ORIGINAL ARTICLE

Association of Circulating Matrix Metalloproteinase-9 and the Risk of Coronary Heart Disease in Young Smokers

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ABSTRACT

Introduction: Smoking causes cardiovascular risk which may alter the stability between the production and degradation of the extracellular matrix. Matrix metalloproteinase-9 (MMP-9) is a zinc-containing endopeptidase that degrades the extracellular matrix and plays a vital role in tissue remodeling. As a result, elevated serum MMP-9 levels produced by smoking, particularly at young age, raise the risk of future CHD. So this study aims to find out the possible relationship between circulating MMP-9 and the risk of cardiovascular disease in young smokers. **Methods:** The study was conducted on smokers with CHD subjects attending cardiology and medicine OP of the SRM Medical College Hospital and research center Tamil Nadu, India. The study group was divided into three groups. Group 1 includes 120 healthy controls as nonsmokers, Group 2 includes 120 smokers with Coronary heart disease (CHD), and Group 3 includes 120 smokers with diabetes and CHD subjects in the age group of 20-55 years. Serum MMP-9, hs-CRP, and APO-E levels were measured using the ELISA method and the lipid level was measured enzymatically using AU480 automatic analyzer (back man coulter). **Results:** The mean serum MMP-9, hs-CRP, and APO-E levels were significantly higher in both groups ($p<0.05$) when compared to controls. The study also shows a significant positive association between MMP-9 with hs-CRP, APO-E, smoking burden, and smoking intensity. **Conclusion:** The study concludes a significant association exists between cigarette smoking with MMP-9 and also relative exposure to circulating inflammation markers plays a potential role in the pathogenesis of CHD.
Malaysian Journal of Medicine and Health Sciences (2023) 19(4):21-27. doi:10.47836/mjmhs19.4.5

Keywords: APO-E, Cardiovascular disease, hs-CRP, Matrix metalloproteinase-9, Smoking

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INTRODUCTION

Smoking is regarded as an environmental risk factor that is associated with different genetic factors and multifactorial disorders (1). The mechanism by which smoking induces cardiovascular risk is still unclear to some extent (2). It is well established that smoking can increase cardiovascular disease which is adversely related to the smoking burden and smoking intensity (3). Other than active smoking, Passive smoking also increases cardiovascular risk by altering the serum lipid levels in young smokers (4). The chemical components in cigarette smoke especially Nicotine can increase the fatty acid level by stimulating the release of adrenaline, leading to an increase the cardiovascular risk (5). The free fatty acid can increase the TGL and LDL secretion and release cholesterol from the hepatic circulation (6). Smoking also produces free radicals which can alter the coagulation system (7), and may accelerate the formation of plaque in the arteries (8). Smoking also causes inflammation, which can lead to the creation of atherosclerotic plaques and contributes to the development of cardiovascular disease. Most recently, a different study shows that the formation of atherosclerotic plaque and subsequent rupture happens due to the development of chronic inflammation (8). Although for the predictor of cardiovascular disease the markers for general inflammation are related to acute phase reactants and pro-inflammatory cytokines including hs-CRP (9). Most particularly the tissue necrosis factor-α (TNF-α) and pro-inflammatory cytokines such as interleukin-1 (IL-1) regulate Matrix metalloproteinase-9 (MMP-9) synthesis by mesenchymal stem cells (10, 11). Recent studies reveal that smoking may alter the stability of the extracellular matrix by altering the balance between the production and degradation of MMP, which may lead to the development of cardiovascular disease (12). Matrix metalloproteinase is the family of zinc-containing zymogene endopeptidases that degrades the extracellular matrix proteins, play an important role in the extracellular matrix for tissue remodeling, and

contribute to a variety of physiological processes (13-15). MMP-9 is a collagenase enzyme with a molecular weight of 92 kDa. In the basement membrane, MMP-9 breaks proteoglycan proteins, type 4 collagen, and interstitial proteins (16, 17). To date, it is the most studied enzyme in relation to cardiovascular disease. A different study shows the high inflammatory condition the MMP-9 activity has been increased in the formation of atherosclerotic plaque (18, 19). So increased MMP-9 levels, particularly in inflammatory conditions caused by smoking, are associated with a greater risk of future cardiovascular disease. Thus MMP-9 can be used as a therapeutic target as well as a biomarker for future CHD (20, 21). So being a part of India in the southern state the smoking rate in Tamil Nadu is vulnerable to increases, which may increase the prevalence of CHD patients in recent times, which may be due to the modification of lifestyle and the increased rate of smoking at an early age. Thus the current study intends to investigate the link between circulating MMP-9 and the risk of CHD in young smokers.

