EVALUATION OF SERUM VITAMIN D LEVELS IN PATIENTS WITH ORAL LEUKOPLAKIA, ORAL SUBMUCOUS FIBROSIS AND ORAL SQUAMOUS CELL CARCINOMA

Dissertation submitted to
Maharashtra University of Health Sciences, Nashik
in the partial fulfillment of regulations
for the award of the degree of

MDS

IN

DEPARTMENT OF ORAL MEDICINE & RADIOLOGY

BRANCH IX

2022

CONTENTS

LIST OF TABLES

LIST OF COLOUR PLATES

ABBREVIATIONS

Serial No.	Abbreviations	Full form
1	OSCC	Oral Squamous Cell Carcinoma
2	OL	Oral Leukoplakia
3	OSMF	Oral Submucous Fibrosis
4	VDR	Vitamin D Receptor
5	25(OH)D	25-hydroxycholecalciferol
6	$1,25(OH)_2D$	1,25-dihydroxycholecalciferol
7	PTH	Parathyroid Hormone
8	ELISA	Enzyme Linked Immunosorbent Assay
9	RIA	Radioimmunoassay
10	LCTMS	Liquid Chromatography Tandem Mass Spectrometry
11	RT-PCR	Real Time Polymerase Chain Reaction
12	SNP	Single Nucleotide Polymorphism
13	HNC	Head and Neck Cancer
14	HPV	Human Papilloma Virus
15	NF-κB	Nuclear Factor-kappa B
16	APM	Antigen Presenting Machinery
17	SIN	Squamous Intraepithelial Neoplasia
18	PBMC	Peripheral Blood Mononuclear Cells
19	HPLC	High-Performance Liquid Chromatography
20	ECL	Electrochemiluminescence Assay
21	CMIA	Chemiluminescent immunoassay
22	NHANES	National Health and Nutrition Examination Survey

Serial No.	Abbreviations	Full form
23	LC-MS/MS	Liquid-Chromatography-Mass Spectrometry/Mass Spectrometry
24	UHPLC – MS/MS	Ultra-High Performance Liquid Chromatography – Tandem Mass Spectrometry
25	ID HPLC-APCI MS/MS	Isotope-Dilution High Performance Liquid Chromatography/ Atmospheric Pressure Chemical Ionization Tandem Mass Spectrometry
26	HDL	High Density Lipoprotein
27	BMI	Body Mass Index
28	H_2SO_4	Sulfuric acid
29	COX-2	Cyclooxygenase 2

INTRODUCTION

'Cancer' is obtained from the Greek word 'Karkinos' meaning crab, stating its extensively spreading nature like the claws of a crab into the neighbouring tissues[(1)]. 'Oral cancer' encompasses all the malignancies that arise in the oral cavity which have high prevalence of mortality and morbidity[(2)]. It is the 'sixth' most prevalent cancer globally as well as among the top 'three' types of cancers in India[(3,4)]. Its prevalence is higher in males and in old age[(5)].

Predominant potentially malignant lesions seen in India are oral leukoplakia (OL) and oral submucous fibrosis (OSMF) having malignant transformation rate of 0.13% to 10% and 1.2% to 23% respectively [(6,7)]. The etiology for oral cancer and potentially malignant lesions is very complex and multifactorial. It develops in patients with genetic predisposition, immunosuppression, chronically exposed to carcinogens, radiation and nutritional deficiency etc. Large proportion of young adults in central India are involved in addictive habits like tobacco, arecanut[(8,9)].

Vitamin D has long been known in controlling homeostasis of calcium in the body and in maintaining healthy bones, along with its function on extraskeletal tissues[10]. But over the past few decades, it has gained significant attention due to its negative correlation with various bone disorders, cardiovascular disorders, diabetes mellitus, autoimmune and neurodegenerative diseases, and even mortality[10]. It is said to be responsible for the overall health of the oral cavity. In 2011, Orell - Kotikangas showed vitamin D deficiency in colon and colorectal cancer, ovarian cancer, breast cancer while Grimm 2015 reported similar result in oral cancer patients[11,12]. Studies have shown that tobacco alters vitamin D metabolism and function while arecanut increases the expression of 25-hydroxylase enzyme which leads to decreased serum vitamin D[13,14]. It is reported to have antineoplastic activity by affecting multiple hallmarks of cancer such as induction of apoptosis, inhibition of angiogenesis, proliferation, invasiveness and metastasis in various types of cancers[12,15]. It also has anti-inflammatory, anti-fibrosis and anti-proliferative properties[16,17]. It acts as a paracrine and autocrine agent and functions via vitamin D receptor (VDR) systemically which is found in various cells and tissues, thus helps in the regulation of cell differentiation and maturation [18].

India is a tropical country which receives abundant sunlight but still more than 70% population suffers with hypovitaminosis D[14]. Large percentage of people in India have low socioeconomic status, are deprived of various basic privileges of life making vitamin D dietary sources unaffordable to them, thus poor nutrition and vitamin D deficiency. Altered lifestyle, use of addictive agents like tobacco, arecanut and indoor working is also responsible to worsen this condition.

Vitamin D is the most underevaluated nutrient in the world. Its importance and possible role in pathophysiological processes of many oral diseases is well established. The development of therapeutic modalities to prevent or treat OSCC can have a substantial influence on patient's survival and quality of life. For this, the potential action of vitamin D in oral cancer is recently been highlighted. But, there has been limited research done to ascertain the effect of vitamin D in potentially malignant conditions like OL, OSMF and oral cancer.

Thus, the present study was planned to compare vitamin D levels in OL, OSMF, OSCC patients as well as individuals with addictive habits.

AIM AND OBJECTIVES

AIM

To evaluate levels of vitamin D in OL, OSMF and OSCC patients.

PRIMARY OBJECTIVES

1] To find vitamin D levels in OL, OSMF and OSCC patients.

2] To compare vitamin D levels in OL, OSMF and OSCC patients.

SECONDARY OBJECTIVES

1] To find vitamin D levels in individuals with habit but no lesions.

2] To compare vitamin D levels in individuals with habit but no lesions with OL, OSMF and OSCC patients.

REVIEW OF LIERATURE

Vitamin D

Vitamin D is also called as 'Sunshine vitamin' or 'Anti-ricketic factor'[19]. This vitamin is fat soluble, also functions as a steroid hormone after its absorption by the body[20].

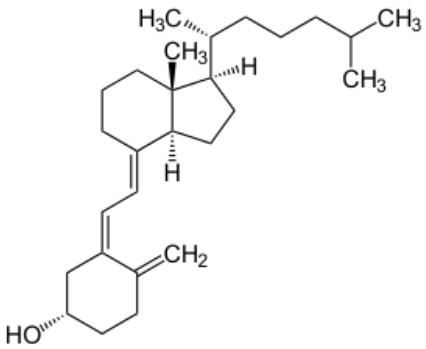


Fig 1 - Chemical structure of Vitamin D (cholecalciferol)[21]

Vitamin D exists in two forms namely Vitamin D_2 (ergocalciferol) and Vitamin D_3 (cholecalciferol) where the former is acquired mainly from the plant sources while the latter is endogenously produced from sterols (7-dehydrocholesterl) in the body due to the photolytic activity of ultraviolet rays on the skin (Fig 1)[10,22].

$$\text{Total Vitamin D} = \text{Vitamin } D_2 + \text{Vitamin } D_3$$

Vitamin D_3 undergoes a variety of sequential hydroxylation steps to form calcitriol (1,25-dihydroxycholecalciferol) which is biologically active form of vitamin D[12,20,22]. Calcitriol binds to vitamin D binding protein and is carried throughout the body along with its main target organs like kidney, intestine and bone while moreover it carries out most of the physiological activities of vitamin D (Fig 2)[17].

Vitamin D actions are imparted by VDR which attaches to vitamin D metabolites like calcitriol[16]. VDR is a protein coding gene formed by VDR gene which is situated on chromosome 12[23]. It contains various polymorphisms and is presented in many normal as well as malignant tissues[24]. In normal cells, it regulates gene transcription that are involved in intestinal and renal transport of calcium and other minerals[23].

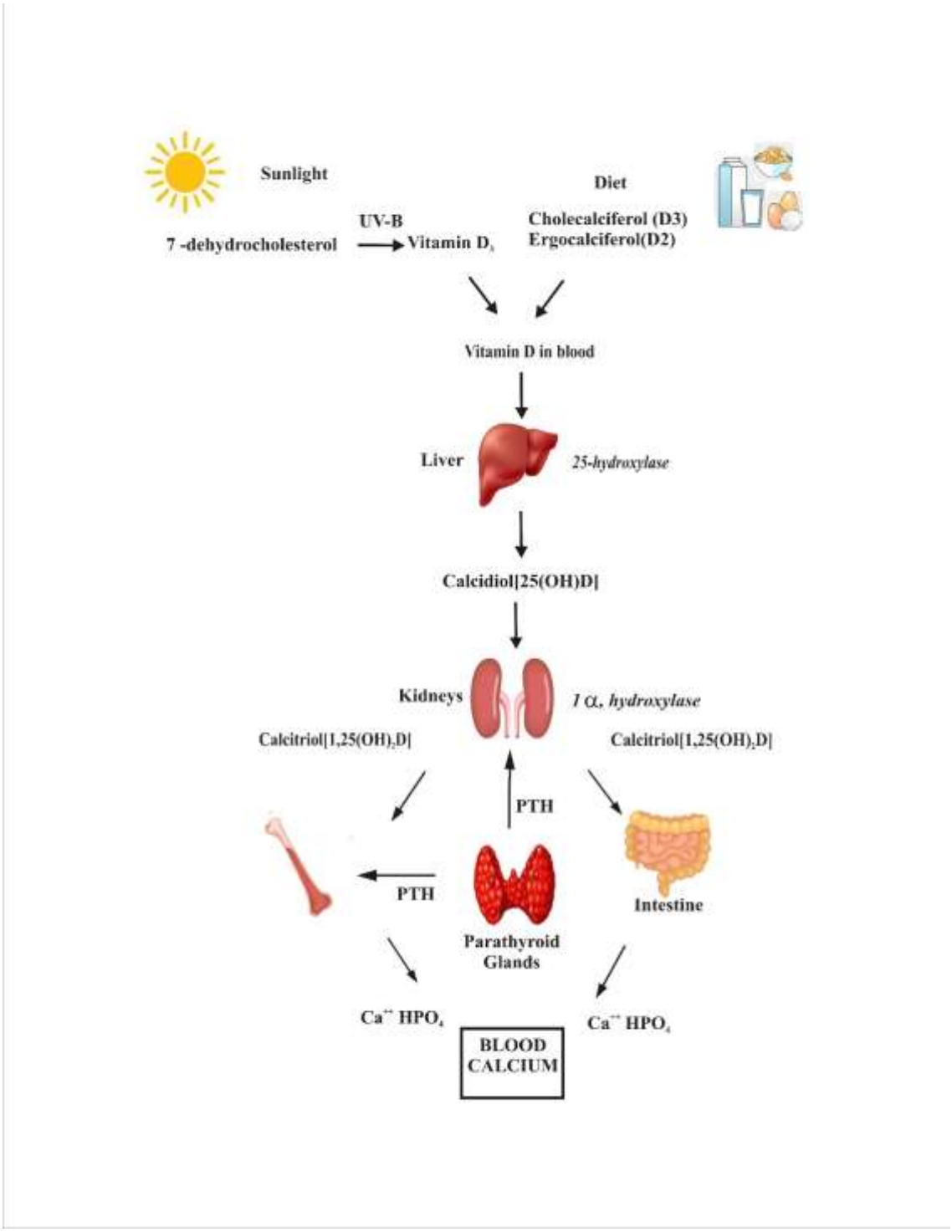


Fig 2 – Vitamin D pathway in the human body

Vitamin D is very crucial for growth of the bones and its metabolism. VDR and 1-α hydroxylase (enzyme that cause activation of vitamin D) have been found in large number of cell types ranging from skeletal muscles, macrophages and immunological cells[25]. Vitamin D serves various functions such as stimulation of innate immunity, regulation of renin-angiotensin system suggesting its variety of roles in whole body functions[18].

In India, currently 490 million individuals are estimated to suffer from deficiency of vitamin D and it is rapidly becoming a major global health concern in both the developed as well as developing countries[26]. Deficient vitamin D in the body causes 'calcemic' disorders like 'Rickets' in children and 'Osteomalacia' as well as 'Osteoporosis' in adults[27]. It has higher risk of developing the 'non-calcemic' disorders like malignancies involving the various parts of the body including the oral cancer, hypertension, upper respiratory tract infections, otitis media, loss of cognitive function, dementia, atopic dermatitis and several autoimmune diseases like type 1 diabetes, multiple sclerosis, rheumatoid arthritis and systemic lupus erythematosus[27,28].

Vitamin D measurement

In the present study, serum level of 25(OH)D was estimated to determine vitamin D status using ELISA test.

It is a challenging task to find out the accurate vitamin D status in the body as it is acquired both from sun exposure as well as from ingested food[10]. Its metabolites circulate in the bloodstream by binding to specific proteins and approach various

target organs, disassociate to gain entry into the cells and carry out the required functions[17].

In liver, ergocalciferol and cholecalciferol is converted to 25(OH)D. It is the principal form of vitamin D that circulates in the body and its detection is advisable than other forms[29,30]. 1-α hydroxylase, serum calcium and parathyroid hormone (PTH) helps in effective regulation of vitamin D level. In the kidneys, only a small quantity of 25(OH)D is transformed to calcitriol. Thus, measuring blood levels of calcitriol does not represent the actual vitamin D levels in the body[10,30]. Importantly, when there is deficiency of vitamin D, it leads to decreased absorption of calcium in the intestine causing lowered ionized calcium. Calcium sensors in the parathyroid glands gets signalled for increasing the production and secretion of PTH which helps to regulate the calcium metabolism by causing its increased reabsorption in the kidneys, increases calcium mobilization from the body and increased renal production of calcitriol. Thus, rise in the PTH levels due to deficient vitamin D causes normal or elevated calcitriol levels[31]. Also, it possess a shorter half-life of 4-15 hours as compared to 2- 3 weeks of 25(OH)D and its levels in serum are 1000 times less than 25(OH)D[10,32,33].

