Original Article

To study the effect of cigarette smoking on anthropometric markers, serum alpha 1 antitrypsin and cotinine levels

Basant Kumar Joshi[1], Sangeeta Singh[2], Sumeru Samanta[1], Tapan Mohapatra[3]

[1]Department of Biochemistry, Rohilkhand Medical College and Hospital, Bareilly, Uttar Pradesh, India, [2]Department of Biochemistry, Soban Singh Jeena Government Institute of Medical Science and Research, Almora, Uttarakhand, India, [3]Department of Biochemistry, Santosh Medical College, Ghaziabad, Uttar Pradesh, India

Corresponding Author:
Dr. Basant Kumar Joshi, Department of Biochemistry, Rohilkhand Medical College and Hospital, Bareilly, Uttar Pradesh, India. E-mail: bjoshi86@gmail.com

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Introduction: In the United States (US) and other countries cigarette smoking (CS) continues to be the more preventable cause of disease and death. To both tobacco use and exposure to environmental tobacco smoke (ETS), cotinine is widely applied as a marker, because it has a longer half-life (average, 18–20 h) than nicotine (average, 2–3 h). Alpha1-proteinase inhibitor or SERPINA1 are other used words for alpha 1 antitrypsin (A1AT), and also the SERPIN (an acronym for serine proteinase inhibitor) family of protease inhibitors, prototypical member. Age, height, weight, body mass index (BMI), waist circumference, and waist-hip ratio are simple and valid anthropometric measures for the assessment of risk of obesity and other systemic diseases in smokers The objectives of the present study were to measure the levels of serum A1AT, cotinine in cigarette smokers and to study the association between these biochemical markers with anthropometric markers and the duration and number of cigarette smoked. **Materials and Methods:** The present study was carried out in the Department of Biochemistry, Santosh Medical College, Ghaziabad. Prior to estimation anthropometric markers (weight, height, BMI, waist circumference, hip circumference, and waist-hip ratio) were done in all subjects followed by serum A1AT by ELISA (Elabscience, Catalog No: E-EL-H0109), serum cotinine by HPLC (high-pressure liquid chromatography). **Results:** The mean serum cotinine level was significantly raised in cigarette smokers as compared to non-smokers whereas mean serum A1AT level was significantly decreased in cigarette smokers as compared to non-smokers. This difference was found to be statistically significant ($P < 0.05$). **Conclusion:** Based on our findings and the other data in the study, we speculate that these biomarkers to the detection of smokers might be useful with a high risk of pulmonary and cardiovascular diseases developed by smoke-induced and will help to clinicians to formulate novel treating protocol and follow-up for their patients.

KEY WORDS: A1AT and anthropometric markers, cigarette smokers, serum cotinine

INTRODUCTION

In most of the world cigarettes are the most common form of tobacco used and each year 443,000 deaths occur in the United States (US). Due to rise of tobacco industry and population growth cigarette smoking (CS) and the use of other tobacco products is increasing in the developing world.[1]

Tobacco smoking is the inhalation of smoke from burned dried or cured leaves of the tobacco plant, most often in the form of cigarette.[2] Different kinds of cigarettes (flavored, hand-rolled, manufactured, filtered, and unfiltered), pipes, and cigars included as smoked forms of tobacco. The main form of tobacco smoked globally is cigarette smoking, particularly manufactured cigarettes, in some developed

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and developing countries other forms of smoked tobacco are predominant.[3]

In developed countries cigarette smoking, hereafter referred to as "smoking," is the largest single risk factor for premature death. In the United States, approximately one-fifth of the deaths are attributable to smoking and 28% of the smoking-attributable deaths involve lung cancer, 37% involve vascular disease and 26% involve other respiratory diseases. Approximately 80% of adult smokers initiate their tobacco use before 18 years of age as per the WHO estimates. Therefore, the fact that many adult smokers make smoking a significant public health problem by initiating their smoking habit as in adolescents age.[4]

Cigarette smoke is separated into the gaseous phase and particulate (tar) phase. a material that is trapped when the smoke stream is passed through the Cambridge glass-fiber filter that retains 99.9% of all particulate material with a size >0.1 μm is defined the tar or particulate phase. The particulate phase of cigarette smoke contains the condensable part of the gas phase. In the particulate phase aldehydes, ketones, organic acid, and alcohol are found.[5]

Body mass index (BMI) and waist-to-hip circumference ratio (WHR) are the simple measures and widely used anthropometric markers in clinical practice. The most widely used method to define thinness and fatness is BMI, a ratio of weight in kilograms divided by height in meters squared (kg/m^2). It has been correlated to morbidity and mortality risk in various populations (Willett *et al*., 1999).