MATERIALS AND METHODS

Study subjects and pattern

The present cross-sectional study was conducted at SRM Medical College Hospital and Research Center, SRMIST, Tamil Nadu, India between October 2019 and September 2021. The study group was divided into three groups. Group 1 includes 120 healthy controls as nonsmokers, group 2 includes 120 young active smokers with CHD, and group 3 includes 120 young active smokers with diabetic CHD subjects who were attending the SRM Medical College Hospital in Tamil Nadu for cardiology and medicine OP. In order to determine whether patients fit the inclusion/exclusion criteria and are qualified to take part in the trial, a simple questionnaire was initially administered to each participant.

Inclusion criteria

Males aged between (20-55 years) who were either non-smoker or at least 5 cigarettes per day for more than one-year duration of smoking. The diagnostic criteria of CHD were based on ECG changes, ST-segment elevated and abnormal coronary angiography with more than 50% stenosis in one or more major arteries.

Exclusion criteria

The study excludes the participants with cardiomyopathy, chronic diseases such as liver failure, alcoholics, cancer patients, heart failure, cardiovascular accidents, significant systemic sickness, and systemic inflammatory disease.

Ascertainment of smoking exposure

The Smoking habits were ascertained through self-reporting. The smoking status, smoking burden (duration of smoking), and smoking intensity (No. of smoking per day), who have smoked conventional cigarettes frequently >5 cigarettes per day for at least the last 12 months were regarded as smokers (22).

Ascertainment of Covariates

Self-reporting is used to determine socio-demographic factors such as age, gender, educational history, and other health and medical histories. Standard equipment and technique were used to measure anthropometric characteristics such as weight and height. After 2 minutes of rest, resting blood pressure was measured three times at one-minute intervals in the sat posture; the average of the second and third measurements was used for analysis. A systolic blood pressure of more than 140 mmHg and diastolic blood pressure of more than 90 mmHg were defined as hypertension. After a 12-hour fast, 5 ml of venous blood was aseptically extracted in a plane tube with a vacutainer. Serum samples were obtained by centrifuging blood samples at 2000 x g for 10 minutes at 40^{o}C and storing them at -200^{o}C until analysis. The AU480 automated analyzer (Beckman coulter) was used to assess the enzymatic levels of fasting blood glucose and lipid parameters. Diabetes mellitus was defined as a previous medical diagnosis of diabetes mellitus or meeting diagnostic criteria for diagnosis based on fasting plasma glucose levels of ≥126 mg/dl, 2-hour plasma glucose levels obtained as part of a 75-g oral glucose tolerance test ≥200, or the glycated hemoglobin test (HbA1c) of ≥6.5 percent.

hs-CRP, MMP-9, and APO-E assay

A sandwich enzyme-linked immunosorbent assay was used to assess the serum hs-CRP, MMP-9 and APO-E concentration in accordance with the manufacturing protocol (Abbkine, Inc. china).

Ethical consideration

Ethical approval for the study was obtained from the Human Research Ethical Committee of SRM Medical College Hospital and Research Center, SRMIST (approval number IEC No: 1763). All study participants provided informed consent.

Statistical analysis

The statistical analysis was performed using IBM Corp.'s Statistical Package for the Social Sciences (SPSS), version 22 (Armonk, NY, USA). The quantitative variables are expressed as mean and standard deviation. Data were examined using a one-way analysis of variance to compare the differences between the three groups (ANOVA). Differences were considered to be highly significant, significant, or non-significant for $P<0.001$, $P<0.05$, or $P>0.05$, respectively. The associations between variables were determined using Pearson's correlation coefficient (r) or Spearman correlations and linear regression analysis was performed to show the relationship between MMP-9 with hs-CRP, APO-E, smoking load, and smoking intensity was affected by smoking status.

RESULTS

Baseline and biochemical characteristics of the study groups

Table I and Table II show the demographic and baseline characteristic data of all three groups. A significant difference was observed between the subjects weight, BMI, W/H ratio, BP (blood pressure), and No. of cigarettes per day (smoking intensity), and smoking duration (smoking burden). Groups 2 and 3 showed substantially higher fasting blood glucose and lipid levels when compared to controls. The study group was not receiving any kind of lipid-lowering treatment.

