JOURNAL OF PURE AND APPLIED MICROBIOLOGY, March 2012. Vol. **6**(1), p. 483-486

Estimation of Serum Vitamin C in Oral Submucous Fibrosis

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(Received: 27 November 2011; accepted: 02 January 2012)

In the present study Serum Vitamin C in Oral submucous fibrosis (OSMF) was estimated. The objective was to evaluate the Serum Vitamin C in OSMF individuals. OSMF is basically a disorder of collagen metabolism where Vitamin C gets utilized in conversion of proline into hydroxyproline, this hydroxylation reaction requires Vitamin C. In recent years OSMF has received considerable attention as a precursor to cancer of oral cavity but till date there is no report suggesting spontaneous regression and no widely accepted treatment. Many studies regarding micronutrients and other antioxidants levels have been emphasized, but very few studies are done on the Serum levels of Vitamin C in OSMF patients.

Twenty five OSMF patients with age and gender matched healthy individuals (controls) were selected. Two ml of venous blood was collected from each individual. Vitamin C level in serum was estimated by 2-4 Dinitrophenylhydrzine method. The level of Serum Vitamin-C was significantly decreased in OSMF patients when compared to controls which were statistically significant. On the basis of these observations, it seems possible that the chemical, thermal and/or mechanical factors associated with the use of areca nut may act in conjunction with the Vitamin C deficiency leading to the development of OSMF. Thus we should consider Vitamin C in management of OSMF.

Key words: Oral Submucous Fibrosis, Serum Vitamin C.

In early 600 B.C a condition similar OSMF was first described by Sushruta & it was named "VIDARI" having features of progressive narrowing of mouth, depigmentation of oral mucosa and pain on taking food.[1] OSMF is defined as insidious chronic disease affecting any part of oral cavity and sometimes pharynx. Although occasionally preceded by and/or associated with vesicle formation, always associated with Juxtaepithelial inflammatory reaction followed by fibro elastic change of lamina propria with epithelial atrophy leading to stiffness of oral mucosa and causing trismus and inability to eat".[2] In recent years, OSMF has received considerable attention as a precursor to cancer of the oral cavity. Its prevalence in India has been reported as 5% for women and 2% for men.[3] The reported risk of malignant transformation varies from 2.3–7.6%.[4]

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Areca nut extract acts as a potent stimulator for collagen synthesis in human fibroblasts culture leading to excessive accumulation of collagen, leading to fibrosis.[5] High levels of copper in areca nuts, a major etiological factor in OSMF plays an initiating role in stimulation of fibrinogenesis by up-regulation of lysyl oxidase and thereby causing inhibition of degradation of collagen and causing its accumulation thereby causing OSMF. The high serum copper levels may also lead to generate high levels of free radicals by metal-catalyzed Haber-Weiss reaction and this can be one of the reasons for the carcinogenesis in tobacco and areca nut users.[6] Antioxidants are micronutrients which have the ability to neutralize free radicals or their actions.[7]

Required daily allowance for Vitamin C is 45 to 95 mg/day, 250 mg/day is required to prevent any type of cancer and it has a protective role in carcinogenesis.[8] (Table 1). Normal serum levels of Vitamin C 0.8-1.2 mg/dl. Studies have shown low serum levels of Vitamin C may be associated with an increased risk of cancers.[9]

Thus, the present study is undertaken to comprehend the association between OSMF and Vitamin C by estimating the levels serum Vitamin C in patients diagnosed with OSMF and comparing the values with that of healthy subjects.

MATERIALS AND METHODS

Source of data

Twenty five OSMF patients with age and gender matched healthy individuals (controls) were selected from the institute.

GROUP A study group

The clinical diagnosis of oral submucous fibrosis in Group A was made by using the criteria as mentioned by Khanna J.N. and Andrade N.N. (1995).[10]

Method of Collection of data

Ethical clearance from the institution was obtained and informed written consent was obtained from individual. Detailed case history of all subjects was recorded which includes deleterious habit, systemic diseases and any subjects undergoing antioxidant/multivitamin therapy were excluded.

Collection of Sample

Two ml of venous blood was obtained from median cubital vein, blood was allowed to clot at room temperature for 1 to 2 hours. The serum was separated by centrifuge machine at 3000 rpm for 10 minutes to get a clear serum sample, The serum thus obtained was pipetted using a micro pipette and transferred into sterile plastic storage vial and was stored at -20°C in a dark container until assay.

Methodology

Vitamin C: 2-4 Dinitrophenylhydrzine method

Principle

Dehydro ascorbic acid was coupled with 2,4 dinitrophenylhydrazine and the resulting derivative is treated with sulphuric acid to produce a newly observed color which is measured at 545 nm.

RESULTS

The results were expressed as Mean ± SD values. The means of the controls and patients were compared using Student's t-test to find the significance of study parameters on a continuous scale for intergroup analysis. The age of patients varied from 20 yrs to 45 yrs, mean age being 32.5 yrs. The OSMF group showed male predominance with 23 males and 2 females.

