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Oral supplementation of vitamin C reverses haemostatic dysfunction in chronic smokers.

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Abstract

Vitamin C is a strong reducing agent and also known to be an antioxidant in vitro and in vivo. It has been suggested that haemostatic dysfunction may be a consequence of excess formation of free radicals. The present study is aimed at estimating the effects of oral vitamin C supplementation on bleeding time (BT), whole blood clotting time (WBCT), total platelet count (TPC), prothrombin time (PT) and activated partial thromboplastin time with kaolin (APTTK) in chronic smokers. The study comprised of 100 chronic smokers and 100 non-smokers (controls). Base line blood samples were collected from all the subjects. One 500 mg tablet of vitamin C (Mekophar ®) was orally administered daily to all the subjects for two consecutive weeks. At the end of two weeks, blood samples were collected from only 156 subjects (78 chronic smokers and 78 controls) who turned up. Standard haemostatic procedures were used. Results showed that BT. WBCT, PT and APTTK coagulation markers were significantly decreased ($p<0.05$) in chronic smokers and TPC was significantly increased ($p<0.05$) when compared with non-smokers. Result also showed the effect of vitamin C in the baseline and post-test results in controls was not significant ($p>0.05$) in all the parameters used. Oral vitamin C supplementation significantly increased ($p<0.05$) BT, WBCT, PT and APTTK and a significant decreased TPC in the chronic smokers compared with their baseline values ($p<0.05$). The study has shown that oral vitamin C has beneficial effect on haemostatic dysfunction in chronic smokers.

Keyword: chronic smoker, haemostatic dysfunction, vitamin C

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Introduction

Tobacco smoking, one of the most potent and prevalent addictive habits, is now increasing rapidly throughout the developing world and is also one of the biggest threats to current and future world health [1]. Tobacco continues to be the second major cause of death in the world and by 2030, if current trends continues, smoking will kill more than 9 million people annually [2]. The hypercoagulablity and hyperthrombotic state apparently induced lends credence to the theory that smoking cigarettes contribute to the comparatively high incidence of acute myocardial infarction among heavy smokers. These blood clots can

block the blood supply and may lead to serious heart conditions, including fatal heart attacks [3]. Chronic smokers are defined as having been smoking for an average of 21 "pack-years" [2]. Chronic smoking refers to persistent, daily, long-term smoking. Chronic cigarette smoking (CCS) is known as one of the major risk factors in the development of atherosclerosis in coronary and peripheral arteries. CCS shows adverse effects on the production of the eicosanoid system, which plays an important role in the protection of haemostatic balance. Many studies agree that smoking increases platelet aggregation (PA) and this plays an important role in smoking-induced vascular injury – a mechanism by which smoking leads to arterial disease [4, 5].

Cigarette smoke has been estimated to contain 10^{15} - 10^{17} free radicals per inhalation and these free radicals can oxidize the fat components of the body and this is quite harmful [6]. Vitamin C, a potent antioxidant, has been shown to help clear the body of cell-damaging oxygen free radicals and may counter the "oxidative stress" on the lining of the bold vessels, caused by smoking. Vitamin C supplements also dramatically combat the oxidative damage caused by smoking and exposure to tobaccos smoke [7, 8]. Teramoto et al [8] found that smokers have a 30 percent lower vitamin C level than nonsmokers and also believed that nicotine may interfere with vitamin C absorption since nicotine boost metabolic rate, thereby increasing the rate that vitamin C is metabolized. The rapid increase in the number of chronic smokers, especially among young people, and the fact that several studies have shown the adverse effect of smoking on the body, especially the blood, necessitates the present study.

This study is therefore aimed at estimating the effect of oral vitamin C supplementation on haemostatic dysfunction induced in chronic smokers. The results of this study will help to substantiate the beneficial effect of oral vitamin C supplementation on clotting dysfunction in chronic smokers.

Materials and Methods

Subjects

The study was conducted in Enugu State, South-East Nigeria. The subjects recruited for the study were chronic smokers who meet our criteria. A total of two hundred (200) subjects (one hundred chronic smokers and one hundred non-smokers) with mean age 41± 20years who meet our criteria were included in the study. However, only one hundred and fifty-six subjects (78%) turned up to complete the study (78 chronic smokers and 78 non-smokers, respectively).

Subjects were made up of medical students from Enugu State University of Science and Technology, ESUT Teaching Hospital, Park Lane G.R.A. Enugu-North Local Government Area, soccer players at Sunshine Football club Enugu, students of Institute of Management Technology (IMT) Enugu and volunteers living in Enugu metropolis. Seminars and talks were conducted to create the awareness and the conviction needed for the subjects' participation in the research. Also incentives were given to the subjects to ensure their total commitment.

