Areca Nut and its Role in Blood Sugar Level

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Abstract

Oral submucous fibrosis(OSMF) is a slowly developing chronic ailment and a complex, highly effectivepremalignant condition of the oral cavity and oropharynx,identified by reddening of juxta-epithelial area and increasing thickening and scarring of connective tissue.Areca nut is a part of betel quid and it is one of the main causative agents which play an important role in the pathogenesis of this disease.Nitrosated products of areca alkaloids can be diabetogenic in mice causing type 2 diabetes with obesity.The aim of the study was to estimate random blood sugar level in patients suffering from OSMF, individuals with habit of areca nut chewing but without OSMF and healthy controls.The objective was to compare blood sugar level between these three study groups.When mean sugar level was compared between these groups,the difference was found to be statistically significant.An elevation in the mean random blood sugar level in patients suffering from OSMF was found.

Keywords:fibrosis, areca nut, oral cavity, diabetes, sugar

1.INTRODUCTION

Oral submucous fibrosis (OSMF) is a long standing, slow growing oral disease with very harmful effects and it has the potential to damage the oral cavity and pharynx.In this disease, the lamina propria and deeper connective tissues undergo fibrosis which results in depressed cheeks and trismus.A sense of burning in the oral cavity and occurrence of fibrous bands in the labial and buccal mucosa, are some of the major features of this disease.This disease mostly affects individuals between 16 to 35 years of age. The causation of this disease involves multiple factors but the main reason is areca nut chewing.[1] Betel pepper leaves,areca nut,calcium hydroxide and spices are the major components of betel quid and this quid,which is a portion of tobacco,is chewable and a deleterious habit in South Asia.Some of the major components of areca nut include arecoline,arecaidine,guvacine,guvacoline,copper ,cadmium,catechin,calcium,lead and nitrosamines.[2] If sufficient insulin is not present in human body then this causes high blood sugar which can lead to Diabetes Mellitus.Diabetes Mellitus is of three types,type 1 Diabetes Mellitus(type 1 DM),type 2 Diabetes Mellitus(type 2 DM), and gestational diabetes.Type 1 DM occurs when body is not able to generate sufficient insulin,type 2 DM occurs when body cells are not able to utilize insulin,and gestational diabetes occurs in pregnant females suffering from elevated blood sugar level.[3] Hyperglycaemia causes polyuria,polyphagia,polydipsia, along with diabetic dermopathy, diabetic bullae, pruritus and psoriasis.[4]The areca alkaloids have nitrosated components which can trigger type2 diabetes in mice and they are also agents which can cause cancer.[5]

2.MATERIAL AND METHOD

This observational study was conducted in the Department of Oral Medicine and Radiology, People's College of Dental Sciences and Research Centre, Bhopal, India from June 2015 till November 2015. Prior to starting the study, an ethical clearance was obtained from the Institutional Ethical Committee.The study included 60 individuals of either sex and was divided in three groups as OSMF group(patients suffering from OSMF) (n=20),habit group(individuals with habit of areca nut chewing but without OSMF) (n=20) and healthy

control group (n=20). Diagnosis of OSMF was made on the basis of characteristic clinical features of the disease. A written informed consent was obtained from all the patients before inclusion in the study.

INCLUSION CRITERIA:

1. Patients with clinically diagnosed OSMF.
2. Individuals with areca habit without any other underlying systemic disease as habit group.
3. Individuals without areca habit and underlying systemic disease as control group.

EXCLUSION CRITERIA:

1. Patients having other oral lesions besides OSMF.
2. Individuals having systemic disorders and chronic inflammatory disorders.
3. Previously treated OSMF patients.

Classification of OSMF was followed as per Khanna and Andrade classification (1995) for selected OSMF individuals.[1]Blood sample was drawn from antecubital vein using a 3ml syringe and it was taken into a vial containing a clot activator and serum gel separator. After that, it was transferred into a centrifuge tube. The centrifugation of blood was done for 10 minutes and serum was obtained. Proper centrifugation is important because if the glucose is measured from unprocessed blood samples, then glycolysis can occur, which may causeglucose level to reduce by approximately 5% per hour and may give incorrect readings of blood glucose. Centrifugation is a procedure of using centrifugal force and the aim is to separate two unmixable liquids.There is sedimentation of heterogeneous mixtures in which more heavy constituents drift away from the axis of centrifuge and less heavy constituents drift towards the axis of centrifuge. The effective gravitational force on a test tube was increased which caused the precipitate to collect on the bottom of tube and the remaining supernatant liquid was withdrawn with the help of a pipette.[6] The purpose was to separate serum and plasma. In separated unhemolyzed serum, the concentration of glucose is stable upto 8 hours at 25 degree Celsius, and bacterial contamination is avoided.

