Effect of Cigarette Smoking on High-Density Lipoprotein Cholesterol Levels: A Biochemical Study

[1]Ranjana Srivastva; [2] Dr Juhi Aggarwal; [3]Mohini Arora

[1]M.Sc Student, department of Biochemistry, Santosh Medical College & Hospital, SDU, Ghaziabad, UP, India

[2] Prof & Head, department of Biochemistry, Santosh Medical College & Hospital, SDU, Ghaziabad, UP, India

[3] Senior Tutor, department of Biochemistry, Santosh Medical College & Hospital, SDU, Ghaziabad, UP, India

DOI: https://doi.org/10.68050/JAMS.968

Received: 12 November 2026; Accepted: 13 November 2026; Published: 17 November 2026

ABSTRACT

Introduction

Cigarette smoking is a major modifiable risk factor for several chronic diseases, including atherosclerosis and cardiovascular disease. In addition to its direct toxic effects, cigarette smoking can adversely influence lipid metabolism and alter the concentration and function of circulating lipoproteins. High-density lipoprotein cholesterol (HDL-C) plays an important cardioprotective role through reverse cholesterol transport and other anti-atherogenic mechanisms. Previous studies have generally reported lower HDL-C concentrations among smokers; however, the influence of different intensities of smoking on HDL-C levels requires further evaluation.

Objective

The present study aimed to assess the effect of cigarette smoking on serum HDL-C levels in male smokers and to compare HDL-C concentrations among mild, moderate, and heavy smokers with those of healthy non-smokers.

Methods

This cross-sectional study was conducted over 12 months in the Department of Biochemistry in association with the Department of Respiratory Medicine at Santosh Medical College and Hospital, Ghaziabad, Uttar Pradesh. A total of 80 healthy male subjects aged 30–40 years were included. Twenty healthy non-smokers served as controls, while 60 smokers were categorized into mild, moderate, and heavy smoking groups, with 20 subjects in each group, according to smoking intensity expressed in pack-years. Subjects with alcohol consumption, previous smoking history, diabetes mellitus, hypertension, renal disease, chronic hepatic dysfunction, endocrine disorders, obesity, a family history of coronary heart disease, or the use of lipid-lowering drugs were excluded. Serum HDL-C was estimated following precipitation of LDL, VLDL, and chylomicrons, followed by enzymatic estimation of cholesterol in the supernatant.

Results

A progressive decline in serum HDL-C levels was observed with increasing smoking intensity. The mean ± SD HDL-C levels were 48.94 ± 3.92 mg/dL in controls, 38.31 ± 3.16 mg/dL in mild smokers, 32.10 ± 2.15 mg/dL in moderate smokers, and 30.71 ± 2.48 mg/dL in heavy smokers. The corresponding 95% confidence intervals were 47.11–50.78, 36.83–39.79, 31.09–33.11, and 29.55–31.87 mg/dL, respectively. HDL-C levels

were significantly lower among smokers compared with controls, with a progressive reduction observed with increasing smoking intensity

Discussion

The findings demonstrate an adverse association between cigarette smoking and serum HDL-C concentration. The progressive decline in HDL-C with increasing smoking intensity supports a possible dose-related effect of smoking on lipid metabolism. Smoking-induced alterations in enzymes and proteins involved in HDL metabolism, including lecithin cholesterol acyltransferase and cholesteryl ester transfer protein, may contribute to reduced HDL-C levels and impaired cardioprotection.

Conclusion

Cigarette smoking was associated with significantly lower serum HDL-C levels, and greater smoking intensity was accompanied by a further reduction in HDL-C. These findings emphasize the importance of smoking prevention and cessation as part of strategies to reduce adverse cardiovascular risk.

Keywords: Cigarette smoking; High-density lipoprotein cholesterol; Lipid profile; Cardiovascular risk.

INTRODUCTION

Cigarette smoking is one of the most important preventable causes of morbidity and mortality worldwide. Tobacco use is associated with a wide range of adverse health outcomes, including respiratory disease, malignancy, atherosclerosis, and cardiovascular disease. The harmful effects of cigarette smoking are mediated by numerous toxic constituents of tobacco smoke, including nicotine, carbon monoxide, hydrogen cyanide, tar, and several oxidant and irritant compounds.

