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Original Article

Comparative Evaluation of Plasma Lycopene Levels in Oral Leukoplakia

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***ABSTRACT*:**

Background - Leukoplakia is the most common pre-cancerous lesion in the oral cavity. Present study aimed to evaluate plasma lycopene levels in patients with clinically/ histopathologically proven leukoplakia with that of control group (smokers/non-smokers) **Materials and Methods**- Total of 180 individuals were included in the study with two main groups namely cases and controls: clinically/ histopathologically proven cases of oral leukoplakia (60) and age and sex matched control group divided into two categories non-smokers (60) and smokers without leukoplakia (60). Determination of lycopene in blood plasma by high performance liquid chromatography. **Results-** The age of the patients varied between 31-70 years. Mean plasma lycopene levels for the leukoplakia patients (smokers) was 32.86±21.42 ng/ml, and for controls who were smokers it was 42.70±26.54 ng/ml, however the mean levels for non-smokers was 146.6±52.45 ng/ml which was significantly more. **Conclusion-** Thus lycopene appears to be a very promising antioxidant as a treatment modality in oral leukoplakia. Results indicate that lycopene can protect cells against cell damage and play a protective role against progression of dysplasia.

Keywords- Antioxidants, Leukoplakia, Plasma Lycopene.

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INTRODUCTION:-

Leukoplakia is the most common pre-cancerous lesion in the oral cavity. According to WHO 1997 Leukoplakia is defined as "a predominantly white lesion of the oral mucosa that cannot be characterized as any other definable lesion".**[1]** It is now categorized under potentially malignant disorders.**[2]** Malignant potential of leukoplakia was hinted by Sugar and Banoczy way back in 1957.[3] Association between tobacco chewing and smoking with oral leukoplakia is established beyond doubt. Tobacco smoke contains NOO radicals, which are carcinogenic. Free radical scavengers should be the necessary part of the treatment regimen in tobacco chewers or smokers to prevent the formation, induce the remission or inhibit the progression of pre-cancerous lesions into malignancies. Lycopene, the carotenoid that gives the ripe tomato its bright red color, is a very effective natural antioxidant and quencher of free radicals. It is also found in various fruits such as watermelons, guava and pink grapefruit.[3,4]

Studies have shown that process of carcinogenesis occurs by generation of reactive oxygen species (ROS) , which act by initiating lipid peroxidation (LPO).**[4]** Reactive oxygen species (ROS) is generated in tissues and can damage DNA, proteins, carbohydrates and lipids .

Antioxidants especially lycopene exhibits highest physical quenching rate constant with singlet oxygen. Lycopene has been found to be least 3-fold more effective than ß-carotene in preventing cell death by quenching of NOO radicals. It also protects DNA damage induced by 1-methyl 3-nitro-1-nitrosoguanidine and H_2O_2.**[5]**

Lycopene is a safe antioxidant of utmost importance. Lycopene is a bright red carotene and carotenoid pigment and a phytochemical found in tomatoes and other red fruits and vegetables, such as red carrots, watermelons and papayas. Preliminary research has shown an inverse

correlation between the consumption of tomatoes and cancer risk. It has been shown to have several potent anticarcinogenic and antioxidant properties and has demonstrated profound benefits in precancerous lesions such as leukoplakia. Lycopene exhibits the highest physical quenching rate constant with singlet oxygen.[6] Present study aimed to estimate and compare plasma lycopene levels in patients with clinically/ histopathologically proven leukoplakia with that of control group.

MATERIALS & METHODS:

The present study was conducted in the Department of Oral Pathology. Ethical permission from Institute and informed consent from patients involved were taken before the commencement of study.

Thirty patients, who were clinically diagnosed with oral leukoplakia were included for the study. Age and sex matched healthy control groups were also enrolled in the study.

Subjects on oral antioxidants, patients with other mucosal disease (eg. Oral cancer, oral lichen planus, oral submucous fibrosis) and patients known to have other systemic disease were excluded from study group.

These patients were randomly categorized in two groups.

