

**Original Article****Effects of cigarette smoking on serum proteins profile in male active and passive smokers**

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*Department of Zoology, University of the Punjab, Lahore, Pakistan***Article history****Received:** March 28, 2017**Revised:** December 10, 2017**Accepted:** December 11, 2017**Authors' Contribution****NR:** Supervise the research;  
**M:** contributed in analytical work, data, interpretation and drafting of article, **SA:** contributed in collection and processing of data**Key words**Albumin  
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Cigarette smokers**Abstract**

The present study deals with the analysis of serum total proteins and fractions (albumin, total globulins, gamma globulins and non-gamma globulins) of active and passive smokers. Blood samples of 180 cigarette smokers were collected from different locations of Lahore and 60 healthy non-smokers from University of the Punjab, Quaid-e-Azam campus Lahore, in terms of comparable age, height, weight and socioeconomic set up. Serum protein profile was analyzed using commercially available kits by using automated clinical chemistry analyzer. Serum total proteins have shown a significant decrease in smokers in comparison with controls. A significant depression in serum albumin was observed in smokers due to increased excretion of albumin in bound state with the chemicals, whereas, total globulins, gamma globulins and non-gamma globulin serum proteins have shown significant increase in smokers. Our findings provide primary information about fractions and total serum proteins of cigarette smokers. The decrease in albumin justifies the reduction in total serum proteins and elevation in globulins. Smoking cessation plays a significant role in recovery of these proteins.

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**INTRODUCTION**

Cigarette smoking is an important risk factor for coronary, peripheral and atherosclerotic vascular disease. It alters the process of cell division in cardiac muscles. Additionally there is dose dependent response between the quantity of cigarettes smoked, cardiovascular morbidity and fatality. Smoking increases absorption and inhalation of numerous detrimental metabolites and compounds (Qulander *et al.*, 2012). Though tobacco has detrimental effects on the human health, it is still highly consumed globally (Benowitz *et al.*, 1988). It has been considered as causative agent of lethal diseases such as cancer, respiratory disorders, variety of infections and heart diseases (Zhong *et al.*, 2008; Mehta *et al.*, 2008). Many biochemical parameters and body systems are affected by cigarette smoke. Moreover, cigarette smoke contains

approximately 4000 compounds, 200 of which are toxicants and 80 are known carcinogens. Cigarette smoke includes large quantities of oxidants, free radicals that generates oxidative stress and polycyclic aromatic hydrocarbons that are highly lipid soluble (Carel and Eviatar, 1985; Abel *et al.*, 2005; De Heens *et al.*, 2008). These chemicals tend to accumulate in the liver progressively (Osman *et al.*, 2017).

Chemicals in cigarette smoke have deleterious effect on liver tissues, therefore, altering some of the serum biochemical parameters related to liver efficiency (Abdul-Razaq and Ahmed, 2013). The risk of death in smokers vary on the basis of number of cigarettes smoked per day and duration of smoking (Janson *et al.*, 2001; Lubin *et al.*, 2007). Proteins are the most important compounds that are present in human serum abundantly and play a key role for the maintenance of subtle acid-alkaline balance of the body. In addition, proteins are source of

energy for the tissues and muscles when adequate amount of carbohydrate is not ingested. The human serum protein level shows a significant decrease in smokers (Alsalhen and Abdalsalam, 2014). Serum total protein contains albumin and globulin (DeGowin *et al.*, 2004). The quantitative determination is usually done by automated analyzer (Gates and Fink, 2008). In human serum, about 50-60% of proteins are represented by human serum albumin (Zunszain *et al.*, 2003). Through numerous studies, remarkable reduction in serum albumin level has been detected in smokers. Globulins are heterogeneous group of protein and second to albumin on the basis of available amount in serum with the solubility rate lower than the albumin (Marieb and Hoehn, 2010). Globulins acts to compensate the reduced level of albumin in the serum by increased production of immunoglobulins by the lymphocytes and failure of these cells to synthesize globulins leads to advanced diseases that have been involved in reduced protein synthesis and accelerates the catabolism of proteins (Pisters and Brennan, 1990; Inui, 2002).

