steps of rehydration, isoelectric focusing (first dimension) and 12% SDS-PAGE (second dimension) and gel was run at constant voltage of 160volts. Comparison of control serum gels with those of patient's pointed towards a 28 kDa ,up-regulated in patients compared to controls as shown in (Figure 2A and 2 B).

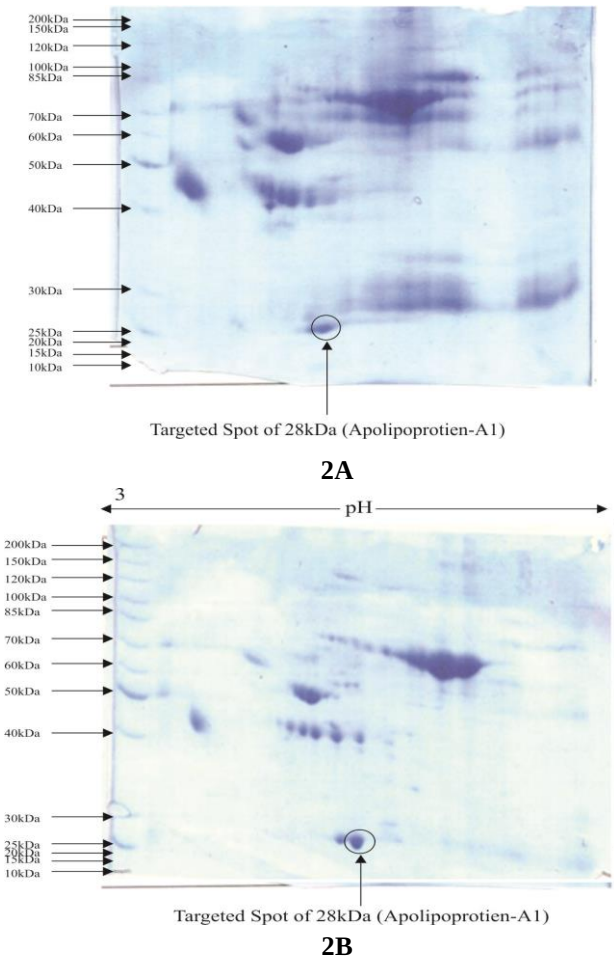

Figure 2:Two dimensional gel electrophoresis resolution of 500µg serum proteins.2A : 2D gel view of lung cancer control serum revealing 28.06kDa protein spot with isoelectric point 5.27, overexpressed versus control sample.. 2B: 2D gel view of lung cancer serum sample. These spots was excised followed by tryptic digestion and processed to peptide mass fingerprinting for absolute protein identity 5µl of unstained protein standard marker (Fermentas, PageRular, SM0661) ranging from (10kDa-200kDa) is on the left sides of gels.

These protein spots (encircled in gels 2A and 2B) were digested to respective constituent peptides with trypsin enzyme and extracted peptides were subjected to peptide mass fingerprinting processing.

Peptide mass fingerprinting and protein identification

In-gel digested peptide extract was applied to Maldi plate and analysis was accomplished following above mentioned conditions. Mascot database results revealed the protein to be Apolipoprotein-A1 precursor by subscribing the analyzed peak data at 149ppm with significant score of 117 and sequence coverage status as 56% and accession no CAA00975. Molecular weight of identified protein was found to be 28.061kDa with isoelectric point of 5.27. The spectra indicating the identified peaks of captured peptides have been shown in (Figure 3) and matched peptides layout has been given in (Figure 4). Protein database used for protein identification was Swiss Prot.