Cardiovascular disease is closely associated with abnormalities in lipid metabolism. Cigarette smoking has been identified as an important environmental and lifestyle factor capable of altering the circulating lipid profile. Previous studies have reported that smoking may be associated with increased concentrations of total cholesterol, triglycerides, low-density lipoprotein cholesterol (LDL-C), and very-low-density lipoprotein cholesterol (VLDL-C), along with reduced concentrations of high-density lipoprotein cholesterol (HDL-C).

HDL-C is traditionally regarded as a protective component of the lipid profile because of its role in reverse cholesterol transport. HDL particles facilitate the transport of excess cholesterol from peripheral tissues, including the arterial wall, to the liver for metabolism and excretion. In addition to its role in cholesterol transport, HDL has been associated with antioxidant and anti-inflammatory properties. Therefore, a reduction in HDL-C concentration or impairment of its normal function may contribute to an increased risk of atherosclerosis and coronary heart disease.

The metabolism of HDL is complex and involves several enzymes, transfer proteins, and lipoprotein particles. Cigarette smoking may adversely affect HDL biosynthesis, maturation, remodeling, and function. Smoking has been reported to reduce the concentration of apolipoprotein A-I, an important structural component of HDL. It may also influence the activity of lecithin cholesterol acyltransferase (LCAT), cholesteryl ester transfer protein (CETP), and hepatic lipase, thereby altering HDL metabolism. Oxidative stress induced by cigarette smoke may further impair the atheroprotective properties of HDL.

Nicotine may also contribute to lipid abnormalities by stimulating the sympathetic-adrenal system and increasing catecholamine release. This can promote lipolysis and increase circulating free fatty acids, resulting in altered hepatic lipid metabolism and increased production of triglycerides and VLDL. These metabolic changes may contribute to an overall atherogenic lipid profile.

Although several studies have demonstrated lower HDL-C levels in smokers compared with non-smokers, the relationship between the intensity of cigarette smoking and the extent of HDL-C reduction remains an area of clinical and biochemical interest. Evaluation of mild, moderate, and heavy smokers may provide additional

information regarding whether increasing exposure to cigarette smoking is associated with progressively greater alterations in HDL-C concentration.

The present study was therefore undertaken to assess the effect of cigarette smoking on serum HDL-C levels in male smokers and to compare HDL-C concentrations among different categories of smoking intensity with those observed in healthy non-smoking controls.

MATERIALS AND METHODS

This cross-sectional study was conducted over a period of 12 months, from July 2025 to June 2026, in the Department of Biochemistry in association with the Department of Respiratory Medicine at Santosh Medical College and Hospital, Ghaziabad, Uttar Pradesh, India. A total of 80 male subjects aged between 30 and 40 years were included in the study.

The study population comprised 20 healthy non-smokers who served as controls and 60 cigarette smokers. The smokers were further divided into three groups according to smoking intensity: mild smokers, moderate smokers, and heavy smokers, with 20 subjects included in each group. Smoking exposure was categorized on the basis of pack-years. Mild smokers were defined as those with less than one pack-year of smoking exposure, moderate smokers as those with one to two pack-years, and heavy smokers as those with more than two pack-years. In the study protocol, one pack-year was described as smoking approximately one pack of cigarettes per day for one year.

Smoking exposure was assessed based on the number of cigarettes smoked per day and the duration of smoking. Pack-years were calculated as the average number of cigarette packs smoked per day multiplied by the duration of smoking in years. One pack-year was defined as smoking one pack of cigarettes per day for one year. Based on the predefined study criteria, participants were categorized into mild, moderate, and heavy smokers according to their cumulative pack-year exposure.

Healthy male subjects aged 30–40 years who had never smoked and avoided passive smoking were selected as controls. Written informed consent was obtained from all participants before their inclusion in the study. Subjects with alcohol consumption, a history of previous smoking, diabetes mellitus, hypertension, renal disease, chronic hepatic dysfunction, endocrine disorders, obesity, a family history of coronary heart disease, or consumption of lipid-lowering medication or other relevant medicinal preparations were excluded to minimize potential confounding influences on lipid metabolism.