Cigarette smoke affects the organs, even those not in direct contact with the smoke and it alters the enzymatic activity of the organs playing an important role in anabolic metabolism of proteins (Abdul-Razaq and Ahmed, 2013). Cigarette smoke contains numerous oxidants and prooxidants that are capable of producing free radicals by neutrophils and generates oxidative stress in the body which in turn produces highly reactive oxygen and nitrogen species (Cedergren *et al.*, 2007; Arinola, 2011). Once formed, these oxidizing species are eliminated by the body's antioxidants like Super oxide dismutase (SOD), Glutathione (GSH), Glutathione peroxidase (GSH-Px) and catalase (Valko *et al.*, 2007; Seven *et al.*, 2008). When free radicals are overproduced, the level of antioxidants begins to reduce and damage biomolecules such as lipids, proteins and DNA (Seven *et al.*, 2008).

Albumin, one of the well-known acute phase protein, has antioxidant properties and it can be considered as a marker of inflammation (Pongpaew *et al.*, 2001).

Globulins are antibodies that are produced by the lymphocytes. Free radicals in cigarettes smoke enhance the production of globulins that help the body to fight against the oxidative stress. Gamma globulins are the most important globulins, synthesized by the B lymphocytes or plasma cells and perform a

number of enzymatic functions in the plasma in addition to natural and acquired immunity against the invading organisms (Al-Bayatee and Shanawa, 2014).

The current study was conducted to assess and evaluate the effect of cigarette smoke on human serum protein.

## MATERIALS AND METHODS

### Subjects

Protein analyses were carried out on serum samples from 240 male subjects (age: 30-50) classified into four groups with 60 subjects in each group. Subjects were categorized according to the number of smoked cigarettes per day into three smokers groups (passive, moderate and heavy smokers, 60 subjects each) and non-smokers (control group, 60 subjects). A proforma was designed to obtain a detailed clinical history for each subject of study. Each subject provided the details of his age, blood pressure, body weight, body height and other physical measurements. Participants were selected on the basis of their clinical history, smoking status and number of consumed cigarettes per day. The exclusion criteria for the study include existing physical diseases like hepatitis, cancer, autoimmune disease, cardiovascular disorders, diabetes *etc.* Sixty healthy non-smokers volunteered were selected as control group. Body mass index (BMI) was measured using the following formula:

$$\text{BMI (Kg/m}^2\text{)} = \text{Weight (Kg)} / (\text{Height (m)})^2$$

### Experimental design

Whole blood was collected in a test tube and allowed to clot by leaving it undisturbed at room temperature for 15-30 minutes. Clot was removed by centrifuging at 3000 rpm for 15 minutes in a refrigerated centrifuge. All the samples were analyzed for albumin and total proteins using Chema Diagnostic kits. Serum gamma globulin proteins were assessed by precipitation from serum with ammonium sulphate-sodium chloride reagent (Wolfson *et al.*, 1948). Chemistry analyzer (Model 5010, Robert Riele GmbH & Co KG, D- 13467 Berlin, Germany) was used for quantitative determination of proteins. Total serum globulins were measured by deducting the values of albumin from total protein whereas, non-gamma globulin proteins were determined by deducting

the values of gamma globulins from globulins for each sample.

### Statistical analysis

Statistical analysis was performed by using GraphPad Prism version 5.00. Data were analyzed statistically using one-way ANOVA and presented as Mean $\pm$ SEM. Tukey's post hoc multiple comparison tests were employed for comparison amongst the groups of the study. Differences were assumed to be significant at  $p < 0.05$ .

## RESULTS

Serum protein profile including total proteins, albumin, total globulins, gamma globulins, non-gamma globulins along with anthropometric parameters were analyzed for

control and smokers groups. Variations were assessed by inter-group comparisons. The mean value of systolic blood pressure showed a significant increase in chain smokers when compared with controls and passive smokers. Diastolic Blood pressure showed a significant decrease in passive smokers in comparison to controls and a significant increase in chain smokers when compared to passive smokers. A significant increase was observed in body mass index of chain smokers when compared with moderate smokers. Significant reduction was observed in total proteins level in passive, moderate and chain smokers when compared to controls and smokers groups. Significant decrease in albumin level was observed in passive and moderate and chain smokers when compared to controls and smokers group.

