

Salivary Amylase, Serum Malondialdehyde, Vitamin – C And Total Proteins In Cases Of Oral Submucous Fibrosis (OSMF) And Diabetes Mellitus With OSMF.

Abstract

Oral Sub mucous Fibrosis (OSMF), has been thought to have some effect on the levels of Salivary Amylase (SA), Serum Malondialdehyde (MDA), Serum Vitamin – C and Total Proteins. Clinically well diagnosed cases of OSMF, Diabetes mellitus (DM) having Oral Sub mucous Fibrosis were included in this study, with view to find out the levels of these parameters. The study was carried out in four phases for every patient, i.e. pre-treatment, post treatment (after 4 - weeks, 6 - weeks and 8 - weeks). The patients were supplemented with "Ala - 100" and "Antoxid" capsules containing antioxidants. Significantly high levels of Serum MDA and total proteins, where as significantly low levels of Salivary Amylase, Serum Vitamin – C were observed in pre - treatment cases in reference to Controls, which were normalized in post treatment i.e. after 8 - weeks. Serum MDA, Serum Vitamin – C and Serum Protein levels were normalized after 6 - weeks in cases of OSMF in comparison with D.M. with OSMF. This observation indicates that out of the three parameters studied in, D.M. with OSMF require more period i.e. 08 - weeks for normalization.

Key Words

Malondialdehyde, Oral Submucous Fibrosis, Antioxidants, Serum.

Introduction:

Oral Sub mucous fibrosis is a chronic progressive, scarring disease of unknown etiology reported mainly in Indians. It has also been reported in Indians living in South Africa, Malaysia, Kenya, Uganda, Fiji and Britain^[1]. In 1952 Schwartz, while examining five Indian women from Kenya first ascribed the descriptive term "atrophia (tropica) mucosae oris"^[2] Later in 1953, Joshi^[3] from Bombay (Mumbai) redesignated the condition as Oral Sub mucous fibrosis, implying predominantly its histological nature. The WHO definition for an oral pre-cancerous condition - "a generalized pathological state of the oral mucosa associated with a significantly increased risk of cancer," accords well with the characteristic of OSF.

Epidemiological studies have shown that the process of carcinogenesis occurs by generation of Reactive Oxygen Species (ROS)^[4], which act by initiating lipid per oxidation (LPO)^[5]. Prevention against LPO mediated damage is done by non-enzymatic antioxidants, especially Beta-carotene and Vitamin – E and C.

Saliva is a complex fluid composed of a wide variety of organic and inorganic constituents that collectively act to modulate the oral environment^[6]. Interest in saliva as a diagnostic fluid has grown exponentially in recent years. Whole saliva can be collected noninvasively. The earliest "sialochemical" studies on oral fluids were conducted by Micheals^[7] and Krik^[8], each of whom examined saliva for specific components that would be diagnostic for various conditions.

Successful treatment with antioxidant supplementation for leukoplakia, an oral pre-malignant condition, has also been reported^[9]. With this understanding the present study was undertaken to ascertain the co-relation between anti-oxidant supplementation and the changes in the levels of Serum MDA, Serum Vitamin – C and Serum Protein in cases only with OSMF, Diabetes mellitus having Oral Sub mucous Fibrosis cases with respect to period.

Material And Methods:

Well diagnosed cases of OSMF (N=100), D.M. with OSMF (N=44), and Healthy Controls (N=50) were studied for

¹ S. J. Makija

² Sanjay Sarode

¹ Associate Professor, Dept. of Biochemistry, S.B. Science College, Aurangabad, Maharashtra.

² Lecturer, Dept. of Biochemistry, C.S.M.S.S. Dental College & Hospital, Kanchanwadi, Aurangabad, Maharashtra.

Address For Correspondence:

Dr. S. J. Makhija, Associate Professor, Dept. of Biochemistry, S.B. Science College, Aurangabad, Maharashtra.
Email ID : srjayme_mail53@rediffmail.com

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Salivary Amylase, Serum MDA, Serum Vitamin – C and Serum Protein levels. Random Blood Sugar levels were studied in the cases of D.M. with OSMF.

