Scholars Journal of Applied Medical Sciences (SJAMS) **ISSN 2320-6691 (Online)**
Sch. J. App. Med. Sci., 2016; 4(11D):4141-4145 **ISSN 2347-954X (Print)**

(An International Publisher for Academic and Scientific Resources)
www.saspublishers.com DOI: 10.36347/sjams.2016.v04i11.055

Original Research Article

Study of atherosclerotic markers in smokeless tobacco users in a teaching hospital

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Abstract: Cigarette smoking is an established cardiovascular risk factor and the leading preventable cause of coronary artery disease in most of the developed world. However, it remains unclear whether the smokeless tobacco is also responsible for same deleterious effect on cardiovascular health. We planned this study with an aim to assess endothelial dysfunction and measurement of hs CRP an inflammatory mediator as an early atherosclerotic marker of cardiovascular risk in smokeless tobacco users. In a hospital based, case control study, fifty smokeless tobacco users for at least 3 years and 50 age gender and BMI-matched healthy volunteers were enrolled. Anthropometric measurements, biochemical parameters, Flow mediated vasodilatation (FMD) and high sensitive C reactive protein (HsCRP) were assessed in all study subjects. The mean duration of smokeless tobacco consumption was 8 ± 3.5 years. HDL cholesterol was low and fasting insulin was significantly higher in smokeless tobacco users than the controls. FMD was found to be reduced in smokeless tobacco users while Hs CRP was not significantly different in two groups. Our study revealed that smokeless tobacco use is associated with endothelial dysfunction which is an indicator of subclinical atherosclerosis. However, further studies with larger sample sizes are needed to clarify the association between smokeless tobacco use and pathogenesis of atherosclerosis.
Keywords: Smokeless tobacco, Flow mediated vasodilatation, Hs CRP, Atherosclerosis

INTRODUCTION

Cigarette smoking is an established cardiovascular risk factor and the leading preventable cause of coronary artery disease in most of the developed world [1]. Prevalence of smoking has declined in most high income countries while practice of smokeless tobacco is rampant in South East Asia [2]. According to World Health Organization (WHO), 90% of smokeless tobacco consumers live in South East Asia [2]. Easy accessibility and affordability, along with unawareness concerning its useful health effects are main contributing elements for augmented smokeless tobacco consumption. However, it remains unclear whether the smokeless tobacco is also responsible for same deleterious effect on cardiovascular health.

Cigarette smoking is responsible for accelerated atherosclerosis by variety of mechanisms such as endothelial dysfunction, inflammation and procoagulant activity [3]. It has been suggested that smokeless tobacco produces vascular damage through similar biological mechanisms [4, 5].

Endothelial dysfunction has been regarded as an early feature of atherosclerosis. Assessment of endothelial dysfunction by measuring flow mediated dilatation (FMD) of the brachial artery is considered as potential tool for predicting coronary atherosclerosis [6] highly sensitive C-reactive protein (hsCRP) is also a well known surrogate marker and good predictor of subclinical atherosclerosis and future cardiovascular events [7].

We planned this study with an aim to assess endothelial dysfunction and measurement of hs CRP an inflammatory mediator as an early atherosclerotic marker of cardiovascular risk in smokeless tobacco users.

MATERIAL AND METHODS

In a hospital based, case control study, fifty smokeless tobacco users visiting to medicine outpatient clinic between January 2015 and March 2015 were enrolled. The control group comprised 50 age gender

and BMI-matched healthy volunteers. The inclusion criterion for cases was using smokeless tobacco for at least 3 years.

Exclusion criteria comprised of hypertension, diabetes mellitus, chronic obstructive lung disease, coronary artery disease, chronic renal failure, Morbid obesity, history of taking medications for the above-mentioned diseases, and history of smoking and alcohol consumption. Informed consent was given by all study participants, and the study was approved by the Institutional Ethics Committee

Medical history was taken and detailed physical examinations were performed in all subjects. Body weight was measured to the nearest 0.5 kg in light clothing without shoes. Height was measured to the nearest 0.5 cm. Body mass index (BMI) was calculated as kilograms divided by square meters (kg/m2). Waist circumference was measured in centimetres with the patient standing, at a point midway between the lower costal margin and the iliac crest in the mid-axillary line.

