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## **Production of Monospecific Anti Alpha-1-Antitrypsin and Its Efficiency in Diagnosis of Human Pulmonary Emphysema**

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**Abstract:** The present study deals with the preparation of pure alpha-1-antitrypsin (AAT) protein and its corresponding antibody in albino rabbits. This antibody was found very useful in the immuno-diagnosis of human pulmonary emphysema. This study also has been concerned with the biochemical changes associated with AAT deficiency in pulmonary emphysema. To fulfill this work, pure AAT antigen was separated from blood of healthy persons. The AAT was used for the preparation of anti-AAT, the purity and potency of antibody was checked by titration methods. The biochemical changes were studied in three groups clinically divided to control, heavy cigarette smokers with pulmonary emphysema and non-smoking subjects with pulmonary emphysema. The results of this study revealed that the level of serum AAT and its tryptic inhibitory capacity (TIC) showed very highly significantly decreased level in both smokers and non-smokers as compared to control, while the elastase activity and hydroxyproline (HP) level in Bronchoalveolar lavage (BAL) as a marker of elastin and collagen breakdown were significantly increased in these patients. Moreover the level of serum ceruloplasmin and transferrin as antioxidant substances; IgA level as predominant immunoglobulins responsible for local immune response in respiratory tract; As well as thiobarbituric acid reactive substances (TBARS) as a direct indicator of oxidative stress showed significantly increased level in such patients as compared to control. In conclusion, the preparation of pure AAT and the production of its corresponding anti-human AAT on the local level is less expensive and less time consuming than the commercial anti-AAT and can be useful in immuno-diagnosis and prognosis of pulmonary emphysema. Also it is clear from the foregoing findings that emphysema especially in smoking subjects would affect on other biochemical parameters.

**Key words:** Alpha-1-antitrypsin deficiency, emphysema, cigarette smoke, elastase activity, hydroxyproline, oxidative stress, liver

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### **INTRODUCTION**

Emphysema is a world wide problem (Lomas and Silverman, 2001), defined as a chronic pathologic condition of the lung that is characterized by destruction of the alveolar walls, with subsequent abnormal permanent enlargement of the respiratory air spaces distal to the terminal bronchiole and it appears as a non uniformity in the pattern of air space enlargement, with irreversible loss of the lung parenchyma (Ofulue and Mary, 1999). Emphysematous patients are at increased risk of contracting recurrent respiratory infections and lung cancer. Also they are at high risk for respiratory

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and coronary failure and often emphysema leads to failure of the right ventricle (Avelar *et al.*, 2005). The association between alpha-1-antitrypsin (AAT) and lung emphysema was first described by Laurel and Eriksson (1963) who observed a link between a reduction in size of  $\alpha_1$ -globulin in electrophoretic analysis of plasma protein.

Many plasma protein components are important for the identification of many metabolic processes and may be helpful in the diagnosis of many human diseases (O'connell *et al.*, 2005). Among these proteins alpha-1-antitrypsin which consists of a single polypeptide chain and three N-linked carbohydrate chains, giving an overall molecular mass of about 52 kDa and is mainly synthesized in the liver and serve as inhibitor for serine proteases, which inhibit neutrophil elastase (Kwon *et al.*, 1995). As serine proteases capable of destroying most connective tissue components. AAT interacts with the enzyme through its Met 358-Ser 359 residues. A deficiency of AAT has been associated with pulmonary emphysema through elastase attacks and damage lung tissue causing potentially fatal emphysema especially among smokers and resulted also in childhood liver cirrhosis (Brantly *et al.*, 1988). The inhalation of environmental pollutants, including tobacco smoke, can lower AAT activity (Insley and Kawasaki, 1987). Human emphysematous condition was found to be relieved by intravenous injection of partially purified AAT (Cabezon *et al.*, 1984).

AAT is currently purified from human blood donor (Chisti, 1998). Antitrypsin purified from blood plasma is commercially sold as Prolastin®. This antigen product was found to be highly expensive when imported from abroad. Patients with hereditary deficiency of AAT are given a dose of 120-180 mg kg<sup>-1</sup> of body weight for treatment every two to three weeks (Nita *et al.*, 2005). Purification of the AAT from blood by previous methods is a complex process requiring removal of albumin by chromatography; ammonium sulfate fractionation; DEAE-cellulose chromatography and ultrafiltration (Pannell *et al.*, 1974).

As antigen-antibody reactions in gel are now widely used in routine as well in research laboratories for the diagnosis of several diseases through qualitative and quantitative changes in the level of individual plasma proteins. This study has been suggested to prepare a highly purified human AAT from the healthy blood donors and its corresponding monospecific antibody from albino rabbits and use this prepared antibody in the diagnosis of pulmonary emphysema. Also this study concerned with evaluation of the biological changes associated with AAT deficiency in pulmonary emphysematic patients.