Information regarding smoking habits was collected using a questionnaire designed in accordance with the study protocol. Age and body mass index (BMI) were also recorded for all participants to assess the comparability of the study groups.

The primary outcome measure was the serum concentration of HDL-C in non-smokers and smokers. HDL-C estimation was performed by first precipitating LDL, VLDL, and chylomicrons. For the precipitation procedure, 250 µL of the sample was mixed with 500 µL of precipitating reagent. The mixture was allowed to stand for 10 minutes at room temperature and was subsequently centrifuged at 4000 rpm for 10 minutes to obtain a clear supernatant.

The HDL-C concentration in the supernatant was then estimated by an enzymatic cholesterol assay. Three tubes—blank, standard, and test—were prepared. The blank consisted of cholesterol working reagent and distilled water, the standard consisted of cholesterol working reagent and HDL standard, and the test consisted of cholesterol working reagent and the prepared sample supernatant. The reaction mixtures were mixed and incubated for 10 minutes at 37°C. Absorbance of the standard and test samples was measured at 505 nm against the blank. HDL-C concentration was calculated using the ratio of test absorbance to standard absorbance according to the assay procedure described in the study protocol.

The collected data were analyzed by calculating the mean and standard deviation of HDL-C concentrations in the different study groups. Statistical significance of differences between groups was assessed using p-values,

with p<0.05 considered statistically significant. The study was initiated after approval from the Institutional Ethics Committee, and all participants provided written informed consent.

Statistical Analysis

Data were expressed as mean ± standard deviation (SD). The normality of continuous variables was assessed before statistical analysis. Differences in mean HDL-C levels among the four study groups were evaluated using one-way analysis of variance (ANOVA). Pairwise comparisons between groups were performed using an appropriate post-hoc test. A p-value of <0.05 was considered statistically significant. The 95% confidence intervals were calculated for the mean HDL-C values

RESULTS

A total of 80 healthy male subjects between 30 and 40 years of age were included in the study. The participants were equally distributed among four groups comprising healthy non-smoker controls, mild smokers, moderate smokers, and heavy smokers, with 20 subjects in each group.

Comparison of Age and Body Mass Index Among Study Groups

The mean age and BMI of the participants were comparable across all four study groups. There was no statistically significant difference in age or BMI between controls and smokers, indicating that the groups were broadly comparable with respect to these variables.

Table 1. Distribution and Baseline Characteristics of Study Participants

Characteristic	Controls (n=20)	Mild Smokers (n=20)	Moderate Smokers (n=20)	Heavy Smokers (n=20)
Number of subjects	20	20	20	20
Age, mean ± SD (years)	36.3 ± 2.05	37.1 ± 1.11	36.25 ± 1.06	36.70 ± 2.01
BMI, mean ± SD (kg/m²)	21.91 ± 0.98	21.94 ± 1.13	21.86 ± 1.07	22.03 ± 1.13

The study groups did not differ significantly with respect to age or BMI (p>0.05).

Comparison of HDL-C Levels Between Controls and Smokers

The principal finding of the study was a marked reduction in serum HDL-C levels among cigarette smokers compared with healthy non-smoker controls. The reported results demonstrated the highest HDL-C concentration among controls and progressively lower levels among mild, moderate, and heavy smokers.

Table 2. Comparison of Mean Serum HDL-C Levels Among the Study Groups

Study Group	N	**Mean HDL-C (mg/dL)**	**SD**
Controls	20	48.94	3.92
Mild smokers	20	38.31	3.16
Moderate smokers	20	32.1	2.15
Heavy smokers	20	30.71	2.48

The findings demonstrate a clear decline in HDL-C concentration from the control group to the groups with progressively greater smoking exposure.

The results section of the dissertation further reports statistically significant differences between controls and all three categories of smokers. The mean HDL-C level in non-smokers was significantly higher than that observed in mild smokers ($p<0.001$), moderate smokers ($p<0.001$), and heavy smokers ($p<0.001$).

Comparison of HDL-C Levels According to Smoking Intensity

Comparison among the smoking groups showed that HDL-C levels declined with increasing smoking intensity. Mild smokers had significantly higher HDL-C levels than moderate smokers ($p<0.001$). HDL-C levels were also significantly lower in heavy smokers compared with mild smokers. The difference between moderate and heavy smokers was reported to be statistically significant ($p<0.01$).

