Journal of Advanced Zoology
ISSN: 0253-7214
Volume 44 Issue 5 Year 2023 Page 1426:1432

Estimation Of Serum Vitamin B_2 Levels In Oral Squamous Cell Carcinoma Patients: A Case-Control Study

Dr.Sangeeta Palaskar[1], Dr.Himali Pachpande[1*], Dr.Dipti Pardeshi[1], Dr.Samruddhi Kamble[1], Shrutika Tele[2], Shreyasi Tade[2]

[1]Department Oral Pathology and Microbiology, Sinhgad Dental College and Hospital, Maharashtra University of Health Sciences, Pune, India
[2]Sinhgad Dental College and Hospital, Maharashtra University of Health Sciences, Pune, India

***Corresponding Author :** Dr.Himali Gopal Pachpande*
***Department Oral Pathology and Microbiology, Sinhgad Dental College and Hospital, S. No. 44/1, Vadgaon Budruk, Off. Sinhgad Road, Pune, India – 411 041*
Email: himalipachpande7@gmail.com

Abstract

Background: Head and neck cancer (HNC) is one of the most prevalent cancers of the upper aerodigestive tract, with squamous cell carcinomas accounting for most cases. Tobacco and alcohol are the foremost etiological factors; however, bioactive food components, including those that modulate DNA methylation, are being linked to susceptibility. Vitamin B_2 (Riboflavin) has been associated with carcinogenesis. Riboflavin is essential for one-carbon metabolism, which involves the transfer of one carbon unit for Deoxyribonucleic Acid (DNA) and Ribonucleic Acid (RNA), amino acid metabolism and methylation.
Aim & Objectives: To evaluate the association of Riboflavin (Vitamin B_2) mediated one-carbon metabolism with Oral Squamous Cell Carcinoma (OSCC)
Materials and Methods: This study involved 10 newly diagnosed cases of oral squamous cell carcinoma and 10 age & gender-matched healthy controls. The cases were made up of 05 males and 05 females same as in controls. Serum obtained from participants' blood was analysed by high-performance liquid chromatography technique for evaluation of Vitamin B_2 levels.
Results: The mean ages of cases and controls were 60.1 and 58.3 years, and the median ages of cases and controls were 62.5 and 59.5 years, respectively. Serum Riboflavin (Vitamin B_2) levels were statistically low in OSCC when compared to controls ($P<0.05$).
Conclusion: Serum Riboflavin levels can differ due to tumour growth and subsequent metabolic changes, or they may precede and accelerate tumour progression.

Keywords: ***Riboflavin, Vitamin B_2, One carbon metabolism, Oral squamous cell carcinoma, Oral cancer.***

INTRODUCTION :

Head and neck cancer, which includes the oral cavity is the 7th most common cancer globally, accounting for more than 660,000 new cases and 325,000 deaths each year [1]. Oral squamous cell carcinoma (OSCC) represents 95% of all forms of head and neck cancer derived from the stratified squamous epithelium of the oralmucosa [2]. Oral cancer risk factors are widely known and include tobacco (chewing/smoking), alcohol, betel quid [3], genetic factors, socioeconomic status, poor oral hygiene, human papillomavirus (HPV), and diet [4]. The incidence of OSCC increases with age, with most of the OSCC cases occurring in patients >40 years. The incidence is higher in men than in women (5.8 and 2.3 per 100,000 individualsrespectively); however, there seems to be an increasing trend in women mainly due to their relatively past exposure to potential risk factors like tobacco or alcohol [5].

Many studies seem to indicate that different food compounds could alter ormodify cancer cells. Certain nutrients and micronutrients in food components can act as protective elements. This protective effect can be procured from fruits, vegetables, and certain vitamins as well as in other foods and products common to our diet. Bioactive components present in the diet appear to have the potentialto prevent various types of cancer, including oral cancer [6]. Vitamins found in numerous dietary products have antioxidant and antiproliferative properties, including immune system enhancement with synthesis and DNA methylation [7].

Riboflavin (7,8-dimethyl-10-ribityl-isoalloxazine) is a water-soluble vitamin present in an ample variety of meats and fortified foods. Detailed biochemical analyses have shown that riboflavin or FAD plays a central role in regulating the activity of phagocytic NADPH oxidase that generates superoxide anions in response to infection [8].

It is an essential element in DNA methylation and has been linked to different tumours such as breast, ovary, cervix, lung, and colon cancer. Poor riboflavin status has also been embroiled as a risk factor for cervical dysplasia, which is a precursor condition for invasive cervical cancer [9]. This study attempts to study the association of Vitamin B_2 in OSCC.