It is also believed that combined use of BMI and WHR parameters of generalized and abdominal obesity may be better in identifying people at risk of cardiovascular disease (CVD) than either of them alone (Ardern *et al*., 2003, Meisinger *et al*., 2006) because they correlate well with each other. World Health Organization include 18.5–24.9 kg/m^2 for normal, 25.0–29.9 for overweight and >30 kg/m^2 for obesity recommended currently cut-offs of BMI (World Health Organization, 1997). International Diabetes Federation Criteria for Central Adiposity waist circumference male ≥90 cm and female ≥80 cm South Asian.

The nicotine absorbed is metabolized to cotinine an average of 70–80%. In adult smokers, a nicotine intake of approximately 1 mg can be estimated from a blood cotinine level of 71 nmol/L (12.5 ng/mL) using a conversion factor of 0.08 mg/24 h per nanogram per milliliter under steady-state conditions. The main biomarker used to distinguish tobacco users from people who do not use tobacco is cotinine, which reflects the extent of exposure, not how the exposure was derived.[6]

Alpha 1 antitrypsin (A1AT), also referred to as alpha1-proteinase inhibitor or SERPINA1 and is the prototypical member of the SERPIN (an acronym for serine proteinase inhibitor) family of protease inhibitors. The term SERPIN was introduced as by Carrell and Travis in 1985 to describe a superfamily of serine protease inhibitors of mammalian plasma.

A1-Antitrypsin (a1-AT) is synthesized mainly by hepatocytes, a 52-kDa glycoprotein. It protects the alveolar matrix from destruction by neutrophil elastase (NE), a serine protease capable of destroying most of the structural components of the alveolar wall in the lung parenchyma its primary function.[7] Accumulation of polymorphonuclear leukocytes and macrophages in the lungs caused by smoking. A1-AT is abundant in human plasma with concentrations in the 20–53 mM range in addition to its presence in the lung. Due to mutations of the a1-AT gene, the anti-NE protection on the alveolar surface is inadequate, resulting in unopposed proteolytic activity, eventually leading to lung destruction and the development of emphysema by the third or fourth decade of life, when plasma levels of a1-AT are below a protective threshold of approximately 11 mM.[8]

Although many studies have been conducted on biochemical markers in cigarette smokers, levels of A1AT and cotinine were not well documented. Their involvement and association in cigarette smokers are still not known and the results obtained by other studies are contradictory and have not been extensively studied. These markers have also been implicated in a several lung diseases, including lung, cancer, and emphysema, etc. Therefore, the objectives of the present study were to measure the levels of serum A1AT, cotinine in cigarette smokers and to study the association between these biochemical markers with anthropometric markers and the duration and number of cigarette smoked.

MATERIALS AND METHODS

The present study was carried out in the Department of Biochemistry, Santosh Medical College, Ghaziabad. Institutional ethical clearance was taken prior to the study (F. No SU/2018/528 {2}.

Inclusion Criteria

The age group of 18–60 years about 284 healthy cigarette smokers (without any systemic diseases) compared with age and sex-matched 284 controls (non-smokers) were included in the study.

Exclusion Criteria

Person with a habit of tobacco chewing along with smoking and taking other forms of smoke (bidi, hookah, cigar, etc.) and patients of tuberculosis, pulmonary disorders, coronary artery diseases, diabetes mellitus, renal failure, chronic liver diseases, thyroid dysfunction, anemia, malnourished individuals, were excluded from the study.

According to prevalence, used in the previous study[9] sample size is calculated

$$n = \frac{Z^2 \times p \times q}{d^2}$$

Where n is the sample size, Z is 1.96 (5% level of significance), p is prevalence, q is 1-p and d is 0.05 (95% of c.f.). According to this formula, sample size was 284 for cigarette smokers.

Cigarette smokers comprising the number of cigarettes per day and duration of cigarette smoking was recorded on participant proforma after taking detailed history. Based on the number of cigarettes per day and duration of cigarette smoking subjects were classified into different groups i.e. smoking 1–15 cigarettes/day <5 years are mild smokers in group I, 15–20 cigarette/day <5 years in group II, 15–20 cigarette/day 5–10 years moderate, and 15–20 cigarette/day >10 years are heavy smokers.[10] Out of total 284 cigarette smokers, 129 were in group I, 42 were in group II which were in mild group, 36 were in moderate and 77 were in heavy group smokers.