After the due consent of patient in writing the study was carried out in four phases i.e. pre-treatment and post-treatment (i.e. after 04, 06 and 08 weeks respectively) All the patients were subjected to a thorough history regarding dietary habits and addiction. All the details were taken in the "Case History" proforma.

Collection of Saliva and Blood Sample

With all aseptic precautions subjects were asked to spat out saliva accumulating freely in their mouth as frequently as desired over duration of 2 - minutes, further its was immediately used for Salivary Amylase estimation {Claycomb, (1956)^[10]}. Also 4ml of blood was collected from antecubital vein of each individual into a plain and fluoride bulb and bulb using EDTA as anticoagulant. The plasma and serum was separated and used for the estimation of Serum Malondialdehyde {Satoh, 1978}^[11], Blood Sugar {(GOD – PAP) End – Point Biolab Diagnostics}, Vitamin – C of {Harris and Ray, (1935)^[12]

Observation Table : Table No. :I Serum Mda, Serum Vitamin - C And Serum Protein Levels In Patients With OSMF,D.M. With OSMF, And Controls.

Parameters	Control	Pre-Treatment		4-Weeks		6-Weeks		8-Weeks	
		OSMF	D.M. c OSMF	OSMF	D.M. c OSMF	OSMF	D.M. c OSMF	OSMF	D.M. c OSMF
Salivary Amylase (mg/ml)	2.8 (±0.2)	1.6 (± 0.3)	1.6 (±0.2)	1.8(±0.2)	2.1(±0.4)	2.0(±0.3)	2.7(±0.1)	2.7(±0.1)	2.7(±0.1)
Blood Sugar (mg %)	115.9 (±5.2)	--	122.7 (±16.6)	--	115.9(±13.3)	--	116.6(±14.3)	--	115.0(±13.1)
Serum MDA (nmole/ml)	5.4 (± 0.5)	6.9 (±0.5)	7.3 (±0.9)	6.1(±0.6)	7.1(±0.9)	5.6(±0.3)	6.6(±0.6)	5.3(±0.1)	5.3(±0.4)
Serum Vit. - C (mg/l)	9.8 (± 1.4)	5.9 (±0.6)	5.8 (±0.9)	7.0(±0.7)	6.5(±1.0)	9.9(±0.6)	7.8(±1.0)	10.0(±0.4)	9.3(±1.1)
Total Serum Protein (gm%)	6.2 (± 0.2)	7.6 (±0.2)	8.2 (±0.2)	7.4(±0.3)	7.7(±0.3)	6.9(±0.3)	6.9(±0.3)	6.3(±0.3)	6.6(±0.3)

} and Serum Total Proteins {Lowry}^[13].

Statistical Analysis:

The data was analyzed with Student's independent 't' test.

Results And Discussion:

Diabetes is the commonest metabolic disease of endocrine origin, characterized by hyperglycemia resulting from defects in insulin secretion, insulin action or both, affecting a large number of people in the world. Multiorgan involvement in diabetes as in retina, blood vessels, kidneys and nerves have been well documented.

The history of all the cases included in the study revealed that significant portion of OSMF cases chewed the processed betel quid preparations. It has been shown that Betel quid extract aercoline is more cytotoxic than (+)-catechin and extracts of Inflorescence of piper betel (IPB) and betel nut (Areca catechu). These might act synergistically on the pathogenesis of OSMF^[14].

In the present study, the levels of Salivary Amylase, Serum MDA, Serum Vitamin - C and Serum total protein were compared between the OSMF group (i.e. OSMF, Diabetes mellitus having Oral Sub mucous Fibrosis) and healthy Controls. OSMF group which was in turn divided as Pre-treatment and Post-treatment (i.e. after 04-weeks, 06-weeks and 08-weeks). Random Blood Sugar levels were analyzed which were normal in the cases of D.M. with OSMF.