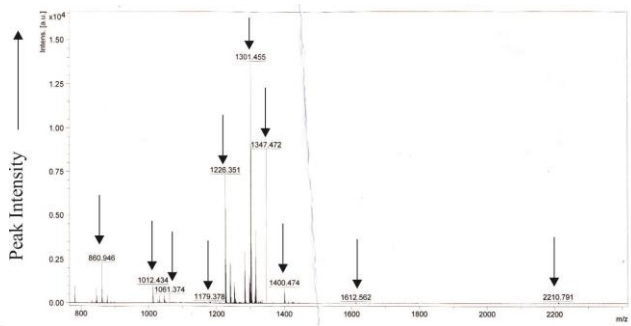

Figure 3: Peptide mass fingerprinting presentation of Apolipoprotein-A1 spectra. Pointed arrows are indicating the identified m/z values of in-gel digested peptides. Resulting peak data was searched against Mascot database for characterization of targeted protein identified as Apolipoprotein -A1.

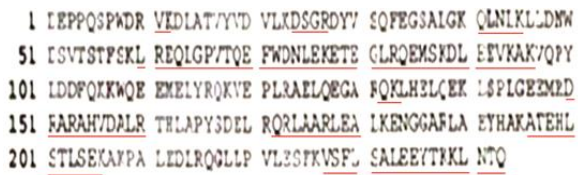

Figure 4: Peptide sequence illustration of Apolipoprotein-A1 representing matched (underlined) and unmached peptides .

Electro-elusion and immunization procedures

As Apolipoprotein A-1 was selected for further immunological screening and validation, maximum amount of this protein was collected by resolving serum samples on 15% SDS- PAGE and electro-eluting the protein by above mentioned protocols. Resulting purified protein bands as shown in (Figure 5) were dried and reconstituted in PBS for preparation of antigenic dose to be injected in 4 weeks old male Albino mice as described earlier and anti-sera was collected and purified using IgG purification protein A column kit according to the manufacturer's instructions.

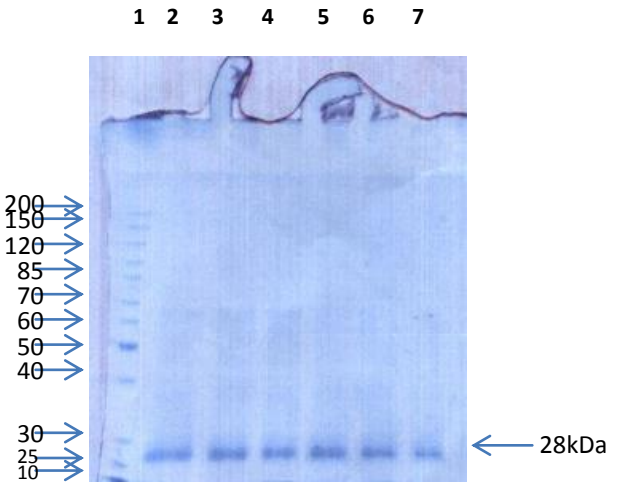

Figure 5: Electro-elusion of Apolipoprotein-A1 from 15% SDS-gel. Lane 1: Standard protein marker (Fermentas, PageRular Cat# SM0661), Lane 2-7 : Eluted and purified protein bands of Apo-A1.

Western blotting

Serum samples of patients' and controls were resolved on 15% SDS-gel by loading 10µg of total proteins following protein transfer on 0.22µm nitrocellulose membrane film (G-Biosciences, Cat# 786-018NC) and blot was processed as stated above and respective bands of 28kDa protein were observed as shown in (Figure 6). Band intensity in patient samples was found to more noticeable as compared to controls giving clue that this protein is up-regulated during the course of lung cancer.

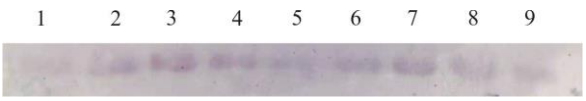

Figure 6: Western blot validation of Apolipoprotein -A1. Serum samples of lung cancer subjects and healthy controls (10µg total proteins) were separated on 15% SDS–gel, electrophoretically transfered to nitrocellulose membranes (0.22µm), blotted with mouse polyclonal anti-Apo-A1primary antibody and detected with AP-substrate. Lane 1: Healthy control serum samples, Lane 2-9: Lung cancer patient samples, significant up-regulation was observed at 28.06kDa, in samples in these samples, authenticating Apo-A1 as lung cancer differentially existing protein compared to healthy control sample expression displaying low band density.