## MATERIALS AND METHODS

This study was started on July 2004 at biochemistry department, National Research Center, Cairo, Egypt. All chemicals used in this study were of the highest purity and purchased from sigma chemical Co. (St. Louis Mo, USA), while antisera for ceruloplasmin, transferrin and IgA were obtained from Dakopatts Company (Copenhagen Denmark).

### Preparation of Anti-AAT

Blood samples were collected from healthy subjects and allowed to clot at room temperature. Serum was obtained by centrifuging the blood at 3000 rpm for 15 min. Serum protein components were assayed as described by Lowry *et al.* (1951) and then separated on agarose gel electrophoresis as described by Carlström and Johansson (1983). The AAT fractions were eluted from the gel into physiological saline solution. This crude solution was further purified by ion exchange chromatography using DEAE Sephadex A-50 column according to the method reported by Pharmacia (1983), where elution was performed using a gradient 0.2-2 mM tris-HCl buffer (pH 8.0) and with rate of flow 10-12 mL h<sup>-1</sup>, the elute was dialyzed at 4°C against distilled water and the product was tested for impurities by two-dimension electrophoresis and immunoelectrophoresis against whole antihuman

serum as described by Goers (1993a) and Kricka (1994). The purified AAT was then lyophilized weight and stored at -20°C till the time of immunization.

The immunization steps were performed in albino Newzland rabbits (3 kg) as described by Goers (1993a), the obtained antiserum of considerable titre were collected. The purification of the antibody from antiserum was carried out according to the method of Goers (1993b) where the successive precipitations with saturated ammonium sulphate at room temperature are sufficient to yield fairly pure gamaglobulin. The latter is further purified by ion exchange chromatography as described by Pharmacia (1983). The resulting elute fraction was dialysed at 4°C against physiological saline solution, then concentrated and stored at -20°C till time of use. The purified antibody was tested for impurities by immunoelectrophoresis and two-dimension immunoelectrophoresis against standard serum protein as described by Goers (1993a) and Kricka (1994). The antibody titre was estimated by using rocket immunoelectrophoresis as described by Harboe and Ingild (1983).

### **Subjects of Study**

Male subjects suffering from pulmonary emphysema with age range 18-65 years were selected from Chest Hospital, El-Omrana, Giza, Egypt and all cases were investigated clinically by chest radiography, forced expiratory volume in the first second (FEV<sub>1</sub>), forced ventilation capacity (FVC) and FEV<sub>1</sub>/FVC. They were categorized into heavy cigarette smokers with pulmonary emphysema, had smoked 37.5±3.5 cigarettes per day for duration of 27.3±2.8 years; Non-smoking subjects pulmonary emphysema mean age 57.6±2.8 years beside a number of normal healthy subjects with without smoking (mean age 52.2±3.6 years). Smoking index was calculated according to Brinkman index based on the product of the number of cigarettes smoked each day and the number of smoking years. Subjects who had smoking index >400 (smoke 30±8 cigarettes per day for duration >10 years) were considered as heavy smokers (Abdel Meguid *et al.*, 1999).

Blood samples were collected from patients and sera were separated by centrifugation at 3000 rpm for 30 min. Broncho alveolar lavage (BAL) fluid was performed after premedication and lidocaine local anesthesia, using the Fujinon bronchofiberscope (system 2000 fully submersible, fujinon INC Wayne, USA). Approximately 50 mL of sterile physiological saline were used to lavage the middle lobe or the lingual of lung. The lavage fluids were centrifuged at 15,000 x g for 30 min at 4°C, to remove any debris. BAL fluid was then concentrated about 20-30 fold using the freeze dryer. The concentrated BAL fluid aliquoted and frozen at -20°C for later assays.

The level of AAT, ceruloplasmin, transferrin and IgA were quantitatively estimated in serum by using single radial immunodiffusion technique as described by Mancini *et al.* (1965), using the corresponding specific antibodies. Serum tryptic inhibition capacity (TIC), which is functional activity of AAT to inhibit the trypsin action, was measured by enzymatic assay as described by Dietz *et al.* (1974). In brief, AAT inhibit the hydrolysis of  $\alpha$ -N-benzoyl DL-arginine-p-nitroanilide by trypsin. Adding acetic acid stops the reaction and the absorbance was read at 400 nm.

Determination of elastase activity in BAL fluid was measured by colorimetric assay as described by Zay *et al.* (1999), using N-succinyl-trialanyl-p-nitroanilide as substrate. Estimation of the hydroxyproline (HP) level in BAL fluid as a marker of collagen breakdown as described by Selman *et al.* (1996), depending on the oxidation of HP to pyrrole and then pyrrole can be converted into a relatively specific chromophore with p-dimethyl aminobenzaldehyde read at 560 nm. The level of serum Thiobarbituric acid reactive substances (TBARS) was assessed as a direct indicator of lipid peroxidation as described by Conrad *et al.* (2000), using 1, 1, 3, 3-tetraethoxypropane as standard.