The serum MMP-9, hs-CRP, and APO-E levels were represented as mean±S.D. in Table 2. The result from the study revealed a gradual increase in serum MMP-9, hs-CRP, and APO-E levels were observed in smokers with CHD followed by CHD with diabetic subjects when compared to controls ("p-value" <0.0001) indicating that smoking induces inflammation.

Correlation Analysis

Table III and figure 1 show the adjusted correlation between serum MMP-9, hs-CRP, and APO-E in smokers in the CHD group. A positive correlation between MMP-9 and hs-CRP (r-value=0.3776), APO-E(r-value=0.4039), TC (r-value=0.3204), TGL (r-value=0.3881), LDL-C (r-value=0.5003), No. of smoking/day (r-value=0.3411) and duration of smoking (r-value=0.3175).

As shown in the table IV and figure 2 studies also show Pearson's correlation between serum MMP-9, hs-CRP, and APO-E in diabetic CHD subjects. A positive correlation was found between MMP-9 with hs-CRP (r-value=0.3776), APO-E (r-value=0.4614), TC (r-value=0.4858), TGL (r-value=0.3917), LDL (r-value=0.4689), No. of smoking/day (r value=0.4287), duration of smoking (r-value=0.3638) and a negative correlation was found with HDL-C levels (r-value=-0.3705).

Table I: Anthropometric measurements of smokers with CHD and normal controls

Parameter	Control group 1 (n=120)	Smokers with CHD group 2 (n=120)	Smokers with diabetic CHD group 3 (n=120)	p-value
Age	31.45±11.57	37.65±9.16	42.45±8.08	<0.0001*
Height(cm)	171.11±3.37	171.15±2.97	171.43±4.31	0.8740
Weight(kg)	67.75±7.97	71.4±4.97	74.31±7.64	<0.0001*
BMI (kg/m2)	23.18±2	24.25±1.5	25.29±1.97	<0.0001*
WC(cm)	86.95±5.54	91.03±4.81	92.15±4.73	<0.0867
HC (cm)	99.8±2.69	100.36±4.54	99.8±5	0.683
W/H ratio	0.86±0.04	0.90±0.04	0.91±0.04	<0.0001*
Systolic BP (mmHg)	117.9±4.2	124.8±4.4	128.3±3.7	<0.0001*
Diastolic BP (mmHg)	80±2.1	79.8±4.1	77.4±5.8	0.0662
No of Smoking /day	0	8.9±3.03	10.05±4.01	<0.0001*
Duration of smoking(years)	0	10.9±6.06	14.61±7.23	<0.0001*

"p-value" <0.05 is statistically significant. One way anova calculation. BMI (body mass index), WC (waist circumference), HC (hip circumference, W/H ratio (waist/hip ratio).

Table II: Biochemical parameters of smokers with CHD and normal controls (non smokers)

Parameter	Control group 1 (n=120)	Smokers with CHD group 2 (n=120)	Smokers with diabetic CHD group 3 (n=120)	p-value
FBG(mg/dl)	96.37±8.11	99.3±11.54	217.76±66.84	<0.0001*
TC(mg/dl)	160.01±22.48	222.86±27.02	230.51±47.67	<0.0001*
TGL(mg/dl)	91.21±34.74	164.45±94.97	200.61±90.44	<0.0001*
HDL(mg/dl)	45.76±8.44	40.45±6.41	39.81±6.88	<0.0001*
LDL(mg/dl)	106.21±17.59	155.03±22.56	157.76±28.08	<0.0001*
VLDL(mg/dl)	18.03±6.97	32.08±15.14	38.56±15.79	<0.0001*
TC/HDL-C	3.56±0.64	5.6±1.03	5.83±1.12	<0.0001*
LDL/HDL-C	2.36±0.51	3.92±0.85	4±0.75	<0.0001*
MMP-9 (ng/ml)	26.10±12.17	58.09±27.78	91.87±31.64	<0.0001*
hs-CRP(mg/L)	0.7789±0.4179	1.9507±0.9531	4.0250±2.2819	<0.0001*
APO-E (ng/ml)	56.78±9.76	46.84±13.19	36.94±6.71	<0.0001*

"p-value" <0.05 is statistically significant. One way anova calculation. FBG(fasting blood glucose), TC(total cholesterol), TGL(triacylglycerides), HDL(high density lipoprotein), LDL (low density lipoprotein), VLDL(very low density lipoprotein), MMP-9(Matrix matelloproteinase-9), hs-CRP(high sensitive C reactive protein), APO-E(Apo lipoprotein-E).