**Table I: Average values (mean $\pm$ SEM) of anthropometric parameters, serum total proteins and its fractions (g/dl) in study groups.**

Parameters	Control	Passive	Moderate	Chain
Systolic BP	116.2 $\pm$ 0.826	114.8 $\pm$ 1.334	118.5 $\pm$ 1.160	122.0 $\pm$ 1.160
Diastolic BP	79.50 $\pm$ 0.837	75.67 $\pm$ 1.019	78.33 $\pm$ 0.757	80.67 $\pm$ 1.059
Body mass index	21.81 $\pm$ 0.218	23.01 $\pm$ 0.531	21.46 $\pm$ 0.403	23.25 $\pm$ 0.540
Total proteins	11.01 $\pm$ 0.168	10.46 $\pm$ 0.136	9.457 $\pm$ 0.084	9.063 $\pm$ 0.080
Albumin	6.542 $\pm$ 0.121	6.020 $\pm$ 0.141	4.640 $\pm$ 0.081	3.943 $\pm$ 0.081
Total globulins	4.445 $\pm$ 0.080	4.272 $\pm$ 0.084	5.207 $\pm$ 0.098	5.145 $\pm$ 0.094
Gamma globulins	2.982 $\pm$ 0.056	3.352 $\pm$ 0.048	3.570 $\pm$ 0.075	3.552 $\pm$ 0.044
Non-gamma globulins	1.563 $\pm$ 0.037	1.077 $\pm$ 0.040	1.330 $\pm$ 0.031	1.925 $\pm$ 0.068

Passive; passive smokers, Moderate; moderate smokers, Chain; chain smokers

**Table II: Percentage differences and 'p' value summary of anthropometric parameters, serum total proteins and its fractions in compared groups.**

Parameters	Control vs Passive	Control vs Moderate	Control vs Chain	Passive vs Moderate	Passive vs Chain	Moderate vs Chain
Systolic BP	2% $\downarrow$	2% $\uparrow$	5% $\uparrow^{**}$	3% $\uparrow$	6% $\uparrow^{***}$	3% $\uparrow$
Diastolic BP	5% $\downarrow^*$	2% $\uparrow$	1% $\uparrow$	3% $\uparrow$	7% $\uparrow^{**}$	3% $\uparrow$
Body mass index	5% $\uparrow$	2% $\downarrow$	7% $\uparrow$	7% $\downarrow$	1% $\uparrow$	8% $\uparrow^*$
Total proteins	5% $\downarrow^*$	14% $\downarrow^{***}$	18% $\downarrow^{***}$	10% $\downarrow^{***}$	13% $\downarrow^{***}$	4% $\downarrow$
Albumin	8% $\downarrow^{**}$	29% $\downarrow^{***}$	40% $\downarrow^{***}$	23% $\downarrow^{***}$	34% $\downarrow^{***}$	15% $\downarrow^{***}$
Total globulins	4% $\downarrow$	17% $\uparrow^{***}$	16% $\uparrow^{***}$	22% $\uparrow^{***}$	20% $\uparrow^{***}$	2% $\downarrow$
Gamma globulins	12% $\uparrow^{***}$	20% $\uparrow^{***}$	19% $\uparrow^{***}$	6% $\uparrow^*$	6% $\uparrow$	1% $\downarrow$
Non-gamma globulins	31% $\downarrow^{***}$	15% $\uparrow^{**}$	23% $\uparrow^{***}$	23% $\uparrow^{**}$	78% $\uparrow^{***}$	45% $\uparrow^{***}$

$\uparrow$  Increase,  $\downarrow$  Decrease, \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$

Total globulins level showed a significant increase in moderate and chain smokers when compared to controls and passive smokers. Gamma globulins showed a significant increase in passive, moderate and chain smokers when compared to controls and an increase was observed in moderate smokers when compared to passive smokers. Significant reduced level of non-gamma globulins was observed in passive smokers when compared to controls and significant increase was observed in non-gamma globulins level of moderate and chain smokers when compared to controls and passive smokers (Table I-II).