Concentrations of different vitamin D metabolites increases proportionately with gain in the uptake of parent vitamin D, thus it is mandatory to estimate the total vitamin D levels in the body[29]. Levels of total serum vitamin D (25(OH)D) is both Vitamin D_2 and Vitamin D_3 [vitamin D acquired through diet and endogenously produced vitamin D] which in turn is the main storage form in the muscles and adipose tissues and is considered as the best indicator and a widely accepted biomarker of true vitamin D status in the body[10,18,30,34]. It is very stable under several

preanalytical laboratory conditions and can be estimated in plasma, whole blood or serum[29]. Also, it can be stored for long period of time and thus is used for diagnosing vitamin D deficiency.

Vitamin D levels are dependent on the method of its assessment. Various terms have been used to define its deficient state as insufficiency, deficiency, severe deficiency etc[27,30,31]. Serum levels of 30–100 ng/ml vitamin D is considered to be the most desired and safe values that are sufficient to carry out the important functions of vitamin D and is sufficiently low enough to cause vitamin D toxicity[27].

Various tests are available to measure 25(OH)D in clinical laboratories that includes immunoassays such as enzyme linked immunosorbent assay (ELISA), radioimmunoassay (RIA), chemiluminescence immunoassay (CMIA), liquid chromatography tandem mass spectrometry (LCTMS), mass spectrometry, competitive protein binding assays[27,29,33,35]. Test like LCTMS that measures vitamin D levels is expensive, tedious, time-consuming procedure and therefore rarely used commercially[27].

ELISA is a basic, popular method of immunoassay to measure serum vitamin D concentration[35]. One of the types of ELISA test is sequential competitive binding. It is a quantitative assay which uses monoclonal antibodies that bind specifically to human vitamin D[33]. The process occurs between the sample antigen and antibody bound to the wells of microplate. The reagents of the ELISA test are immobilized to the microplate surface that causes easy separation of bound material from unbound material, thus washing away any non-specifically bound materials during the assay[35]. This binding and immobilization of reagents makes ELISA easy to design and

perform. It is a versatile test providing high thorough output using small serum sample volumes, is easy to use and has low cost[33].

Studies have shown that ELISA method is reliable to evaluate vitamin D levels in various conditions[36-43].

Vitamin D role in various malignancies of the body

Available literature shows deficient vitamin D status contributes in malignancies affecting various parts of the body. It includes cancer of pancreas, prostate, ovary, breast, lung, colorectal, hepatocellular carcinoma, melanoma, chronic lymphocytic leukemia[44-52]. Authors have discussed the possible mechanisms of anticancer effects by vitamin D[13,15,16,24,25,53-57].

But few studies have shown no correlation between vitamin D and antineoplastic activity[39,58,59].

As oral addiction habits are practiced immensely in India, it causes several potentially malignant oral lesions and conditions. The main component in tobacco products is nicotine which has a major metabolite called cotinine[60]. Cotinine helps to distinguish the tobacco users from nonusers and their extent of exposure. Its levels are increased in saliva and urine as it is rapidly excreted from the body[61]. It has been shown that the chemicals in the tobacco smoke can influence vitamin D metabolism and function and conversely carcinogenicity of tobacco smoke chemicals may be modified by vitamin D[53]. So, increased research is carrying out to assess vitamin D levels in these patients.

Vitamin D in Oral cancer

Lipworth et al (2009)[62] assessed the impact of dietary vitamin D on oesophageal, oral as well as pharyngeal cancer patients caused by smoking and alcohol consumption. Information was obtained from cases and controls on sociodemographic characteristics, addictive habits by standard structured questionnaire and about diet by food frequency questionnaire. Cases had increased frequency of smoking and alcohol consumption. There was significant association between decreased oesophageal, oral and pharyngeal cancer caused by addictive habits after intake of vitamin D. Heavy current smokers with poor intake of vitamin D had a greater chance of developing oesophageal and oral, pharyngeal cancer. Similar finding was observed in heavy alcohol consumers. Thus, they concluded that patients with habits of smoking and alcohol consumption along with poor intake of vitamin D had a greater chance of oral and pharyngeal cancers.

Bektas-Kayhan et al (2010)[23] investigated the function of TaqI polymorphism of VDR gene in patients with OSCC and compared it with healthy subjects. TaqI polymorphism was detected by polymerase chain based reaction polymorphism which showed a difference that was important statistically in the distribution of this TaqI genotypes of VDR in OSCC female population when compared to controls but no significant difference in male population or in total population was found. Significantly greater risk of OSCC was found in patients with VDR Tt genotype which was higher in female patients with OSCC. However, in the distribution of VDR TaqI polymorphism, no such difference was found according to differentiation, grade and nodal metastasis of OSCC. Of the demographic and addictive habits of the patient like the age, family history, smoking and alcohol

consumption status, TT genotype was associated with people with habit of alcohol consumption along with the greater risk of OSCC. Thus, this study confirmed the association between higher risk of OSCC with VDR Tt genotype.

Arem et al (2011)[63] explored the relation of vitamin D serum concentration with HNC risk including oral cavity, pharynx and larynx. Detailed history and smoking habit of patients was obtained through questionnaire where majority of the patients had laryngeal cancer followed by oral and pharyngeal cancer. Vitamin D concentrations were measured using chemiluminescence assay and compared with the controls which showed statistically nonsignificant connection of serum vitamin D concentrations with the chance of HNC while cases had statistically significant lower BMI, serum beta-carotene and alpha-tocopherol levels as well as high serum HDL, smoking and alcohol consumption than controls. Thus, no relation was discovered between concentrations of serum vitamin D and HNC risk.

Dev et al (2011)[64] aimed to find the correlation of deficiency of vitamin D, other symptoms and blood levels abnormalities in advanced cancer patients with intensified symptoms like poor appetite and fatigue. Levels of serum vitamin D was estimated by chemiluminescent immunoassay. Patients with gastrointestinal and lung cancer were the most common followed by few HNC patients. Majority of patients had insufficient vitamin D while some had its deficiency. Females were more likely to be vitamin D deficient than males which was significant statistically while vitamin D insufficiency was not significant with regards to gender. On correcting calcium levels, it was nonsignificantly linked to serum vitamin D, similarly vitamin D with other symptoms of advanced cancer was not significantly associated. Some patients had hypothyroidism while majority of male patients had hypogonadism. Patients not

receiving corticosteroids showed hypoadrenalism while vitamin D levels showed positive relation with serum testosterone levels in patients taking opioids. Thus, conclusion was made that advanced patients of cancer with cachexia or fatigue along with hypogonadic males had higher frequency of deficient vitamin D status.

Orell- Kotikangas et al (2012)[11] evaluated concentrations of serum vitamin D, calcium as well as phosphate status in Finnish HNC patients. Details about weight, height, BMI, smoking history were obtained from the patients. Oropharyngeal cancer was the most common cancer followed by oral cancer. Serum vitamin D concentrations were detected by liquid chromatography. 20% HNC patients had hypovitaminosis, 45% patients were vitamin D deficient while 35% of patients had sufficient vitamin D. 3 patients had optimal levels of vitamin D. However, smokers had markedly reduced vitamin D concentrations as compared to non-smokers. There was high percentage of hypovitaminosis D as well as deficiency of vitamin D in patients but no subnormal values. Thus, the study found high frequency of deficient vitamin D status in HNC patients.

Afzal et al (2013)[13] tested the hypothesis regarding lowered plasma vitamin D concentration and an increased probability of tobacco related malignancies than other malignancies such as cervical, oesophageal cancer and myleiod leukemia. 25(OH)D total assay was used to calculate plasma level of 25-hydroxyvitamin D. Patient's smoking habits were obtained which showed decreased concentration of vitamin D as they grew old, developed a smoking habit, increased current and cumulative tobacco use and reduced physical activity. Increased incidences of tobacco related malignancies were found with decreased concentrations of plasma vitamin D. Furthermore, there was greater probability of tobacco related malignancies with

higher cumulative tobacco consumption whereas smoking had no effect on vitamin D concentrations and probability of tobacco related malignancies. So, they proved that deficient vitamin D status increases the probability of tobacco related malignancies and not of other malignancies.

Grimm et al (2013)[65] analysed VDR expression in OSCC patients as well as in normal mucosal tissues of the oral cavity where staining and immunohistochemistry of their specimens was done. RT-PCR was done to deduce VDR gene expression and western blot to confirm its specificity. Expression of VDR was presented in half of the OSCC patients out of which some of them showed high expression. CD44++/VDR+ tumour cells were dominant in the basal epithelial layer of OSCC tissues by immunohistochemistry with single as well as double staining. There was a weak but statistically important inverse connection between proliferating tumour cells Ki-67 and VDR expression in OSCC patients. Also, patients with low VDR expression had statistically significant low survival rate in comparison to high VDR expression patients. Hence, crucial role of VDR expression in OSCC patients was discovered in the study.

Zhang et al (2015)[48] estimated serum vitamin D concentrations, calcium as well as PTH values in OSCC patients as well as healthy control individuals. ELISA was utilized to calculate serum vitamin D concentrations, automatic biochemical analyzer for calcium values along with automated chemiluminescence immunoassay for PTH values. OSCC patients showed significantly increased values of serum PTH than healthy controls while serum vitamin D concentrations and calcium in them showed no remarkable difference. These findings suggest no possible correlation of serum vitamin D concentrations and OSCC.

Deschasaux et al (2015)[53] searched for any relation among vitamin D concentration, SNPs of genes associated with its metabolism along with the risk of cancers related to tobacco use. Patients detailed personal, medical history and smoking habits were obtained by self-administered questionnaires. Electrochemiluminescent total 25(OH)D assay was utilized to detect vitamin D concentrations. SNPs selected were VDR, CYP24A1, GC, RXR, and CASR for each gene and alleleic discrimination was used to determine genetic polymorphisms. T allele i.e CT as well as TT genotypes of the RXR rs7861779 polymorphism was found in cases who were more obese, smoked and consumed less dietary vitamin D. Former or current smokers with increased vitamin D concentrations had a decreased chance of developing cancers related to tobacco use than never smokers while the VDR FokI with genotype AA and RXR rs7861779 polymorphism with genotype TT was correlated with greater chance of developing cancers related to tobacco use. SNPs and concentration of vitamin D or smoking status showed no interaction. Hence, an important relation was observed between decreased concentrations of vitamin D and greater chance of developing cancers related to tobacco use in smokers.

Mostafa et al (2016)[54] assessed serum vitamin D concentrations, calcium as well as phosphate concentrations in HNC patients before treatment with subgroups of smokers and non-smokers and compared it with controls. ELISA was used to evaluate levels of serum vitamin D and Synchron CX-9 autoanalyzer to measure calcium and phosphate levels. Cases had significantly lowered vitamin D concentrations as compared to controls along with less optimal vitamin D concentrations. Serum concentrations of calcium and phosphate showed no difference of statistical significance in cases and controls. Similarly, serum vitamin D concentrations, calcium

as well as phosphate showed statistically nonsignificant difference with regards to various tumour sites. So, the prospective study showed significant prevalence of deficiency of vitamin D in HNC patients.

Fanidi et al (2016)[66] studied the involvement of circulating levels of vitamin D for possibly developing HNC and oesophagus cancer as well as survival in them and controls. Active smokers had lowered values of vitamin D whereas former smokers had higher values. Patients with higher concentrations of vitamin D had decreased possibility of HNC while some of the HNC patients died during the follow-up. Higher pre-diagnostic circulating values of vitamin D was found to be related to increased survival post HNC diagnosis. While, risk or survival in oesophageal cancer patients did not show any clear association. The expected 5-year chance of survival was higher in cases with higher vitamin D concentrations than with lowered values in them, thus describing the effect of greater vitamin D values in lowering HNC risk as well as improving the chance of survival.

Adisa et al (2017)[67] compared the VDR expression in OSCC as well as skin SCC in black African sub-population. Immunohistochemistry was done of nineteen blocks of OSCC and fifteen skin SCC for VDR where among 15 cases of moderately differentiated OSCC and 10 well differentiated SCC of skin, 7 (46.7%) and 8 (80%) cases respectively were moderate to strong positive for VDR while 8 and 2 cases respectively were negative which showed that approximately the same number of OSCC and skin SCC cases expressed moderate to strong positivity for VDR.

Yokosawa et al (2018)[24] explored the relation of vitamin D consumption with survival rates in HNC patients. Vitamin D consumption data was obtained from

patients by pretreatment food frequency questionnaire with variants of dietary as well as supplementary intakes. Consumption of vitamin D and HNC recurrence showed an inverse ratio of statistical significance especially in stage 4 disease patients. Dietary as well as supplementary consumption showed no relation with regards to survival and recurrence. So, it was concluded that decreased vitamin D consumption leads to increased HNC recurrence risk. Thus, increased consumption of vitamin D through diet or supplementations would help in prevention of recurrence of cancer.

Bochen et al (2018)[3] studied the incidence of deficient vitamin D in HNC patients as well as compared its levels with patient's clinical and pathological data like BMI, calcium levels and overall survival. Immunomodulatory property of vitamin D was also looked for. The median serum vitamin D values were notably decreased in HNC patients as that of control individuals. BMI, calcium levels in cases did not show significant difference when compared to serum vitamin D values in them. Reduced serum vitamin D values were significantly related with positive lymph node status and overall shorter survival rate. Higher intratumoural and stromal infiltration of T cells, helper T cells etc was observed in patients with greater vitamin D values leading to improved prognosis in them. Overall, HNC patients showed greater frequency of occurrence of deficient vitamin D which can thus be a predictor of short survival rate.

Laczmanski et al (2019)[68] explored the effect of VDR polymorphisms as one of the risk factors in tobacco-related malignancies. Total 12 articles with 26 studies were included and it had five studies regarding FokI polymorphism, six studies regarding BsmI polymorphism and ApoI polymorphism respectively and nine studies regarding TaqI polymorphism. Occurrence of 't' allele in the TaqI polymorphism of VDR showed a significant relation with tobacco related malignancies which reduced

the probability of lung cancer and statistically nonsignificant effects on other cancers like the oesophagus, oral, HNC was found. ApaI, BsmI and FokI polymorphism with cancer risk was not significantly related. Thus, it was concluded that TaqI polymorphism of VDR increases the risk of tobacco-related malignancies.