1. Clinically/ histopathologically proven cases of oral leukoplakia (60)
2. Control group divided into two categories: Non-smokers (60) and Smokers without leukoplakia (60)

Diagnosis of oral leukoplakia was first made on the basis of history and clinical features described as follows:

A. i. Homogenous

ii.Non Homogenous which includes: Speckled, Nodular, Verrucous or Proliferative verrucous leukoplakia.[3]

B. Histopathological grading according to severity of dysplasia as follows: Mild/Moderate/Severe epithelial dysplasia or Carcinoma in situ.

Method of collection of blood sample

After obtaining detailed case history patients were asked to report next day morning with fasting for 8 hour before arrival**.** This was to avoid any dietary influence on plasma lycopene levels. Then 4ml of venous blood was withdrawn and put into sodium citrate vial (3.2%) of AkuSet^{TM} and was assigned a number with the help of marker. Blood samples were stored on ice until centrifuged for 10 min at 4000 rpm, plasma was separated[16] and collected in a new vial assigned a same number. Vials were then wrapped with the tin foil and blood samples chilled in ice[17,18] packs were transported on the same day after collection for analysis and all analysis were done under dim light.

RESULTS:

The study was designed to assess the Plasma lycopene levels in patients with oral leukoplakia and to compare the results with age/sex matched controls. The control group was further divided into smokers and non smokers. Total of 180 individuals were included in the study. Out of which 60 patients were of clinically/histopathologically proven oral leukoplakia with the positive history of smoking and all the patients were males. The age of the patients varied between 31-70 years.

In the present study mean plasma lycopene levels for the leukoplakia patients (smokers) was 32.86±21.42 ng/ml, and for controls who were smokers it was 42.70±26.54 ng/ml, however the mean levels for non-smokers was 146.6±52.45 ng/ml which was significantly more. (Table 2)

Table 1.1: Descriptive analysis of Age

		N	Mean	Std. Deviation	p-value
Age	Non-Smokers	60	51.62	8.16	0.943
	Smokers Without Leukoplakia	60	46.57	8.20	
	Smokers With Leukoplakia	60	42.67	10.42	
	Total	180	57.63	10.98	

Table 2: Plasma Lycopene Levels in different groups

		N	Mean	Std. Deviation	p-value
Plasma lycopene Levels	Non-Smokers	60	146.6	52.45	<0.001**
	Smokers Without Leukoplakia	60	42.70	26.54	
	Smokers With Leukoplakia	60	32.86	21.42	
	Total	180	74.84	66.28	

** The p-value is significant at 5% level

Table 3: Mean Comparison between Smokers without Leukoplakia and Smokers with Leukoplakia.

	Group	N	Mean± Std. Deviation	p-value
Frequency	Smokers Without Leukoplakia	60	12.2±2.421	0.568
	Smokers With Leukoplakia	60	12.28± 4.05	
Duration	Smokers Without Leukoplakia	60	21.33±6.39	0.528
	Smokers With Leukoplakia	60	23.47±6.30	
Plasma lycopene Levels	Smokers Without Leukoplakia	60	42.27±22.56	0.056
	Smokers With Leukoplakia	60	28.66±16.45	
Age	Smokers Without Leukoplakia	60	50.42±8.26	0.648
	Smokers With Leukoplakia	60	49.36±10.32	

Table 4: Mean Comparison between Unilateral and Bilateral

	Distribution	N	Mean±Std. Deviation	p-value
Frequency	Unilateral	46	12.6±6.62	0.152
	Bilateral	44	15.26±6.46	
Duration	Unilateral	46	20.08±6.59	0.068
	Bilateral	44	24.54±8.84	
Plasma lycopene Levels	Unilateral	46	33.56±16.72	0.46
	Bilateral	44	29.32±14.37	
Age	Unilateral	46	48.46±12.67	0.56
	Bilateral	44	50.66±12.42	

Table 5: Mean Comparison between Homogeneous and Speckled.