DISCUSSION

Smoking habits continuously elevate the risk of cardiovascular disease and peripheral vascular disease (23). Modifiable risk factor such as high blood pressure or high cholesterol level does not explain clearly the relation between cigarette smoke and the occurrence of cardiovascular disease risk (24, 25). However certain studies give evidence that smoking increases the circulating MMP-9 concentration, which increases the risk of cardiovascular disease (26, 27).

This current study shows a significant positive correlation between cardiovascular risk factors especially smoking with (hs-CRP) and MMP-9. Moreover, different studies show that elevated levels of MMP-9 were positively associated with smoking status and inflammatory markers (including hs-CRP, and IL-6) (27, 28). The study also shows that MMP-9 is significantly correlated with APO-E levels. This may be due to the shedding of the lipoprotein receptor by MMP-9. MMP9 is an endopeptidase that can bind and proteolyze (i.e., shedding) lipoprotein receptors (29, 30).

In this study, MMP-9 levels of serum were found to be considerably higher in smokers with CHD (with and without diabetes) than in the control group (p<0.0001). MMP-9 levels increased considerably in group 3 when compared to groups 2 and group 1, with a significant value of (0.001). This may reflect abnormal extracellular matrix metabolism in the diabetic CHD group (31, 32). MMP-9 is also identified as a novel regulator of

Table III: Correlation of all different parameters in smokers with CHD subjects

parameter	Smokers with CHD subjects					
	MMP-9		hs-CRP		APO-E	
	r -value	p- value	r- value	p- value	r- value	p value
TC(mg/dl)	0.3204	0.0003*	0.3180	0.0004*	0.4651	<0.0001*
TGL(mg/dl)	0.3881	<0.0001*	0.3278	0.0003*	0.3805	<0.0001*
HDL(mg/dl)	-0.1774	0.0525	-0.1701	0.0428*	-0.1843	0.051
LDL(mg/dl)	0.5003	<0.0001*	0.5065	<0.0001*	0.5058	<0.0001*
VLDL(mg/dl)	0.1177	0.370	0.1455	0.0767	0.2033	0.0259*
MMP-9(ng/ml)	-----	-----	0.3776	<0.0001*	0.4039	<0.0001*
hs-CRP(mg/L)	0.3776	<0.0001*	------	------	0.4039	<0.0001*
APO-E(ng/ml)	0.4039	<0.0001*	0.4039	<0.0001*	-----	-----
Smoking /day	0.3411	0.00013*	0.3362	0.00017*	0.4854	<0.0001*
Duration of Smoking (years)	0.3175	0.0004*	0.2825	0.0017*	0.374	<0.0001*

"p-value" <0.05 is statistically significant.

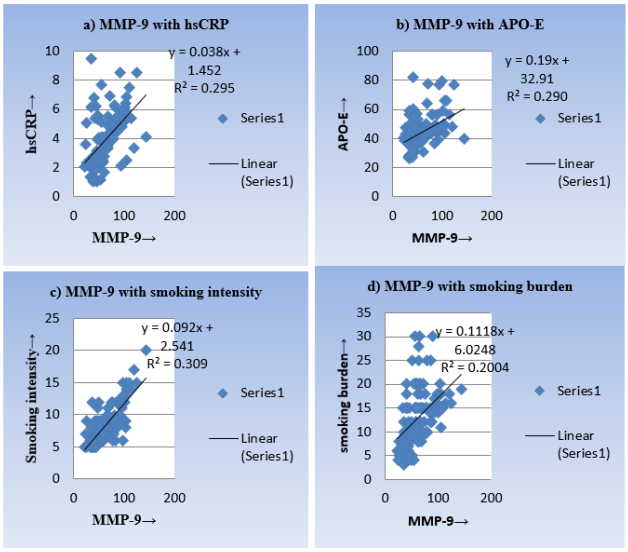

Figure 1: Linear regression analysis of MMP-9 with hsCRP, APO-E, smoking burden and smoking intensity in smokers with CHD subjects.

cholesterol metabolism in the study. Furthermore, the findings show that dysregulation of MMP-9 activity alters hepatic transcriptional responses to dietary cholesterol, potentially leading to metabolic disorders such as atherosclerosis and coronary heart disease (33, 34).

