Original Research Article

Study design of Serum Protein levels in Oral Submucous Fibrosis Patients

Abstract

Background and Aim: Serum total protein, albumin and uric acid are plasma's potent antioxidant defense; it can be used as a dependable marker of oxidative stress in the body and can untimely recognize oral submucous fibrosis (OSMF), beside the other intrusive and not so successful demonstrative aides. Hence the present study was aimed to compare the serum levels of total protein, albumin and uric acid in oral submucous fibrosis patients.

Material & Methods: Patients were allotted into three categories: Group I comprised 25 healthy individuals, Group II comprised of 25 individuals with chewing tobacco habit without OSMF, and Group III comprised of 25 patients with chewing tobacco habit with OSMF. The inclusive patients were asked to fast overnight. The very next day between 8-10a.m, 5 ml of blood was drawn from each subject intravenously, using a sterile disposable syringe. Assay of albumin & total protein were carried out using the bromocresol green method and that for uric acid was carried out by biuret method.

Results: The mean serum levels of Total protein fell from group I to group III; variation was not found to be statistically considerable. Serum levels of Albumin fell from group I to group III; dissimilarity was found to be statistically considerable ($p < 0.05$). Serum levels of Uric acid decreased from group I to group II; difference was found to be statistically significant ($p < 0.05$).

Conclusion: Our study proposes that the degree of oxidative damage in OSMF can be evaluated by estimating the levels of serum Total Protein, Albumin & Uric Acid in pretentious patients.

The basic deficiency of antioxidants can be remedied by adding a diet of these antioxidants. This can help in successful management of OSMF and avoid the negative consequences.

Key Words: Serum, Albumins, Total Protein, Uric Acid, Oral submucous fibrosis,

Introduction

With the increase in current globalization and continuous transformation of metro cities into mega cities, surveys have found upsurge in the rate of use of various habitual products in India and various other Southeast Asian countries. Of all the products betel quid Gutkha are one of the most commonly practiced habits.[1] In India betel quids & Gutkha are socially accepted habits which are openly distributed, easily available and popular among youngsters. Around 26% of Indian population consumes betel quid & Gutkha. Bihar and Jarkhand have the highest percentage whereas Goa and Himachal Pradesh have the least amount of betel quid and Gutkha chewers. Betel quid is the combination of fresh betel leaf, fresh areca nut, slaked lime, catechu and tobacco; whereas Gutkha is industiral manufactured item contaning tobacco [2].

Betel quid and Gutkha consumption are considered as the leading causative agents for oral submucous fibrosis, oral cancer and upto some extent liver diseases [3]. OSMF first described by Schwartz in 1952, is considered as one of the potentially malignant condition that hampers the normal functional activity of oral cavity and sometime pharynx [4].OSMF is multifactorial in origin, which varies from local irritants like capsaicin, tobacco, betel nut, punget and spicy food to other factors like chronic candidiasis, iron and nutritional deficiencies, papilloma virus (HPV), genetic abnormalities, Herpes simplex virus (HSV), Human autoimmunity. Various case control studies have shown the association of areca nut, a constituent of betel quid towards exaggerating the occurrence of OSMF [5].

The International Agency for Research on Cancer has considered areca nut in betel quid as group I carcinogen to humans [6]. Aerca nut is known to contain many alkaloids and several polyphenols. Aercoline is the major alkaloid found in aerca nut. Several evidences conclude that aercoline is genotoxic and mutagenetic component for many cells and inhibits the growth of oral mucosal fibroblasts, gingival fibroblasts and keratinocytes [7]. Reactive oxygen species (ROS) are generated during chewing of areca nut, it is implicated in the process of multistage carcinogenesis. Chang et al. 2001 in the study concluded that ingredients present in the areca nut are critical in the pathogenesis of OSMF & oral cancer [1].