Glucose oxidase(GOD) was used to measure glucose level in blood. GOD is an enzyme consisting of two polypeptide chains which are identical in order and number. Flavin adenine dinucleotide (FAD) is present in its each subunit. There are two major structural and functional units of each GOD monomer, one that connects non-covalently the FAD portion and another that attaches the beta-D-glucose substrate. In the presence of GOD,glucose was converted into gluconate and hydrogen peroxide. Phenol and 4-aminophenazone(4-AP)reacted with hydrogen peroxideand the reaction took place in the presence of peroxidase(POD), and caused the formation of red colored dye quinone.[7] We measured the glucose concentration according to the intensity of the color produced due to the dye.So, the reagents used were GOD, which was used 15ku/L (kilounits per liter),POD 1.0 ku/L, phenol 0.3mmol/L (millimoles per liter), 4-AP 2.6 mmol/L, stabilizers, activators, glucose aqueous primary standard solution 100 mg/dL(milligrams per deciliter). The appliances used were colorimeter, cuvettes and general laboratory equipment. The wavelength was kept 505 nanometer and the temperature was kept between 15 to 25 degree Celsius. Clean, dry test tubes were taken and they were labeled as Blank(B), Standard (S) and Sample. Proper mixing was done. Incubation was done at 25 degree Celsius for ten minutes. The absorbance of the standard and test sample against blank was measured. After incubation, the color was stable for sometime.We divided the absorbance of test sample by absorbance of the standard and the product is multiplied by 100. We obtained glucose concentration in mg/dL.

3.RESULTS

Comparison of mean RBS (random blood sugar) levels between OSMF group and habit group(individuals with habit of areca nut chewing but without OSMF) was made, and the

difference was found to be statistically significant.(Table 1)

TABLE 1

RBS level	OSMF group	Habit group	T test value	P value
Mean	95.40	85.15		
Standard Deviation	14.84	8.24	2.6	0.01

Comparison of mean RBS levels between OSMF group and healthy control group was made, and the difference was not found to be statistically significant (Table 2)

TABLE 2

RBS level	OSMF group	Healthy control group	T test value	P value
Mean	95.40	87.15		
Standard Deviation	14.84	12.00	1.9	0.06

Comparison of mean RBS levels between habit group and healthy control group was made, and the difference was not found to be statistically significant (Table 3)

TABLE 3

RBS level	Habit group	Healthy control group	T test value	P value
Mean	85.15	87.15		
Standard Deviation	8.24	12.00	0.6	0.5

Comparison of mean RBS levels between all the three groups was made, and the difference was found to be statistically significant (Table 4)

The mean RBS level was 95.4 mg/dL in OSMF group, 85.15 mg/dL in habit group and 87.15 mg/dL in healthy control group.

TABLE 4

RBS level	OSMF group	Habit group	Healthy control group	Total
Mean	95.40	85.15	87.15	89.23
Standard Deviation	14.84	8.24	12.00	12.62
ANOVA F value	4.09			
P value	0.02			

4.DISCUSSION

OSMF is a persistent disease, which was first explained by Schwartz in 1952 and he formulated the phrase "atrophiaidiopathicamucosaoris" but the disease was called "Oral submucous fibrosis" by Joshi in 1953.[8]Blisters,dryness, tenderness, and erythema can occur in the oral cavity.The presence of rigid fibrous tissue in juxtaepithelial area of mucosa in oral cavity causes difficulty in mouth opening, difficulty in tongue movement and such patients are also unable to blow whistle. The other major symptoms include continuous fibrosis of the thin, vascular layer of connective tissue beneath the epithelium in the oral cavity, which further leads to difficulty in chewing, deglutition and speech. When the patient consumes hot and peppery food, he feels a sense of burning in the oral cavity and long-lasting ulceration can develop in such patients. The buccal mucosa has a spotted white surface and the floor of the mouth develops a faded exterior.[9] The faucial pillars and palate are also affected due the fibrous deposit which can cause whitish area on the soft palate as well as shrinking and distortion of uvula . In severe cases, there can be damage to the tonsils and

sunken cheeks are present. Areca nut can trigger allergy in the mucosa.The response of the blood vessels can lead to development of petechiae on tongue and other sites of oral mucosa.[10]

In areca nut tree, the fruit has a dry outer covering, and on taking off this outer covering, the betel nut is obtained, which comprises of arecoline.This arecoline is an alkaloid which activates the fibroblasts. This causes excessive generation of collagen.[11]The areca nut is an addictive, chewable material which helps the human body to relax by developing euphoria and reducing fatigue and hunger. The connective tissue develops elevated levels of cytokines which causes reduction in serum albumin concentration in the body.[12] The collagen undergoes a metabolic process in the body, which is strongly influenced by collagenases and matrix metalloproteinases. Arecoline controls these enzymes which later, leads to excessive fibrosis, inflammation and infection in the oral cavity.[13] The fibroblasts cause phagocytosis of collagen but arecoline suppresses this phagocytosis. The resistance of collagen fiber to collagenase is elevated due to tannin, which is present in areca nut.[2]