Laboratory investigations, including lipid profile, fasting blood glucose, fasting insulin, uric acid were done in all subjects.

The hsCRP concentration was determined using an immunoturbidimetric method (Randox, Mauguio, France) in mg/dl.

FMD was performed using a linear 7 MHz transducer (Vivid 7, GE Healthcare), and both groups were directed to abstain from Tea/caffeine 12 hours prior to the study. A longitudinal image was used to measure brachial artery diameter (1st baseline image), and a blood-pressure cuff was inflated on the upper arm (2–5 cm above the cubital fossa) to 50 mmHg above systolic pressure for 5 minutes and then deflated after 1 minute. A second longitudinal scan was obtained (from the same position) to calculate the brachial artery diameter (post-occlusion value). Flow-mediated dilation (FMD) was calculated as: maximum diameter during reactive hyperemia−diameter at baseline)× 100/(diameter at baseline). All measurements of the brachial artery lumen diameter were assessed at end diastole.

Statistical analysis

Statistical analysis was performed using Microsoft Excel (Microsoft Office 2011; Microsoft, Inc., Redmond, WA, USA).

All data were expressed as mean ± standard deviation. The difference between two means was statistically analyzed using the Student *t* test. Categorical variables are defined as percentages. Pearson's correlation coefficient (r) was calculated to test the association between two variables. Significance determined as $p < 0.05$.

RESULTS

In this hospital based study we included 50 smokeless tobacco users who consumed tobacco in form of pan masala or pure tobacco chewing for more than 3 years. The mean duration of smokeless tobacco consumption was 8 ± 3.5 years. The characteristics of study participants have been shown in Table 1. Anthropometric and demographic profile was similar in two groups.

The traditional cardiovascular risk factors including waist-hip ratio, waist circumference, systolic and diastolic blood pressure were studied and all were not significantly different between cases and controls except the diastolic blood pressure which was higher in cases than controls.

Table 2 is showing biochemical parameters of the study participants. There were no significant differences in terms of total cholesterol, LDL cholesterol, triglycerides, and fasting blood glucose levels between the controls and smokeless tobacco users (p>0.05). However, HDL cholesterol was low and fasting insulin was significantly higher in them than the controls.

Flow mediated vasodilatation was found to be reduced in smokeless tobacco users than controls (Table 3) while hs CRP was not significantly different in two groups. FMD reduction had significant negative correlation with duration of consumption of smokeless tobacco(r= 0.42, p=0.001).

Table-1: Clinical characteristics of smokeless tobacco users and control subjects

Variables	**smokeless tobacco users** (N=50)	**Controls**(N=50)	P value
Age(years)	34.82± 3.26	35.7± 4.6	0.54
BMI(kg/m^2)	26.2±4.8	25.7± 4.2	0.2
Waist circumference(cm)	96.2±6.8	98.3± 6.2	0.62
Hip circumference(cm)	107.6±12.4	109.2±7.8	0.5
Waist/Hip ratio	0.90±0.1	0.89±0.08	0.12
Systolic BP(mmHg)	126±20	118±16	0.08
Diastolic BP(mmHg)	86.4±7.2	70±7.6	0.001

Data is expressed in mean±SD

Table-2: Biochemical parameters in smokeless tobacco users and control subjects

Variables	**smokeless tobacco users** (N=50)	Controls(N=50)	P value
Fasting glucose(mg/dl)	86±7.6	82.2±6.0	0.08
Triglyceride(mg/dl)	122 ±54	119±32	0.12
LDL-cholesterol(mg/dl)	102±39.2	96± 27	0.6
HDL-cholesterol(mg/dl)	46.8±7.6	48± 9.7	0.07
Insulin(mu/ml)	10.2±3.4	6.1 ±1.8	0.05

Data is expressed in mean±SD

Table-3: Atherosclerosis markers in smokeless tobacco users and control subjects

Variables	**smokeless tobacco users** (N=50)	Controls(N=50)	P value
HsCRP(mg/dl)	3.6± 2.12	3.08 ±2.4	0.06
FMD (%)	12.18± 2.3	8.3± 2.23	0.01

Data is expressed in mean±SD

DISCUSSION

Smoking and smokeless tobacco use is an important public health problem. Prevalence of this health hazard is high in low income countries and the highest in South Asia and South eastern Asian regions [8].