ROS is a collective term which include molecules like Hydroxyl radical (OH^-), Superoxide anion (O_2) and Hydrogen peroxide (H_2O_2). According to Pryor 1986, ROS causes tissue damage by different mechanisms which include protein damage, cancer-causing mutation, lipid damage, DNA damage and alter cellular antioxidant defense system [8]. Damage to the DNA and other cellular molecules, by reactive oxygen species are considered as major culprits in occurrence of cancer.

Antioxidants are the substances which inhibits the adverse effect of ROS. They are present in all body fluids. Antioxidants play their role by hunting the various reactive oxygen species, by hindering their formation. A wide range of antioxidants are present in the plasma including Albumin, Uric acid and Total protein. The thiol group present in them is known to act as important scavengers of various free radicals [9] [10]. Oxidative stress is caused by the imbalance between the oxidants and the antioxidants which results in the formation of Oxidative stress. This has a major role in promotion of a variety of pathological processes [11-13].

Despite of advancement in therapy and diagnostic aids, the rate at which the prevalence of OSMF and its malignant transformation rate are spreading is alarming. According to the study

done by Pillai et al. in 2005 [14] malignant transformation rate of OSMF is recorded to vary from 7.6% to 15 %.(Teklal patel) This highlights that there is need to discover suitable biomarker for early detection of oxidative stress which can adjuvant in diagnosis of this potentially malignant disorder [15]. Moreover, the responsibility of oxidative stress in the initiation, promotion and progression of various pathological entities has been subject of much speculation; hence the aim of the present study was conducted to evaluate and monitor Total Serum Protein, Albumin & Uric acid values in healthy individuals, those chewing tobacco without Oral Submucous Fibrosis and those chewing tobacco suffering from Oral Submucous Fibrosis.

Methods &Material

Study Population:

The study was conducted at the Outdoor Patient Department of Pathology in collaboration with the Department of Dentistry and Hospital laboratory services at the medical college for one year. Patients were allotted into one of three categories: The Group I consisted of 25 healthy people, the Group II comprised of 25 individuals with chewing tobacco habit without OSMF, and Group III comprised of 25 patients with chewing tobacco habit with OSMF.

Data Collection:

None of the research team was in the treatment regimen or was suffering from any systemic conditions that could affect albumin levels in the body. Also, chronic alcoholics were excluded from the study to prevent possible changes in liver function that could alter serum levels of albumin, uric acid and total protein. Participants were informed in detail about the planned study and informed written consent. A certificate of ethics from the institutional ethics committee was obtained prior to the study. Participants were instructed to fast all night. The

next day between 8 and 10 a.m., 5 ml of dangerous blood was withdrawn from each subject using a disposable syringe that was used to prevent hemolysis. Serum is separated by ultracentrifugation with care to prevent hemolysis. Examination of albumin and total protein was performed using the raw method of bromocresol and that uric acid was made in the form of a biuret.

Statistical Analysis

Data were analyzed by one-way analysis of variance (ANOVA) using SPSS software version 24, to differentiate between the 3 groups. The $p < 0.05$ values were considered statistically significant. Data validity check prior to statistical analysis was performed.

Results

The value of the Mean serum levels of Total protein decreased from 7.42 g/dl in normal individuals to 6.10 g/dl in individuals with chewing tobacco without OSMF and 5.83 g/dl in individuals with chewing tobacco with OSMF; difference was not found to be statistically significant ($p > 0.05$) (Table 1). Serum levels of Albumin decreased from 4.70 g/dl in healthy individuals to 3.01 g/dl in patients detected with chewing tobacco without OSMF and 2.30 g/dl in patients detected with patients diagnosed with chewing tobacco with OSMF; difference was found to be statistically significant ($p < 0.05$) (Table 2). Serum levels of Uric acid decreased from 6.05 g/dl in normal individuals to 3.06 g/dl in patients detected with chewing tobacco without OSMF and 2.70 g/dl in patients detected with patients diagnosed with chewing tobacco without OSMF; difference was found to be statistically noteworthy ($p < 0.05$) (Table 3).