### **Statistical Analysis**

Results were expressed as the mean±SE using Microcal Excel™ for windows (Microcal Software, 2000) and statistical differences between groups were assessed by Student's t-test. Values of p<0.05 were considered significantly different.

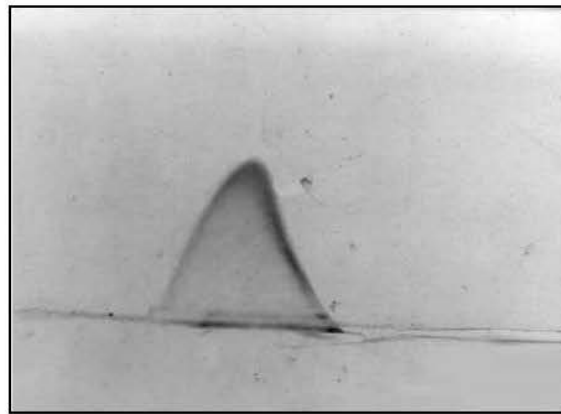
## RESULTS

### Isolation and Purification of AAT

AAT was isolated from pooled normal human serum by simple electrophoresis, followed by purification by ion exchange chromatography. The concentration of AAT after different separation and purification steps was shown in Table 1.

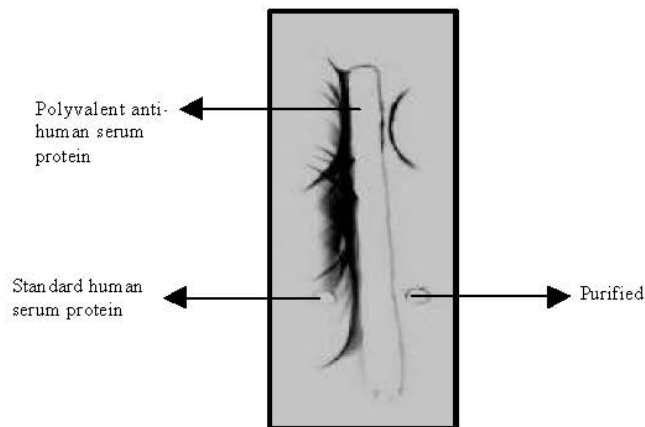
### Detection of AAT Purity

The purity of AAT as antigen was checked by two different methods; Two-dimension electrophoresis and immunoelectrophoresis. Only one peak was obtained for AAT by the two-dimension technique (Fig. 1a), indicates that the antigen is free from any interference. Immunoelectrophoresis technique also showed only one arc of AAT at its expected position compared to standard human serum protein (Fig. 1b).



(a)

Fig. 1a: Only one peak of AAT was obtained by two-dimension electrophoresis, where the gel contains 1.25  $\mu\text{L}$  per  $\text{cm}^2$  of antihuman serum against 5  $\mu\text{L}$  of purified AAT



(b)

Fig. 1b: Only one arc was obtained in position of AAT by immunoelectrophoresis

Table 1: The concentration of AAT during different purification steps

Separation and purification of AAT	AAT (mg per 10 mL)
Initial concentration in serum	20.00
Concentration after simple electrophoresis	16.40 (82)
Concentration after ion exchange chromatography	14.80 (74)
Concentration after lypholyzation	13.90 (70)

The values between brackets represent the percentage of recovery to initial concentration

Table 2: Shows the values of serum AAT, TIC, IgA, TBARS, ceruloplasmin and transferrin, as well as elastase activity and hydroxyproline level in BAL fluid of the pulmonary emphysematic group (nonsmokers or smokers) as compared to the mean normal values

Groups	AAT (mg dL <sup>-1</sup> )	TIC ( $\mu\text{m min}^{-1}\text{ mL}^{-1}$ )	Elastase activity (U mL <sup>-1</sup> )	Hydroxyproline (mg dL <sup>-1</sup> )
Control	246.59±11.41	4.76±0.21	1.06±0.22	1.82±6.13
Pulmonary emphysema nonsmokers	177.52±12.24 (28)	2.50±0.26* (48)	3.19±0.32* (200)	227.55±3.75* (270)
Pulmonary emphysema smokers	143.00±4.86* (41)	1.17±0.15* (75)	7.64±0.91* (600)	283.08±10.88* (350)

Table 2: Continued

Groups	IgA (mg dL <sup>-1</sup> )	TBARS (nm mL <sup>-1</sup> )	Ceruloplasmin (mg dL <sup>-1</sup> )	Transferrin (mg mL <sup>-1</sup> )
Control	233.25±18.75	20.89±1.68	40.22±2.91	187.06±8.73
Pulmonary emphysema nonsmokers	305.93±28.92* (31)	48.00±1.16* (129)	60.69±5.45* (50)	206.46±10.20* (10)
Pulmonary emphysema smokers	352.89±24.75* (51)	62.00±6.38* (197)	83.80±4.96* (108)	220.43±6.74* (18)

The data represented as the mean value±SE of different studied groups. \*Significantly different from the control group at (p<0.05). The values between brackets represent the percentage change from the control

### Evaluation of the Anti Human AAT after Purification

The purification of antibody arised in the rabbits could be detected by two different ways; Two-dimension electrophoresis and immunoelectrophoresis. The two-dimension technique revealed that only one peak was obtained for the antibody indicating that the obtained antibody is free from any other substances as shown in Fig. 2a. Immunoelectrophoresis technique also revealed only one arc of AAT as shown in Fig. 2b.