The global epidemic of obesity and diabetes mellitus has resulted in an increasing number of patients suffering from metabolic syndrome. This is of particular concern because of the close association between metabolic syndrome and cardiovascular disease [9]. There has been increasing trend in prevalence of coronary artery disease [10].

Smoking is a well-known cardiovascular risk factor but smokeless tobacco has been less studied as far as its effect on cardio vascular health is concerned. We conducted this study in a teaching hospital to assess the surrogate atherosclerotic markers in smokeless tobacco users and compared with nonusers as controls.

Various studies have shown contradictory results with regard to the cardiovascular effects of smokeless tobacco. Some have reported an association between smokeless tobacco and cardiovascular complications [11, 12]. Some studies showed lower risk in comparison to those who smoked tobacco [13], while some studies failed to find such an association [14, 15].

According to the INTERHEART Study carried out in 52 countries, individuals who only chewed tobacco had a significantly increased risk [Odds ratio 2.23] of first myocardial infarction compared to those who never used tobacco [16]. Current use of smokeless tobacco was associated with increased risk of CVD incidence among nonsmokers in the ARIC Study and authors concluded that current users of smokeless tobacco should be informed of its harm [17].

Smokeless tobacco has been reported to contain more than 2,000 chemical compounds, and the major addicting substance is nicotine which is found to reach a higher concentration in smokeless tobacco users in comparison to smokers [18].

Mechanisms by which smokeless tobacco might cause CVD have been studied [5] and include acute activation of the sympathetic nervous system and acute elevation of blood pressure [19] and chronic hypertension [20]. Nicotine has been reported to injure endothelial cells and to release growth factors to promote angiogenesis, which could contribute to atherogenesis [21].

In addition to hemodynamic effects mediated by sympathetic effects, nicotine may also contribute to endothelial dysfunction [22]. Endothelial dysfunction is a systemic pathological condition which can be broadly defined as an imbalance between vasodilating and vasoconstricting substances produced by the endothelium. Endothelial dysfunction plays a major role as an early marker of cardiovascular risk, and there is a close relationship between endothelial function in human coronary and peripheral circulation [23]. Endothelial-dependent vasodilation is impaired by nicotine use through catecholamine release and oxidative stress [24].

Flow-mediated Dilation (FMD) is a non-invasive radiological imaging method used for endothelial dysfunction determination [25]. It is applicable for screening asymptomatic patients. FMD was significantly decreased in patients with CVD [26]. The studies have been conducted in chronic smokeless tobacco users or cigarette smokers and have shown that smokeless tobacco users have the same endothelial dysfunction as smokers [27, 28]. In the present study, we also observed reduced FMD in smokeless tobacco users than nonusers.

We found higher diastolic blood pressure and low HDL in smokeless tobacco users than controls. In another study, involving 90 patients from India, smokeless tobacco users had lower HDL and higher triglyceride levels [29].

In a population-based study of 3128 men, there was a higher prevalence of diabetes among long-term users of smokeless tobacco than among nonusers [30]. Eliasson et al demonstrated that smokeless tobacco users had higher insulin levels than nonusers, suggesting a similar link between smokeless tobacco and insulin resistance Although the mechanism is not entirely clear, it may be related to increased levels of norepinephrine or other counterregulatory hormones [31]. Our study also showed similar findings.

Some studies conducted on healthy populations reported that cigarette smoking was positively correlated with serum hsCRP concentration [32, 33]. To the best of our knowledge, no study has yet investigated the associations between smokeless tobacco and serum hsCRP level in our population so our study was unique in this respect. In the present study we did not find any difference in hsCRP levels between the two study groups.

Effects of smokeless tobacco on cardiovascular system have not adequately studied in our population so there is a requirement of large appropriately designed studies to address the issue. Limitations of present study are its cross sectional design and small sample size.

CONCLUSION

Our study revealed that smokeless tobacco use is associated with endothelial dysfunction which is an indicator of subclinical atherosclerosis. However, further studies with larger sample sizes are needed to clarify the association between smokeless tobacco use and pathogenesis of atherosclerosis. In addition, development of public health policies is of great importance to discourage its use and increase awareness about its adverse effect on cardiovascular health.

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