Izreig et al (2020)[15] discussed the etiopathogenesis of HNC along with the effect of vitamin D on it. Tobacco smoke, alcohol, HPV and NF-κB transcriptional pathway activation are the various predisposing factors for developing OSCC. Immune system has the capacity to mount an adaptive response to tumour cells which is hampered due to disruption of tumour antigen presentation caused by mutation in the antigen presenting machinery (APM) while decreased T lymphocyte levels in circulation causes state of immunosuppression due to increase in the levels of circulating cytokines like transforming growth factor-beta and interleukin 10. Vitamin D induces antiproliferative effects, upregulates antioxidative pathway, thereby reducing oxidative damage to DNA. It helps in the regulation of immune system and limits inflammation, improves the sensitivity of tumour cells to radiotherapy. In vitro studies of vitamin D on HNC have shown that it promotes cellular differentiation, inhibits the invasive and metastatic activity of cancer cells. Also, genetic polymorphisms in the VDR genes involved in vitamin D metabolism pathway plays an important role. Degree of affected cells to become malignant is related to the degree of enhanced VDR expression. Thus, the review helps in recognizing the effect of vitamin D in HNC.

Udeabor et al (2020)[55] aimed to calculate the serum values of vitamin D in OSCC patients and control subjects. Most common cancer site was the tongue cancer (45%). 74.51% of cancer patients had moderate to severe vitamin D deficiencies

which was statistically significant. Only 20.35% of the controls had moderate vitamin D deficiency but none of them had severe deficiency, thus concluding that OSCC patients have decreased vitamin D values in them.

Pandey et al (2020)[69] determined the rate of deficiency of vitamin D in untreated cancer patients like the solid organ cancer of breast, gall bladder, HNC etc and haematological cancer with most stage IV cancer. Levels of vitamin D was estimated by chemiluminescent immunoassay (CMIA). Majority of patients had deficient vitamin D followed by insufficient and severe deficient values. Younger patients, female population and patients with solid organ cancer had higher rates of deficiency. Oesophageal and stomach cancer patients showed the highest vitamin D deficiency followed by breast, colorectal and ovarian cancer patients and stage III and IV solid cancer patients. More stage IV solid organ cancer patients showed severe vitamin D deficient status than stage III patients. Majority of the patients with deficient as well as insufficient values received vitamin D supplementations. Many of them received one-time intramuscular preparation and remaining patients received supplementations of vitamin D. Also, majority of patients showed compliance to therapy. Thus, greater rate of occurrence of deficient vitamin D was confirmed in cancer patients.

Sufiawati et al (2021)[56] evaluated the concentrations of serum vitamin D in oral malignancy patients along with its relationship with cancer stages. ELISA was used to estimate concentrations of vitamin D which showed its statistically significant reduced levels in oral malignancy patients than in healthy control group. Levels of serum vitamin D remained unchanged according to cancer stages which indicated the

prevalence of deficient vitamin D in oral malignancy patients but no relationship between the levels and stages of cancer.

Makitie et al (2021)[25] reviewed systematically to learn the impact of vitamin D in HNC. Out of 276 studies identified after thorough search, 13 studies were included in the study. 5 were prospective studies that estimated concentrations of vitamin D prior to diagnosis of malignancy along with its effect on chance of developing HNC. In 8 studies, vitamin D was estimated after the malignancy was diagnosed. In 2 studies, a negative correlation was observed among vitamin D concentrations with the chance of developing HNC whereas 2 studies did not report any connection. Several studies demonstrated deficiency of vitamin D in oral cancer patients than controls. In terms of mortality and post treatment survival, mortality in HNC was unrelated to concentrations of vitamin D in 2 studies while 2 studies showed longer post-treatment survival rate with increased concentrations of vitamin D before treatment. One study showed more cancer complications with reduced concentrations of vitamin D while one study showed no association between it and HNC recurrence. Thus, conclusion was made that deficient vitamin D status was more frequent in HNC patients along with its increased chance of occurrence and decreased overall survival.

Pu et al (2021)[57] conducted meta-analysis and reviewed systematically to find a relationship between vitamin D exposure as well as incidence and death in HNC patients based on diet intake, circulated levels and genomic phenotype. After thorough searching for the articles to meet the inclusion criteria, finally sixteen articles were included out of which three articles were based on vitamin D intake, nine studies on circulating blood levels of vitamin D and four studies on VDR gene

polymorphisms. Intake of vitamin D in all studies was assessed by food frequency questionnaires while vitamin D levels were detected by automated immunoassay, radioimmunoassay and chromatographic methods. Statistically significant connection was observed among polymorphism of VDR gene with reduced probability of HNC incidence as well as highly inverse relation of vitamin D exposures i.e vitamin D consumption and circulating vitamin D levels was found. Also, greater circulating vitamin D levels in cases resulted in significantly higher survival rates during a 4-5 follow up years. Thus, increase in the vitamin D gene polymorphism activity, its consumption and circulating levels can reduce the incidence and mortality in HNC patients.

Vitamin D in Potentially malignant disorders – OSMF and OL

Grimm et al (2015)[12] investigated expression of VDR and levels of vitamin D in healthy individuals, precancerous lesions (simple hyperplasia; squamous intraepithelial neoplasia, SIN I-III) as well as correlated it with OSCC patients. VDR expression was analysed by immunohistochemistry and levels of serum vitamin D was estimated by radioimmunoassay. VDR was detected in normal, precancerous and OSCC specimens but VDR expression in tumour cells was significantly increased than in normal tissues. However, VDR expression was highly decreased in them as compared to patients with SIN I-III. Moderate vitamin D deficiency was detected in SIN I-III patients. Oral cancer patients showed severe deficient vitamin D status but its levels and VDR expression in OSCC patients showed nonsignificant connection. Hence, the study concluded that deficiency of vitamin prevails in oral precancer and cancer patients.

Kumar et al (2017)[70] aimed to evaluate the serum malonialdehyde and calcitriol levels in OSMF patients and compare it with healthy control cases. Malonialdehyde and calcitriol levels were estimated by thiobartituric acid reactive species method and immunoassay method respectively which showed a marked increase in malonialdehyde level of statistical significance and mild decrease in calcitriol levels with statistically nonsignificant difference. So, no remarkable correlation among OSMF patients and vitamin D deficiency was established.

Anand et al (2017)[2] analysed range of vitamin D and VDR expression in healthy participants, leukoplakia as well as oral cancer patients. Among them, some patients with advanced oral malignancy who had chemoradiation were checked for supplementations of vitamin D. Serum vitamin D was estimated by CMIA method which showed lowered mean vitamin D levels in cases of statistical significance. Increased expression of VDR was observed in neoplastic oral tissue than normal mucosa of the oral cavity with nonsignificant difference. Cancer patients showed deficient vitamin D levels during pre-supplementation stage which substantially increased after its supplementation. Also, supplementation of vitamin D significantly improved the symptoms related to oral cancer like erythema, ulceration, pain and swallowing after 3 months. It leads to conclusion that patients with neoplastic oral lesions have deficient vitamin D, and providing supplementation of vitamin D to these individuals helps to improve the deficiency greatly.

Vitamin D in Oral addictive habit patients

Ogunkolade et al (2006)[71] hypothesized that addictive habits like betelnut chewing have a crucial role in hypovitaminosis D by modulating concentration of

vitamin D in a pilot research of healthy participants. Detailed information on diet, smoking and betel usage was obtained from patients, 24(OH)ase mRNA concentrations as well as PBMC 1 alpha (OH)ase was measured. 25(OH) D-24(OH)ase expression was directly related to betel usage. PBMC and 24(OH)ase mRNA had a positive association and serum vitamin D had an inverse association with betelnut chewing per day which concluded that betelnut chewing causes increased 25(OH)ase expression leading to reduced serum levels of vitamin D, thus supporting the hypothesis that habit like betel chewing aggravates the outcomes of vitamin D deficiency.

Grimnes et al (2010)[72] compared the differences of six distinct serum vitamin D assays performed in patients with smoking habit and non-smokers used in a validation study as well as Tromso (A city in northern Norway) study. The Tromso study was conducted six times where patient's serum from second visit was thawed and vitamin D was estimated by electrochemiluminescence immunoassay (ECL). Validation study was done as a further study to the sixth survey of the Tromso study. Vitamin D levels were measured by LC-MS/MS, RIA HPLC and ECL. Tromso study showed significant rise in concentrations of serum vitamin D in current smokers but slight rise in vitamin D in the former smokers than never smokers. Concentrations of serum vitamin D increased with described number and years of cigarettes smoked in current smokers while not in regards to former smokers. While, as the number of years since they stopped smoking increased, serum levels in them reduced slightly. Five methods showed statistically nonsignificantly lowered vitamin D levels in smokers than non-smokers in the validation study. ELC that measured serum vitamin D concentrations was significantly greater in smokers as compared to non-smokers

while it varied significantly with regards to effect of smoking from other methods. Thus, it was concluded that smoking has an impact on serum vitamin D levels using ELC as compared to other methods.

Cutillas - Marco et al (2012)[28] aimed to detect the values of vitamin D in healthy individuals as well as corelate it with their habits of smoking and frequency of sun exposure. Demographic data was obtained from the participants and values of serum vitamin D, phosphorus, calcium and PTH were calculated. Vitamin D and parathyroid values were calculated by chemiluminescence immunoassay which showed vitamin D deficiency in 4.5% of participants and the risk was highest in smokers. Youngest age group individuals and who had the habit of everyday sun exposure showed higher mean vitamin D values of statistical significant difference. Vitamin D values were not related with consumption of dietary as well as supplementary vitamin D while smoking and parathyroid values correlated negatively. No association was found between smokers and non-smokers with regards to serum calcium values. Hence, it was concluded that smoking is a major risk factor that causes deficiency of vitamin D.

Hysi et al (2013)[73] studied the action of smoking on serum concentrations of vitamin D, calcium as well as PTH in postmenopausal women who were former or current smokers and had osteoporosis. Concentrations were analysed by ECL and compared with non-smoker control individuals. Mean vitamin D concentrations and PTH were reduced in smokers that was of statistical significant difference than the former smokers and non-smokers while the concentrations of calcium in all the three groups showed statistically nonsignificant difference leading to a conclusion that smoking causes lowered vitamin D concentration.

Garg et al (2014)[74] reviewed systematically and corelated the published data on systemic effects of arecanut in 62 studies which covered the effect of arecanut on metabolism of food in the body. Consumption of arecanut was associated with effects on various systems of the body like the cardiovascular, gastrointestinal, endocrine, respiratory, on blood as well as its components. Vitamin D deficiency aggravated in arecanut users due to increased expression of 25(OH)ase which leads to lowered serum vitamin D levels. So, they concluded that chronic arecanut consumption causes euphoria, hypothyroidism, hyperlipidemia, cardiac arrhythmias, prostate hyperplasia, infertility, interfers with immune system, fat metabolism and vitamin D deficiency.

Manavi et al (2015)[61] evaluated the action of smoking measured by levels of cotinine on serum vitamin D values in people surveyed by NHANES in 6 years for two-year cycle between 2001 to 2006. Demographic data of each individual like the age, gender and ethnicity was recorded. The results showed reduced mean serum vitamin D values in black females with active smoking than the passive/light black female smokers which in turn had slightly lowered mean serum vitamin D values than black female non-smokers. However, there was inadequate vitamin D values in all the three groups with trending deficiency status in the active smokers. Similar findings were seen in the Hispanic and white females with lowest vitamin D values in active-smokers than the passive/light smokers and non-smokers. But the values were higher in white passive/light female smokers than the non-smokers. In all the three groups of Hispanic females, serum vitamin D values were trending to inadequate state rather than deficiency while in white females, mean values of serum vitamin D were of adequate level yet below it. Black females had the lowest mean serum vitamin D values while highest values were found in the white females with similar findings

reported in males. Black active female smokers had the most deficient serum vitamin D status followed by Hispanic females and white females with similar pattern observed in male active smokers. Also, black female active smokers had the most inadequate vitamin D followed by Hispanic females and white females with similar pattern observed in male active smokers. Another observation was that black female passive/light smokers had the greatest frequency of vitamin D inadequacy as compared to Hispanic and white females. Thus, a strong negative correlation was observed regarding smoking and serum vitamin D values.

Kassi et al (2015)[75] studied the rate of occurrence of vitamin D insufficiency (D2 as well as D3 independently) in healthy young/middle aged men and its relationship with bone markers like osteocalcin, parathyroid function and demographic details of patients like age, BMI, smoking, alcohol consumption. Vitamin D levels were detected using LC-MS/MS, calcium levels using LC while PTH levels using sandwich immunoassay. Nonsignificant correlation was seen among vitamin D with above mentioned parameters while half of the participants had deficient vitamin D, some had insufficient levels, thus smokers had statistically significantly lowered vitamin D levels than non-smokers. Also, slightly increased chance of vitamin D deficiency was observed for the 20-29 years age subgroup while it was high for the 40-50 years age subgroup which was statistically nonsignificant leading to a conclusion that a strong correlation exits among vitamin D and smoking in healthy young/ middle aged males.

Shinkov et al (2015)[76] investigated vitamin D concentrations in adult individuals in relation to factors that may affect its synthesis as well as metabolism like individual's demographic and personal details, where vitamin D concentrations

were estimated by LCTMS. Deficiency of vitamin D was more common in young males with higher education than with elementary and secondary education while no difference was reported in females irrespective of their education. Concentrations of vitamin D in either gender were unrelated according to their marital status. Deficiency of vitamin D occurred more frequently in male smokers with secondary and higher education than non-smokers while female smokers showed statistically nonsignificant difference as compared to non-smoking females. Obese females had markedly reduced concentrations of vitamin D than their healthy counterparts while the levels did not differ in males. Thus, it was concluded that the demographic and personal details of an individual like smoking and higher education in males and obesity in females acts as a significant predisposing factor for deficient vitamin D status.

Jiang et al (2016)[77] investigated the correlation among smoking and serum vitamin D concentrations. Personal history including habits of patients were recorded by a questionnaire. Serum vitamin D concentrations were assessed by ELISA and analysed by Uranus auto analyser which showed lower levels of BMI, less physical activity and reduced serum concentrations of vitamin D in current smokers in comparison to former or non-smokers. Concentrations of serum vitamin D decreased as there was increase in the number of cigarettes consumed each day. Increased serum vitamin D concentrations was seen in patients who had quit the habit long ago. Current smokers with greater intensity of exposure repeatedly showed statistically significant reduced vitamin D concentrations than smokers with lowest intensity of exposure. So, they concluded that smoking leads to lowered serum concentrations of vitamin D and was positively related with high intensity, frequency and duration of smoking.

