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Effect of vitamin C supplementation on hematological parameters in smokeless tobacco chewers

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ABSTRACT

The present study was aimed to evaluate the effect of Vitamin C (VC) supplementation on hematological parameters in Smokeless Tobacco (ST) chewers. A total of 338 subjects aged between 31 to 60 years (168 ST chewers and 170 ST non-chewers) participated in the present study. ST chewers were further divided into 3 subgroups with respect to ST chewing duration in years. Subjects of both the groups were examined at the baseline study and after 45 days of supplementation of 1 g of Vitamin C (VC) for hematological parameters. WBC count (p=0.04), granulocytes % (p=0.0007), HCT (p=0.01) and MCV (p=0.04) were significantly increased whereas, monocytes % (p=0.002) and platelet count (p<0.0001) were significantly decreased in ST chewers as compared to controls. After supplementation of VC, WBC count (p<0.001) and granulocytes % (p<0.0001) were significantly decreased and lymphocytes % (p=0.008), monocytes % (p<0.0001), RBC count (p=0.01) and Hb content (p=0.006) were significantly increased in ST chewers as compared to their baseline values. In conclusion, the use of ST had deleterious effects on hematological parameters; however, supplementation of 1 g of VC showed protective effects on hematological parameters.

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INTRODUCTION

According to World Health Organization (WHO), Smokeless Tobacco (ST) is the single greatest cause of preventable death all over the world (WHO, 2008). The various forms of tobacco are smoked, chewed, sniffed, sucked and applied to teeth and gums (National Sample Survey Organization, 1999). Forms of chewing tobacco include crushed tobacco leaves mixed with slaked lime paste, khaini, gutkha, mishri (a roasted and powdered tobacco), mawa (a mixture of sliced areca nut, lime, kiwam and tobacco leaves), pan (piper betel leaf filled with sliced areca nut, lime and tobacco leaves) and dry snuff or tapkeer (use for inhalation) (Zakiullah *et al.*, 2012). ST largely used in South Asia and mainly in India as compared to the Western world (WHO, 2009). In India, millions of people die every year due to tobacco use (Thomsen *et al.*, 2008). The majority of cardiovascular diseases (CVD), cancers and lung diseases are directly credited to tobacco use (Thomsen *et al.*, 2008).

ST use is documented in 120 nations of the world (Sinha *et al.*, 2010). India has the largest number of ST users in the world. Out of the 346 million worldwide tobacco users, India alone has 152.4 million people and out of that, 80.8 million ST users (US NCI and WHO, 2016) and there has been a considerable rise in ST users among all age groups (Bhan *et al.*, 2016). The financial burden due to tobacco-

related diseases in India was US$ 22.4 billion in the year 2011- 2012 (MoHFW and WHO, 2014; Pednekar *et al.*, 2016). The economic burden was more in females as compared to males due to ST use (US NCI and WHO, 2016).

In view of the various pharmacological actions of nicotine and its wide use in many regions of the world, chronic consumption of ST may affect the status of hematological parameters. However, there are limited studies available on the effect of ST use on hematological parameters in humans. The present study was undertaken to find out whether 'smokeless tobacco' causes any adverse effect on hematological parameters as well to evaluate the beneficial effects of vitamin C supplementation.

MATERIALS AND METHODS

The present interventional study has an experimental approach and the case-control design was carried out in the Krishna Institute of Medical Sciences, Karad, during 2015–2019. The study protocol was approved by the Institutional Ethical Committee of KIMSDU, Karad (Ref. No: KIMSDU/IEC/01/2015). A total of 338 subjects aged between 31 to 60 years (168 ST chewers and 170 ST non-chewers) participated in the study. Experimental protocol and study objectives were explained to the participants and their written informed consent were obtained according to the Helsinki declaration (World Medical Association, 2013).

Inclusion Criteria

Healthy adults aged between 31 to 60 years with at least last one or more years of history of ST chewing (dried tobacco leaves mixed with slaked lime) were selected as ST chewers. Age and sex-matched healthy adults with no history of tobacco use in any form were selected as controls.

Exclusion Criteria

Individuals with any type of CVD, autonomic, endocrine, metabolic dysfunction, liver disease, cancer, any type of long term and regular medication and alcoholic individuals were excluded from the present study.

EDTA anticoagulated blood was used for estimation of hematological parameters on SYSMEX XT- 1800 fully auto analyzer. Subjects of both groups were examined at the study baseline for hematological parameters. All study participants, ST chewers and controls were asked to consume 1gm of VC (Celin, Glaxo SmithKline) daily after a meal, respectively, for 45 days.