## DISCUSSION

Smoking has been considered as one of the 10 greatest contributors to global health problems and it is considered as harmful agent causing disease with multiple organ dysfunction and deaths. If present trends were allowed to continue unchecked and the government continued to be a silent spectator, the number of deaths annually could touch the 10 million marks (Leone, 2005).

Proteins are major extracellular component of the circulatory system and acts as a dynamic system with various biological functions (Gorinstein *et al.*, 2000). Modern proteomics and improvement in assay of protein markers are useful for the study of proteins in controls and smokers samples (Mathelin *et al.*, 2006). Marked variation has been observed in smokers on the basis of serum proteins level, it is not the amount of total proteins but the changes at individual protein level that are important (DeGowin *et al.*, 2004). Serum total protein level is constant; the decrease in one type of protein (albumin) through catabolism is compensated by the increase in the second (globulin) by increase synthesis (Al-Joudi, 2005). It provides information regarding a patient's general status. It was observed that serum protein concentration showed a marked reduction in smokers with an increase in the degree of dose and consumption rate (Salive *et al.*, 1992).

Most of the proteins are synthesized in the liver and by mature lymphocytes. Cigarette smoke causes adverse effects on the organs that are not in direct contact with the smoke, such as the liver, as it is involved in the processing of toxins to be eliminated from the

body (Yu *et al.*, 1997 and Pessione *et al.*, 2001). Albumin is the most abundant protein in the serum, it comprises more than half of the blood serum proteins (Sokolowska *et al.*, 2009). Non-smokers have normal level of albumin in the blood that plays a role in the transport of biomolecules. Chemicals in cigarette smoke have both direct and indirect effect on serum protein profile. These directly alters the binding properties of albumin and results in its degradation in the liver and loss of albumin by the kidney (hypo-albuminuria) (Chase *et al.*, 1991; Ramamurthy *et al.*, 2012). Most of the proteins are present in the serum in free and bound state. Albumin binds water, cations, fatty acids, bilirubin, thyroxine, hormones and various drugs that are metabolized in the liver. Reduced albumin in serum is a sign of poor health status (Farrugia, 2010). Albumin synthesis takes place in the liver and a marked reduction in serum albumin is observed in moderate and chain smokers as compared to controls. It has a well-marked clearance rate of chemicals from the blood. Albumin is degraded into amino acids in the serum and hence is available for energy (Thakore *et al.*, 1994).

Albumin has antioxidant properties that are modified by carbonylation induced by cigarette smoke chemicals (Clerici *et al.*, 2014). High level of free radicals results into increased proteolytic activity (Tetley, 2006). Albumin excretion rate is about 2.8 times higher in smokers than non-smokers with development and progression of diabetic renal damage (Chase *et al.*, 1991). The human serum protein includes globulin in addition to albumin. Globulins are antibodies that are produced in response to stimulation of the immune system. Total globulins level is elevated in moderate and chain smokers in comparison to controls and passive smokers. It consists of gamma and non-gamma fraction of globulins. Globulins are produced by the lymphocytes that are activated by immune system when a body becomes prone to infection by the exposure of volatile and polycyclic aromatic hydrocarbons released from the cigarette smoke (De-Gowin *et al.*, 2004; Al-Joudi, 2005; Marieb and Hoehn, 2010). Globulins are present in free or bound state in plasma, it is metabolized and excreted in free-state (Al-Joudi, 2005). Globulins bind with hemoglobin, transport metals, such as iron, in the blood. They are present in free or bound state in the plasma. Non-gamma globulins include IgA, IgM, IgE and IgG that show a

marked increase in moderate and chain smokers as compared to controls (DeGowin *et al.*, 2004).

### Conclusion

In this study a marked decrease in serum protein level was observed in cigarette smokers when compared to control subjects. Heavy smoking was found to be associated with low albumin concentration, low total protein levels and higher globulins levels. Cigarette smoke can affect hepatic functions due to deleterious effect of free radicals and toxicants on liver that alter the synthesis of proteins and reduces the protein content of the serum. Increased globulins levels in smokers compensate for the reduced albumin level in serum and hence total protein level remains constant. Albumin and globulins level vary as the duration and dose of smoking changes. Thus the study concludes that smokers have higher risk of liver dysfunction than that of non-smokers.

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