

Original article

Effect of Vitamin C Supplementation on Lipid Profile in Smokeless Tobacco Chewers

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ABSTRACT:

Background: Smokeless Tobacco (ST) may have a considerable influence in the derangement of lipoprotein fractions, which may result in atherosclerosis. The present study was aimed to evaluate the effect of Vitamin C (VC) supplementation on serum cotinine (CTN) level, lipoprotein fractions and atherogenic indices in ST chewers.

Materials and Methods: A total 338 healthy participants of aged between 31 to 60 years were divided into two groups; comprising ST chewers and ST non chewers. Participants were asked to take 1 g of VC daily for 45 days. Serum CTN and lipoprotein fractions were measured at baseline study and after supplementation of VC.

Results: Serum CTN ($p < 0.0001$), Triglyceride (TG) ($p < 0.0001$), Very low density lipoprotein cholesterol (VLDL-C) ($p < 0.0001$) and atherogenic indices were significantly increased whereas, High density lipoprotein cholesterol (HDL-C) ($p < 0.0001$) level was significantly decreased in ST chewer as compared to controls at the baseline study. However, deranged lipoprotein fractions were improved after supplementation of VC.

Conclusion: ST consumption is associated with increased levels of serum CTN, TG, VLDL-C, atherogenic indices and decreased level of HDL-C. Daily consumption of 1 g of VC may be helpful in lowering the deranged levels of lipoprotein fractions.

Key words: Atherogenic index, Cotinine, Lipoprotein fractions, Vitamin C

Introduction:

Smokeless Tobacco (ST) use is at large scale in South Asia, especially in India as compared to rest of the world [6]. In the world, India is the largest consumer and third largest producer of tobacco [1]. Global Adult Tobacco Survey-2 (GATS-2) reports that 28.6% of the people use tobacco in different forms. Every year, millions of people die in India due to cancers, cardiovascular diseases (CVD) and lung diseases, which are directly credited to tobacco use [2]. Since the toxic properties of nicotine and other constituents of ST, there is apprehension that ST products may cause cardiovascular disease (CVD) or lead to death [3]. In short, nicotine modifies cell morphology which facilitates passage and attack of cells that line the blood vessels. This enables a change in morphology called

podosomes, which lead to deprived vessels and can root the formation of plaque. Ultimately, plaque can cause arteries to harden, called atherosclerosis. It can also block blood flow to the brain or heart, which causing stroke or myocardial infarction [4].

Atherogenic indices such as CRI-I (Castelli's Risk Index I), CRI-II, AC (Atherogenic Coefficient) and AIP (Atherogenic Index of Plasma) [5] are used and found better to predict cardiovascular health than independently determined Triglycerides (TG), LDL-C or HDL-C. However, there is hardly any study available which has seen the effect of smokeless tobacco (mixed with slaked lime) on atherogenic indices. VC is a natural antioxidant and its biological effects have been extensively studied. Its intake reduces the lipid peroxidation and improves the antioxidant

status [6]. VC takes part in catabolism of cholesterol and therefore in the regulation of lipoprotein fractions [7]. Therefore present study was undertaken to evaluate the effect of smokeless tobacco and to study the effect of vitamin C supplementation on atherogenic indices in smokeless tobacco chewers.

Materials & Methods:

The present study was carried out in the Krishna Institute of Medical Sciences Deemed University, Karad. Study protocol was approved by Institutional Ethics Committee of KIMSDU, Karad (Ref. No: KIMSDU/IEC/01/2015, Dated: 05/03/2015). Apparently healthy tobacco chewers and tobacco non-chewers of age group between 31 to 60 years were included in the study after their written consent obtained. Total 338 subjects participated in the present study. They were categorized into tobacco chewers and tobacco non chewers (control group). ST chewers were further divided into three sub groups according to smokeless tobacco chewing (STC) duration in years (1 -10 years, 11 -20 years and 21 -30 years). Participants enrolled in the study received 1 gm of vitamin C / day for 45 days. Compliance was monitored at timely intervals through cell phone or meeting them.

Inclusion Criteria: Adult males of age group of 31 to 60 years, who had been chewing ST at least from last one year or more and age, sex matched controls.

Exclusion Criteria: Subjects with any type of disease, regular medication and alcoholic individuals were excluded from the study.

Investigations

1) Serum cotinine level was estimated by cotinine ELISA CALBIOTECH kit method on Elisa reader.

2) Lipid profile: Estimation of serum TC, TG and HDL-C was done on the ERBA 360 fully automated analyzer [8].