All aseptic precautions were taken; with a disposable syringe, about five mL of blood was drawn by veinpuncture from a peripheral vein. For the retraction of clot collected blood in clean dry glass tubes was allowed to stand for 30 min at room temperature. Then, it was centrifuged at 3000 r.p.m. for ten minutes to obtained the serum. The serum was stored at 4°C in the refrigerator for analysis.

Prior to estimation anthropometric markers (weight, height, BMI, WHR) were done in all subjects followed by serum A1AT by ELISA (Elabscience, Catalog No: E-EL-H0109), serum cotinine by HPLC (high-pressure liquid chromatography).

Statistical Analysis

Unpaired "t" test and one-way ANOVA were used to analyze all the data for statistical significance, using the SPSS 19.0.2 program for windows.

RESULTS

In the present study, out of total of 284 cigarette smokers, 272 were male and 12 were female. Mean age of the cigarette smokers and nonsmokers was 40.66 ± 11.08 years and 37.42 ± 9.73 years, respectively.

The cigarette smokers had significantly higher mean weight, BMI, waist circumference, and waist-hip ratio as compared to non-smokers. These differences were found to be statistically significant ($P < 0.05$).

The mean serum cotinine level was significantly raised in cigarette smokers as compared to non-smokers whereas mean serum A1AT level was significantly decreased in cigarette smokers as compared to non-smokers. This difference was found to be statistically significant ($P < 0.05$).

The mean serum cotinine level was significantly raised in cigarette smokers when adjusted with duration and number of cigarette smoked as compared to non-smokers. This difference was found to be statistically significant ($P < 0.05$).

The $P < 0.05$ which signifies significant variation in levels of serum cotinine and A1AT when compared among each other in terms of group means.

A1AT had negative correlation with cotinine ($r = -0.143$; $P < 0.000$) and ($r = -0.212$; $P < 0.000$) in cigarette smokers and non-smokers. Linear relationships were observed between the parameters.

DISCUSSION

Cigarette smoking that has spread all over the world is a reprehensible habit. Considerable research attests the adverse effects of chronic smoking on human health. In the development of many cardiovascular, pulmonary, and ocular diseases and also neurological disorders smoking has been implicated.[11]

Cigarette consumption has risen over the past two decades in India and most other countries. Reports reveal that the smoking rate is on continuous increasing. The WHO estimates suggest that if the current pattern of smoking continues, this 21st century is about to see 1 billion tobacco deaths.[12]

In the present study, Out of total 284 cigarette smokers, 272 were males and 12 were females. 15.14% of the cigarette smokers were in the age group 18-29 years followed by 27.11% within 30-41 years, 29.93% within 42-53 years, and 27.82 within 54–60years [Table 1]. According to study prevalence, our study indicates that male are more prone for smoke-related diseases than females. Same results were also obtained by other studies. Our finding is similar to the Townsend *et al*. who reported in the sub-Saharan Africa that the prevalence of cigarettes use was higher among males than females across all countries in the region.

About 20% of men aged 18 and over smoked compared with 17% of women according to ash fact sheet in 2016. Smoking prevalence is highest among young adults 23% of those aged 16–24 and 24% among the 25–34 age groups. Smoking continues to be the lowest among people aged 60 and over. Although they are more likely than younger people to have ever been smokers, they are more likely to have stopped smoking.

In the present study prevalence of cigarette smoking among people aged 30 years and above and this was much higher among males (95.77%) than females (4.23%). A clustered community –based study in 2001 with about 50% of adult males between 30 and 60 years found to be smokers and very few females admitting to smoking similar finding has been reported from

Table 1: Distribution of age group among cigarette smokers (n=284)

Age group (years)	Male		Female		Total	
	No	%	No	%	No	%
18–29	41	15.07	02	16.67	43	15.14
30–41	75	27.57	02	16.67	77	27.11
42–53	81	29.79	04	33.33	85	29.93
54–60	75	27.57	04	33.33	79	27.82
Total	272	95.77	12	4.23	284	100

Delhi by Chhabra *et al*.[13] The young often smoke because their peers smoke reported by these studies in urban areas. Their most common reason was their film hero who smokes. In the rural areas, many people were unaware of the hazards of smoking.[14]