Khalid et al (2018)[78] determined the frequency of deficiency of vitamin D occurring in young smokers and its relation with smoking, BMI, alcohol consumption as well as dietary consumption. Details of calcium consumption and vitamin D was collected through diet which showed that majority of smokers had insufficient vitamin D followed by deficiency state and some of them with severe deficiency of vitamin D. Also, deficient vitamin D was highly common in male population, obese smokers of age 20-31 years, with high HDL level while it was lowest in 37-48 years of age smokers leading to conclusion that there is increased frequency of deficient vitamin D in young smokers along with other risk factors like low physical activity and high HDL levels.

Kamboj et al (2018)[79] investigated the frequency of occurrence of hypovitaminosis D in India as it is widespread irrespective of gender, age, race and geographic location and is considered as the public health problem in India. Studies included in this literature review showed a widespread occurrence of deficient vitamin D in all age groups ranging from 44.3 - 66.7% among infants, 84.9 - 100% in school-aged children, 42 – 74% in pregnant women, 70 – 81.1% among lactating mothers and about 30 - 91.2% in adults with a high prevalence of > 65% in infants, pregnant and lactating mothers. It was present in all obese children of 6 -17 years of age, in 93% of children of 6 -18 years of age and in 93 - 85% of 10-18 years of age children belonging to lower and upper socioeconomic groups respectively. Randomized controlled trials included in the review showed cholecalciferol as the main supplement along with calcium supplementation given for a duration of three months to one year. An efficient and safest method of improving serum vitamin D concentrations in India is fortifying milk with it. Thus, the study revealed high

occurrence of deficiency of vitamin D in India with highest among infants, pregnant and lactating women and supplementary nutrition programmes launched by the government may help in improving the hypovitaminosis D problem.

Rahman et al (2019)[14] assessed the role of common addictive habits like smoking and betelnut chewing on hypovitaminosis D in young adults with these habits. Sociodemographic details were obtained by self-reported questionnaires which showed statistically significant association between hypovitaminosis D and smoking, betelnut chewers. Thus, the findings demonstrated that smoking, betelnut chewing causes increased risk of vitamin D deficiency.

Paladugu et al (2020)[80] tested occurrence of vitamin D (D_2 as well as D_3 independently) deficiency in young and middle aged healthy male smokers. Details about patient's weight, height, BMI, bone mineral densitometry, period of smoking were obtained which included some smokers with smoking duration of less than 3 years, some with duration greater than 7 years while majority of them with smoking duration of 3-6 years. Levels of serum calcium, serum vitamin D_2 and D_3 was analysed by LC-MS/MS assay and the sum of vitamin D_2 and D_3 was calculated as vitamin D. It was observed that majority of smokers had levels of vitamin D below 20 ng/ml followed by some smokers with more than 20 ng/ml levels while few with levels less than 10 ng/ml. Also, many smokers with smoking duration of 3-6 years and few smokers with smoking duration of greater than 7 years had vitamin D level below 20 ng/ml which showed notable correlation of vitamin D deficiency with smoking. For calcium levels, majority of smokers smoked for greater than 7 years while some of them smoked for 3-6 years which showed its nonsignificant correlation with levels of serum vitamin D. Also, the levels of serum vitamin D were unrelated with BMI.

Hence, study concluded that deficient state of vitamin D prevails in smokers in comparison to non-smokers which were unaffected by other lifestyle factors of the patients.

Meshram et al (2020)[9] assessed the result of smoking and chewing tobacco on serum calcium, vitamin D metabolism as well as on bone metabolism in healthy rural men which showed statistically nonsignificant difference in serum ionized calcium levels among tobacco and non-tobacco consumers while statistically marked difference was observed in alkaline phosphatase levels in both groups. Increased serum phosphate level was found among tobacco consumers. Tobacco chewing and vitamin D deficiency along with PTH levels differed significantly while a negative correlation was seen among tobacco consumption and serum osteocalcin. Hence, the findings concluded that smoking affects vitamin D, PTH and calcium metabolism.

Jawad et al (2020)[81] identified a relationship between cigarette smoking and serum vitamin D concentrations in elderly participants who's sociodemographic and cigarette smoking details were obtained by pretested questionnaire. Serum vitamin D concentration was measured by chemoimmunoassay method. Vitamin D concentration in smokers was lower than non-smokers. Majority of the smokers had vitamin D deficiency or insufficiency with highly significant inverse association among smoking duration and vitamin D concentrations while some elderly patients had hypovitaminosis D. The study thus successfully explained the role of cigarette smoking in decreased vitamin D concentrations.

Bakan et al (2020)[60] investigated the relation of urine cotinine levels with vitamin D in students of eastern Turkey university. Detailed history was obtained

from the students through a questionnaire and nicotine dependence was calculated by Fagerstrom test. Urine cotinine and serum vitamin D levels were analysed by Human ELISA urine kit and Human ELISA serum kit respectively. Average urine cotinine level 1.60 ± 0.32 ng/L with highest value of 2.34 ng/L and lowest value of 1.25 ng/L. Average level of serum vitamin D was 32.4 ± 15.3 ng/ml with highest value of 67.3 ng/ml and lowest value of 1.3 ng/ml which showed statistically nonsignificant difference among both these levels, thus not signifying any relation of urine cotinine levels with serum vitamin D.

Suchanecka et al (2020)[20] analysed the effect of three SNPs in VDR gene i.e rs2228570 (Fok I), rs7975232 (Apa I), and rs1544410 (BsmI) on oral health with regards to pH, periodontitis, gingival index (GI), dry mouth, inflamed oral mucosa, dry socket, OL in cigarette smokers and control subjects. Also, relation between these polymorphisms and smoking was assessed. Genotyping for VDR gene was performed with RT-PCR where a statistically important difference for VDR rs1544410 gene was found in smokers than in controls and statistically nonsignificant difference for VDR rs1544410 allele frequency between smokers and controls was found. Statistically nonsignificant difference was found for alleles frequency of VDR rs797532 and VDR rs2228570 and genes between smokers and controls. pH of the saliva in smokers was statistically significantly decreased in smokers with increased gingival index and high frequency of dry socket, dry mouth, inflammation of oral mucosa and periodontitis. However, statistically nonsignificant difference was found for OL in smokers and controls which concluded that genetic and lifestyle parameters greatly affect the health of the oral cavity.

Yang et al (2021)[82] carried out a meta-analysis to discover the impact of smoking on vitamin D values. 124 articles were finally included in the study after thorough search where vitamin D values were detected using ELC immunoassay, RIA, ELISA, LC-MS/MS and CMIA. Smokers were more likely to be vitamin D deficient than non-smokers. Further, taking supplements of vitamin D was unrelated with any changes (low levels) in vitamin D values in smokers than non-smokers. Vitamin D values in smokers in different age subgroup was found to be reduced than in control group which confirms the inverse relation of smoking behavior with vitamin D values.

Yuan et al (2021)[83] explored the impact of tobacco smoke exposure on concentrations of vitamin D in patients enrolled in NHANES over 14-years period. Vitamin D and serum levels of cotinine were calculated by UHPLC-MS/MS and ID HPLC-APCI MS/MS respectively. Active and passive smoking exposure had a positive correlation with vitamin D concentration which was more pronounced in females. Also, this positive association was seen for male adolescents. Adults showed consistent decrease in vitamin D concentrations with increased levels of cotinine and marked evidence in females. Also, there was an enhanced risk of vitamin D inadequacy in active adult smokers. Thus, it was concluded that exposure to tobacco smoke may lead to decreased vitamin D concentrations.

MATERIALS AND METHODS

This is a hospital based, observational, cross-sectional study that began after receiving Institutional Ethics Committee approval and was conducted in the Department of Oral Medicine and Radiology and in collaboration with the Department of Biochemistry.

Total 150 subjects in the age range of 20 - 60 years were recruited as per inclusion criteria from the outpatient Oral Medicine and Radiology department after obtaining their consent.

The study group comprised of 120 patients that were categorized into 4 groups of 30 patients each, whereas, control group comprised of 30 clinically healthy individuals with no addiction habits.

STUDY GROUP

- Group 1 – Healthy individuals with no habit and no lesions.
- Group 2 – Individuals with habit but no lesions
- Group 3 – OL patients
- Group 4 – OSMF patients
    - A (Group II)
    - B (Group III)
    - C (Group IV)
- Group 5 – OSCC patients

Patient's selection criteria

INCLUSION CRITERIA

1] Clinically diagnosed patients with OSMF.

2] Histopathologically diagnosed patients with OL and OSCC

3] Individuals with history of addiction habits like arecanut, kharra, tobacco chewing, smoking since 1 year.

4] Individuals between 20-60 years of age.

5] Individuals who were ready to take part in the study.

EXCLUSION CRITERIA

1] Individuals with history of treatment for OL, OSMF and OSCC.

2] Individuals with any systemic diseases and/or any medications.

3] Individuals diagnosed and/or under treatment for the disorders of Vitamin D.

4] Individuals with malignancy in any other region of body.

5] Pregnant and lactating women.

MATERIALS

1] Armamentarium for clinical examination.

- Disposable gloves.
- Kidney tray.
- Mouth mirror, tweezer, straight probe
- Sterile gauze piece and cotton.
- Digital vernier calliper.

2] Armamentarium for blood sample collection

- Disposable gloves
- Tourniquet
- Sterile gauze piece and cotton.
- Spirit
- 2 ml sterile disposable syringe with 26 gauge disposable needle
- Sodium citrate bulb.

➢ *Armamentarium for serum sample separation and storage*

- Pipette
- Disposable tube heads
- Disposable aliquot tubes

3] Biopsy instruments

- 5 ml sterile disposable syringe with 26 gauge disposable needle.
- 2% lignocaine hydrochloride with 1:80,000 adrenaline.
- A sterile biopsy tray.

- BP handle no.3 with no.15 blade.
- Biopsy punch no. 5,6,7.
- Sterile gauze and cotton.
- Tissue holder.
- Non resorbable suture material and needle.
- Needle holder.
- Formalin 10% for tissue collection.

4] Centrifuge machine

5] ELISA AccuBind Kit (Tosoh India Pvt. Ltd)
Mfg. Lic No.: 28-KD/526, Product code: 9425 – 300, Lot No.: eia-94KH021

6] ELISA reader.

REAGENTS

ELISA kit of vitamin D contains calibrators, controls and reagents. The kit was stored at 2-8^{0}C.

1] Vitamin D calibrators

- It contains six vials of human serum albumin reference calibrators for 25-OH Vitamin D at approximate concentrations of 0 (A), 5 (B), 10 (C), 25 (D), 85 (E) and 150 (F) in ng/ml with a preservative.

2] Vitamin D Controls

- It contains two vials of human serum reference controls at an established concentration and with an added preservative.

3] Vitamin D releasing agent

- It contains one vial of vitamin D binding protein releasing instant agents.

4] Wash solution concentrate

- It contains one vial of surfactant in buffered saline with a preservative (useful in manual technique).

5] Vitamin D Enzyme reagent

- It contains one vial of 25-OH Vitamin D (Antigen)-horseradish peroxidase (HRP) conjugate in a protein-stabilizing matrix.

6] Substrate reagent

- It contains one vial of tetramethylbenzidine (TMB) and hydrogen peroxide (H_2O_2) in buffer.

7] Stop solution

- It contains one vial of sulfuric acid (H_2SO_4).

8] Vitamin D Antibody coated plate

- It contains microplate with 96 – wells coated by < 1.0 µg/ml anti-Vitamin D sheep IgG and packaged in an aluminium bag with a drying agent.

METHODOLOGY

Clinical examination

A detailed case history of each patient was recorded in structured proforma designed for recording all the relevant information and observations. (Annexure I).

OSMF patients were categorized according to the Khanna and Andrade classification system[84]. Patients with OL were classified as homogenous and non-

homogenous leukoplakia while patients with oral malignancy were categorized as per clinical TNM staging[85].

Routine blood investigation was performed before carrying out biopsy procedure. Biopsy was performed in patients with OL and OSCC for histopathological confirmation. 6 mm punch was used for biopsy in OL patients while incisional biopsy was performed for malignant lesion. After confirmation of diagnosis, vitamin D levels were measured.

For evaluation of vitamin D levels, venepuncture was done under aseptic precautions to draw 2 ml of fasting venous blood from the veins in antecubital fossa - median cubital vein and collected in citrate bulb. The bulbs were left to remain for one hour at room temperature followed by its centrifugation at 3000 rpm for 12 -15 mins to separate the serum. Vitamin D levels were evaluated by ELISA technique using AccuBind Kit (Tosoh India Pvt. Ltd.) at biochemistry department.

PRINCIPLE OF ELISA[86]

ELISA kit is based on the sequential competitive assay principle and is solid phase reaction. Vitamin D in the body is in the bound form, so vitamin D releasing agent is used to separate the vitamin D antigen. Wells coated with anti-vitamin D antibody contain primary antibodies that reacts with the sample vitamin D antigen to form antigen-antibody complex and any unbound antigens are washed off. As the sample vitamin D antigen is present in variable quantity, it bounds likewise to the antibodies on the wells. If the sample antigen bounds less, some unoccupied sites will be left in the complex. Enzyme conjugate (secondary antigen) will react with these

unoccupied sites and form antigen-antibody complex. Availability of more antigens in the sample causes more primary antibodies to become bound to the sample antigen reducing the availability of secondary antigen to bind to the antibody coated wells.

Secondary antigens bound to the antibody coated wells decreases as the vitamin D concentration in serum sample of patient increases. After addition of substrate, enzyme conjugated (secondary) antigen will convert substrate into product resulting in signal reduction. Stop solution is added to the wells to terminate the reaction process (development of colour is stopped). The enzyme activity in the antibody-bound fraction is inversely proportional to the concentration of sample antigen i.e the yellow colour change (absorbance) occurs at the end of the procedure in the wells of the microplate is indirectly proportional to the vitamin D concentration in the serum sample.

Absorbance is measured spectrophotometrically at 450 nm using ELISA reader. The results of the test are reported in ng/ml or nmol/l (1 ng/ml = 2.496 nmol/l).

Following table shows the vitamin D values in test and sample after calibration and standardization and using dose response curve which is provided with the kit.