MATERIALS AND METHODS:

This was a cross-sectional study between 2020 and 2022. It involved 10 recently diagnosed Oral squamous cell carcinoma cases and 10 healthy counterparts. The age of the participants involved in the study ranged from 45 to 75 years. The study was explained and informed consent was obtained from allthe individuals before collecting the blood sample from them. Five millilitres of blood were taken from the antecubital fossa under aseptic conditions for cases of OSCC and allowed to clot and retract. The blood sample was centrifuged at 3000 rpm for 10 minutes and Sera were obtained from all the samples. The sera were stored at -20 degrees until the time of analysis.

Analysis was accomplished by high-performance liquid chromatography (HPLC) technique. The column effluents were monitored at 290nm for Vitamin B_2. The study protocol was ratified by the Institutional ethics committee review board. (Letter No.:-SDCH/IEC/OUT/2022/39)

RESULTS:

The mean ± SD of the age of cases in Group 1(OSCC) and Group 2(Controls) was 58.30± 10.42 years and 60.10± 7.86 years, respectively (Table 1). The minimum–maximum age range in Group 1 and Group 2 was 43-69 years and 47-68 years, respectively.

Table 1. Mean Age of participant					
	N	**Minimum**	**Maximum**	**Mean**	**Standard Deviation**
Control	10	43.00	71.00	58.30	10.42
OSCC	10	47.00	68.00	60.10	7.86

The Inter-group comparison of the type of diet among both groups wasstudied. Of 10 OSCC cases in Group 1, 03 (30.0% had a vegetarian diet and 08 (80.0%)had a non-vegetarian diet. Of 10 Controls in Group 2, 05 (50.0%) had a vegetariandiet and 05 (50.0%) had a non-vegetarian diet. The distribution of type of diet didnot differ significantly between the two study groups (*P* value >0.05) (Table 2).

Table 2. Distribution of Participants based on Diet.					
	Control		**OSCC**		**P-value**
	N	**Percent**	**N**	**Percent**	
Vegetarian	03	50.0	05	50.0	0.35
Non-Vegetarian	08	80.0	05	50.0	
	10	100	10	100	

Inter-group comparison of tobacco use was performed between OSCC cases andControls. Of 10 controls, none had any habits. Out of 10 OSCC cases, 05 (50.0%) had smokeless tobacco, 04(40.0%) had smoking tobacco, and 01(10.0%) used Mishri. The distribution of tobacco use among the cases andcontrols studied did not differ significantly between the two study groups (*P* value<0.05) (Table 3).

Table 3. Distribution of Participants based on Tobacco Habit for OSCC group (Group 1)				
		N	**Percent**	**P-Value**
Tobacco Habit	Smoking	04	40.0	0.33
	Mishri	01	10.0	
	Smokeless	05	50.0	
	Total	10	100.0	

Inter-group comparison of average serum vitamin B_2 levels was analyzed. The Mean and Standard deviation of Serum Vitamin B_2 levels were lower in the OSCC cases as compared to controls (Table 4 and Figure 1).

Table 4. Mean B2 levels in OSCC and Control group				
		N	**Mean**	**Standard Deviation**
Vitamin B2	**OSCC**	10	3.22	2.16
	Conrol	10	7.97	4.63

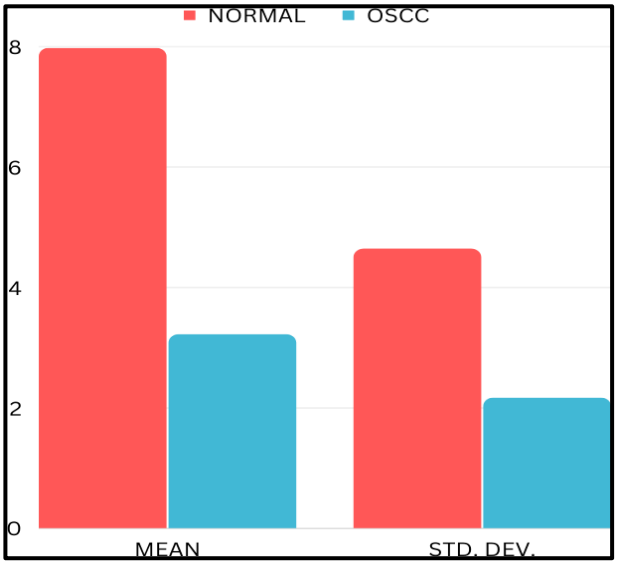

Figure 1: The inter-group distribution between the Mean and Standard Deviation of Vitamin B_2 levels in Oral Squamous Cell Carcinoma Patients and Healthy Individuals.

Serum levels of Vitamin B_2 were lower in OSCC cases and statistically significant between the study group and controls (*P* value= 0.009) (Table 5 and Figure 2).