3) Atherogenic indices of lipids were calculated as Castelli's Risk Index -I (CRI-I) = TC/HDL-C, Castelli's Risk Index- II (CRI II) = LDL-C/ HDL-C, Atherogenic Coefficient (AC) = (TC- HDL-C) /HDL-C and Atherogenic Index of Plasma (AIP) = log (TG/HDL-C) [9, 10].

Statistical analysis: The study data were analyzed on statistical software SPSS 20, $p < 0.05$ was considered as significant.

Results

Table 1: Comparison of serum CTN and lipoprotein fractions (Mean±SD) between controls and ST chewers.

Parameters	Tobacco non chewers	Tobacco chewers	Unpaired t test	p value
	(N=170)	(N=168)		
Serum CTN (ng/ml)	4.00±2.64	181.55±99.33***	23.29	<0.0001
TC (mg/dl)	166.58±31.51	168.79±33.43	0.624	0.532
TG (mg/dl)	109.66±46.06	141.25±57.62***	5.570	<0.0001
VLDL-C (mg/dl)	21.96±9.28	28.25±11.52***	5.522	<0.0001
LDL-C (mg/dl)	99.68±28.37	101.88±30.50	0.685	0.493
HDL-C (mg/dl)	44.83±9.12	39.06±8.82***	5.910	<0.0001

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ as compared to controls.

Table 2: Comparison of Atherogenic index between controls and ST chewers.

Atherogenic Ratio	Controls (N=170)	ST chewers (N=168)	unpaired t value	p value
CRI- I (TC/HDL-C)	3.83±0.95	4.46±1.08	5.69	<0.0001
CRI- II (LDL/HDL-C)	2.31±0.83	2.71±0.93	4.17	<0.0001
AC (Non HDL/HDL-C)	2.88±1.00	3.46±1.08	5.12	<0.0001
AIP Log (TG/HDL-C)	0.27±0.22	0.33±0.22	2.50	0.01

Table 2-A: Comparison of Atherogenic Indices between before and after supplementation of VC in ST chewers.

Atherogenic Ratio	ST chewers (N=168)		Paired t test	p value
	Before VC	After VC		
CRI- I (TC/HDL-C)	4.46±1.08	4.09±0.84	0.84	0.39
CRI- II	2.71±0.9	2.55±0.8	0.47	0.63

(LDL/HDL-C)	3	6		
AC (Non HDL/HDL-C)	3.46±1.08	3.27±0.85	0.36	0.71
AIP Log (TG/HDL-C)	0.33±0.22	0.29±0.20	1.47	0.14

Table 3: Comparison of serum CTN and lipoprotein fractions with respect to Smokeless Tobacco chewing (STC) duration.

Parameters	Control	ST chewers (STC duration in years)			ANOVA F value (p value)
	(N=170)	1-10 years (N=50)	11-20 years (N=65)	21-30 years (N=53)	
	Group A	Group B	Group C	Group D	
Serum CTN (ng/ml)	4.00±2.64	166.08±116.05***	174.48±89.21***	189.46±96.33***	169.92 (<0.0001)
TC (mg/dl)	166.58±31.51	165.77±32.54	168.34±38.45	170.45±30.73	0.19 (0.89)
TG (mg/dl)	109.66±46.06	134.78±42.26***	139.57±47.95***	148.76±78.46***	10.83 (<0.0001)
VLDL-C (mg/dl)	21.96±9.28	26.95±15.69*	27.91±9.59**	29.57±15.60***	8.38 (<0.0001)
LDL-C (mg/dl)	99.68±28.37	98.97±34.31	101.49±30.81	102.65±28.07	0.17 (0.91)
HDL-C (mg/dl)	44.83±9.12	39.85±9.30*	38.94±8.93***	38.23±8.01***	11.86 (<0.0001)

*p<0.05, **p<0.01, ***p<0.001 compared with control.

Table 4: Comparison of serum CTN and Lipoprotein fractions with respect to STC duration in controls and ST chewer sub groups.

Parameters	p and t values	STC duration wise comparison					
		A vs B	A vs C	A vs D	B vs C	B vs D	C vs D
Serum CTN	p value	<0.0001	<0.0001	<0.0001	0.62	0.32	0.41
	t value	18.29	24.94	25.28	0.488	0.997	0.825
TC	p value	0.87	0.70	0.49	0.69	0.49	0.76
	t value	0.158	0.384	0.679	0.393	0.678	0.293
TG	p value	0.0007	<0.0001	<0.0001	0.56	0.28	0.43
	t value	3.452	4.746	4.052	0.580	1.069	0.783
VLDL-C	p value	0.005	<0.0001	0.0001	0.66	0.44	0.47
	t value	2.807	4.697	3.933	0.435	0.771	0.709

LDL-C	p value	0.88	0.64	0.56	0.66	0.59	0.84
	t value	0.148	0.459	0.578	0.435	0.533	0.195
HDL-C	p value	0.0009	<0.0001	<0.0001	0.57	0.390	0.68
	t value	3.739	4.816	4.072	0.557	0.851	0.413

Group A: Controls, **Group B:** STC duration 1-10 years, **Group C:** STC duration 11-20 years, **Group D:** STC duration 21-30 years.