	Clinical Type	N	Mean±Std. Deviation	p-value
Frequency	Homogeneous	46	9.88±4.951	0.016
	Speckled	44	14.14±4.258	
Duration	Homogeneous	46	17.38±7.154	0.014
	Speckled	44	23.36±5.458	
Plasma lycopene Levels	Homogeneous	46	35.95±18.813	0.025
	Speckled	44	22.05±12.791	
Age	Homogeneous	46	45.5±10.218	0.286
	Speckled	44	49.5±9.967	

The p-value is significant at 5% level

Table 6: Mean Comparison with respect to Histopathological Grading

	Histopathological Grading	N	Mean±Std. Deviation	p-value
Frequency	Mild	43	9.62±4.805	0.04
	Moderate	47	13.59±±4.651	
Duration	Mild	43	17.54±7.902	0.076
	Moderate	47	22.18±5.67	
Plasma lycopene Levels	Mild	43	40.49±17.987	0.005
	Moderate	47	21.04±11.75	
Age	Mild	43	45.31±10.483	0.346
	Moderate	47	48.94±9.877	

The p-value is significant at 5% level

DISCUSSION:-

The association between oral leukoplakia and tobacco habits is well established in numerous epidemiologic studies. The association has generally been found to be strong and therefore the habits of tobacco smoking and chewing are accepted as the principal aetiologic factors for oral leukoplakia (Gupta PC, 1980)[7] Reports indicate that 15.8-48.0% of Oral squamous cell carcinoma patients were associated with Oral leukoplakia when diagnosed.[8] More than 70% of patients in the present study were belonging to the age range of 31–70 years. Thoma[9] reported similar incidence, out of 321 patients within this age range, 70% of them had leukoplakia. Leukoplakia is now being reported in patients under 20 years of age.

Various treatment modalities have been suggested in the literature for management of leukoplakia by various

authors. These include both pharmacological and non pharmacological approaches for the same. As the association of leukoplakia and tobacco has been strongly suggested so the free radical scavengers should be the necessary part of the treatment regimen in tobacco chewers or smokers to prevent the formation and to induce remission or inhibition of progression of precancerous lesions into malignany.[10]

Bhuvaneswari[11] in their study checked the chemopreventive efficacy of lycopene on 7,12-dimethyl benz[a]anthracene (DMBA)-induced hamster buccal pouch (HBP) carcinogenesis. The results suggested that lycopene was very efficacious in preventing neoplasia.

The present biochemical study is an attempt to analyse plasma lycopene levels in patients with oral leukoplakia and compare with normal healthy individuals thereby analyzing the oxidative stress..

In the present study mean plasma lycopene levels for the leukoplakia patients (smokers) was 32.86±21.42 ng/ml, and for controls who were smokers it was 42.70±26.54 ng/ml, however the mean levels for non-smokers was 146.6±52.45 ng/ml which was significantly more. This was supported by many studies that smokers have lower plasma concentrations of most carotenoids than non-smoker.[12] The possible explanation is that smoking is well known for introducing a source of free radicals, which can increase lipid peroxidation and DNA damaging. Among the cellular molecules, lipids that contain unsaturated fatty acids with more than one double bond are particularly susceptible to action of free radicals, The resulting reaction known as lipid peroxidation, disrupts biological membranes and is thereby highly deleterious to their structure and function.[13] Lycopene may be used to neutralize the free radicals generated from smoking.

In the study conducted by Nagao T (2000)[14] the mean serum lycopene and β-carotene levels among male cases were significantly lower than those detected for controls. The results of the population-based study suggested that high serum levels of β-carotene and lycopene reduce the risk of leukoplakia in Japanese males. In their study this was confirmed in the bivariate analysis but the significance of lycopene was not apparent in the logistic regression analysis.