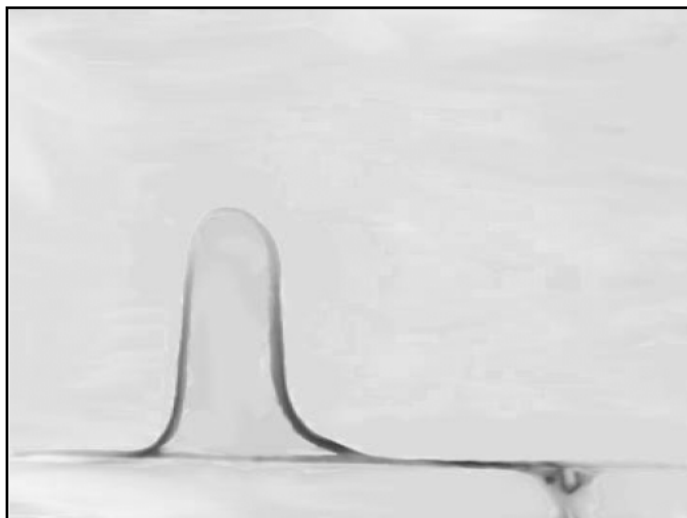
### Titration of Anti-Human AAT

The antibody titre was measured by rocket immunoelectrophoresis as shown in Fig. 2c. The antibody titre of AAT before the purification was found to be 1278  $\mu\text{g mL}^{-1}$  while the titre was 748  $\mu\text{g mL}^{-1}$  after its purification.

### Biochemical Parameters

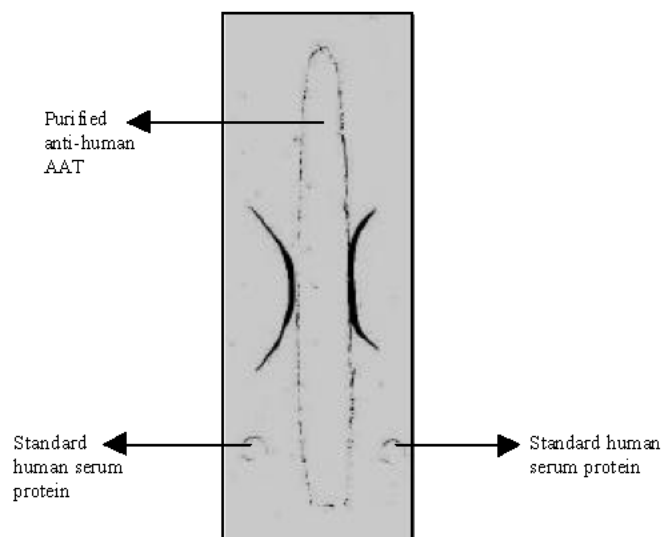
The concentration of AAT and its TIC were measured in normal healthy subjects of the control group and in pulmonary emphysematous patients, either nonsmokers or smokers. Table 2 revealed that the concentration of serum AAT and TIC showed significantly decreased (p<0.05) in emphysematous nonsmokers group and emphysematous smokers as compared with the control group by 28 and 41%, respectively for AAT and by 48 and 75%, respectively for TIC. As shown in Table 2 the level of serum IgA was measured in pulmonary emphysematous patients either nonsmokers or smokers showed very significantly increased (p<0.05) as compared with the control group. The level of serum IgA was higher than the control group by 31 and 51%, respectively. Evaluation of elastase activity and hydroxyproline level in BAL fluid showed significantly increased values (p<0.05) in the group with pulmonary emphysema as compared with control group. The enzyme activity was increased by 200 and 600% in emphysematous nonsmokers and emphysematous smokers, respectively as compared to mean normal values. Additionally, hydroxyproline level was increased by 270 and 350% as compared to mean normal values of the control in both groups, respectively (Table 2). The level of

serum TBARS, ceruloplasmin and transferrin were measured in pulmonary emphysematous patients either nonsmokers or smokers showed very highly significantly increased ( $p < 0.001$ ) as compared with the control group. The level of serum TBARS was higher than the control group by 129 and 197%, respectively. On the other hand the level of serum ceruloplasmin was higher than the control group by 50 and 108%, respectively. In addition, our results revealed that the concentration of serum transferrin was higher than the control group by 10 and 18%, respectively (Table 2).