Discussion

Oxygen is an important substance for life on earth especially for human life and act like double edged sword. Oxygen is required for its cellular function and about 5% of inhaled oxygen is

converted into production free radicals through univalent reduction of O2.(plant) According to Halliwell, 2007 “free radicals are constantly produced during the normal cellular metabolism, mainly in the form of Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS)” [16]. These toxicity of oxygen molecule is itself beneficial in performing a function in inter and intracellular signalling, in therapeutics aid like hyperbaric oxygen therapy, and on other hand over production of free radicals causes formation of oxidative stress and further lead to damage the cellular metabolism, which would result in triggering or transforming normal cells into malignant ones [10].

Substances that neutralize the oxidative stress and neutralize the ill effect of free radicals are grouped in Antioxidant Defense System (ADS).[17] Such system encompasses many substances which are often called as Antioxidants, Free radical scavengers, Chain terminators [6][17]. Based on their function ADS is classified into four classes;

First: - Preventive antioxidants (suppress formation of free radicals).

Second: - Scavenging antioxidants (suppress chain reaction).

Third: - Repair and denovo antioxidants.

Fourth: - Adaptation where the signal for the production & actions of free radicals induces formation and transport of the appropriate antioxidant.

Free radicals are highly reactive molecules containing one or more unpaired electrons in their outermost shell. Antioxidants get oxidized in this reaction but are stable in their reduced form.[18]

Betel quid one of the causative agent in occurrence of OSMF is a mixture of Betel leaf, Areca nut, Slaked lime and tobacco pieces. When combination of areca nut and slaked lime occurs there is production of higher percentage of alkaloids. Arecoline is the major alkaloid and constitute about 0.15% to 0.67% of dry weight of betel quid [6][19]. In many studies the

genotoxicity and mutagenicity of these alkaloids have been detected [6][20]. Tobacco consists of tar phase and smoke phase. Smokeless tobacco consists of tar phase. The tar phase of tobcco contains stable free radicals of which principle is quinone/hydroquinone. This stable free radical when combine with alkaloids produce active redox system which reduce molecular oxygen to produce hydroxyl radicals [21][22].

Areca nut contains a higher level of copper (mean 302 nmol/g) in comparison to other edible nuts, (22–173 nmol/g) following chewing areca for 5–30 min soluble copper is released into whole mouth [7]. Data from the study done by **Trivedy CR et al. 2000** [23] demonstrated a high content of copper in the oral biopsy of OSF tissues using the element spectrum obtained by analytical scanning electron microscopy, nonetheless, The precise means of copper-induced mutagenesis lacks proper research. DNA damage caused by copper has been reported [24] theories suggest that copper may bind to the protein product of p53, causing modifications in its conformation [25][28] p53 aberrations is found in OSF tissues. Enhancement of P53, which is not detrimental to the development of potentially fatal lesions in oral squamous cell carcinoma [37], may result from DNA damage caused by chewing a copper containing Areca nut [29][34].

Active redox systems along with copper content of areca contribute in generation of free radicals by Fenton & Haber – Weiss reaction.

Haber – Weiss reaction

$Cu^{2+} + O_2^{-} = Cu^{+} + O_2$

Fenton Reaction

$Cu^{+} + H_2O_2 = Cu^{2+} + OH + OH^{-}$

Copper combine with this oxygen radical & hydrogen peroxide to produce free hydroxyl radicals. These are quenched by body's second line of non enzymatic antioxidants like Total

Protein, Albumin & Uric Acid.[35] a wide range of important anti-oxidants including total protein, albumin & uric acid are present in the plasma; possesses antioxidant property owing through free thiol group. Normal concentration range of each of these parameters in serum is as follows total protein is 6.6 – 8.3 gm/dl; Albumin: 3.5 – 5.0 gm/dl; Uric acid: 3.5 – 7.2 mg/dl.