Table 1 – Absorbance and vitamin D values of test/sample

Sample I.D	Well number in microplate	Abs (A)	Mean Abs (B)	Value (ng/ml)
Cal A	A1	2.559	2.548	0
	B1	2.537		
Cal B	C1	2.041	2.047	5
	D1	2.054		
Cal C	E1	1.848	1.826	10
	F1	1.804		
Cal D	G1	1.286	1.267	25
	H1	1.249		
Cal F	A2	0.654	0.663	85
	B2	0.712		
Cal G	C2	0.511	0.529	150
	D2	0.546		
Pat# 1	A3	1.027	1.039	37.5
	A4	1.039		

.

where, Cal – Calibrators, A1 to H1 – well numbers in duplicates of calibrators, A2 to D2 - well numbers in duplicates of controls with known antigen concentration (provided with kit), Abs – Absorbance measured at 450 nm,
Pat# 1 – Patient 1 that refers to Test/Sample with unknown antigen concentration.

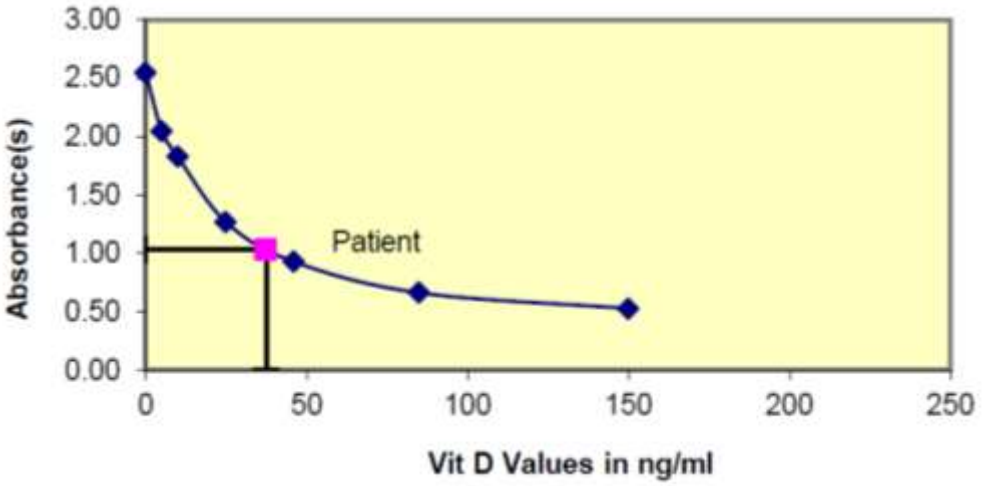


Fig 3 – Reference dose response curve to determine the vitamin D levels.

TEST PROCEDURE

Following steps were carried out to estimate vitamin D in the present study,

- The reagents and samples used were brought to room temperature before performing the test.
- For calibration, standardization of the test procedure was performed. It was done by formatting the microplates' wells for six serum reference calibrators and two controls.
- The absorbance obtained for each calibrator of known antigen concentration was correlated with the provided mean values in the kit (Table 1) and was marked with the corresponding 25-OH vitamin D concentration in ng/ml to get a dose response curve on a linear graph (Fig 3). This curve was used to ascertain the 25-OH vitamin D concentration in each unknown serum samples of the patients (study samples).

1] Before starting the procedure, all the sample serum collected in the aliquot tube were well shaken for proper mix and about 25 μL of each sample was added in the given wells with a pipette.

2] 100 μL of the *25-OH Vitamin D releasing agent* was added in all wells in the same order as the serum was added.

3] The contents in the wells of the microplate were mixed for 20-30 seconds to be homogenous.

4] Next step was to cover the microplate and incubate for 30 minutes at room temperature. Precaution was taken so that the contents were not disturbed.

5] The contents of the microplate wells were discarded by decantation and the wells were blotted dry with an absorbent paper to remove any excess liquid in order to prevent the dilution of the reagents added in the next step.

6] With an automatic plate washer, decantation of wash was done for a total of three times to remove all unbound antigens.

7] 100 µL of *25-OH Vitamin D enzyme reagent* was added to all the wells.

8] Step 4, 5 and 6 were repeated.

9] 100 µL of *substrate reagent* was added to all the wells followed by covering the microplate and incubating it for 20 minutes at room temperature. Precaution was taken so that the contents were not mixed.

10] 50 µL of *stop solution* was added to each well and mixed gently for 15-20 seconds.

11] The absorbance in each well was read at 450 nm within 15 minutes of addition of the stop solution.

Interpretation of results

To find the 25-OH vitamin D concentration of an unknown serum sample of patients (study sample), the average absorbance of the duplicates for each serum sample was pointed on the vertical axis of the graph, its intersecting point was found on the reference curve and the vitamin D concentration was read from the horizontal axis of the graph (in ng/ml).

Vitamin D status of each unknown serum sample (study sample) was interpreted as per the expected vitamin D value chart provided with ELISA kit (Table 2).

Table 2- Vitamin D status

Level	Range (ng/mL)
Very severe Vitamin D deficiency	<5
Severe Vitamin D deficiency	5 - 10
Vitamin D deficiency	10 - 20
Suboptimal Vitamin D provision	20 - 30
Optimal Vitamin D level	30 - 50
Upper norm	50 - 70
Overdose, but not toxic	70 - 150
Vitamin D intoxication	>150

- Following example shows how vitamin D was determined.

  An absorbance of 1.4 of an unknown serum sample intersects the dose response curve at 15.5 on horizontal axis.

  So, the concentration of 25-OH vitamin in this sample is 15.5 ng/ml and the patient is said to have vitamin D deficiency.

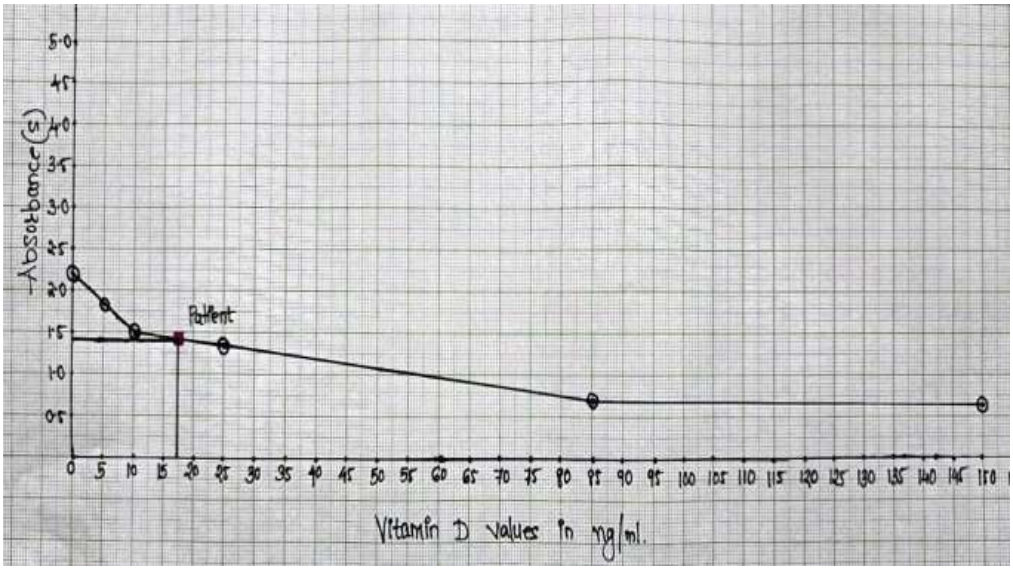


Fig 4 - Dose response curve for vitamin D assessment in study sample on graph.

STATISTICAL METHODS

SAMPLE SIZE

According to study by Orell-Kotikangas et al (2012)**,** the authors reported the mean vitamin D levels in different study groups of individuals[11]. The mean for control group was 118.75 (Range: 55-175) and for head and neck cancer was 40.35 (Range: 31.9-55).

The proposed study also aims at determining the vitamin D levels in five different groups of individuals exposed to different risk factors as well as patients with potentially malignant lesions. In the absence of data on similar design, the above published data for two groups was referred, which resulted into an effect size of 3.26; however, a more stringent effect size of 1.0 was targeted in the proposed study, that resulted into a sample size of 25 subject per group (total: 125 subjects) that can provide the desired effect with 95% confidence and 90% power.

The formula used for estimating the sample size was:

$$n = \frac{2(Z_{1-\alpha/2} + Z_{1-\beta})^2}{ES^2}$$

$$n = \frac{2(1.96 + 1.28)^2}{(1)^2} = 20.99$$

where, $Z_{1-\alpha/2}$ is standardized normal value for α=5% and $Z_{1-\beta}$ is the value for β=10%. *ES* is the effect size (1.0) and *n* is the sample size.

The demographic parameters age and sex were summarized according to scale of measurement. Age was expressed in terms of mean, standard deviation, minimum

and maximum, while sex distribution was obtained in terms of numbers and percentage. The vitamin D levels of subjects across different study groups were also summarized in terms of mean, standard deviation, minimum and maximum. The statistical significance of difference of means was tested using one-way analysis of variance. The paired comparison of levels between groups was performed using Tukey's post-hoc test.

All the analyses were performed using SPSS ver 26.0 (IBM Corp. USA) software and the statistical significance was tested at 5% level.

The description of various formulae and the methods used is as below:

Arithmetic Mean

If x_1, x_2,…x_n are the observations on a random variable X, then following measures of central tendency can be obtained:

Mean for a set of observations is given by

$$\bar{x} = \frac{1}{n}\sum_{i=1}^{n} x_i$$

Standard deviation

Standard deviation for a set of observations is given by

$$s = \sqrt{\frac{1}{(n-1)}\sum_{i=1}^{n}(x_i - \bar{x})^2}$$

Where, x_i = observation on each object (i=1,2,…n) and n = number of objects

One-way Analysis of variance

Analysis of variance (ANOVA) is used to test the significance of difference in the mean of three or more groups. The basic assumption is that the variable of interest is normally distributed in the population under study.

Here the interest is to test the null hypothesis that the population means are same, i.e.

$H_0 : \mu_1 = \mu_2 = ... \mu_m$ against the alternative H_1 that they are not same.

Some of the statistics computed to test the hypothesis are as below:

i) **Grand mean**: It is the mean of set of all observations in the studied groups $\bar{x}_{GM} = \frac{1}{N}\sum_{i=1}^{N} x_i$ and is given by:

ii) **Total sum of squares:** It is the sum of squares of each observation from the grand mean and is given by:

$$TSS = \sum_{i=1}^{N} (x_i - \bar{x}_{GM})^2$$

Total sums of squares is the sum of two components i.e., variation between groups and within groups.

iii) **Between group sum of squares**

$$SSB = \sum_{j=1}^{m} n_j (\bar{x}_j - \bar{x}_{GM})^2$$

iv) **Within group sum of squares**

$$SSW = \sum_{j=1}^{m}\sum_{i=1}^{n} (x_{ij} - \bar{x}_j)^2$$

The mean sum of squares is obtained by dividing the above sum of squares with the respective degrees of freedom, i.e. *N*-1, *p*-1 and *p*(*n*-1).

v) F-statistic: It is the ratio of between and within mean sum of squares

$$F = \frac{MS_{Between}}{MS_{Within}}$$

If the *p*-value based on F-statistic is greater than 0.05, H_0 is accepted, otherwise H_1 is accepted.

vi) Tukey's post-hoc test

After performing ANOVA, if alternative hypothesis H_1 is accepted, then the subsequent interest is to determine the pair wise significance of difference in the means of study groups. This could be carried using Tukey's post-hoc test. The difference between the means of all groups are determined and compared with this critical difference called the honest significant difference (HSD). It is given by:

$$HSD = q\sqrt{\frac{MS_{within}}{n}}$$

where, *q* is the studentized range statistic derived from the tables, *n* is the sample size and the mean square value is from the ANOVA analysis. If the critical difference exceeds the absolute difference between any two sample means, then the corresponding means differ significantly.

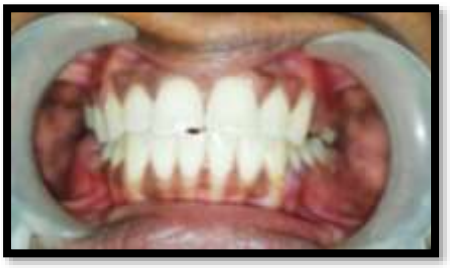
PHOTO PLATE I - Armamentarium

For clinical examination

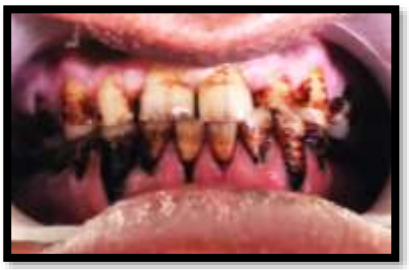
For blood sample collection

For ELISA procedure

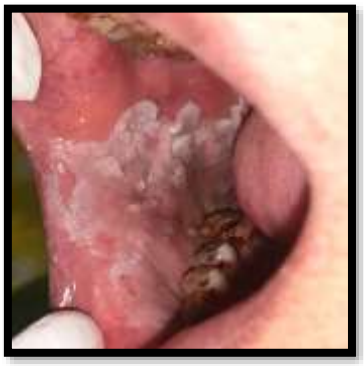
PHOTO PLATE II – Clinical photos of study group patients.



Group I - Healthy individuals with no habit and no lesions.

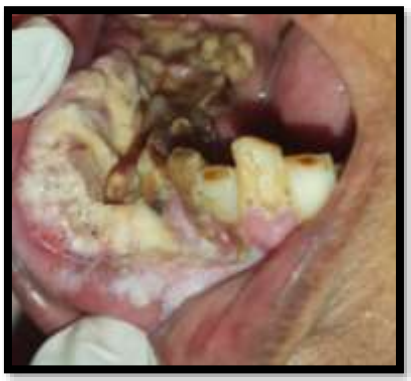


Group II – Individuals with habit but no lesions.