Table 5. Comparison of Vitamin B2 between OSCC and Control Group

	t	p-value	Mean Difference	95% Confidence interval of the Difference	
				Lower	Upper
Vitamin B2	-2.93	**0.009***	-4.74	-8.15	-1.34

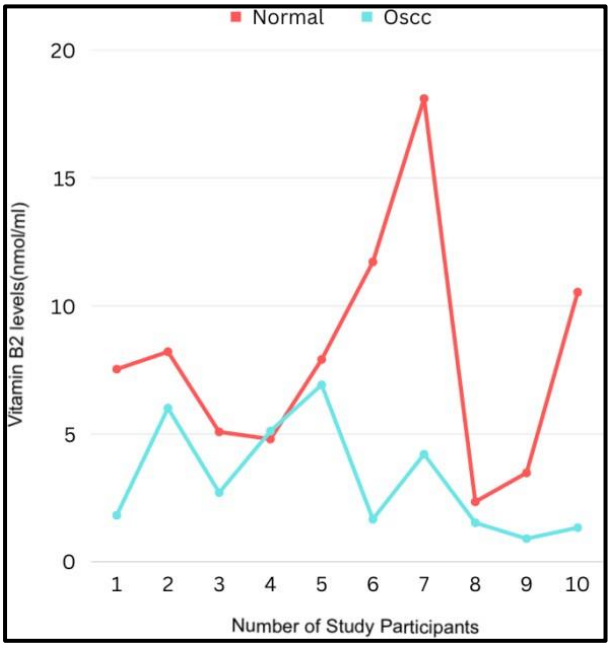

Figure 2: The inter-group distribution between Serum Vitamin B_2 levels.

DISCUSSION:

Oral squamous cell carcinoma (OSCC) is the most common head and neck squamous cell carcinoma, accounting for 90% of all oral cancers [10], with an estimated 5-year overall survival rate of only approximately 50% [11]. The consequential associated risk factors of OSCC are tobacco, alcohol, viral infectionssuch as EBV, HPV, and herpes simplex virus, poor oral hygiene (including sharp teeth and decay), ill-fitting denture, ultraviolet (UV) exposure, nutrition, and genetic predisposition. The etiology of oral cancer varies in different populationsdue to area-specific etiological factors [12]. A delay in OSCC diagnosis is often associated with increased disfigurement and poorer survival rates [13].

In oral squamous cell carcinogenesis, multiple genetic events leading to the alteration of normal functions of both oncogenes and tumour suppressor genes. The histologic progression of oral carcinogenesis from hyperplasia to dysplasia, followed by severe dysplasia and eventual invasion and metastases, is believed to reflect the accumulation of these changes [14].

The most important dietary sources of vitamin B_2 are milk and dairy products [15]. Vitamin B_2 serves as a co-factor in fat, amino acid, carbohydrate, and vitamin metabolism [16]. It is a major co-factor for the one-carbon metabolism pathway. One-carbon metabolism has a vital role in nucleic acid synthesis pathways, making it a high-octane therapeutic target for the treatment of cancer. Disturbances in one-carbon metabolism can lead to decreased DNA synthesis, genomic instability, and decreased methyl donor production.

Decreased levels of riboflavin in the blood can be due to various internal and external factors with genetic polymorphism being the formal factor and diet beingthe latter one. Alterations in riboflavin metabolism have been documented in various types of cancer, which can affect cellular processes and contribute to cancer progression. This raises the possibility of utilizing Riboflavin as an essential etiopathological marker for cancer.

In a study by Zhao et al (2016), Decreased levels of Riboflavin were associated with poor prognosis, and alterations in Riboflavin metabolism were linked to increased cellular proliferation and survival, which was observed in breast cancer patients [17]. Similarly in oral cancer, decreased expression of riboflavin transporter proteins has been associated with poor prognosis [18]. Another study published in the American Journal of Clinical Nutrition in 2011 found that high riboflavin intake was associated with a reduced risk of oesophagealal squamous cell carcinoma in Chinese adults [19]. In a study conducted by Yoon et al (2016), Vitamin B_2 is inversely correlated with colorectal cancer risk [20]. Literature also supports altered Riboflavin metabolism in gastric cancers [21] and lung cancers [22].

In our pilot study distribution of mean serum, and riboflavin levels were significantly different between the group of cases and the group of controls studied [Table 4]; [Figure 1]. The negative t-value and low p-value (0.009) suggest that there is a significant difference in the mean Vitamin B2 levels between the two groups [Table 5]. The results of our study are consistent with the studies reported in the literature.

CONCLUSION:

This study investigated Serum Riboflavin (Vitamin B_2) levels in OSCC patients and healthy individuals using the chemiluminescence immunoassay method. Decreased serum Riboflavin levels were observed in OSCC compared to healthy individuals. Based on our results we also suspect that Riboflavin deficiency could be one of the factors responsible for the etiopathogenesis of Oral Squamous Cell Carcinoma. Further research with a larger sample size and prospective studies should be carried done to validate the findings.