Table 5: Comparison of serum CTN and Lipid profile in ST chewers between baseline study and after supplementation of VC.

Parameters	Smokeless Tobacco chewers (N= 168)		Paired t test	p value
	Baseline study	After supplementation of VC		
Serum CTN (ng/ml)	181.55±99.33	156.17±82.56	7.86	<0.0001
TC (mg/dl)	168.79±33.43	165.88±26.50	2.64	0.008
TG (mg/dl)	141.25±57.62	135.05±47.75	4.72	<0.0001
VLDL-C (mg/dl)	28.25±11.52	27.01±9.55	4.71	<0.0001
LDL-C (mg/dl)	101.88±30.50	95.80±23.63	5.20	<0.0001
HDL-C (mg/dl)	39.06±8.82	43.16±8.84	12.32	<0.0001

Discussion:

TG (p<0.0001), VLDL-C (p<0.0001) and serum CTN (<0.0001) were significantly increased and HDL-C (p<0.0001) was significantly decreased in tobacco chewers as compared to controls (table 1). To the best of our knowledge this is the first study finding the effects of ST use mixed with slaked lime on lipoprotein fractions. However, there are several studies on other ST forms such as gutkha [11], Maras powder [12] and naswar [13] in which increased levels of TC, LDL-C and TG however, decreased levels of HDL-C had been observed in ST chewers when compared with controls.

In STC duration wise (in years) comparison, it was found that, serum CTN (p<0.0001), TG (p<0.0001) and VLDL-C (p <0.05, <0.01 and <0.001 respectively) were progressively and significantly increased. However, HDL-C (p<0.05, <0.001, <0.001 respectively) was progressively and significantly decreased with respect to increased STC duration (table 3).

However, STC duration wise comparison between different ST chewer groups, there was found no any significant change (table 4). Similar findings were recorded by the study of Jaywant T et al. [14], Poonam G et al. [15] and Axelson A et al. [16], all those findings also showed significant decrease in HDL-C with gradual rise in STC duration. Triglyceride-rich lipid is most significant for the succession of early atherosclerotic plaques, while cholesterol-rich lipoproteins mainly affect the late atherosclerotic development [17].

Atherogenic indices such as CRI- I (TC/HDL-C), CRI- II (LDL/HDL-C), AC (Non HDL-C/HDL-C) (p<0.0001) and AIP (p=0.01) were increased significantly in ST chewers as compared to controls (table 2). It was suggested that tobacco chewers are at the risk of atherosclerosis [3, 4]. After supplementation of VC all atherogenic indices were reduced in ST chewers but, not to the significant extent (table 2-A). However in the recent study, supplementation of VC lowers

atherogenic indices to the significant level in obese females [18].

In addition, daily supplementation of 1 gm VC for 45 days resulted in significant decrease in serum CTN ($p < 0.0001$), TC level ($p < 0.008$), TG ($p < 0.0001$), VLDL-C ($p < 0.0001$) and LDL-C ($p < 0.0001$) and significant increase in HDL-C (< 0.0001) in ST chewers as compared to controls (table 5). In a study of Afkhami M et al. [19] and Ghanwat G et al. [18], daily supplementation of 1 gm and 1500 mg of VC for 6 weeks and 3 months respectively caused significant decrease in TG and LDL-C. However, Bishop et al. [20] observed no significant change in TC, TG and HbA1c after the supplementation of 500 mg/day of VC. In a study performed by Paolisso et al. [21] on type 2 diabetes patients, daily intervention with 1 gm VC for 4 months significantly decreased the levels of TC, TG and LDL-C.

Conclusion:

Use of ST is associated with increased levels of serum CTN, TG and VLDL-C whereas, decreased level of HDL-C. The atherogenic indices as indicated by various risk ratios found to be increased significantly in ST chewers than in controls, which indicated that, ST chewers were at a pro- atherogenic state. Increased ST chewing duration produced increased adverse effects on lipoprotein fractions. Daily consumption of 1 gm of vitamin C may be beneficial in revised the deranged levels of lipoprotein fractions in ST chewers.

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Conflicts of Interest

The authors affirm that they have no conflict of interest.

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