Regular follow up were taken twice in a week and subjects were advised to maintain their normal diet and continue their habitual physical activity. Subjects of both groups were examined at the end of the study (after supplementation of VC) for hematological parameters. Participants of the ST chewers group were further divided with respect to ST chewing duration in years. The data obtained from both study groups were analyzed and compared by using a suitable statistical test.

RESULTS AND DISCUSSION

The present study showed that tobacco chewers had significantly deranged hematological parameters. In the present study, we observed that, WBC count (p=0.04), granulocytes % (p=0.0007), HCT (p=0.01) and MCV (p=0.04) were significantly increased whereas, monocytes % (p=0.002) and platelet count (p<0.0001) was significantly decreased in ST chewers as compared to controls (Table 1). Selected hematological parameters showed increased derangement with respect to increased ST chewing duration in years (Table 2). Lymphocytes % (p=0.02) and monocytes % (p=0.001) were significantly decreased with respect to increased ST chewing duration whereas granulocytes % (p=0.01) and platelets count (p<0.0001) were significantly decreased with respect to increased ST chewing duration.

In the present study, monocytes % (p= 0.008) shows a significant positive correlation with serum VC. WBC count shows a significant positive correlation with serum CTN (p=0.04) and a negative correlation with serum VC (p=0.05). However, platelet count (p=0.004) shows a significant negative correlation with serum CTN in ST chewers (Table 4).

In previous similar studies, it was concluded that Hb level and WBC count were increased whereas monocytes and basophils count were decreased in gutkha consumers as compared to controls (Rajsekhar *et al.*, 2007; Jaganmohan and Sarma, 2011; Dass *et al.*, 2013).

In another previous study (Kumar *et al.*, 2017) observed that Hb, RBC and WBC count were significantly increased, whereas; lymphocytes, monocytes and platelet count were significantly decreased in tobacco chewers as compared to controls. The findings of Ukoha indicated that chronic tobacco consumption might put the body at some risk of adverse hematological and haemostatic conditions (Ukoha *et al.*, 2012). Roan Mukherjee reported that the adverse effects of gutkha on hematological parameters are not less than smoking (Mukherjee and Chatterjee, 2013).

Table 1: Comparison of hematological parameters between controls and ST chewers

Parameters	Controls N= 170	ST Chewers N= 168	Unpaired 't' test	p-value
WBC (1000/ cumm)	7568±1369	7851±1204	2.01	0.04
Lymphocytes (%)	27.28±4.70	25.90±4.81	2.70	0.07
Monocytes (%)	6.00±1.07	5.57±1.09	3.78	0.002
Granulocytes (%)	66.70±4.68	68.52±5.14	3.40	0.0007
RBC (million/cumm)	5.20±0.46	5.27±0.50	1.33	0.18
Hb (g/dl)	14.86±1.164	14.98±0.91	1.05	0.29
HCT (%)	44.67±4.03	45.70±3.81	2.41	0.01
MCV (fl)	84.86±5.01	85.92±4.74	1.99	0.04
MCH (pg)	29.14±2.11	29.35±1.92	0.51	0.60
MCHC (g/dl)	32.35±1.53	32.54±1.31	1.22	0.21
Platelets (lakh/ cumm)	2.63±0.55	2.31±0.64	4.93	<0.0001

WBC- White Blood Cells, RBC- Red Blood Cells, Hb-Hemoglobin HCT- Hemtocrit, MCV- Mean corpuscular Volume, MCH- Mean Corpuscular Hemoglobin, MCHC- Mean Corpuscular Hemoglobin Concentration. cumm- Cubicmillimeter, %- percentage, g/dl-grams per deciliter, fl- femtoliter(10^{-15}), pg- picogram

Table 2: Comparison of hematological parameters with respect to ST chewing duration in ST chewer groups as compared to controls