Group III – Oral leukoplakia

Group IV – OSMF



Group V - OSCC

PHOTO PLATE III - Machines

Centrifuge machine for serum separation

Serum separation and storage

Washer used during the ELISA procedure

ELISA reader

PHOTO PLATE IV - ELISA Kit

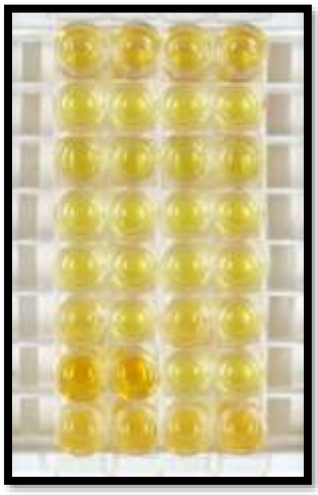
ELISA kit with microplate

Reagents

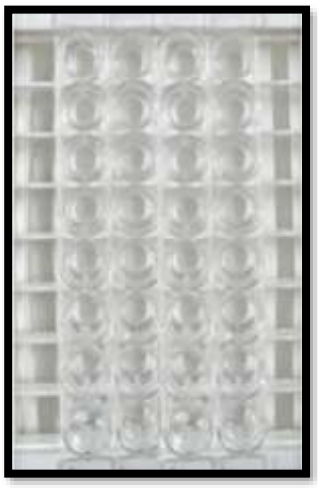
Calibrators

Controls

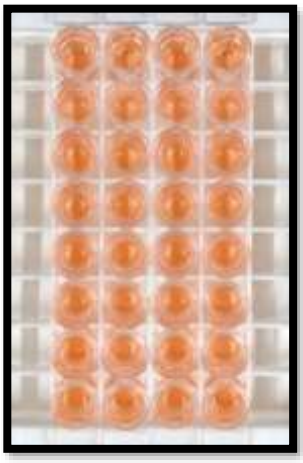
PHOTO PLATE V – Steps for assessing vitamin D levels with ELISA technique



After addition of releasing agent

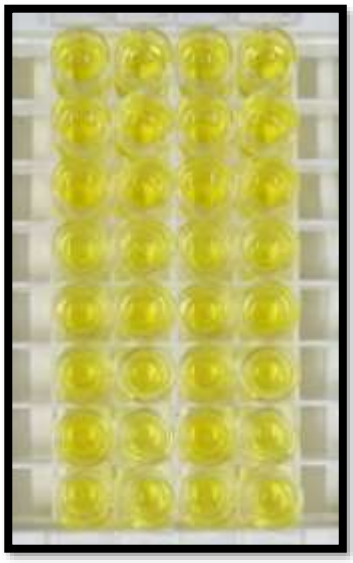


After decantation



After addition of enzyme conjugate

After addition of substrate reagent



After addition of stop solution and absorbance was measured by ELISA reader

OBSERVATIONS AND RESULTS

A hospital based, cross-sectional study was undertaken to evaluate the levels of serum vitamin D in OL, OSMF and OSCC patients. Total 150 subjects were selected randomly from the department OPD and categorized into **FIVE** groups of 30 subjects each and are as follows:

- Group 1 – Healthy individuals with no habit and no lesions.
- Group 2 – Individuals with habit but no lesions.
- Group 3 – OL patients
- Group 4 – OSMF patients
  - A (Group II)
  - B (Group III)
  - C (Group IV)
- Group 5 – OSCC patients.

The results and observation of the study were evaluated and interpreted according to following parameters-

Distribution of age

Table 3 provides the descriptive statistics for age of subjects in different study groups. There were 30 subjects in each group. The mean age of healthy individuals without habits and no lesions was 37.93 (SD: 9.01) years with a minimum of 22 years while maximum of 55 years. The mean age of subjects with habits but no lesions was 34.07 (SD: 10.64) years with a minimum of 21 years and maximum of 60 years. The mean age of patients with OL was 44.6 (SD: 6.8) years with minimum age of 32 years and maximum of 57 years. The mean age of patients with OSMF was 38.27 (SD: 10.1) years with a minimum of 23 years and maximum of 55 years. The mean age of patients with OSCC was 45.43 (SD: 7.37) years with a minimum of 32 years and maximum of 60 years. The difference in the mean age of subjects across groups was statistically significant with a p-value < 0.0001. The mean age of subjects with habits and no lesions was significantly lower than that of other groups. A graphical representation of the mean age of patients in five groups is given in Graph 1.

Distribution of gender

Table 4 provides the distribution of subjects according to gender in different groups. In the healthy individuals with no habits and no lesion group, there were 16 (53.3%) males and 14 (46.7%) females, while in habits and no lesions group, there were 18 (60%) males and 12 (40%) females. In the OL patient group, there were 22 (73.3%) males and 8 (26.7%) females. In the OSMF patient group, there were 17 (56.7%) males and 13 (43.3%) females, while in the OSCC patient group, there were

20 (66.7%) males and 10 (33.3%) females. The difference in the distribution of subjects as per gender differed insignificantly across groups as indicated by p-value of 0.512. A graphical representation of the gender distribution in five groups is given in Graph 2.

Vitamin D levels in different groups.

Table 5 provides the descriptive statistics for vitamin D levels of subjects in different study groups. The mean level of vitamin D in healthy individuals without habits and no lesions was 31.78 (SD: 9.20) ng/ml with a minimum of 18.5 ng/ml while maximum of 46.6 ng/ml. Its mean level in subjects with habits but no lesions was 20.2 (SD: 7.59) ng/ml with a minimum of 6.5 ng/ml and maximum of 33.5 ng/ml. Its mean level in patients with OL was 14.36 ng/ml (SD: 4.22) ng/ml with minimum of 5.5 ng/ml and maximum of 22.2 ng/ml. Its mean level in patients with OSMF was 15.01 (SD: 6.16) ng/ml with a minimum of 5.8 ng/ml and maximum of 28 ng/ml. Its mean level in patients with OSCC was 11.98 (SD: 4.61) ng/ml with a minimum of 4.5 ng/ml and maximum 19.7 ng/ml. The difference in the mean vitamin D level of subjects across groups was statistically significant with a p-value < 0.0001. A graphical representation of vitamin D levels in five groups is given in Graph 3.

Comparison of vitamin D levels in different groups.

Table 6a provides the paired comparison of vitamin D levels between different groups. It is evident from the table that the mean difference of level between healthy individuals with no habits & no lesions and individuals with habit but no lesions was 11.58 [95% CI: 6.858, 16.302] and was statistically significant with a p-value < 0.0001. Similarly, the mean difference between healthy individuals with no habits & no lesions and OL patients was 17.423 [95% CI: 12.702, 22.145] and was

significant with a p-value < 0.0001. The mean difference of vitamin D between healthy individuals with no habits & no lesions and OSMF patients was 16.776 [95% CI: 12.055, 21.498] and was significant with a p-value < 0.0001. Further, the mean difference between healthy individuals with no habits & no lesions and OSCC patients was 19.803 [95% CI: 15.082, 24.525] and was significant with a p-value < 0.0001.

On similar lines, the mean difference of vitamin D between individuals with habit but no lesions and OL patients was 5.843 [95% CI: 1.121, 10.565] and was significant with a p-value 0.007. The mean difference between individuals with habits but no lesion and OSMF patients was 5.196 [95% CI: 0.475, 9.918] and was significant with a p-value 0.023. Further, the mean difference of vitamin D between individuals with habits but no lesion and OSCC was 8.223 [95% CI: 3.501, 12.945] and was significant with a p-value < 0.0001.

Table 6b shows the one-way analysis of variance table for Vitamin D levels of subjects in five study groups. The mean sum of squares between groups and within groups resulted into a F-value of 42.968 with (4, 145) degrees of freedom and corresponding p-value < 0.0001. This suggested statistically significant difference of mean vitamin D levels across study groups.

Vitamin D levels in sub-groups of OSMF patients

Table 7 provides the descriptive statistics for vitamin D levels for the sub-groups of OSMF patients. There were 10 patients in each sub-group. The mean level for sub-group II was 15.49 (SD: 5.78) ng/ml with a minimum of 7.90 ng/ml and maximum of 26.1 ng/ml. In sub-group III, the mean was 15.22 (SD: 7.28) ng/ml with

a minimum of 5.8 ng/ml and maximum 26.8 ng/ml. In sub-group IV, the mean was 14.31 (SD: 5.91) ng/ml with a minimum of 9.7 ng/ml and maximum of 28 ng/ml. A graphical representation of vitamin D levels in sub-groups of OSMF patients is given in Graph 4.

Comparison of vitamin D levels in sub-groups of OSMF patients

Table 8a gives the paired comparison of vitamin D levels between sub-groups of OSMF patients. It is evident from the table that the mean difference between any two sub-groups was statistically insignificant with $p > 0.05$.

Table 8b gives the one-way analysis of variance results for vitamin D levels across the sub-groups of OSMF patients. The mean sum of squares between and within groups resulted into a F-value of 0.095 with (2, 27) degrees of freedom, with a corresponding p-value of 0.91. This suggested that the difference of mean vitamin D levels across sub-groups of OSMF patients was statistically insignificant.

DISCUSSION

The term 'vitamin D' as a whole refers to a group of steroids like, active and inactive molecules that includes ergocalciferol (vitamin D_2), cholecalciferol (vitamin D_3), calcidol (25-hydroxycholecalciferol) and calcitriol (1,25-dihydroxycholecalciferol)[87]. Vitamin D is different from other vitamins as it functions like a hormone. In human, predominant form of vitamin D is cholecalciferol which is synthesized in the skin after exposure by the ultraviolet rays, while approximately 20% is obtained through dietary sources and supplements[22].

Vitamin D interacts with the its receptor (VDR) and undergoes variety of sequential metabolic steps to become active. The physiological activity of vitamin D is mainly carried out by calcitriol[25]. It regulates the absorption of calcium in the small intestine which along with parathyroid hormone helps in the mineralization of bone, thus maintaining calcium homeostasis in the blood[15]. It helps to regulate cell

differentiation and maturation, causes reduced immunoglobulins, plasma cell and memory cell production and thus maintain innate immune responses[18].

Considering the multifarious functions of vitamin D in the body, its deficiency or insufficiency must have alarming consequences on the overall health of an individual[10]. Marked alterations in its levels have been reported in various oral lesions[17,18]. So, there has been an increased demand for determining the role of vitamin D in systemic and oral pathologies.

Hence, the present study was planned to evaluate levels of vitamin D in oral cancer, potentially malignant lesions and individuals with habits but no lesions.

Among the various methods of evaluation of vitamin D, ELISA was used in the present study. It is a gold standard diagnostic test to estimate vitamin D levels[35]. It evaluates serum 25-hydroxycholecalciferol that represents a true, major circulating form of vitamin D[86,88]. ELISA test is advantageous due to its high sensitivity arising from the differences in the composition of complex antigen mixtures even with the presence of small amount of specific detecting antibody[35]. It is easy to use and has low cost too[33].

The present health care centre-based study was done on total 150 patients which were categorized into five groups of 30 patients each. Patients with oral addiction habits, OL, OSMF and OSCC were compared with clinically healthy individuals with no addiction habits.

Individuals in control group of the present study were predominantly male of age range 20 - 60 years. They were healthy and without any addiction habit with apparently normal mucosa. The mean level of vitamin D in them was 31.78 ng/ml i.e

optimal level (Table 5, Graph 3). Most of the individuals were indoor workers and obese who are likely to get low vitamin D. But as central region of India receives abundant sunshine, these healthy individuals showed optimum vitamin D. Near optimum value of vitamin D might be the result of altered lifestyle and dietary deficiency of vitamin D which is not similar to the findings of the previous studies[89-93].

In the present study, group II individuals with addiction habits without oral lesions had 20.20 ng/ml mean vitamin D level (Table 5, Graph 3). Vitamin D level in them was significantly lower when compared to control group (Table 6a). The above finding is in accordance to studies showing hypovitaminosis D in tobacco users[9,14,28,61,71-83]. While ***Bakan et al (2020)*** observed no association of vitamin D levels in smoker[60].

Decreased vitamin D levels in oral addictive habit individuals of the present study may be due to the association of carcinogens like tobacco and areca nut. It is considered that smokeless tobacco products cause increased expression of enzyme 25-hydroxylase which catalyzes the conversion of vitamin D to its relatively inactive form[14]. Tobacco can cause dysfunction of vitamin D-parathyroid hormones (PTH) axis, leading to disturbed vitamin D metabolism. Heavy metals in the tobacco may accumulate in kidneys causing dysfunction of renal tubules, ultimately hampering vitamin D activation due to impaired kidney function[83]. There may be dysregulation in the expression of some genes and enzymes like 25-hydroxylase involved in the metabolic pathway of vitamin D due to smoking which can inhibit translocation of VDR from the nucleus to the cell membrane[80]. The present study results is similar to

study by ***Yuan et al (2021)*** which showed increased cotinine levels in urine and hypovitaminosis D in tobacco users [83].

The mean level of vitamin D in patients with OL (group III) of the present study was 14.36 ng/ml (Table 5, Graph 3). It was significantly lower compared to control and individuals with addictive habits while decreased level were nonsignificant with OSMF and OSCC patients (Table 6a). The result of the present study is in accordance with the studies reported by ***Grimm et al (2015) and Anand et al (2017)*** who found decreased vitamin D levels in OL patients when compared to healthy controls and OSCC[2,12]. Hypovitaminosis D in OL patients may be due to increased expression of VDR leading to reduced availability of active vitamin D[12]. Tobacco use is considered as a sole cause of hypovitaminosis D which is main etiologic agent in OL[94].

Mean vitamin D levels in OSMF patients (group IV) of the present study was found to be 15.02 ng/ml (Table 5, Graph 3). It was significantly lower when compared to controls and individuals with addiction habits but the levels were not significant with OL and OSCC (Table 6a). This finding is not in accordance with study by ***Kumar et al (2017)*** who observed no association of vitamin D levels between healthy controls and OSMF[70].

The present study assessed vitamin D levels in various subgroups of OSMF which was not performed earlier. Though vitamin D level was decreased as per severity of lesion, it was nonsignificant when assessed within subgroups (Table 8b, Graph 4). Decreased level of vitamin D in the present study may be due to arecanut which is the main causative agent in OSMF and affects VDR activity[70,95]. Secondly,

OSMF is a chronic inflammatory condition which affects the oral cavity and sometimes pharynx. Vitamin D also acts as an anti-inflammatory and antifibrotic agent[16,17,80]. Also, hypovitaminosis D is more common in young population, so age in OSMF (mean age – 38.27 years) may also be responsible for its lowered levels (Table 3, Graph 1).

In the present study, group V consisted of patients with OSCC which showed mean vitamin D level as 11.98 ng/ml suggesting its significant deficiency when compared with controls while it was comparatively lower in individuals with addiction habits (Table 5, Graph 3, Table 6a). These findings of the present study are in accordance with the studies reported by ***Dev et al (2011), Orell- Kotikangas et al (2012), Mostafa BE et al (2016), Bochen et al (2018), Udeabor et al (2020), Pandey et al (2020), Sufiawati et al (2021)*** while it is in contrast with the studies by ***Arem et al (2011), Zhang et al (2015)***[3,11,48,54,55,56,63,64,69]**.** In the present study, vitamin D level in OSCC was nonsignificant when compared with OL and OSMF.