All subjects gave informed consent and the study protocol was approved by the Ethics Committee of Enugu State University of Science and Technology Teaching Hospital (ESUTTH) Park Lane G.R.A. Enugu. Well structured questionnaires were also administered.

Chronic smokers were included in the study if they had a history of smoking 10±5 cigarette sticks per day for one year. Subjects having arterial hypertension, sugar in their urine (tests were done using urinalysis strip) or those currently being administered antioxidants were excluded from the study.

Sample collection and processing

The subjects came to the laboratory at 7.30 to 10am. From each subject 10ml of blood was drawn, delivered into different test tubes and well labeled for the parameters studied. Immediately after blood collection, 4.5ml of patient's blood were gently mixed with 0.05ml of sodium citrate (that is 9 parts of blood to 1 part of the anticoagulant) in glass test tube and centrifuged for 10-15min at 1500 to 3000 rpm in bucket centrifuge. The plasma was immediately removed and transferred into another set of 2ml glass tube and kept in plastic racks at room temperature for PT and APTTK processing. About 3.5ml anticoagulated blood used for total platelet count, the remaining 2ml for whole blood clotting time and the results were compared. The whole process was repeated after two weeks of oral supplementation of vitamin C to the subjects who turned up to complete the study.

Analytical Method

The determination of PT was made by Quick time method (one-stage) using Plasmacann Reagent Test Kit manufactured by Quimica Clinica Aplicada S.A (QCA). Determination of Activated Partial Thrombin Time With Kaolin (Apttk) was done using the Hemoscann Test Kit, manufactured by Quimica Clinica Aplicada S.A (QCA). Determination of Bleeding Time was done by Duke's Method whereas Whole Blood Clotting Time determination was made by using Lee and White Method (Dacie & Lewis [9]. Visual Total Platelet Counts was done using Improved Neubauer Chamber [9].

Statistical Analysis

Graph pad prism software (Statmate) version 2.0 and SPSS version 20.0 were used for the data analysis and the test of significance was calculated using paired student's t-test. Results were presented as mean ± standard error of mean (mean value ± SEM) and $p < 0.05$ was considered significant.

Results

Table 1 shows the baseline coagulation values in chronic smokers and controls. A statistically significant decrease ($p<0.05$) in BT, WBCT, PT, and APTTK was observed in the chronic smokers compared to the controls. Conversely, a significant increase ($p<0.05$) in total platelet count was recorded in chronic smokers as compared with the controls.

The effect of vitamin C supplementation on the test and control subjects was shown in Table 2. After two weeks of vitamin C oral supplementation in chronic smokers, values of BT, WBCT, PT, APTTK and TPC were reversed back to control values (Table 2).

Table 1. *The baseline coagulation parameters of the subjects.*

Parameters	**Normal Values**	**Control results** (n=100)	**Chronic Smokers** (pre-sample results: n=100)
Bleeding time (min)	1.3 - 4 minutes	1.64 ± 0.02	0.74 ± 0.03*
Whole blood clotting time (min)	5 - 11 minutes	7.10 ± 0.07	5.10 ± 0.05*
Total platelet count (mm^3)	150,000 - 350,000 mm^3	262100 ± 3450	276800 ± 2969*
Prothrombin time (s)	11 - 16 seconds	12.70 ± 0.08	9.8 ± 0.06*
Activated partial Thrombin time with kaolin (s)	30 - 40 seconds	33.04 ± 0.12	27.00 ± 0.17*

** = Statistically significant ($p<0.05$).*

Table 2. *Effect of vitamin c on chronic smokers in some clotting factors*

Baseline results		**Results after 2 weeks**			
Parameters	**Normal values**	**Controls** (pre-sample) n=78	**Chronic smokers** (pre-samples) n= 78	**Controls** (post-samples) n=78	**Chronic smokers** (post-sample) n=78
Bleeding time(min)	1.3 - 4 minutes	1.62±0.02	0.73±0.05	1.63±0.03 ŋ	1.64± 0.05*
Whole blood clotting time (min)	5- 11 minutes	7.40±0.16	5.2±0.11	7.44±0.09 ŋ	7.4± 0.17*
Total platelet counts (mm3)	150,000 – 350,000 mm^3	255144±4448	269480±7283	255064±4463 ŋ	255200±7802*
Prothrombin time(s)	11 - 16 seconds	12.90±0.11	10.0±0.16	12.80±0.11 ŋ	12.9±0.41*
Activated partial thrombin time with kaolin (s)	30 - 40 seconds	32.71±0.12	26.9±0.41	32.70±0.12 ŋ	32.6±0.22*

= Statistically significant ($p<0.05$).*

Discussion

The effect of oral supplementation of vitamin C on haemostatic dysfunction in chronic smokers was estimated. The significant increase ($P<0.05$) in total platelet count (TPC) (262100 ± 3450 mm^3 Vs 276800 ± 2969mm^3) of the chronic smokers compared with the non-smokers (Table 2) is consistent with the findings of other investigators [10, 11].