Parameters	Controls	ST chewers (tobacco chewing duration in years)			ANOVA F-value (p-value)
		1-10 years	11-20 years	21-30 years	
	N=170	N= 50	N= 81	N= 37	
WBC (1000/ cmm)	7568±1369	7729±1176	7838±1204	7986±1071	1.55 (0.19)
Lymphocytes (%)	27.28±4.70	26.83±5.13	25.78±4.62	25.09±4.97	3.12 (0.02)
Monocytes (%)	6.00±1.07	5.84±1.08	5.49±1.02	5.38±0.98	5.48 (0.001)
Granulocytes (%)	66.70±4.68	68.13±5.64	68.51±5.19	68.92±4.90	3.75 (0.01)
RBC (million/cmm)	5.20±0.46	5.21±0.48	5.28±0.56	5.30±0.52	1.91 (0.12)
Hb (g/dl)	14.86±1.164	14.89±1.03	15.02±0.91	15.05±0.89	0.70 (0.54)
HCT (%)	44.67±4.03	45.81±3.68	45.74±3.98	45.55±4.03	2.00 (0.11)
MCV (fl)	84.86±5.01	85.86±5.13	85.90±4.68	86.04±4.58	1.32 (0.26)
MCH (pg)	29.14±2.11	29.18±1.83	29.30±1.98	29.27±2.05	0.170 (0.84)
MCHC (g/dl)	32.35±1.53	32.57±1.39	32.51±1.28	32.53±1.42	0.62 (0.53)
Platelets (lakh/ cmm)	2.63±0.55	2.35±0.70	2.33±0.60	2.29±0.74	7.049 (<0.0001)

*p<0.05, **p<0.01, ***p<0.001 significant as compared with controls

Table 3: Comparison of hematological parameters before and after supplementation of VC in controls and ST chewers

Parameters	Tobacco non-chewers (controls) N =170			Tobacco chewers (subjects) N =168		
	Before VC	After VC	Paired t (p-value)	Before VC	After VC	Paired t (p-value)
WBC (1000/ cumm)	7568±1369	7420±1124	2.56 (0.01)	7851±1204	7456±1137	8.47 (<0.001)
Lymphocytes (%)	27.28±4.70	27.32±4.43	009 (0.92)	25.90±4.70	27.81±4.20	2.65 (0.008)
Monocytes (%)	6.00±1.07	6.12±1.14	1.58 (0.11)	5.57±1.09	5.98±0.90	5.69 (<0.001)
Granulocytes (%)	66.70±4.68	66.56±4.54	0.50 (0.61)	68.52±5.14	66.19±4.58	6.562 (<0.001)
RBC (million/cumm)	5.20±0.46	5.24±0.44	1.65 (0.10)	5.27±0.50	5.35±0.44	2.576 (0.01)
Hb (g/dl)	14.86±1.164	14.96±0.96	3.00 (0.003)	14.98±0.91	15.24±0.84	2.72 (0.006)
HCT (%)	44.67±4.03	44.89±3.26	1.16 (0.24)	45.70±3.81	45.86±2.95	0.43 (0.66)
MCV (fl)	84.86±5.01	85.07±4.30	0.93 (0.35)	85.92±4.74	86.30±3.95	0.79 (0.45)
MCH (pg)	29.14±2.11	29.12±1.65	1.06 (0.28)	29.35±1.92	29.21±1.57	0.80 (0.42)
MCHC (g/dl)	32.35±1.53	32.41±1.02	1.30 (0.19)	32.54±1.31	32.52±0.91	1.70 (0.09)
Platelets (lakh/ cumm)	2.63±0.55	2.60±0.48	0.53 (0.59)	2.31±0.64	2.30±0.62	0.14 (0.88)

Nicotine present in tobacco might affect the suprarenal glands to release more catecholamine, which may influence leukocytosis causing damage and inflammation to tissues (Ewing and Clarke, 1982). In the present study, WBC count was significantly increased in tobacco chewers as compared to controls. A similar significant increase in WBC count was also seen in previous studies (Jaganmohan and Sarma, 2011; Saeed *et al.*, 2005).

The granulocytes, mainly neutrophils %, significantly increased in the present study, may be linked with continuous inflammation of tissues. Neutrophils are well known to produce cytotoxic material which harmful to lung functions (Gillum, 1991; Carel *et al.*, 1988).

In our study, monocytes % was significantly decreased in tobacco chewers than controls; this variation of monocytes % might be due to placid adverse effects on lungs (Jensen *et al.*, 1998).

However, lymphocytes % did not show any significant change in tobacco chewers in the present study. According to previous studies, inadequate pulmonary function in tobacco and gutkha chewers might be responsible for stimulating erythropoiesis for fulfilling the demands of oxygen to the tissues (Shahla *et al.*, 2007).

Blood indices such as MCV, MCHC, and MCH relate to individual RBCs as well as indicators of anemia. A lowered level of MCV related to iron deficiency anemia and increased levels are related to vitamin deficiency anemia (Kalburgi *et al.*, 2013).