Smoking accelerates inflammation and oxidative modification of lipids and prospectively slows down the matrix metalloproteinase activity at various different levels. By activating inflammatory transcription factors smoking increases the MMP expression (12). Along with this smoking also elevate the monocyte expression of IL-Beta cells (35). Cigarette smoke contains nicotine and the predominant metabolite cotinine increases the production of vascular smooth muscle cell collagenase and gelatinase may lead to plaque rupture (36).

Different mechanisms have been anticipated to explain by which smoking induces the stimulation of MMP, both in vitro and in vivo (37, 38). In vitro tobacco smoke induces MMP-9 expression through the endothelial cell (39). Similarly, exposure to smoking induces MMP-1 expression through the human fibroblast. While tobacco smoke also induces proteolysis by inhaling the cadmium present in smoke (13), which may lead to cardiovascular disease as it increases in the aorta of smokers (40).

The key limitation of this study is the limited sample size that gives a solid conclusion on the link between smoking and CHD risk. Some factors such as age, gender, individual condition, environment, and experimental procedure may disrupt the results and this may influence the interpretation of results in this study. It is very important to analyze the activity of endogenous tissue inhibitors of metalloproteinase 1 (TIMP1) because it creates a balance between the MMP-9, and TIMP-1 (41-43). But our study did not analyze the TIMP-1 concentration.

So our finding on MMP-9 in young smokers gives evidence that cigarette smoking accelerates the circulating MMP-9 levels at a young age.

CONCLUSION

The current investigation revealed a substantial link between serum MMP-9 and the risk of coronary heart disease among young smokers. According to the findings, an increase in MMP-9 levels, particularly in inflammatory conditions produced by smoking, is

Table IV: Correlation of all different parameters in smokers with Diabetic CHD subjects

parameter	Smokers with CHD subjects					
	MMP-9		hs-CRP		APO-E	
	r -value	p- value	r- value	p- value	r- value	p value
TC(mg/dl)	0.4858	<0.0001*	0.4773	<0.0001*	0.513	<0.0001*
TGL(mg/dl)	0.3917	<0.0001*	0.2257	0.013*	0.3021	0.0189*
HDL(mg/dl)	-0.3705	<0.0001*	-1762	0.0542	-0.1903	0.0007*
LDL(mg/dl)	0.4689	<0.0001*	0.7314	<0.0001*	0.6983	<0.0001*
VLDL(mg/dl)	0.1287	0.161	0.1964	0.0315*	0.1579	0.084
MMP-9(ng/ml)	-----	-----	0.3686	<0.0001*	0.4614	<0.0001*
hs-CRP(mg/L)	0.3686	<0.0001*	-----	-----	0.5253	<0.0001*
APO-E(ng/ml)	0.4614	<0.0001*	0.5253	<0.0001*	-----	-----
Smoking /day	0.4287	<0.0001*	0.3845	<0.0001*	0.7335	<0.0001*
Duration of Smoking (years)	0.3638	<0.0001*	0.3899	<0.0001*	0.7629	<0.0001*

"p-value" <0.05 is statistically significant.

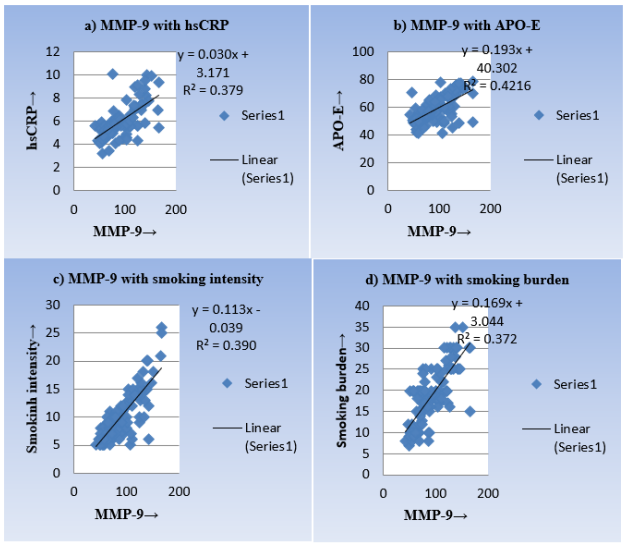

Figure 2: Linear regression analysis of MMP-9 with hsCRP, APO-E, smoking burden and smoking intensity in smokers with diabetic CHD subjects.

associated with a greater risk of future cardiovascular disease.

ACKNOWLEDGEMENTS

The authors appreciate the permission and cooperation from the Department of Medicine, Cardiology, and the Master Health Check-up Unit of SRM Medical College Hospital and Research Center, SRMIST, Tamil Nadu, India.

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