(a)

Fig. 2a: Only one peak of AAT was obtained by two-dimension electrophoresis, the gel contains  $1.25 \mu\text{L}$  per  $\text{cm}^2$  of the prepared anti-AAT against  $5 \mu\text{L}$  of standard human serum



(b)

Fig. 2b: Only one arc of AAT was obtained in its position by immunoelectrophoresis technique;  $5 \mu\text{L}$  standard human serum protein was placed in two wells against  $150 \mu\text{L}$  purified anti-human AAT

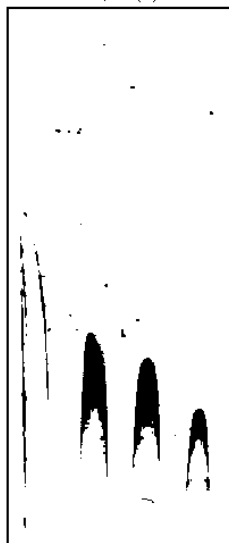


Fig. 2c: Rocket immunoelectrophoresis of the purified anti-human AAT. Gel contains 1.5  $\mu\text{L cm}^2$  of the prepared anti-AAT against different dilutions of the standard human serum protein (1 + 150, 1 + 250, 1 + 400, 1 + 500)

## DISCUSSION

Many plasma proteins can be serving as important indices in clinical laboratories for the identification of diseases. This may give the impetus to elaborate procedures for the production of some specific antibodies against individual proteins that would be help in their detection by immunochemical techniques (O'connell *et al.*, 2005). The protein to be used as immunogen must be as pure as possible. Separation of serum proteins on gel electrophoresis was found as the simplest powerful tools available for the separation of individual proteins from various body fluids (Wells and Weil, 2003).

From our data, it has been found that the percentage recovery of AAT was 82% as separated on agarose gel electrophoresis. Anion exchange chromatography on DEAE have been widely used for the fractionation of serum proteins due to high efficiency of desalting, high reliability and high protein recovery (Pharmacia, 1983). The recovery percentage of AAT after electrophoresis on agarose gel followed by anion exchange chromatography on DEAE Sephadex-A50 was amounted to 74%, whereas the yield after complete purification was found to be about 70% of recovery. The pure antigen was injection in rabbits to produce the antibody (Meager, 1994). The monospecific antiserum of AAT was further purified by using the successive procedure of ammonium sulphate precipitation followed by ion exchange chromatography on DEAE Sephadex-A50. The potency of anti-AAT as titrated by rocket immunoelectrophoresis before and after antibody purification was 1279 and 748  $\mu\text{g mL}^{-1}$ , respectively. Anti-AAT was then used as an important diagnostic test for patients suffering from pulmonary emphysema.

Furthermore, the association between deficiency of serum AAT and pulmonary emphysema represent the major medical problem where the rate of progression in emphysema is inversely proportional to the AAT level (Gottlieb *et al.*, 2000). In our data the analysis of serum AAT in patients suffering from pulmonary emphysema showed that 28 and 41% decreased in the level of AAT in both non smokers and smokers as compared to control. The level of AAT in smokers was markedly less than nonsmokers. This fact coincides with Calabrese *et al.* (2005) who proposed that cigarette



smoking might lead to an inactivation of AAT in the lungs leaving the lower respiratory tract without its normal anti-elastase defense. Moreover Gadek *et al.* (1979) also reported that antiprotease activity in smokers was about half that of nonsmokers. The likely explanation of this is that cigarette smoke easily oxidizes the methionine residue, which is the active site of AAT that combines with the serine residue of neutrophil elastase (Mastrangeli and Crystal, 1996). Also, the function of AAT as expressed by TIC was assayed and the data revealed that there is a markedly significant decreased 48 and 75%, respectively, in TIC values in both nonsmoker and smoker groups as compared to normal. It is obviously that values for TIC in smoking subjects are markedly lower than that in nonsmokers, due to smoke contributes to inactivation of AAT and thereby decreasing its inhibitory capacity (Sveger *et al.*, 1994).

Selman *et al.* (1996) studied hypothesis concerning the pathogenesis of human pulmonary emphysema and reported that emphysema results from proteolytic lung injury caused by an imbalance between proteases and antiproteases in the lower respiratory tract particularly elastase. Normally neutrophil elastase is regulated by the plasma antiproteinase AAT by rapidly trapping and destroying elastase before damaging the lungs (Le *et al.*, 1992). Regarding to our data, it can be suggested that the elevation of elastase activity in comparison to normal may be attributed to the deficiency of AAT in both emphysematous groups. Perlmutter *et al.* (1989) confirmed that patients suffering from AAT deficiency have no alternative anti-elastase in their lower respiratory tract, so that neutrophil elastase is free to degrade alveolar structures. In addition smoke can debris the function of elastase inhibitors and could increase the binding of elastase to elastin (Li *et al.*, 2003), by increasing the elastase burden in the lower respiratory tract and stimulating alveolar macrophages to produce neutrophil chemoattractants (Sveger *et al.*, 1994), this in turn increase the recruitment of elastase-containing neutrophils into the alveolar interstitium (Zay *et al.*, 1999).