In the present study, the MCV level (p=0.04) was significantly increased in tobacco chewers as compared to controls. A similar increase in the MCV level was found in the previous study (Biswas *et al.*, 2015).

However, after VC supplementation, the MCV level was decreased. It suggested that tobacco chewers had vitamin deficiency due to nicotine-induced oxidative stress. Whereas another study reported a significant decrease in the level of MCV in tobacco users (Dass *et al.*, 2013; Shukla *et al.*, 2019).

Table 4: Correlations of serum CTN and serum VC with hematological parameters in controls and ST chewers

	Tobacco non-chewers (controls) N=170				ST chewers (subjects) N= 168			
	Sr. CTN		Sr. VC		Sr. CTN		Sr. VC	
	r-value	p-value	r-value	p-value	r-value	p-value	r-value	p-value
WBC 1000/cumm	-0.01	0.88	0.06	0.40	0.55	0.04*	-0.51	0.05*
Lymphocytes %	-0.43	0.58	-0.09	0.22	-0.05	0.44	-0.41	0.60
Monocytes %	-0.01	0.81	0.10	0.17	0.05	0.48	0.20	0.008**
Granulocytes %	0.04	0.54	0.07	0.36	0.04	0.57	-0.03	0.65
RBC Millions/cumm	0.10	0.16	0.10	0.15	-0.03	0.66	0.11	0.12
Hb g/dl	0.18	0.07	0.14	0.06	0.06	0.37	0.04	0.59
HCT %	0.01	0.82	0.08	0.28	0.05	0.46	0.11	0.13
MCV Fl	-0.01	0.87	-0.01	0.83	-0.07	0.31	0.01	0.85
MCH Pg	0.13	0.08	-0.01	0.82	0.007	0.93	0.01	0.85
MCHC g/dl	0.14	0.06	0.08	0.27	-0.10	0.19	-0.01	0.80
Platelets Lakh/cumm	-0.24	0.06	-0.005	0.95	-0.22	0.004**	0.08	0.27

*p<0.05, **p<0.01, ***p<0.001 significant correlations of serum CTN and serum VC with hematological parameters

In the present study, MCH and MCHC level did not show a significant change in tobacco chewers as compared to controls. However, some previous studies reported that the MCH level was found to be significantly increased in their studies (Dass *et al.*, 2013; Biswas *et al.*, 2015; Shukla *et al.*, 2019).

We studied the effect of VC supplementation in controls as well as in tobacco chewers independently for hematological parameters (Table 3).

In the control group, WBC count (p=0.01) significantly decreased and Hb content (p<0.003) was significantly increased after supplementation of VC as compared to their baseline values.

Whereas, in tobacco chewers group WBC count (p<0.001) and granulocytes % (p<0.0001) were significantly decreased and lymphocytes % (p=0.008), monocytes % (p<0.0001), RBC count (p=0.01) and Hb content (p=0.006) were significantly increased after supplementation of VC as compared to baseline values.

Vitamin C enhances the absorption of iron which is helpful to improve hemoglobin level in girls (Tutu and Rohimah, 2014). Vitamin C involves a hydrogen transfer rather than electron transfer and neutralizes harmful free radicals and reverted hemotoxicity (Ashraf *et al.*, 2017).

In the previous study, Hounkpatin reported the protective effect of VC supplementation on deranged hematological parameters in Wistar rats (Hounkpatin *et al.*, 2012).

Ibitoroko reported that hemotoxicity caused by gasoline was reversed by supplementation VC in Albino rats (George and Adegoke, 2011), the similar result reported in rabbits (Ovuru and Ekweozor, 2004), in Herring gulls and Atlantic puffins (Leighton *et al.*, 1985), in African catfish (Sunmonu and Oloyede, 2008) and in Wistar rats (Akaninwor and Okeke, 2007).

Soronnadi reported that platelet count was significantly decreased after supplementation of VC (Soronnadi *et al.*, 2013). This suggested that oral VC supplementation has beneficial effects on haemostatic dysfunction in chronic tobacco users.

CONCLUSION

ST has adverse effects on hematological parameters such as an increase in WBC count, granulocytes %, HCT and MCV and decreases monocytes % and platelets count whereas, insignificant changes were observed in lymphocytes %, RBC count, Hb, MCH and MCHC. Increased tobacco chewing duration produce increased adverse effects on health. In addition, it was concluded that long term supplementation with 1 g/day of vitamin C may help in improving deranged hematological parameters in ST chewers.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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