A decrease of 75% in the levels of Salivary amylase was observed in Pre - treatment cases of OSMF, D.M. with OSMF with reference to Control. In Post treatment period after 06 - weeks OSMF cases showed an increase in the levels of Salivary amylase i.e. 2.0 mg/ml which was low as compared to DM with OSMF i.e. 2.7mg/ml. The levels were normalized after 08 - weeks with

reference to Control. The decrease in the levels of Salivary Amylase must be due to the reduction in the total salivary secretion seen in fever and other pathologic conditions that affect oral secretions^[15].

A increase of 35% in the levels of Serum MDA was observed in Pre-treatment cases of D.M. with OSMF with reference to Control. This increase in the level was higher as compared to OSMF cases, which were 28% with reference to Control. The mean Serum MDA level in OSMF Pre-treatment cases was 6.9 (±0.5), which showed the lowest increase in reference to Control i.e. 5.4 (±0.5). A steady decrease in these levels was observed after starting the anti-oxidant supplementation i.e. in Post treatment cases in both the groups. A normal level of 5.6 (±0.3) was observed within the period of 06 - weeks in OSMF cases in reference to Control, as compared to the other group, which required a period of 08 - weeks to reach the normal levels. Increase in MDA levels in OSMF cases has also been reported by Suryakant Metkari et al^[16]. They have reported that there is increase in the level from OSMF grade-I, grade-II, grade-III and grade-IV. The increase in lipid per oxidation product MDA in OSMF pre-treatment cases as compared to post treatment i.e. after 08-weeks and control group may be due to the poor antioxidant system, excessive free radical formation due to various tissue abuse habits and decomposition of polyunsaturated fatty acid present in membranes.

Diabetes Mellitus with OSMF Pre-treatment cases showed the highest decrease in Serum Vitamin - C levels with reference to the Controls, and this decrease was 41%. The cases of OSMF and D.M. with OSMF also showed a comparable decrease of 40% in each. A steady increase in the levels of Serum Vitamin - C was observed after starting the anti-oxidant supplementation i.e. in Post treatment cases in both groups. A

normal level of 9.9 (±0.6) was observed within the period of 06 - weeks in OSMF cases in reference to Controls i.e. 9.8 (±1.4) as compared to the other group, which required a period of 08 - weeks to reach the normal levels. This clearly shows that the anti - oxidant load for this Vitamin was lowered in all the cases studied and hence the levels of this vitamin showed a steady increase in Post-treatment cases.

A highest of 32% increase in total Serum Protein levels was observed in Diabetes Mellitus with OSMF cases in reference to Controls. A period of 08 - weeks was required for OSMF and Diabetes Mellitus with OSMF cases to achieve normal Serum Protein levels. A value of 6.6 (±0.3) and 6.3 (±0.3) was observed in OSMF and Diabetes Mellitus with OSMF cases respectively.

The increase in total serum protein levels in pre-treatment cases compared to post treatment i.e. after 08-weeks and control group may be due to the increase in globulin fractions and other serum proteins^[17]. The increase in lipid per oxidation product MDA, Serum total protein and decreased antioxidant Vitamin-C observed in present studies are also supported by similar findings reported in various pathological conditions including OSMF.

The study reveals that a period of 08-weeks is required to normalize the parameters studied in OSMF with diabetes cases after starting the Antioxidant Capsules. Antioxidant is a molecule capable of showing or preventing the oxidation of other molecules. Ascorbic acid is a reducing agent and can reduce, and thereby neutralize, reactive oxygen species (ROS) such as hydrogen peroxide^[18]. Our studies are supported by similar results observed by Soma Gupta et al^[19] who reported that the treatment with the "antioxid" capsules showed some improvements and amelioration of the

symptoms along with increased in the level of vitamin – E and beta carotene which are the antioxidants, the period required was 06-weeks in OSMF cases, however present.

Thus the present study shows possibility of ROS playing a part in aetiopathogenesis of the disease and further Salivary amylase, Serum Vitamin C, and MDA levels as the biochemical marker of the disease state. OSMF and administration of antioxidants may have protecting effect with clinical improvement. Long term follow up study of the more number of patients is required with some more antioxidant markers for analysis in saliva and blood, to validate the use of such therapy in OSMF cases and prevent malignant conversion and also the role of any of above studied parameters as a bio-chemical marker.

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