Diabetes Mellitus is a long-lasting,multifactorial disease affecting the endocrine system of the body and it affects all age groups. This disorder can be related to human genetics. The type 1 DM, which occurs mostly in children, is insulin dependent and type 2 DM, which occurs mostly in obese adults(usually between 45 to 64 years), is not insulin dependent but the metabolic process involving carbohydrates, proteins and fats is distorted.[14]Patients who avoid physical exercise, consume diet high in saturated and trans-fat and low fiber diet, having deleterious habits such as cigarette smoking and alcohol consumption, and patients suffering from obesity, are mostly prone to this disorder.There is a decrease in efficacy of the physiological reaction of insulin to the body cells because there is distortion at the attachment sites of insulin receptors, so the insulin cannot connect to its receptor.[17]Hyperglycaemia is one of the commonest and major symptom of this disease, in which the blood glucose level is mostly above 200 mg/dL. There is reduction in carrying of glucose to various cells of the body,such as muscle cells,fat cells, because there is damage of pancreatic beta cells,which further causes reduced insulin. There is marked elevation in the disintegration of fats and increase in glucagon levels in the body.[15] Nausea, fatigue, vomiting, reduction in body weight, dehydration,Kussmaul breathing and coma are some of the clinical features of this disease.[16]Nephropathy, neuropathy, atherosclerosis, diabetic ketoacidosis, retinopathycausing excessive blood loss from retina leading to defected vision or even complete loss of vision, can strongly affect a diabetic patient.[17] Diabetes disturb the generation of cytokines and the movement by the cells triggered by chemical stimulus is also affected. The engulfment of foreign particles is influenced by this disease leading to reduction in immunity and rise in collagenase. Such a patient has to undergo delayed healing of periodontium if there is any trauma. There is weakening of gingiva and alveolar bone, which causes the teeth to become mobile and such patients suffer from dental caries due to excessive glucose present in saliva. This glucose provides suitable environment for various pathogens in the oral cavity.[14]Patients who smoke are more prone to this disease because the equilibrium of glucose level in the body is disturbed and there is a marked elevation of insulin resistance. Smoking causes rise in plasminogen activator inhibitor 1 functioning, lipoprotein elements,fibrinogen and triglycerides.[18] The American Cancer Society did a cohort study in which one million individuals participated,including both males and females and their age was more than thirty years. It was found that the diabetes rate showed an elevation when there was rise in the deleterious habit of smoking. The smoker males had 45% greater rate of diabetes as compared to non-smoker males. Similarly, the smoker females had 74% greater rate of diabetes as compared to non-smoker females.[19] Some alkaloids, originating from areca nut, strongly influence central nervous system, respiratory system and cardiovascular system and they can cause the development of tumors in the body. These alkaloids block the proper functioning of GABA(Gamma aminobutyric acid) neurotransmitters in brain and their nitroso-

compounds have been shown to abnormally elevate glucose levels in mice.[5] In the present study, we compared mean RBS levels between OSMF group and habit group and found that the difference was statistically significant (P value=0.01) (Table 1). We compared mean RBS levels between OSMF group and healthy control group and found that the difference was not statistically significant. (P value=0.06) (Table 2). We compared mean RBS levels between habit group and healthy control group and found that the difference was not statistically significant (P value=0.5) (Table 3). We compared mean RBS levels between all the three groups and found that the difference was statistically significant (P value=0.02) (Table 4). There was a rise in mean RBS level in OSMF group and it was found to be 95.4 mg/dL whereas mean RBS level in habit group was 85.15 mg/dL . In healthy control group, it was 87.15 mg/dL . Tseng conducted a study in Taiwan, between 1992 to 1996, to establish the link between type 2 diabetes mellitus and chewing of betel nut. The participants included 93,484 patients, who were selected from a total of 256,036 patients, suffering from diabetes mellitus. The odds ratio and rate of incidence was estimated. They also measured ratios for incidence rate. It was found that there was an increased risk of this disease in young patients, who had the deleterious habit of areca nut chewing, and were suffering from obesity (all p values were less than 0.001). Such findings could not be proved in individuals who never chewed areca nut.[20]

5.CONCLUSION

The present study provides useful information about association of areca nut chewing and blood sugar level in humans. The OSMF patients have a considerable increase in their mean RBS level, irrespective of the obesity,which may be an indicator for development of diabetes in future.However, the study had few limitations. The glucose oxidase method depends on the utilization of oxygen. Different altitudes have different oxygen levels,which can result in incorrect glucose level readings.In the past, a study was done at Mount Kilimanjaro in Tanzania and it was found that the same patient had two different blood glucose readings. Hematocrit values can also affect blood glucose reading because if these values are less than 35%, they can lead to falsely increased glucose level estimation.Incorrect estimation of low glucose levels can also be observed, if the hematocrit reading is abnormally elevated.[21]The findings of the study can also be affected if there considerable difference in the mean age among study groups.

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