Decreased vitamin D levels in OSCC patients of the present study may be the result of malignancy or due to the less availability of vitamin D from dietary sources and consumption of addictive agents. Vitamin D in dietary sources is already limited and unaffordable to some underprivileged and socioeconomically backward individuals. Studies reported the protective nature of vitamin D against malignancy suggesting that individuals with its deficiency status is likely to be more prone for malignancy[2,12,16,56,96].

Vitamin D expresses antineoplastic activities by many ways[11,17,23,25,54,66]. It stimulates pro-apoptotic genes, stimulates upregulation of p21 and p27 genes and by increase in the expression of growth inhibitors[17,23]. Vitamin D induces pro-

differentiating effect by keeping the cancer cells in a stage where they have little or no proliferative potential but a more normal phenotype[17,97]. Angiogenesis occurs at increased levels in cancer. It inhibits the expression of vascular endothelial growth factor and arrest angiogenesis[11,13,23]. Vitamin D regulates the expression of plasminogen activator system and matrix metalloproteinases which are crucial promotors of cancer invasion and metastasis and increases the expression of tumor suppressor gene, E-cadherin which has a preventive role in metastasis[16,17,24,66].

Present study population belongs to low-moderate socioeconomic background and malnourished and may be in deficient state of vitamin D and consumption of tobacco, arecanut like carcinogens add to it and likely to aggravate malignancy in them and increase in vitamin D deficiency.

Emphasis was given on assessment of vitamin D levels in various conditions like patients with various oral addictive habits without any lesions, patients with potentially malignant lesions like OL, OSMF and OSCC which none of the previous studies have done. Significant vitamin D deficiency was found in these patients with various oral conditions as compared to control individuals. However, there is still not much evidence of the role of poor vitamin D status in development of oral cancer and its effectiveness in decreasing the risk of oral cancer and thus prolong life. Preexisting vitamin D deficiency can lead to oral cancer and thus malnutrition while malnutrition in oral cancer patients leads to their decreased vitamin D levels and related symptoms and the vivacious cycle goes on[11]. So, oral addictive habit patients should be counselled for discontinuing their habits and be regularly looked for several potentially malignant lesions and oral cancer. This study highlights the significance of vitamin D in tobacco/areca nut users and in patients with OL,OSMF,OSCC.

So, it is necessary to screen apparently healthy as well as diseased individuals for vitamin D so as to maintain its adequate levels and to include it in the management protocol of these lesions.

SUMMARY

The present study was a cross-sectional, hospital based observational study done in the Oral Medicine and Radiology department. Its goal was to assess the levels of vitamin D as well as find its association in patients with oral addictive habits and various oral lesions. A total of 150 individuals were selected and categorized into five groups i.e. healthy individuals with no habits (Group I), individuals with habits and normal mucosa (Group II), individuals with habits having OL (Group III), individuals with habits having OSMF (Group IV) and individuals with habit having OSCC (Group V).

Considering the high prevalence of patients with oral addictive habits like kharra, ghutka, arecanut in central India population and the various ill effects associated with it, the present study represents the best attempt to evaluate levels of vitamin D in patients with habit induced oral lesions.

The present study results are summarized as:

1] The mean age of healthy control group and individuals with habit and no lesion, OL, OSMF and OSCC groups was 37.93, 34.07, 44.60, 38.27 and 45.43 years respectively.

2] All the groups showed male predominance.

3] The mean level of vitamin D in healthy controls and individuals with habit and no lesion, OL, OSMF and OSCC was 31.78 ng/ml, 20.20 ng/ml, 14.36 ng/ml, 15.01 ng/ml, 11.98 ng/ml respectively.

4] On comparison with the healthy control group, individuals with habit and no lesion, OL, OSMF and OSCC showed highly significant difference in levels of vitamin D.

5] On comparing individuals with habit and no lesion, individuals with OL, OSMF and OSCC showed highly significant difference in levels of vitamin D.

6] On comparing individuals with OL, individuals with OSMF and OSCC showed nonsignificant difference in levels of vitamin D.

7] On comparing individuals with OSMF, individuals with OSCC showed nonsignificant difference in levels of vitamin D.

8] The mean vitamin D level in individuals in various subgroups i.e. group II, III and IV of OSMF was 15.49 ng/ml, 15.22 ng/ml and 14.31 ng/ml.

9] On comparing individuals in various subgroups i.e. group II, III and IV of OSMF, nonsignificant difference was observed in levels of vitamin D.

The present study showed optimum vitamin D levels in healthy controls and deficiency in individuals with habit and no lesion, OL, OSMF and OSCC suggesting greater prevalence of hypovitaminosis D in central India population who are involved in various addictive habits and habit induced oral lesions.

Limitations and future perspectives

1] The sample size in the present study was limited to 150 subjects. Further, larger sample size studies would be desirable to substantiate the present study results and to confirm and reveal the exact pathophysiology underlying such changes.

2] Association between frequency and duration of several oral addictive habits among the study population may facilitate further research for evaluating its effect on vitamin D levels.

3] As the study population includes predominantly males, though studies have reported higher frequency of hypovitaminosis D in female population. Age and sex matched study may accurately assess the vitamin D levels in them.

4] Further research should be carried out by delivering vitamin D as a treatment modality in patients with habit induced oral lesions.

CONCLUSION

The micronutrient ‘vitamin D’ has acquired great importance in the recent years especially in oral health keeping in mind its diverse roles in proper functioning of various systems of the human body. Its deficiency or insufficiency sometimes have non-specific clinical presentation depicting a silent condition but it can lead to an increased risk of certain diseases.

From the present study, we can conclude that deficiency of vitamin D is present in individuals with various oral addictive habits, OL, OSMF and OSCC. However, healthy individuals in the present study also showed near adequate vitamin D levels suggesting widespread hypovitaminosis D in central India population. Also, there is high prevalence of various oral addiction habits in Indians. These habits in them make any condition worse, thus increasing the risk of various habit induced oral lesions.

So, levels of vitamin D should be taken into consideration to ensure a healthy and balanced health of the oral cavity and it should be checked and tightly regulated before treatment of several oral lesions to avoid any complications and guarantee long term, fruitful treatment outcomes.

TABLES AND FIGURES

TABLES

Table 3: Descriptive statistics for age of subjects in control and study groups

Statistical parameters	Control group	Study groups			
	Healthy individuals - No habits and no lesions	Individuals - Habits but no lesions	Oral leukoplakia patients	Oral submucous fibrosis patients	Oral squamous cell carcinoma patients
N	30	30	30	30	30
Mean (years)	37.93	34.07	44.60	38.27	45.43
SD (years)	9.01	10.64	6.80	10.10	7.37

SD: Standard deviation

Table 4: Distribution of patients according to gender in different groups

	Control group	Study groups			
Gender	Healthy individuals - No habits and no lesions	Individuals - Habits but no lesions	Oral leukoplakia patients	Oral submucous fibrosis patients	Oral squamous cell carcinoma patients
Male [n (%)]	16 (53.3)	18 (60.0)	22 (73.3)	17 (56.7)	20 (66.7)
Female [n (%)]	14 (46.7)	12 (40.0)	8 (26.7)	13 (43.3)	10 (33.3)
Total	30	30	30	30	30

Table 5: Descriptive statistics for Vitamin D levels of patients in control and study groups

Statistical parameters	Control group	Study groups			
	Healthy individuals - No habits and no lesions	Individuals - Habits but no lesions	Oral leukoplakia patients	Oral submucous fibrosis patients	Oral squamous cell carcinoma patients
N	30	30	30	30	30
Mean [ng/ml]	31.78	20.20	14.36	15.01	11.98
SD [ng/ml]	9.20	7.59	4.22	6.16	4.61
Minimum [ng/ml]	18.50	6.50	5.50	5.80	4.50
Maximum [ng/ml]	46.60	33.50	22.20	28.00	19.70

SD: Standard deviation

Table 6a: Pair wise comparison of Vitamin D levels between different groups

(I) Group	(J) Group	Mean Difference (I-J)	P-value	95% Confidence Interval	
				Lower Bound	Upper Bound
Healthy individuals - No habits and no lesions (Control group)	Individuals - Habits but no lesions	11.580	**< 0.0001 (S)**	6.858	16.302
	Oral leukoplakia patients	17.423	**< 0.0001 (S)**	12.702	22.145
	Oral submucous fibrosis patients	16.776	**< 0.0001 (S)**	12.055	21.498
	Oral squamous cell carcinoma patients	19.803	**< 0.0001 (S)**	15.082	24.525
Individuals - Habits but no lesions	Oral leukoplakia patients	5.843	**0.007 (S)**	1.121	10.565
	Oral submucous fibrosis patients	5.196	**0.023 (S)**	0.475	9.918
	Oral squamous cell carcinoma patients	8.223	**< 0.0001 (S)**	3.501	12.945
Oral leukoplakia patients	Oral submucous fibrosis patients	-0.646	0.996 (NS)	-5.368	4.075
	Oral squamous carcinoma patients	2.380	0.634 (NS)	-2.342	7.102
Oral submucous fibrosis patients	Oral squamous cell carcinoma patients	3.026	0.395 (NS)	-1.695	7.748

**Obtained using Tukey's post-hoc test; S: Significant; NS: Not significant*

Table 6b: One-way analysis of variance for Vitamin D levels across five study groups

Source of variation	Sum of Squares	DF	Mean Square	F-value	P-value
Between Groups	7531.853	4	1882.963	42.968	**< 0.0001 (S)**
Within Groups	6354.246	145	43.822		
Total	13886.099	149			

S: Significant; DF: Degrees of freedom

Table 7: Descriptive statistics for Vitamin D levels of sub-groups of OSMF patients

		OSMF patients		
		Sub-group II	Sub-group III	Sub-group IV
Vitamin D (ng/ml)	n	10	10	10
	Mean	15.49	15.22	14.31
	Standard Deviation	5.78	7.28	5.91
	Minimum	7.90	5.80	9.70
	Maximum	26.10	26.80	28.00

Table 8a: Pair wise comparison of Vitamin D levels between sub-groups of OSMF patients

(I)	(J)	Mean Difference (I-J)	P-value*	95% Confidence Interval	
				Lower Bound	Upper Bound
Sub-group II	Sub-group III	0.270	0.995 (NS)	-6.781	7.321
	Sub-group IV	1.180	0.910 (NS)	-5.871	8.230
Sub-Group III	Sub-Group IV	0.910	0.945 (NS)	-6.141	7.960

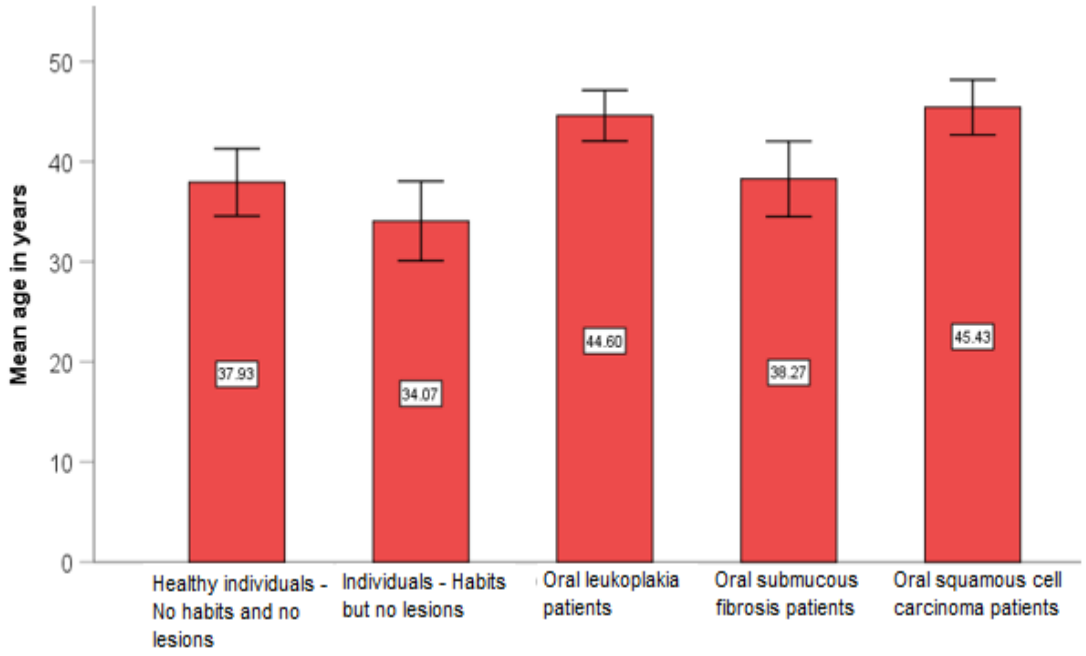
**Obtained using Tukey's post-hoc test; NS: Not significant*