In developing and developed countries females start smoking later than males due to the smoking has been considered socially unacceptable for women (with exceptions in certain areas of India, Nepal, Papua New Guinea, northern Thailand, and for Maoris). Religious constraints may be the reason, for example, to buy cigarettes in Muslim countries women have had less spending power than men; traditional methods of smoking are adhere by rural women, for example., hubble-bubble pipes, and are therefore a lower dosage of tobacco they exposed; and women use tobacco in other forms as chewing tobacco is used in some areas, such as parts of India and the Middle East (Subramanian, 2004). Where it is culturally less acceptable for women to smoke, there may be significant underreporting of smoking among women in countries.[15]

Health effects associated with reproductive health such as problems associated with pregnancy, use of oral contraceptive, menstrual function, and cancers of the cervix and bladder are more prone in women smoker or who exposed with smokers. irregular menstrual cycles and increased menstrual discomfort may also caused by smoking. Women who are smokers may also have an earlier menopause, which increases chances of getting osteoporosis, heart disease, and other conditions for which estrogen provides a protective effect. The risk of sudden infant death syndrome may also increase when a pregnant woman smokes.

Age, height, weight, BMI, Waist Circumference, and Waist-hip ratio are simple and valid anthropometric measures for the assessment of risk of obesity and other systemic diseases in smokers. In the present study, the cigarette smokers had significantly higher mean BMI, waist circumference, and waist-hip ratio as compared to non-smokers [Table 2].

Some studies reported that measures of abdominal adiposity such as WC and WHR are better and simple markers of cigarette smoked in smokers, other studies claim that central adiposity measures such as WC and WHR do not provide additional prognostic information than BMI alone.

The amount of visceral adipose tissue (VAT) is indicated by Waist circumference or waist-to-hip ratio (WHR). WHR is higher in smokers than in nonsmokers indicated by several cross-sectional studies. The number of pack years of smoking is positively associated with WHR and between WHR and the number of cigarettes smoked there is a dose-response relation. WHR is negatively associated with the time since smoking cessation in former smokers.[16]

A study found that CS can lower testosterone levels in men. Smoking caused a significant drop in serum testosterone levels in male dogs. Overall, these findings imply that, in addition to elevated cortisol, an imbalance in male and female sex hormones, as well as a decrease in testosterone in males, may contribute to the effect of smoking on VAT.[17] Smoking has been linked to lower weight and BMI in a number of studies. In addition, smoking has been linked to insulin resistance and type 2 diabetes. Current smokers had lower mean BMI, WC, and body fat percentage than nonsmokers among Caucasian men and women. In smokers, age-adjusted mean WC and body fat rose with the number of cigarettes smoked per day, but not in a way that was connected to BMI. Over a 50-month follow-up period, Basterra-Gortari *et al*. discovered that active smokers gained more weight than never smokers. Nicotine is a well-known appetite suppressor, with numerous studies demonstrating that it reduces food intake in rats.[18] As a result, one may predict smokers with slower nicotine metabolism to have a lower BMI, however, our hypothesis was not confirmed.

It is possible that persons who like cigarettes may also like foods that are rich and high in fat or sugar, which counteracts any appetite suppressant characteristics of nicotine. Heavy smokers tend to have greater body weight than light smokers or nonsmokers remains unanswered. One explanation could be that heavy smokers are more likely to adopt behaviors favoring weight gain (e.g., low physical activity, unhealthy diet, and high alcohol intake) than are light smokers or nonsmokers. Smokers eat less fruit and vegetables adopt unhealthy patterns of nutrient and calorie intake than do nonsmokers.[19]

Number of biochemical markers such as nicotine, cotinine, and carbon monoxide in the expired air and carboxyhemoglobin in blood has been used to validate claims of non-smoking. Levels of nicotine and carbon monoxide/carboxyhemoglobin are easier to determine but can be raised through exposures unrelated to smoking such as traffic emissions and diet. Cotinine is possibly the best marker for situations where accuracy is paramount. It is one of the most frequently used biomarkers for exposure

Table 2: Anthropometric measurements of cigarette smokers and non-smokers (*n*=568)