ACKNOWLEDGEMENTS: None.

CONFLICT OF INTEREST: None.

FINANCIAL SUPPORT: None.

ETHICS STATEMENT: The study protocol was ratified by the Institutional ethics committee review board. (LetterNo.:-SDCH/IEC/OUT/2022/39).

REFERENCES LIST:

1. Gormley M, Gray E, Richards C, Gormley A, Richmond RC, Vincent EE, et al. An update on oral cavity cancer: epidemiological trends, prevention strategies and novel approaches in diagnosis and prognosis. Community Dental Health. 2022, 39, 197–205.
2. Tumuluri V, Thomas GA, Fraser IS. Analysis of the Ki-67 antigen at the invasive tumour front of human oral squamous cell carcinoma. J Oral PatholMed. 2002, 31, 598–604.
3. Wilkey JF, Buchberger G, Saucier K, et al. Cyclin D1 overexpression increases susceptibility to 4-nitroquinoline-1 -oxide-induced dysplasia and neoplasia in murine squamous oral epithelium. Mol Carcinog. 2009, 48, 853–861.
4. Conway D.I., Purkayastha M., Chestnutt I.G. The Changing Epidemiology of Oral Cancer: Definitions, Trends, and Risk Factors. Br. Dent. J. 2018, 225, 867–73.
5. Sarode G., Maniyar N., Sarode S.C., Jafer M., Patil S., Awan K.H. Epidemiologic Aspects of Oral.2020, 66, 100988.
6. De Kok T.M., van Breda S.G., Manson M.M. Mechanisms of Combined Action of Different Chemo preventive Dietary Compounds: A Review. Eur. J. Nutr. 2008, 47, 51–59.
7. Grosso G. et al. Effects of Vitamin C on Health: A Review of Evidence. Front. Biosci. 2013, 18, 1017–1029.
8. Hashida S., Yuzawa S., Suzuki N.N., Fujioka Y., Takikawa T., Sumimoto H., Inagaki F., Fujii H. Binding of FAD to cytochrome b558 is facilitated during activation of the phagocyte NADPH oxidase, leading to superoxide production. J. Biol. Chem. 2004, 279, 26378–26386.
9. Lui T, Soong SJ, Wilson NP, et al. A case-control study of nutritional factors and cervical dysplasia. Cancer Epidemiol Biomarkers Prev.1993, 2, 525–30
10. Montero, P.H.; Patel, S.G. Cancer of the Oral Cavity. Surg. Oncol. Clin. N. Am. 2015, 24, 491–508
11. Ren, Z.H.; Hu, C.Y.; He, H.R.; Li, Y.J.; Lyu, J. Global and regional burdens of oral cancer from 1990 to 2017: Results from the global burden of disease study. Cancer Commun. 2020, 40, 81–92.
12. Meydi R., Shaikh F., Biag S., Qureshi M., Lakhani M., Zia I. et al. Oral squamous cell carcinoma and tobacco use. Jammer. 2018, 28, 1-5.
13. Imbesi Bellantoni M, Picciolo G, Pirrotta I, Irrera N, Vaccaro M, Vaccaro F, et al. Oral Cavity Squamous Cell Carcinoma: An Update of the Pharmacological Treatment. Biomedicines. 2023, 11, 1112.
14. Williams HK. Molecular pathogenesis of oral squamous carcinoma. J Clin and Mol Pathol. 2000, 53, 165-172.
15. Powers HJ. Riboflavin (Vitamin B2) and Health. Am J Clin Nutr. 2003, 77, 1352-60.
16. Rivlin RS. Riboflavin metabolism. N Engl J Med.1970, 283, 463–72.
17. Zhao et al. Riboflavin transporter expression is associated withprognosis in breast cancer. BMC Cancer. 2021, 2, 49.
18. Shukla et al. Altered expression of riboflavin transporter 2 in oralcancer progression. Cancer Invest. 2016, 34, 235-45.
19. Wu QJ et al. Dietary riboflavin intake and the risk of esophageal squamous cell carcinoma in Chinese population: a case-control study. Am J Clin Nutr. 2011, 94, 568-75.
20. Yoon YS, Jung S, Zhang X, Ogino S, Giovannucci EL, Cho E. Vitamin B2 intake and colorectal cancer risk; results from the Nurses' Health Study and the Health Professionals Follow-Up Study

cohort. Int J Cancer. 2016, 139, 996-1008.
21. Zhang et al. High expression of riboflavin transporter 3 (SLC52A3) is associated with a better prognosis in gastric cancer. Cancer Biomark. 2018, 22, 121-128.
22. Liu et al. Inhibition of riboflavin metabolism enhances doxorubicin efficacy in lung cancer cells. Cancer Chemother Pharmacol. 2020, 85, 465-47.