The most abundant plasma protein in humans is albumin. It consists of 55 – 60% of total serum proteins. Synthesized in the liver, it possesses antioxidant properties [25][26]. Albumin helps in the scavenging action of already produced free radicals and inhibits the production of free radicals with the help of polymorphonuclear leukocytes. In the present study Serum Albumin Levels statistically fall from 4.70 g/dl in healthy control to 3.01 g/dl in Betel Quid & Gutkha chewers without OSMF to 2.30 g/dl in patient's Betel Quid & Gutkha chewers with OSMF. Our result is in consistent with various author **Sharan R et al. 2012,** [8][9] found the level of serum albumin to decrease in premalignancy as compared to health individuals [25].

Total serum protein is the combination of albumin and globulin. In the present study Serum Total Protein Levels decrease from 7.42 g/dl in healthy control to 6.10 g/dl in Betel Quid & Gutkha chewers without OSMF to 5.83 g/dl in Betel Quid & Gutkha chewers with OSMF. The difference was not found to be statistically significant. The result is in accordance with various other authors like Change Be et al. 2001, [1] and Hans et al. 2006, [30] found serum total protein level to decrease in premalignancy as compared to healthy individuals. One of the reasons for decrease in total protein is decrease in serum albumin level and other reason is tobacco effect on liver which results in decrease in synthesis of protein and ultimately total protein level decreases.

Uric acid is one of the key radical trapping antioxidants present in plasma. Theories suggest it protects the erythrocyte membrane adjacent to lipid peroxidation [3]. Thus uric acid could be expected to protect against oxidative stresses [6]. In the present study Serum Uric Acid Levels

statistically decreased from 6.05 mg/dl in healthy control to 3.06mg/dl in Betel Quid & Gutkha chewers without OSMF to 2.7mg/dl in Betel Quid & Gutkha chewers with OSMF. This result is in accordance with Mazza A et al. 2001, [33]. Lawal AO et al. 2012, [34] Tumor necrosis factor and interleukins cause decrease in appetite of the patient which results into decreased intake of nutritious food which results in decreased level of uric acid in the body because major portion of uric acid in the body is derived from the diet [35].

Conclusion

Decrease in level of serum proteins can be considered as an important event by which oxidative stress can cause toxic effects on antioxidant defense system in our body and initiate precancerous transformation and other oral diseases. Results of our study will not only be useful in understanding the pathogenesis of OSMF but it also focuses on antioxidant therapeutic approaches and in treatment of OSMF. However, further researches are necessary to accept the efficacy of the antioxidants such as serum albumin, total protein and uric acid in providing a scientific ground for their use in monitoring the disease activity and use as prognostic marker. Stem cell therapy the paradigm for future medicine has opened up new possibilities of treatment of oral lesions. To ascertain the role of stem cells more and more research is needed, animal models have been confirmed but human trials still need more and more advancements [36].

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Table 1: Evaluation of Total Protein, between Normal Control group, Chronic Tobacco Users group and OSMF group

GROUPS	N	Mean	Std. Deviation	P value
1	15	7.42	0.67	0.006
2	15	6.10	0.49	
3	15	5.83	0.36	

Table 2: Evaluation of Albumin, between Normal Control group, Chronic Tobacco Users group and OSMF group

GROUPS	N	Mean	Std. Deviation	P value
1	15	4.70	0.55	0.003*
2	15	3.01	0.32	
3	15	2.30	0.52	

*** indicates statistically significant difference at P < 0.005**

Table 3: Evaluation of Uric Acid, between Normal Control group, Chronic Tobacco Users group and OSMF group

GROUPS	N	Mean	Std. Deviation	P value
1	15	**6.05**	**0.88**	0.002*
2	15	**3.06**	**0.34**	
3	15	**2.70**	**0.38**	

*indicates statistically significant difference at $P < 0.005$