Parameters	Non-smokers (*n*=284) Mean±SD	Cigarette smokers (*n*=284) Mean±SD	*P* value	*t* value	95% Confidence interval for mean	
					Lower	Upper
Height (cm)	166.23±3.73	165.89±3.52	0.425	0.599	158.0	178.0
Weight (kg)	63.56±6.82	66.18±5.16	0.001	5.16*	48.0	76.0
BMI (kg/m^2)	23.00±2.10	24.09±1.63	0.001	6.93*	17.60	27.90
WC (cm)	78.57±4.51	83.16±4.62	0.189	11.98*	70.0	91.0
HC (cm)	90.92±4.44	89.37±3.76	0.006	−0.446	80.0	98.0
WHR	0.86±0.03	0.93±0.03	0.162*	27.39*	0.84	0.98

to environmental tobacco smoke in body fluids and widely practical as a biomarker of nicotine uptake and exposure to both active and secondhand tobacco smoke.[20]

In the present study, the mean serum cotinine level was significantly raised in cigarette smokers as compared to non-smokers and also increased when smokers adjusted with age and duration and number of cigarettes smoked [Tables 3-5].

The present study also shows the correlation of cotinine with serum A1AT level in mild group I, mild group II, Moderate and heavy cigarette smokers, respectively. Cotinine had a negative correlation serum A1AT ($r = 0.458$, $P < 0.006$) in mild group II and moderate cigarette smokers, respectively Linear relationships were observed between the parameters [Table 6].

Balhara YPS, Jain R 2013 detected urinary cotinine by the highest sensitivity and specificity method for smoking using ELISA kits of Calbiotech.[21] Kulza *et al*. 2012 found that the concentration of salivary cotinine was increased in cigarette smokers, detected by using high-performance liquid chromatography with diode array detection. Mean concentrations of cotinine were found to be highly increased suggested that saliva cotinine is useful in the assessment of tobacco smoke.[22] Nuca *et al*. 2012 By using NicAlert™ Saliva tests and found that 44.06% were active smokers, 16.43% were non-smokers, and 39.50% were passive smokers.[23]

In the coronary artery, Risk Development in (Young) Adults study, serum cotinine levels were higher in black smokers than in white smokers, despite lower estimated daily nicotine exposure among black smokers.[24]

Cotinine levels have earlier been used to validate the smoking status of an individual. These biomarkers have also been used in epidemiological studies, to assess the effects of tobacco use on human health, as measures to estimate the exposure to environmental tobacco smoking, and for assessment of the efficacy of interventional methods on cessation of smoking.

Alpha-1 antitrypsin deficiency is a genetic disorder that causes deficiency of the protein, A1AT. The lack of this protein may

Table 3: Serum levels of cotinine and A1AT in cigarette smokers and non-smokers (*n*=568)

Parameter	Non-smokers (*n*=284) Mean±SD	Cigarette smokers (n=284) Mean±SD	*t* value	*P* value	95% Confidence interval for mean		Normal value
					Lower	Upper	
Serum cotinine	14.20±5.15	24.20±5.00	2.38	0.013	11.20	36.50	3–20 ng/mL
Serum A1AT	20.80±11.98	13.06±18.00	−1.06	0.027	3.31	77.53	18.59–81.15 ng/mL

Table 4: Distribution of serum cotinine and A1AT levels according to duration and number of cigarette smoked in cigarette smokers (*n*=284)

Parameter	1–15 C/D <5 years Mean±SD	15–20 C/D <5 years Mean±SD	15–20 C/D 10 years Mean±SD	15–20 C/D >10 years Mean±SD	*P* value
Serum cotinine (mg/dl)	24.16±5.00	24.66±5.00	23.71±5.00	24.62±5.00	0.348
95% confidence interval for mean					
Lower	21.79	22.76	25.10	22.73	
Upper	24.29	25.59	27.46	25.50	
A1AT	13.68±20.75	12.34±17.41	12.21±8.28	13.19±15.86	0.098
Lower	12.41	10.03	10.34	11.80	
Upper	14.26	12.71	14.80	19.48	

Table 5: ANOVA analysis and unpaired "*t*" test of serum cotinine and A1AT level according to duration and number of cigarette smoked in cigarette smokers

Parameter	1–15 C/D<5 years	15–20 C/D<5 years	15–20 C/D 5–10 years	15–20 C/D>10 years	F critical
	Sum of squares	Mean square	F value	*P* value	
Serum cotinine					
Between groups	391.490	130.497	5.565	0.001	3.032
Within groups	6542.533	23.448			
A1AT					
Between groups	1509.215	503.072	3.96	0.098	
Within groups	99833.705	357.827			

Table 6: Correlation between serum A1AT with serum Lp (a) and cotinine levels in cigarette smokers and non-smokers (*n*=568)

Parameter	Correlation analysis	SerumCotinine
Serum A1AT	Correlation coefficient (r)	−0.212
	S.E.OF "r"	0.041
	t statistic	−5.159
	P value	0.000
	R -square	0.043

cause lung disease over time. Alpha-1 antitrypsin deficiency is often undetected for many years, and although treatable, the disease is incurable.