DISCUSSION

Lung cancer prevention is a thoughtful global task. Evidently, productive lung cancer eradication will be resulted by decreasing the possible threat elements associated with improved detection approaches and prompt assessments [14]. In present study, we have described the identification, proteomic characterization and validation of Apolipoprotein A-1 in lung cancer serum samples (n=100) compared to healthy controls (n=50). Experimental procedures involved one dimensional SDS-PAGE of patients and control serum samples at first step. Differentially expressed protein bands were remarked leading to two dimensional gel electrophoresis for improved resolution and characterization of proteins versus both parameters of isoelectric point on X-axis and molecular weight on Y-axis. Comparison of two dimensional gel images of both lung cancer patients and control subjects pointed out an overexpressed protein spot of approximately 28kDa focused at 5.27 isoelectric point. This protein was chosen for further screening procedures (in-gel digestion, Maldi-Tof-Tof, electro-elusion, antisera generation and final validation by western blotting for complete interpretation of suggested opinion. Apolipoprotein-A1 is a principle part of high-density lipoproteins (HDL) in human plasma, responsible for cholesterol transport from the tissues to the liver[15]. Normal levels of Apolipoprotein -A1 in human plasma ranges between 90 and 130mg/dl[16]. Apolipoprotein A-1 has only one polymeric chain having 243 monomeric amino acids, resulting from Apo- A1 precursor. Its gene is found to be present on chromosome 11 adjacent to the the genes encoding Apo- C-111 and Apo- A –IV[8].

Investigative significance of Apo-A1 in early detection of cancers has been indicated in past research studies, showing elevation of Apo-A1 in vast range of malignancies[17]. Giusti et al[18] indicated the overexpression of Apo-A1 precursor in thyroid cancer proposing that it might be a prospective biomarker protein for analytical taxonomy of the disease and results were verified by comparison of expression of Apo-A1, in control and patient's urine samples by western blotting . Topete et al[19] reported two isoforms of Apo- A1 of 28kDa and isoelectric point range of 5.2-7.8, by mass spectrometric analysis by analysing serum proteome changes in acromegalic patients after transphenoidal surgery .Up till now, 9 isoforms of Apo-A-1 in molecular weight range of ~7.49–23.45kDa and isoelectric point ~4.79–7.27 have been reported. Down-regulation of Apo A-1 in breast cancer has been reported by Huang et al[20]. Apo-A1 has been recognized as a prospective biomarker protein of colorectal, ovarian and pancreatic cancers resulted from mass spectrometric procedures. In contrast to these studies, up-regulation of Apo-A1 has also been reported in a wide range of malignancies of liver, breast and ovary[17, 18].

Maciel et al[21] observed down regulation of Apo-A1 in pulmonary adenocarcinoma by 2-DE coupled to MALDI-TOF peptide mass fingerprinting and currently recognized as a new biomarker for pulmonary adenocarcinoma. Zhang et al[22] reported an up-regulation of Apo-A1 in lung cancer tissues based upon the similar pathway of detecting differentially

expressed proteins. Our results also showed an elevated pattern of protein spot of Apolipoprotein A-1 in squamous lung cancer sera compared to healthy control.

Li et al[23] detected up-regulation of a set of 5 proteins, comprising of fibrinogen, lactate dehydrogenase B, apolipoprotein-A1, clusterin and haptoglobin which were found to be overly expressed in low malignant or aggressive bladder cancer patient's urine specimens. Additional evaluation procedures of urine samples of aggressive BTCC illustrated remarkable rise in Apo-A1 expression compared to low malignant BTCC. Quantitative Elisa studies of Apo-A1 provided clinical efficacy to differentiate bladder cancer patients from controls at 18.22 ng/ml and discriminate low malignant BTCC cases from those with aggressive BTCC at 29.86 ng/ml respectively.

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