Table 3. Pattern of HDL-C Changes According to Smoking Intensity

Comparison	Overall Finding	Reported Statistical Significance (p value)
Controls vs Mild smokers	HDL-C significantly lower in mild smokers	<0.001
Controls vs Moderate smokers	HDL-C significantly lower in moderate smokers	<0.001
Controls vs Heavy smokers	HDL-C significantly lower in heavy smokers	<0.001
Mild vs Moderate smokers	HDL-C significantly lower in moderate smokers	<0.001
Moderate vs Heavy smokers	HDL-C lower in heavy smokers	<0.01
Mild vs Heavy smokers	HDL-C significantly lower in heavy smokers	significant

Overall, the results suggest an inverse association between smoking intensity and serum HDL-C concentration.

DISCUSSION

The present cross-sectional study was undertaken to evaluate the effect of cigarette smoking on serum HDL-C levels in healthy male smokers and to compare the effect of different smoking intensities. The major finding of the study was that serum HDL-C concentrations were significantly lower in smokers than in healthy non-smoker controls. Furthermore, a progressive decline in HDL-C levels was observed from mild to moderate and heavy smoking categories.

HDL-C has an important role in maintaining cholesterol homeostasis. Its cardioprotective action has traditionally been attributed to reverse cholesterol transport, through which excess cholesterol is transported from peripheral tissues to the liver. HDL also possesses antioxidant and anti-inflammatory properties that may contribute to protection against the development of atherosclerosis. Therefore, a reduction in HDL-C may contribute to an unfavorable cardiovascular risk profile.

The results of the present study showed that the mean HDL-C concentration was highest in the control group and lower in all categories of smokers. The significant difference between non-smokers and smokers supports the hypothesis that cigarette smoking adversely influences HDL-C concentration. The finding is consistent with the observations of previous investigators who have reported lower HDL-C levels and a more atherogenic lipid profile among cigarette smokers.

An important observation was the progressive decline in HDL-C with increasing smoking intensity. Mild smokers had higher HDL-C levels than moderate smokers, while heavy smokers demonstrated the lowest HDL-C concentrations. This pattern suggests that increasing exposure to cigarette smoke may be associated with a greater adverse effect on HDL metabolism.

Several mechanisms may explain the observed reduction in HDL-C among smokers. Nicotine stimulates the sympathetic-adrenal system and promotes the release of catecholamines. Increased catecholamine activity enhances lipolysis and raises circulating free fatty acids. Increased delivery of free fatty acids to the liver can

alter hepatic lipid metabolism and promote the production of triglyceride-rich lipoproteins, including VLDL. Such changes may indirectly influence HDL metabolism and contribute to an unfavorable lipid profile.

Cigarette smoking may also affect the enzymes and proteins involved in HDL synthesis and maturation. Smoking has been associated with reduced apolipoprotein A-I concentration, which may impair the formation of normal HDL particles. Reduced LCAT activity may interfere with the esterification of free cholesterol and maturation of HDL particles. Alterations in CETP and hepatic lipase activity may further affect HDL remodeling and catabolism.

Oxidative stress represents another possible mechanism through which cigarette smoking may impair HDL function. Tobacco smoke contains numerous oxidants and can promote lipid peroxidation. Oxidative modification of HDL may alter its structure and reduce its normal atheroprotective properties. Thus, smoking may influence not only the circulating concentration of HDL-C but also its functional capacity.

The findings of the present study are consistent with previous reports describing cigarette smoking as an important modifiable risk factor for dyslipidemia and cardiovascular disease. Studies cited in the dissertation have reported lower HDL-C concentrations and other lipid abnormalities among smokers. However, the literature also contains conflicting observations, with some studies reporting no significant differences in selected lipid parameters between smokers and non-smokers. Such variation may be related to differences in smoking intensity, duration of exposure, age, dietary habits, physical activity, alcohol consumption, body composition, genetic factors, and sample characteristics.

Age and BMI were comparable among the four groups in the present study. This is an important observation because both age and body composition can influence lipid metabolism. The absence of a significant difference in these parameters between the groups strengthens the interpretation that the observed differences in HDL-C were associated with smoking exposure rather than major differences in these baseline characteristics.