The results showed that vitamin C has a beneficial effect on the TPC of the smokers and hence can reduce the cardiovascular diseases associated with this habit.

A statistically significant decrease ($p<0.05$) in bleeding time (BT) of chronic smokers in minutes (0.74 ± 0.03) compared with its control group (1.64 ± 0.02) is also in agreement with the study of Pilegeram and Pickard [12] who reported that prolonged cigarette smoke intake causes an increased amount of fibrinogen, hence increased circulating platelets leading to hastened bleeding arrest in smokers. Kampman et al. [13] also reported a shortened bleeding time in smokers when compared with the non-smokers. However, after two weeks of vitamin C supple-

mentation the reversal of BT of chronic smokers to control value (1.64 ± 0.05 Vs 0.73 ± 0.05) is very much encouraging.

A statistically significant reduction ($p<0.05$) in whole blood clotting time (WBCT) was also observed (5.10 ± 0.05 minutes) when compared with the controls (7.10 ± 0.07 minutes) (Table 1) and this may be due to increased inhalation of carbon monoxide from the cigarette smoke [14].

Smoking increases activation of platelets by100 times, which can lead to a significant increase in blood clots [14]. A shortened whole blood clotting time resulting from increasing platelet aggregation in chronic smokers has also been reported [15,16].

After two weeks of vitamin C supplementation, a significant increase ($p<0.05$) in WBCT was found in chronic smokers (7.4 ± 0.17 minutes) compared with their control group (5.20 ± 0.11 minutes) (Table 2) but similar to that of the control subjects (7.4 ± 0.16 minutes). In table 2, a significant shortened time ($p<0.05$) in prothrombin time (PT) and activated partial thrombin time with kaoline (APTTK) [PT (9.8±0.06 seconds) and APTTK (27.0±0.17 seconds)] in chronic smokers compared to the non-smokers [PT (12.7±0.08 seconds), APTTK (33.0±0.12 seconds)] was observed. This may be due to increased stimulation and synthesis of fibrinogen by the liver caused by chronic cigarette smoking, although the mechanism by which smoking increases the plasma fibrinogen concentration is not clear yet. Vyssoulis et al. [17] in their study on 4000 patients (2572 non-smokers and 1428 chronic smokers) reported a significant decrease in coagulation indices such as PT, APTTK, TT, serum fibrinogen, and PAS1-1. The higher levels of fibrinogen in chronic smokers may promote cardiovascular disease by affecting blood viscosity, platelet aggregation and general fibrin formation [17]. Takajo et al. [18] reported that each cigarette smoked per day increases mean plasma fibrinogen by 0.35g/l, whereas Akpotuzor et al. [19] reported significant lower values ($p<0.05$) on PT and APTTK of smokers when compared with the non-smokers.

Although fibrinogen levels were not measured in our subjects, the short time value in the four coagulation parameters (the time required for thrombin to convert fibrinogen to an insoluble fibrin clot) reported in this study is a strong indicator of elevated levels of fibrinogen in smokers. Samples obtained after two weeks of vitamin C supplementation in the chronic smokers showed a significant increase ($p<0.05$) in PT (12.9±0.41seconds) and APTTK (32.6±0.22 seconds) when compared with the baseline values [PT (10.0±0.16 seconds), APTTK (26.9±0.41 seconds)], (Table 2). The table also shows no significant difference ($p>0.05$) in the post vitamin C sample values [PT (12.80±0.11 seconds), APTTK (32.70±0.12 seconds) of the controls compared with their baseline values [PT (12.9±0.11), APTTK (32.70±0.12) seconds].

Conclusion

The present study shows that oral vitamin C supplementation for 2 consecutive weeks, leads to a correction of the hematological dysfunction resulting from chronic cigarette smoking. Vitamin C, being a strong antioxidant appears to be a hope for chronic smokers to minimize their coagulation parameters and prevent the far-reaching consequences of coagulation disorders. Food rich in vitamin C should be encouraged in chronic smokers, however, smoking should be strictly discouraged.

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