



ESTIMATION OF SERUM VITAMIN C AND IRON LEVELS IN ORAL SUBMUCOUS FIBROSIS- A CASE CONTROL STUDY

Dentistry

Dr. Abheet

Assistant Professor, Department of Dentistry, Madhubani Medical College, Madhubani, Bihar

ABSTRACT

Background: Serum Iron content can be a predictor for the progression of this condition. OSMF is basically a disorder of collagen metabolism where Vitamin C gets utilized in conversion of proline into hydroxyproline; this hydroxylation reaction requires ferrous Iron and Vitamin C. **Aims & Objective:** Serum Vitamin C and Iron levels in Oral submucous fibrosis (OSMF) were estimated. The objective was to evaluate the correlation between Serum Vitamin C and Iron levels in OSMF individuals. **Methods And Materials:** The study was done from September 2023 to February 2024 after obtaining clearance from Institutional ethical Research Committee and written informed consent was obtained from all participants. Sample obtained from median cubital vein and serum was separated by centrifuge machine at 3000 rpm for 10 minutes to get a clear serum sample. Estimation of Vitamin C done by 2-4 dinitrophenylhydrazine method and Iron estimation was done by Ramsay's method to form a colour complex whose absorbance was read in colorimeter. **Results:** Mean values of serum Vitamin C and Iron of control group were $(1.07 \pm 0.14 \text{ mg/dl})$ and $(134.20 \pm 11 \text{ } \mu\text{g/dl})$ respectively whereas in OSMF group the values were $(0.5 \pm 0.22 \text{ mg/dl})$ and $(96.75 \pm 16 \text{ } \mu\text{g/dl})$ respectively. OSMF group showed significantly lower levels of serum Vitamin C and Iron ($P < 0.001$). **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 resulted by this disease.

KEYWORDS

Oral Submucous Fibrosis, Serum Iron And Vitamin C.

INTRODUCTION:

Oral Cancer accounts approximately 40% of all cancers in the Indian subcontinent [1]. A significant proportion of oral squamous cell carcinomas (OSCC) develop from premalignant lesions such as leukoplakia and conditions such as oral submucous fibrosis [2]. 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" [3]. In recent years, OSMF has received considerable attention as a precursor to cancer and occurs in younger age group individuals [4]. Its reported risk of malignant transformation varies from 7 to 13% [5]. Habit of chewing areca nut is the major etiological factor of OSMF. Its extract acts as a potent stimulator for collagen synthesis in human fibroblasts culture leading to excessive accumulation of collagen, leading to fibrosis [6]. 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 [7]. Antioxidants are enzymes or other organic molecules that can counteract the damaging effects of reactive oxygen species in tissues [8]. Antioxidant nutrients such as vitamin C, beta-carotene, zinc and selenium are regularly found to reduce the risk of oral cancer and precancers [9]. Iron is required for the functioning of numerous enzymes and it is reasonable to assume that variation in important biochemical markers like serum Iron and Vitamin C levels may be associated with the pathogenesis of OSMF. Iron metabolism is important to maintain the health of oral mucosa, and many disease states, including cancers, are associated with Iron depletion [10]. Serum Iron content can be a predictor for the progression of OSMF. There appears to be an association between Serum Iron content and oral carcinogenesis [11]. It has been also postulated that Vitamin C and Iron is interrelated as Vitamin C plays important role in absorption of Iron from the gut [12]. Vitamin C helps the body to absorb non-heme Iron [13]. Vitamin C enhances Iron absorption by reducing dietary Iron from ferric form to the ferrous form. Thus, Vitamin C deficiency may reduce the availability of intracellular Iron. Vitamin C is also necessary to convert folic acid to its active metabolite, folinic acid. [14,15,16]

AIMS AND OBJECTIVE:

The objective was to evaluate the correlation between Serum Vitamin C and Iron levels in OSMF individuals.

MATERIAL AND METHODS:

The study was approved by the Institutional ethical Research

Committee and written informed consent was obtained from all participants.

Study Duration:- From September 2023 to February 2024

Inclusion criteria for study group

- Subjects with definitive habit of Areca nut/tobacco in any form and Alcohol
- Subjects with clinical signs and symptoms of OSMF.
- Subjects without any clinical Oral lesions
- Subjects with no deleterious habits
- Subjects without systemic diseases.

Exclusion criteria

- Subjects with any other Precancerous lesions or Conditions other than OSMF
- Subjects suffering from any systemic diseases like diabetes, cardiac diseases, renal diseases, liver diseases and other malignancies
- Subjects who are taking Antioxidants/multivitamin preparations.

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

Estimation of Vitamin C done by 2-4 dinitrophenylhydrazine method. The Principle of this method is that Dehydro ascorbic acid was coupled with 2,4 dinitrophenylhydrazine and the resulting derivative is with sulphuric acid to produce a newly observed color which is measured at 545 nm. Iron estimation was done by ramsays method and the principle of this method is that in the presence of sodium sulphate, iron reacts with 2,2' tripyridyl reagent to form a colour complex whose absorbance was read in colorimeter.

Statistical Analysis:

Statistical analysis Mean values, median standard deviation (SDM), and standard error of the mean were calculated. All statistical tests were analyzed to a significance level of 0.05.

RESULT:

Mean values of serum Vitamin C and Iron of control group were $(1.07 \pm 0.14 \text{ mg/dl})$ and $(134.20 \pm 11 \text{ } \mu\text{g/dl})$ respectively whereas in OSMF group the values were $(0.5 \pm 0.22 \text{ mg/dl})$ and $(96.75 \pm 16 \text{ } \mu\text{g/dl})$ respectively. OSMF group showed significantly lower levels of serum Vitamin C and Iron ($P < 0.001$).

Table 1 Comparison of vitamin C and iron level in serum between study and control group

Groups	Vitamin C (mg/dl)	Iron (µg/dl)
Control	1.07	134.2
OSMF	0.5	96.75

DISCUSSION:

In the present study, decrease in Serum Vitamin C levels in OSMF group which is in accordance with the studies conducted by other authors [18,19]. They attributed poor nutritional status [20] and oxidative stress in precancerous lesions and conditions were responsible for decrease in serum and salivary Vitamin C and Vitamin E levels [19]. 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 [21]. Vitamin C levels decreases perhaps because of its utilization in collagen synthesis [7]. 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 [22]. Poor nutrition is one of the causative factors of OSMF [23]. Diet rich in high fiber and Vitamin C both has protective effect on development of OSMF and leukoplakia [24]. In fact, case control studies have shown that consumption of carotene rich vegetables and Vitamin C rich fruits markedly reduced risk of oral cancer [25].

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 resulted by this disease. Further correcting the underlying deficiency of Vitamin C along with Iron supplements 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 these parameter in the initiation and promotion of carcinogenesis.

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