In our study, the mean Serum A1AT level was significantly decreased in cigarette smokers as compared to non-smokers. The mean serum A1AT level also was significantly decreased in cigarette smokers when adjusted with duration and number of cigarettes smoked as compared to non-smokers. This difference was found to be statistically significant ($P < 0.05$) [Tables 2-5].

Different studies demonstrated change in A1AT activities in serum of patients with COPD; which are more commonly associated with cigarette smoking habit.[25] One more study also shows increased A1AT activity in cigarette smokers compared to healthy nonsmokers which suggest its role in mediating some of the chronic health hazards of smoking such as COPD, TB, and other lung diseases; which were found to be associated with an increased levels of A1AT and also reported a positive correlation between pack size and A1ATactivity.[26]

A1AT level was lower in COPD patients with smoking as compared to COPD patients without smoking. The present study shows the significant difference in serum AAT level between the two groups ($P < 0.05$). This study supports the data of previous studies Ogushi *et al*. 1991, Senn *et al*. 2008 and Deore Deepmala *et al*. 2012.[27,28]

Higashimoto *et al*. reported in a cross-sectional study that serum alpha-1 antitrypsin levels were inversely correlated with cigarette smoke in Japanese patients with COPD.[29] Furthermore, Senn *et al*. reported that serum alpha-1 antitrypsin levels were inversely correlated with cigarette smoke and FEV1 in the general population.[28]

These findings could also explain the role of AAT in the development of COPD; its decreased levels in the blood resulting from systemic inflammation, meeting the elevated levels of alveolar neutrophil elastase, and proving to be a marker for increased risk of COPD development, as a result of low-grade lung inflammation. Direct exposure of α1-antitrypsin to gas phase causes loss of elastase inhibitory capacity leading to the formation of reactive free radicals from smoke that inactivate a 1-antitrypsin by oxidizing methionine 358 terminal amino acid.[30]

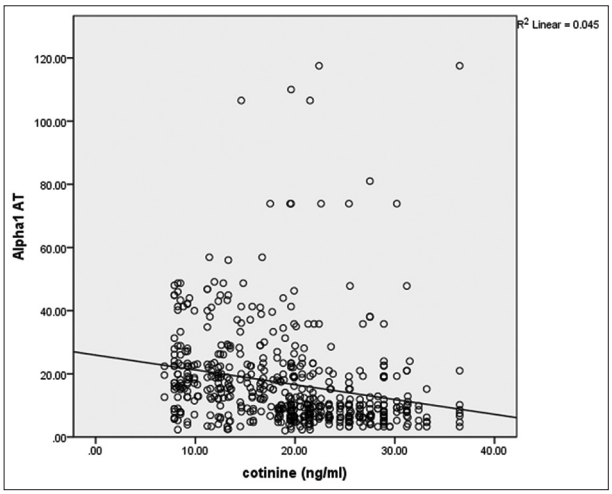

Figure 1: Correlation of serum A1AT with serum cotinine in cigarette smokers and non-smokers

CONCLUSION

Cigarette smoking may increased serum cotinine represents exposure of smoke, decreased serum A1AT modify the deleterious effect of smoking on these markers, indicating genetic susceptibility to smoking-related diseases.

Based on our findings and the other data in the study, we speculate that these biochemical markers might be useful biomarkers for the detection of smokers with a high risk of developing smoke-induced pulmonary and CVD and will help to clinicians to formulate novel treating protocol and follow-up for their patients.

Limitations

The study was limited to the population residing in and around Haldwani and their involvement in the study especially in case of females. Lack of fund, time, and manpower prevented the inclusion of a large study group and other sensitive biochemical markers of cigarette smoke.

Future cross-sectional, longitudinal, and mechanistic studies are needed to determine how CS, anthropometric markers, A1AT and cotinine are useful in large populations of cigarette smokers with the inclusion of clinically relevant endpoints are needed to extend these findings.

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