Foronjy and D' Arminto (2001) had emerged to implicate collagenase and collagen breakdown in the destruction of lung tissue in emphysema. The presence of collagen adds tensile strength to the lung tissue and allows the lung maintain the structure needed to carry out the physiologic function of gas exchange. Christner *et al.* (1985) reported that degradation of fibrillar collagen by collagenase may upset the balance of forces in the lung and leading to emphysema and they also judged this degradation by increased level of hydroxyproline in BAL fluid. In present study, collagen breakdown was increased 4 and 5-folds comparing to control in non-smokers and smokers, respectively. Such excessive collagenolytic activity may cause a direct effect on hydrolyzing and inactivating AAT. Emphysematic smokers have shown that hydroxyproline was slightly exceeded than nonsmokers in BAL fluid, this finding have been also coincides the observation reported by Churg *et al.* (2003) who mentioned that smoking induce much elevations in the matrix breakdown.

Since reactive oxygen species are produced intracellularly by lung parenchymal cells and extracellularly by lung macrophages as well as by infiltrating neutrophils, or originate exogenously as components of tobacco smoke, they have the ability to alter all principal components of the cell including nucleotides in DNA and polyunsaturated fatty acids in cellular membranes (Rahman and Macnee, 1996). In the present study serum TBARS revealed that very highly significant increased values were obtained in nonsmoking and smoking patients and indicating that smokers with emphysema are more susceptible to oxidative stress than nonsmokers.

The oxidants can further mediate functional inactivation of AAT causing the alveolar wall highly vulnerable to elastolytic attack, with a complete destruction of the interstitial connective tissue (Campbell *et al.*, 1999). As BAL fluid contains significant concentrations of enzymatic and non-enzymatic antioxidants (Bell *et al.*, 1981). In present study ceruloplasmin and transferrin, the major serum inhibitors of lipid peroxidation were showed higher values ceruloplasmin level was increased by 50 and 108% than the normal in non-smokers and smokers while transferrin level was 10 and 18% increased over the control, respectively. It can be postulated that this increased level of serum

ceruloplasmin and transferrin may help for protecting lung from oxidative injury associated with the increased lung oxidant burden in patients with pulmonary emphysema. But, in fact this increase could not completely compensate for the increased oxidative stress. The significantly high level of serum ceruloplasmin levels in smokers may be attributed to its acute phase reactant property (Kricka, 1994). Moreover, Galdstone *et al.* (1984) showed that smoking can cause partial inactivation of serum antioxidant activity accompanied by insufficient compensatory increase in ceruloplasmin. Otherwise, it has been found that ceruloplasmin and transferrin as antioxidant were significantly increased in emphysema to neutralize the effect of oxygen radicals release by neutrophils. In addition, ceruloplasmin and transferrin act coordinately in extracellular defense against the damaging effects of  $Fe^{2+}$ , through catalysis of  $Fe^{2+}$  oxidation to  $Fe^{3+}$  by ceruloplasmin followed by strong binding of  $Fe^{3+}$  to transferrin. However, this protective mechanism may be disturbed in the lungs of emphysema patients, since cigarette smoke and oxidants can release iron from iron-binding proteins (Repine *et al.*, 1997).

In present result serum IgA was significantly increased in both groups with emphysema. Silverman and Christenson (1994) reported that, the increases of serum immunoglobulins are the normal response to infections, but IgA tends to predominate in respiratory infections. In lung diseased patient's colonization of the airways by bacteria that stimulate phagocytic cell oxidant production (Sethi, 2000). Bacterial adherence may favor bacterial persistence and colonization of the respiratory tract associated with induction of inflammation and the elevation of IgA, demonstrating an active local immune response (Repine *et al.*, 1997).

In conclusion, the preparation of pure AAT and the production of its corresponding anti-human AAT on the local level is less expensive and less time consuming than the commercial anti-AAT and can be useful in immuno-diagnosis and prognosis of pulmonary emphysema. Also it is clear from the foregoing findings that emphysema especially in smoking subjects would affect on other biochemical parameters.