The present study focused specifically on male subjects between 30 and 40 years of age. This relatively narrow age range helped reduce variation attributable to age and sex but also limits the generalizability of the findings. Nevertheless, the results provide evidence of an adverse association between cigarette smoking and HDL-C even among relatively young adult male smokers.

The progressive reduction in HDL-C with increasing smoking intensity has important clinical and public-health implications. A reduction in this anti-atherogenic lipoprotein may contribute to the long-term cardiovascular consequences of continued smoking. Smoking cessation may therefore offer benefits beyond the prevention of respiratory disease and cancer and may help improve the lipid profile and overall cardiovascular health.

RELATIONSHIP AMONG FACTORS OR PARAMETERS

The present study primarily evaluated the relationship between cigarette smoking intensity and serum HDL-C concentration. The results demonstrated a consistent inverse relationship: as the intensity of smoking increased, the mean HDL-C concentration decreased.

The control group, consisting of healthy non-smokers, demonstrated the highest mean HDL-C levels. Mild smokers showed a substantial reduction in HDL-C compared with controls, while moderate smokers demonstrated a further decline. Heavy smokers had the lowest HDL-C levels among all study groups. This pattern supports the presence of an adverse relationship between smoking exposure and HDL-C concentration.

Age and BMI were also assessed because both factors can influence lipid metabolism. The mean age of participants was similar across the four groups, and no significant difference was reported. Similarly, BMI values were comparable among controls, mild smokers, moderate smokers, and heavy smokers. Therefore, age and BMI were unlikely to account for the marked differences in HDL-C observed among the groups.

Table 4. Relationship Between Smoking Exposure and Study Parameters

Parameter	Observed Relationship
Smoking intensity and HDL-C	Inverse relationship; HDL-C decreased with increasing smoking intensity
Smoking and cardiovascular risk	Reduced HDL-C suggests a more unfavorable atherogenic profile
Age and HDL-C comparison	Age was comparable among groups; no significant group difference
BMI and HDL-C comparison	BMI was comparable among groups; no significant group difference

The relationship observed in this study is biologically plausible. Smoking may alter lipid metabolism through nicotine-induced sympathetic activation, increased free fatty acid mobilization, altered hepatic lipid handling, and changes in enzymes involved in HDL metabolism. The relationship between smoking and HDL-C may therefore contribute to the increased cardiovascular risk associated with long-term cigarette smoking.

Limitations of the Study

The study was limited by its cross-sectional design, which does not establish a definite cause-and-effect relationship between smoking and reduced HDL-C levels. The relatively small sample size and inclusion of only males aged 30–40 years limit the generalizability of the findings. Furthermore, the study focused primarily on HDL-C without evaluating the complete lipid profile, other cardiovascular risk markers, or HDL functionality. Smoking history was self-reported, and potential confounding factors such as dietary habits, physical activity, and stress were not comprehensively assessed. Larger prospective studies involving diverse populations and additional biochemical parameters are therefore recommended.

CONCLUSION

The present study demonstrated a significant adverse association between cigarette smoking and serum high-density lipoprotein cholesterol (HDL-C) levels. Smokers showed significantly lower HDL-C concentrations than healthy non-smokers, with a progressive decline in HDL-C observed as smoking intensity increased. Since age and body mass index were comparable among the study groups, the observed differences in HDL-C are likely to be associated primarily with cigarette smoking.

The reduction in HDL-C may be attributed to smoking-induced alterations in lipid metabolism, including changes in apolipoprotein synthesis, HDL maturation and remodeling, hepatic lipid metabolism, and oxidative modification of lipoproteins. Reduced HDL-C may contribute to a more atherogenic lipid profile and increase the long-term risk of cardiovascular disease.

These findings highlight cigarette smoking as an important modifiable cardiovascular risk factor and emphasize the importance of smoking prevention and cessation programmes. Health education should focus on the metabolic and cardiovascular consequences of smoking in addition to its well-known respiratory and carcinogenic effects. Assessment of lipid parameters in chronic smokers may also help identify individuals at increased cardiovascular risk and facilitate early intervention.

Further large-scale prospective studies involving both sexes and wider age groups are needed to evaluate the complete lipid profile and other cardiovascular risk markers. Such studies may provide a better understanding of the long-term effects of smoking on lipid metabolism and the potential benefits of smoking cessation.