Mean values of serum Vitamin C in control group was (1.08±0.1 7 mg/dl) whereas in OSMF group the values were (0.4±0.23 mg/dl) respectively. OSMF group showed significantly lower levels of serum Vitamin C {$p < 0.001$} (Fig. 1).

Interstage comparison showed a significantly lower serum Vitamin C with progression from stage I (vitamin C= 0.70 ± 0.2 mg/dl) to stage II (vitamin C= 0.6 ± 0.2 mg/dl) to stage

Table 1. Vitamin C:
A protective role in carcinogenesis

Is an antioxidant thus scavenging free radical
Reduces vitamin E degradation
It inhibits nitrosamine formation
Enhances detoxification via cytochrome P450

Table 2. Results showed Serum Vitamin C level is less in OSMF grade III than in grade II

Parameter VITAMIN C (mg/dl)	Controls (n=25)	OSMF Grade : I (n = 2)	OSMF Grade : II (n=13)	OSMF Grade : III (n = 9)	OSMF Grade : IV (n=1)
0.8-1.2	1.08 ± 0.1	0.70 ± 0.2	0.6 ± 0.2	0.3 ± 0.08	0.14

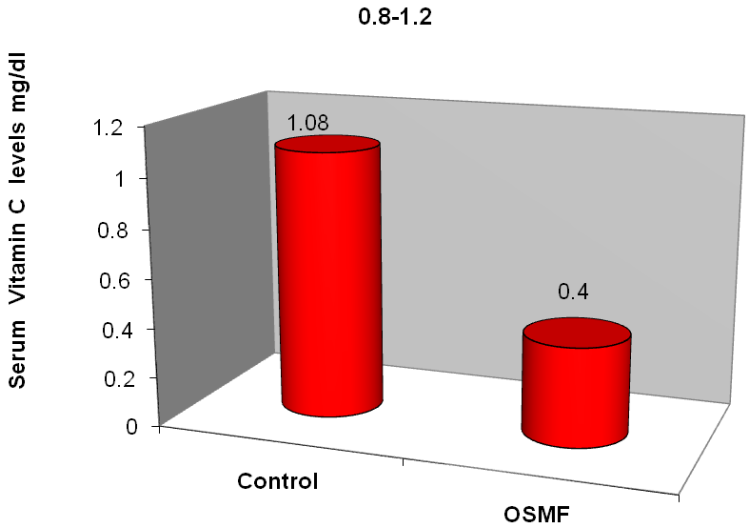

Fig. 1. Level of Vitamin C in Healthy controls & OSMF

III (vitamin C= 0.3 ± 0.08 mg/dl) and finally to stage IV (vitamin C= 0.14mg/dl).Value of $p < 0.001$ (Table 2).

DISCUSSION

In the present study, decrease in Serum Vitamin C levels in OSMF group which is in accordance with the studies by Wahi P.N. et al[11] and Rai Balwant et al.[12] They attributed poor nutritional status[12]and oxidative stress in precancer lesions and conditions like OSMF were responsible for decrease in serum and salivary Vitamin C and Vitamin E levels.[13]

In OSMF patients, there is an increase in the production of highly cross linked insoluble collagen type I, loss of more soluble pro-collagen type III and collagen type VI. The cross linking of collagen due to the up-regulation of lysyl oxidase, plays a crucial role in the development and progression of the condition from stage I to stage II.[13] Vitamin C levels decreases perhaps because of its utilization in collagen synthesis.[6] Rajendran et al who reported that Vitamins and Iron deficiency together with malnourished state of the host leads to derangement in the inflammatory reparative response of the lamina propria with resultant defective healing and scarification which ultimately leads to OSMF.[14] Poor nutrition is one of the causative factor of OSMF.[15] Diet rich in high fiber and Vitamin C both has protective effect on development of OSMF and leukoplakia.[16] In fact, case control studies have shown that consumption of carotene rich vegetables and Vitamin C rich fruits markedly reduced risk of oral cancer.[17] Thomas et al reported that high intake of fruits and vegetables can act as protective shield for OSMF.[15]

In one study by Maher Rehana et al where OSMF individuals were supplemented with a combination of micronutrients (Vitamins A, C, D, E, all eight B Vitamins, calcium, magnesium, iron, phosphorus, copper, manganese, zinc, and molybdenum) there was a significant improvement in symptoms, especially intolerance to spicy food,

burning sensation, and difficulty in mouth opening. The results indicated that supplementation with multiple micronutrients including Vitamin C and Iron can produce a clinical response in patients with OSMF.[18]

CONCLUSION

From the present study, it is evident that by estimation of Serum Vitamin C levels in OSMF patients, one can assess the degree of oxidative damage of the disease.

Further correcting the underlying deficiency of Vitamin C along improves treatment thereby arresting it in early stages and avoiding the possible consequences of malignant transformation. However further elaborate studies with a larger sample size including OSMF with coexisting oral cancer along with follow-up are needed to ascertain the actual role of this parameter in the initiation and promotion of carcinogenesis.

ACKNOWELEDGEMENTS

Dr. Subhash Bose Reddy Phd Biochemistry, Jawaharlal Nehru, Bhopal for guiding and helping in biochemical analysis of the blood samples.

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