## REFERENCES

- Abdel Meguid, A.M., R.M.A. Meki, A.A. Fahmy and D.S. Sayed, 1999. Cotinine concentration in seminal plasma and its influence on sperm parameters, free radicals and antioxidants. *Egypt J. Derm. Ven.*, 19: 69-80.
- Avelar, E., T. Jalili, L. Dong, J. Arvizo, P. Hu, S.E. Litwin and J.P. Mattson, 2005. PKC translocation and ERK1/2 activation in compensated right ventricular hypertrophy secondary to chronic emphysema. *Biol. Med. Centre Physiol.*, 5: 6-17.
- Bell, D.Y., J.A. Haseman, A. Spock, G. McLennan and G.E. Hook, 1981. Plasma proteins of the bronchoalveolar surface of the lungs of smokers and non-smokers. *Am. Rev. Respir. Dis.*, 124: 72-79.
- Brantly, M., T. Nukiwa and R.G. Crystal, 1988. Molecular basis of alpha-1-antitrypsin deficiency. *Am. J. Med.*, 84: 13-31.
- Cabezón, T., M. DeWilde, P. Herion, R. Lorian and A. Bollen, 1984. Expression of human  $\alpha$ 1-antitrypsin cDNA in the yeast *Saccharomyces cerevisiae*. *Proc. Nat. Acad. Sci. USA. Biochem.*, 81: 6594-6598.
- Calibrese, F., C. Giacometti, B. Beghe, F. Rea, M. Loy, R. Zuin, G. Marulli, S. Baraldo, M. Saetta and M. Valente, 2005. Marked alveolar apoptosis/proliferation imbalance in end-stage emphysema. *Respir. Res.*, 6: 14-26.
- Campbell, E.J., M.A. Campbell and S.S. Boukedes, 1999. Quantum proteolysis by neutrophils: Implications for pulmonary emphysema in alpha-1-antitrypsin deficiency. *J. Clin. Invest.*, 104: 337-344.

- Carlström, A. and B.G. Johansson, 1983. Agarose gel electrophoresis-immunofixation. *Scand. J. Immunol.*, 17: 23-32.
- Chisti, Y., 1998. Strategies in Downstream Processing. In: *Bioseparation and Bioprocessing*. Subramanian, G. (Ed.), Wiley-VCH. New York, pp: 3-30.
- Christner, P., A. Fein, S. Goldberg, M. Lippmann, W. Abrams and G. Winbaum, 1985. Collagenase in the lower respiratory tract of patients with adult respiratory distress syndrome. *Am. Rev. Respir. Dis.*, 131: 690-695.
- Churg, A., R.D. Wang, A. Xie and J.L. Wright, 2003. Alpha-1-antitrypsin ameliorates cigarette smoke induced emphysema in the mouse. *Am. J. Respir. Crit. Care Med.*, 168: 199-207.
- Conrad, C.C., D.T. Grabowski, C.A. Walter, M. Sabia and A. Richardson, 2000. Using MT<sup>-/-</sup> to study metallothionein and oxidative stress. *Free Rad. Biol. Med.*, 28: 447-462.
- Dietz, A.A., H.M. Rubinstein and L.V. Hoges, 1974. Measurement of alpha-1-antitrypsin in serum by immunodiffusion and by enzymatic assay. *Clin. Chem.*, 20: 396-399.
- Foronjy, R. and J. D' Arminto, 2001. The role of collagenase in emphysema. *Respir. Dis.*, 2: 348-352.
- Gadek, J.E., G.A. Fells and R.G. Crystal, 1979. Cigarette smoking induces functional antiprotease deficiency in the lower respiratory tract of humans. *Science*, 206: 1315-1316.
- Galdstone, M., V. Levytska, M.S. Schwartz and B. Magnússon, 1984. Ceruloplasmin: Increased serum concentration and impaired antioxidant activity in cigarette smokers and ability to prevent suppression of elastase inhibitory capacity of alpha-1-proteinase inhibitor. *Am. Rev. Respir. Dis.*, 129: 258-263.
- Goers, J., 1993a. Immunization procedure. In: *Immunochemical Techniques Laboratory Manual*, 1st Edn., Academic press, New York, pp: 175-178.
- Goers, J., 1993b. Purification of IgG by ammonium sulphate precipitation. In: *Immunochemical Techniques Laboratory Manual*, 1st Edn., Academic Press, New York, pp: 15-17.
- Gottlieb, D.J., M. Luisetti, P.J. Stone, L. Allegra, J. Cantey-Kiser, C. Grassi and G.L. Snider, 2000. Short-term supplementation therapy does not affect elastin degradation in severe  $\alpha_1$ -antitrypsin deficiency. *Am. J. Respir. Crit. Care Med.*, 162: 2069-2072.
- Harboe, N.H. and A. Ingild, 1983. Immunization, isolation of immunoglobulins and antibody titre determination. *Scand. J. Immunol.*, 17: 345-351.
- Innsley, M.Y. and G. Kawasaki, 1987. Site-specific mutagenesis in  $\alpha_1$ -antitrypsin. US patent, pp: 711- 848.
- Kricka, J., 1994. Principle of Immunochemical Techniques. In: *Teitz Textbook of Clinical Chemistry*. Burtis, C.A. and E.R. Ashwood (Eds.), 2nd Edn., W.B. Saunders Company, Philadelphia, pp: 1974-2005.
- Kwon, K.S., M. Song and M.H. Yu, 1995. Purification and characterization of  $\alpha_1$ -antitrypsin secreted by recombinant yeast *Saccharomyces diastaticus*. *J. Biotechnol.*, 42: 191-195.
- Laurel, C.B. and S. Eriksson, 1963. Globulin pattern of serum in alpha-1-antitrypsin deficiency. *Scand. J. Clin. Lab. Invest.*, 15: 132-138.
- Le, A., G.A. Ferrell, D.S. Dishon, Q. Le and R.N. Sifers, 1992. Soluble aggregates of the human PIZ alpha-1-antitrypsin variant are degraded within the endoplasmic reticulum by a mechanism sensitive to inhibitors of protein synthesis. *J. Biol. Chem.*, 267: 1072-1080.
- Li, T., A. Molteni, P. Latkovich, W. Castellani and R.C. Baybutt, 2003. Vitamin A depletion induced by cigarette smoke is associated with the development of Emphysema in rats. *J. Nutr.*, 133: 2629-2634.
- Lomas, D.A. and E.K. Silverman, 2001. The genetics of chronic obstructive pulmonary disease. *Respir. Res.*, 2: 20-26.
- Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall, 1951. Protein measurement with the folin phenol reagent. *J. Biol. Chem.*, 193: 265-275.