REFERENCES

1. Reddy KS. Cardiovascular disease in India. *World Health Stat Q*. 1993;46(2):101–107.
2. American Heart Association. Cigarette smoking and cardiovascular disease.

3. Gossett LK, Johnson HM, Piper ME, Fiore MC, Baker TB, et al. Smoking intensity and lipoprotein abnormalities in active smokers. *J Clin Lipidol*. 2009;3:372–378.
4. Chelland Campbell S, Moffatt RJ, Stamford BA. Smoking and smoking cessation: the relationship between cardiovascular disease and lipoprotein metabolism. *Atherosclerosis*. 2008;201:225–235.
5. Craig WY, Palomaki GE, Haddow JE. Cigarette smoking and serum lipid and lipoprotein concentrations: an analysis of published data. *BMJ*. 1989;298:784–788.
6. Gepner AD, Piper ME, Johnson HM, Fiore MC, Baker TB, et al. Effects of smoking and smoking cessation on lipids and lipoproteins: outcomes from a randomized clinical trial. *Am Heart J*. 2011;161:145–151.
7. Austin MA. Plasma triglyceride and coronary heart disease. *Arterioscler Thromb*. 1991;11:2–14.
8. Fagerström K. The epidemiology of smoking: health consequences and benefits of cessation. *Drugs*. 2002;62(Suppl 2):1–9.
9. Pyrgakis VN. Smoking and cardiovascular disease. *Hellenic J Cardiol*. 2009;50:234.
10. Wynder EL, Harris RE, Haley NJ. Population screening for plasma cholesterol: community-based results from Connecticut. *Am Heart J*. 1989;117:649–656.
11. Pagan K, Hou J, Goldenberg RL, Cliver SP, Tamura T. Effect of smoking on serum concentrations of total homocysteine and B vitamins in mid-pregnancy. *Clin Chim Acta*. 2001;306:103–109.
12. McCarty MF. Increased homocysteine associated with smoking, chronic inflammation and aging may reflect acute phase induction of pyridoxal phosphatase activity. *Med Hypotheses*. 2000;55:289–293.
13. GBD Tobacco Collaborators. Smoking prevalence and attributable disease burden in 195 countries and territories, 1990–2015. *Lancet*. 2017;389:1885–1906.
14. Howard G, Evans GW, et al. Cigarette smoking and progression of atherosclerosis. *JAMA*. 1998;279:119–124.
15. National Center for Chronic Disease Prevention and Health Promotion. *The Health Consequences of Smoking—50 Years of Progress: A Report of the Surgeon General*. Atlanta, GA; 2014.
16. Mayhan WG, Patel KP. Effect of nicotine on endothelium-dependent arteriolar dilation in vivo. *Am J Physiol Heart Circ Physiol*. 1997;272–H2342.
17. Brewer HB. High-density lipoproteins: a new potential therapeutic target for the prevention of cardiovascular disease. *Arterioscler Thromb Vasc Biol*. 2004.
18. Freeman DJ, Murray GD, et al. Smoking and plasma lipoproteins in man: effect on levels of HDL-C and subfraction distribution. *Eur J Clin Invest*. 1993;23(10):630–640.
19. Souliman S, Simon D, Harman WH, et al. Glycated hemoglobin, fasting and 2-hour plasma glucose in current, ex- and never-smokers. *Diabetologia*. 2014;57(1):30–39.
20. Taniguchi A, Nakai Y, Shakali M, et al. Relationship of regional obesity to insulin resistance in obese men. *Am J Physiol Endocrinol Metab*. 2002;282–E663.
21. Lean MEF, Hankey CR. Relationship between cigarette smoking and BMI. *Int J Obes*. 2005;29(2):236–243.
22. Haslam DW, James WPT. Obesity. *Lancet*. 2005;366:1197–1209.
23. Potter BK, Pederson LL, Chan SS. Does a relationship exist between body weight, concerns about weight and smoking among adolescents? *Nicotine Tob Res*. 2004;6(3):397–425.
24. Wild SH. ABC of obesity: risk factors for diabetes and coronary heart disease. *BMJ*. 2006;333:1009–1011.