The present study reveal no significant difference in plasma lycopene levels of cases of leukoplakia when compared with age, gender, frequency and duration of associated habit matched controls. Whereas a definite significant difference in plasma lycopene levels is observed between smokers and non smokers, which shows inverse relation of smoking and plasma lycopene levels. Thus the role of lycopene in managing the oral leukoplakia is attributed to its role as antioxidant in decreasing free radical damage and its ability to quench singlet oxygen, which is a reactive unstable molecule. Role of lycopene in leukoplakia is empirical but it should be supplemented in leukoplakia as leukoplakia is potentially malignant lesion and the role of lycopene in inhibition and progression of cancer has been proven.

The efficacy of lycopene increases with dose. 8 mg lycopene per day was found to be more efficacious than 4 mg lycopene per day. 4 mg lycopene per day also showed significant clinical efficacy and it could be used for treating oral leukoplakia but probably a longer duration of therapy may be required. It also appears to be a safe drug.

CONCLUSION:

Thus lycopene appears to be a very promising antioxidant as a treatment modality in oral leukoplakia. Results indicate that lycopene can protect cells against cell damage and play a protective role against progression of dysplasia. Lycopene being important carotenoid, further studies with large sample size are necessary to investigate lycopene levels and its association with leukoplakia associated with various habits, Also according to clinical and histopathological types and impact of dietary sources is required as not much of the work has been done on plasma lycopene levels and its relation with oral leukoplakia.

REFERENCES:-

1. Pindborg JJ, Reichart PA, Smith CJ, Vander Waal I. World Health Organization International Histological classification of Tumors. Histological typing of cancer and precancer of the oral mucosa. Berlin: Springer, 1997
2. Warnakulasuriya S, Johnson NW, Vander Waal I. Nomenclature and classification of potentially malignant disorders of the oral mucosa. J Oral Pathol Med 2007; 36: 575-80
3. Banoczy MA. Oral leukoplakia and other white lesions of oral mucosa. Cutaneous Pathol 1983;10(4):238–56.
4. Klaunig JE, Kamendulis LM, Hocevar BA. Oxidative stress and oxidative damage in carcinogenesis. Toxicologic Pathology 2010; 38: 96-109
5. Di Mascio P, Kairer S, Sies H. Lycopene as the most efficient biological carotenoid singlet oxygen quencher. Arch Biochem Biophys 1989;274(2):532–8.
6. Singh Mohitpal, Krishanappa R, Bagewadi A, Keluskar V. Efficacy of oral lycopene in the treatment of oral leukoplakia. Oral Oncology 2004; 40: 591-596.
7. Gupta PC. A study of dose-response relationship between tobacco habits and oral leukoplakia. Br. J. Cancer 1984; 50: 527-531
8. Schepman K, Vander Meij E, Smeele L, VanderWaal I.Concomitant leukoplakia in patients with oral squamous cell carcinoma. Oral Dis 1999; 5: 206-209
9. Thoma. Text book of oral pathology. 6th ed. St. Louis: CV Mosby; 1970. p. 56–63
10. Panwar Y, Choudhary V. Estimation of plasma lycopene in oral leukoplakia. J Adv Med Dent Scie Res 2017;5(2):34-40.
11. Bhuvaneswari V, Velmurugan B, Balasenthil S, Ramachandran CR, Nagini S. Chemopreventive efficacy of lycopene on 7,12-Dimethyl benz[a]anthracene induced Hamster buccal pouch carcinogenesis. Fitoterpia 2001;72(8):865–74.
12. Nayak A, B Anitha, Bhattacharya A, Podder S. Efficacy of Lycopene in Combination with Vitamin E in Management of

Oral Submucous Fibrosis - A Clinical Prospective Study. J Adv Med Dent Scie Res 2015;3(3):21-25.
13. Gupta PC, Hebert JR, Bhonsle RB. Dietary factors in oral leukoplakia and submucous fibrosis in a population based case control study in Gujarat, India. Oral Dis 1998;4(3):200–6
14. Nagao T, Ikeda N, Warnakulasuriya S, Fukano H, Yuasa H, Yano M et al. Serum antioxidant micronutrients and the risk of oral leukoplakia among Japanese. Oral Oncology 2000; 36: 466-470.

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