- Mancini, G.A., A.O. Carbonara and J.F. Heremans, 1965. Immunochemical quantitation of antigens by single radial immunodiffusion. *Immunochemistry*, 2: 235-238.
- Mastrangeli, A. and R.G. Crystal, 1996. Alpha-1-antitrypsin Deficiency. In: Alpha-1-antitrypsin Deficiency. Crystal, R.G. (Ed.), 1st Edn., Marcel Dekker, New York, pp: 3-13.
- Meager, A., 1994. Antibodies. In: *Immunochemistry*, 1st Edn., Andrew's Books, San Diego, pp: 106-110.
- Nita, I., C. Hollander, U. Westin and S.M. Janciauskiene, 2005. Prolastin, a pharmaceutical preparation of purified human  $\alpha$ 1-antitrypsin, blocks endotoxin-mediated cytokine release. *Respir. Res.*, 6: 12-16.
- O'connell, T.X., T.J. Horita and B. Kasravi, 2005. Understanding and interpreting serum protein electrophoresis. *Am. Fam. Physician*, 71: 105-112.
- Ofulue, A.F. and K.O. Mary, 1999. Effects of depletion of neutrophils or macrophages on development of cigarette smoke-induced emphysema. *Am. J. Physiol.*, 271: 97-105.
- Pannell, R., D. Johnson and J. Travis, 1974. Isolation and properties of human plasma  $\alpha$ 1-proteinase inhibitor. *Biochemistry*, 13: 5439-5445.
- Perlmutter, D.H., M.N. Schlesinger, J.A. Pierce, P.I. Punsal and A.L. Schwartz, 1989. Synthesis of stress proteins is increased in individuals with homozygous PIZZ  $\alpha$ -1-antitrypsin deficiency and liver disease. *J. Clin. Invest.*, 84: 1555-1561.
- Pharmacia, 1983. Ion exchange chromatography. Principle and Methods, Pharmacia, Laboratory Separation Division. Sweden, pp: 3-27.
- Rahman, I. and W. Macnee, 1996. Role of oxidants in smoking-induced lung disease. *Free Rad. Biol. Med. USA.*, 21: 669-681.
- Repine, J.E., A.L.T. Bast and I.D.A. Lankhorst, 1997. Oxidative stress in chronic obstructive pulmonary disease. *Am. J. Respir. Crit. Care. Med.*, 156: 341-357.
- Selman, M., M. Montaña, C. Ramos, B. Vanda, C. Becerril, J. Delgado, R. Barrios and A. Pardo, 1996. Tobacco smoke-induced lung emphysema in guinea pigs is associated with increased interstitial collagenase. *Am. J. Physiol.*, 271: L734-L743.
- Sethi, S., 2000. Bacterial infection and the pathogenesis of COPD. *Chest*, 117: 286S-291S.
- Silverman, I. and M. Christenson, 1994. Proteins of Body Fluid. In: *Teitz Book of Clinical Chemistry*. Burtis, C.A. and R.A. Edward (Eds.), 2nd Edn., W.B. Saunders Company, Philadelphia, pp: 671-734.
- Sveger, T., M. Piitulainen and J. Arborelius, 1994. Lung function in adolescents with alpha-1-antitrypsin deficiency. *Acta Paediatr.*, 83: 1170-1173.
- Wells, D.A. and D.A. Weil, 2003. Directions in automated sample preparation of proteins. *Pharma Genomics*, 21: 522-538.
- Zay, K., S. Loo, C. Xie, D.V. Devine, J. Wright and A. Churg, 1999. Role of neutrophils and alpha-1-antitrypsin in coal- and silica-induced connective tissue breakdown. *Am. J. Physiol.*, 276: L269-L279.