Table 8b: Comparison of Vitamin D levels between sub-groups of OSMF patients

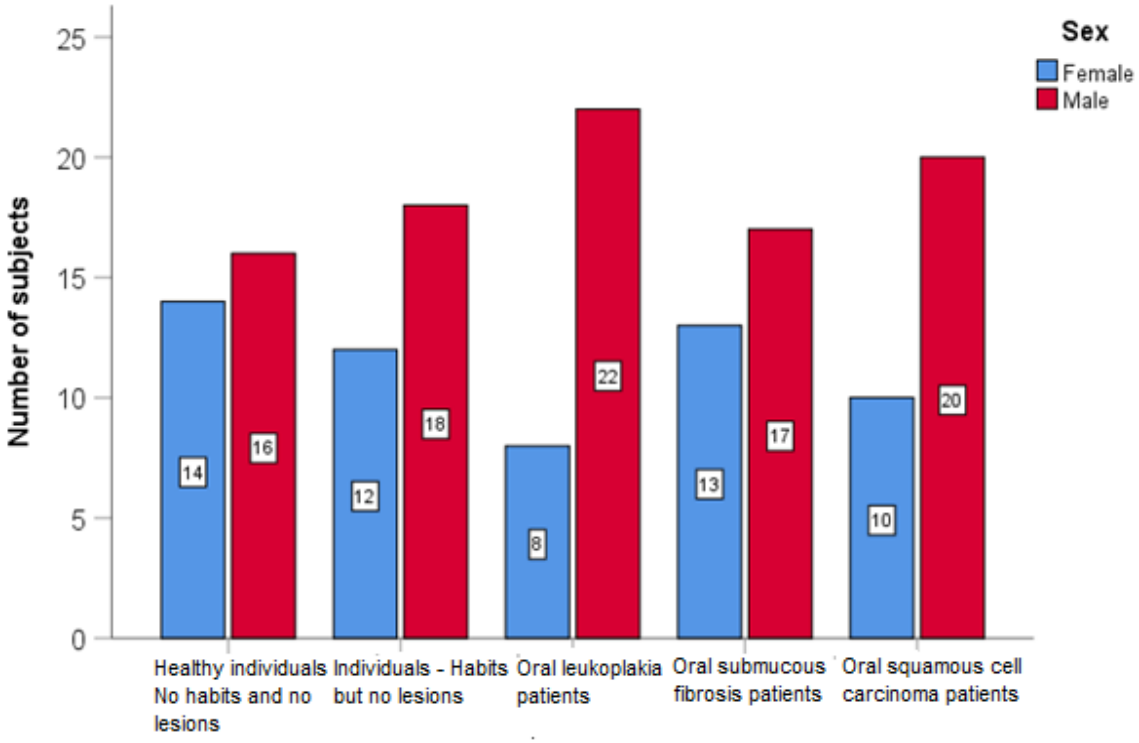
Source of variation	Sum of Squares	DF	Mean Square	F-value	P-value*
Between Groups	7.645	2	3.822	0.095	0.910 (NS)
Within Groups	1091.734	27	40.435		
Total	1099.379	29			

**Obtained using one-way analysis of variance; DF: Degrees of freedom; NS: Not significant*

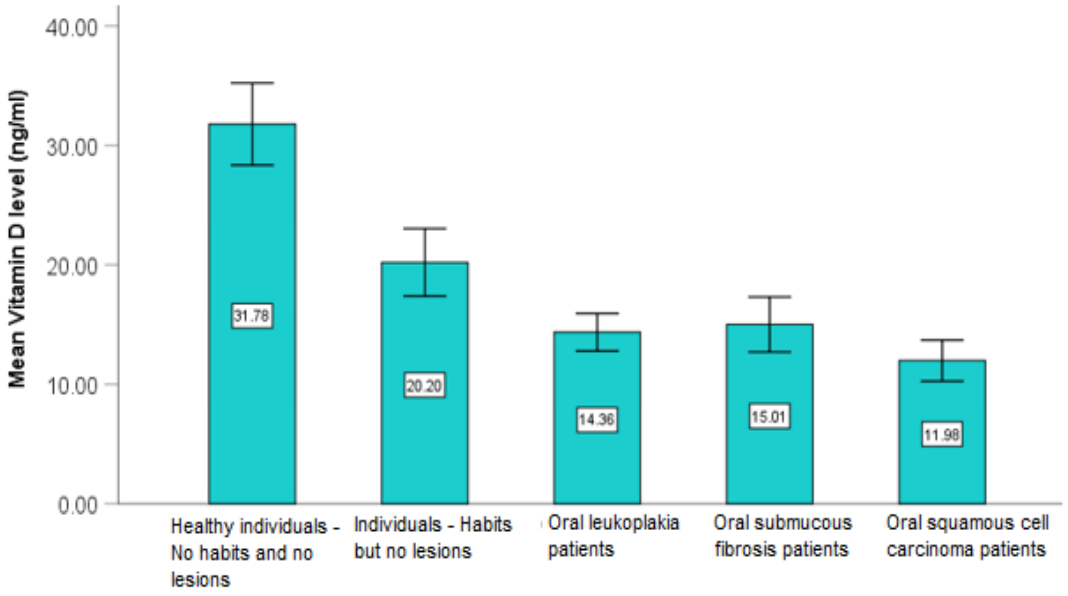
GRAPHS



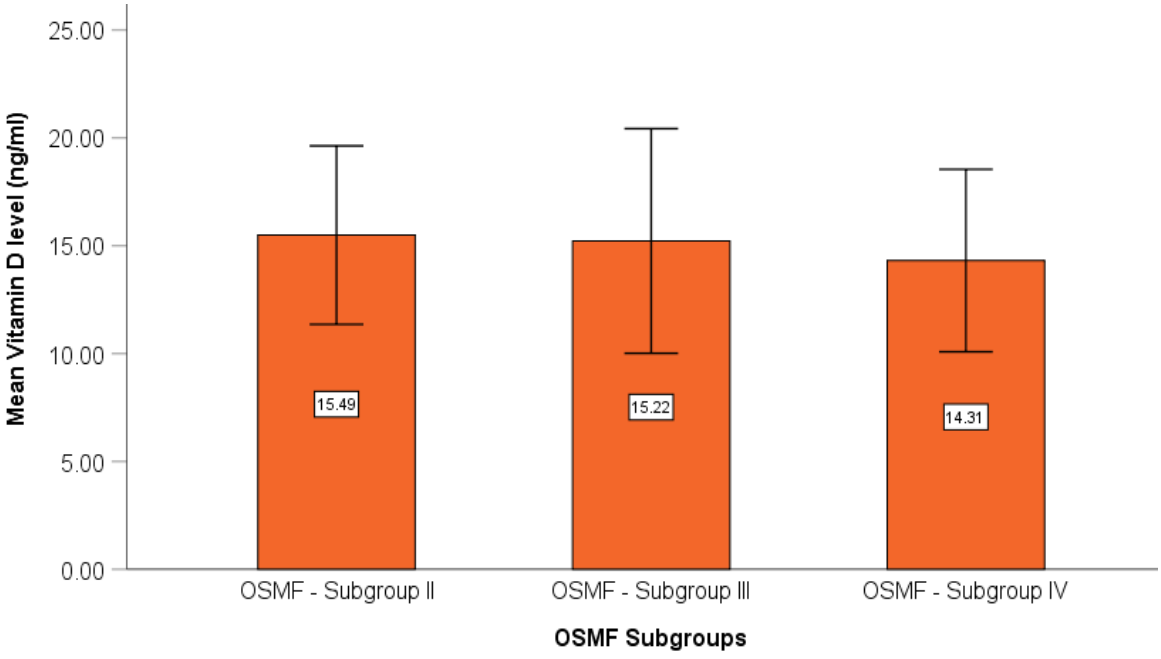
Graph 1: Column chart with error bar showing mean age of subjects in each group



Graph 2: Column chart showing number of subjects according to gender in each group



Graph 3: Column chart with error bar showing mean Vitamin D levels of subjects in each group



Graph 4: Column chart with error bar showing mean Vitamin D levels of OSMF subjects according to subgroups.

REFERENCES

1] George A, Sreenivasan BS, Sunil S, Varghese SS, Thomas J, Gopakumar D, Mani V. Potentially malignant disorders of oral cavity. Oral Maxillofac Pathol J. 2011 Jan 1;2(1):95-100.

2] Anand A, Singh S, Sonkar AA, Husain N, Singh KR, Singh S, Kushwaha JK. Expression of vitamin D receptor and vitamin D status in patients with oral neoplasms and effect of vitamin D supplementation on quality of life in advanced cancer treatment. Contemporary Oncology. 2017;21(2):145.

3] Bochen F, Balensiefer B, Körner S, Bittenbring JT, Neumann F, Koch A, Bumm K, Marx A, Wemmert S, Papaspyrou G, Zuschlag D. Vitamin D deficiency in head and neck cancer patients–prevalence, prognostic value and impact on immune function. Oncoimmunology. 2018 Sept 2;7(9):e1476817.

4] Varshitha A. Prevalence of oral cancer in India. Journal of Pharmaceutical Sciences and Research. 2015 Oct 1;7(10):845.

5] Mortazavi H, Baharvand M, Mehdipour M. Oral potentially malignant disorders: an overview of more than 20 entities. Journal of dental research, dental clinics, dental prospects. 2014;8(1):6.

6] Petti S. Pooled estimate of world leukoplakia prevalence: a systematic review. Oral oncology. 2003 Dec 1;39(8):770-80.

7] Shen YW, Shih YH, Fuh LJ, Shieh TM. Oral Submucous Fibrosis: A Review on Biomarkers, Pathogenic Mechanisms, and Treatments. International Journal of Molecular Sciences. 2020 Jan;21(19):7231.

8] Madani AH, Dikshit M, Bhaduri D. Risk for oral cancer associated to smoking, smokeless and oral dip products. Indian journal of public health. 2012 Jan 1;56(1):57.

9] Meshram A, Meshram K, Ambad R, Kacchua K, Kanyal L, Ingle S, Vagga A, Jha RK. Vit D Status and Osteoporosis in Tobacco Consuming Men in Rural Region Surrounding Wardha City, Maharashtra, Central Part of India. Indian Journal of Forensic Medicine & Toxicology. 2020 Oct 1;14(4):6737-42.

10] Aoun A, Maalouf J, Fahed M, El Jabbour F. When and how to diagnose and treat vitamin D deficiency in adults: a practical and clinical update. Journal of dietary supplements. 2020 May 3;17(3):336-54.

11] Orell–Kotikangas H, Schwab U, Osterlund P, Saarilahti K, Mäkitie O, Mäkitie AA. High prevalence of vitamin D insufficiency in patients with head and neck cancer at diagnosis. Head & neck. 2012 Oct;34(10):1450-5.

12] Grimm M, Cetindis M, Biegner T, Lehman M, Munz A, Teriete P, Reinert S. Serum vitamin D levels of patients with oral squamous cell carcinoma (OSCC) and expression of vitamin D receptor in oral precancerous lesions and OSCC. Medicina oral, patologia oral y cirugia buccal. 2015 Mar;20(2):e188.

13] Afzal S, Bojesen SE, Nordestgaard BG. Low plasma 25-hydroxyvitamin D and risk of tobacco-related cancer. Clinical chemistry. 2013 May 1;59(5):771-80.

14] Rahman AH, Haque ME, Naznin L, Ahmed NU. Role of Common Addictive Habits on Hypovitaminosis D among Bangladeshi People. Medicine Today. 2019 Jun 26;31(2):89-92.

15] Izreig S, Hajek M, Edwards HA, Mehra S, Sasaki C, Judson BL, Rahmati RW. The role of vitamin D in head and neck cancer. Laryngoscope Investigative Otolaryngology. 2020 Dec;5(6):1079-88.

16**]** Fathi N, Ahmadian E, Shahi S, Roshangar L, Khan H, Kouhsoltani M, Dizaj SM, Sharifi S. Role of vitamin D and vitamin D receptor (VDR) in oral cancer. Biomedicine & Pharmacotherapy. 2019 Jan 1;109:391-401.

17] Patil DJ. Clinical Implications of Vitamin D in Oral Diseases-A Review. J. Pharm. Res. Int. 2021:103-10.

18] Botelho J, Machado V, Proença L, Delgado AS, Mendes JJ. Vitamin D deficiency and oral health: A comprehensive review. Nutrients. 2020 May; 12(5):1471.

19] Shrihari TG. Vitamin D role in prevention of cancer: Overview. Indian Journal of Medical and Paediatric Oncology. 2018 Jan;39(01):75-8.

20] Suchanecka A, Chmielowiec K, Chmielowiec J, Trybek G, Masiak J, Michałowska-Sawczyn M, Nowicka R, Grocholewicz K, Grzywacz A. Vitamin D Receptor Gene Polymorphisms and Cigarette Smoking Impact on Oral Health: A Case-Control Study. International journal of environmental research and public health. 2020 Jan;17(9):3192.

21] Satyanarayana U. Essentials of Biochemistry. 2nd edition, Elsevier Health Sciences; 2013.123.

22] Bjelakovic G, Gluud LL, Nikolova D, Whitfield K, Krstic G, Wetterslev J, Gluud C. Vitamin D supplementation for prevention of cancer in adults. Cochrane Database of Systematic Reviews. 2014(6).

23] Bektas- Kayhan KI, Unur M, Yaylim-Eraltan I, Ergen HA, Toptas B, Hafiz G, Karadeniz A, Isbir T. Association of vitamin D receptor Taq I polymorphism and susceptibility to oral squamous cell carcinoma. in vivo. 2010 Sep 1;24(5):755-9.

24] Yokosawa EB, Arthur AE, Rentschler KM, Wolf GT, Rozek LS, Mondul AM. Vitamin D intake and survival and recurrence in head and neck cancer patients. The Laryngoscope. 2018 Nov;128(11):E371-6.

25] Makitie A, Tuokkola I, Laurell G, Mäkitie O, Olsen K, Takes RP, Florek E, Szyfter K, Sier CF, Ferlito A. Vitamin D in head and neck cancer: a systematic review. Current oncology reports. 2021 Jan;23(1):1-8.

26] Amrein K, Scherkl M, Hoffmann M, Neuwersch-Sommeregger S, Kostenberger M, Berisha AT, Martucci G, Pilz S, Malle O. Vitamin D deficiency 2.0: an update on the current status worldwide. European journal of clinical nutrition. 2020 Nov;74(11):1498-513.

27] Gupta A. Vitamin D deficiency in India: prevalence, causalities and interventions. Nutrients. 2014 Feb;6(2):729-75.

28] Cutillas-Marco E, Fuertes-Prosper A, Grant WB, Morales-Suárez-Varela M. Vitamin D deficiency in South Europe: effect of smoking and aging. Photodermatology, photoimmunology & photomedicine. 2012 Jun;28(3):159-61.

29] Jukic AM, Hoofnagle AN, Lutsey PL. Measurement of vitamin D for epidemiologic and clinical research: shining light on a complex decision. American journal of epidemiology. 2018 Apr 1;187(4):879-90.30.

30] Alshahrani F, Aljohani N. Vitamin D: deficiency, sufficiency and toxicity. Nutrients. 2013 Sep;5(9):3605-16.

31] Holick MF. Vitamin D status: measurement, interpretation, and clinical application. Annals of epidemiology. 2009 Feb 1;19(2):73-8.

32] Enko D, Kriegshäuser G, Stolba R, Worf E, Halwachs-Baumann G. Method evaluation study of a new generation of vitamin D assays. Biochemia medica. 2015 Jun 15;25(2):203-12.

33] He CS, Gleeson M, Fraser WD. Measurement of circulating 25-hydroxy vitamin d using three commercial enzyme-linked immunosorbent assay kits with comparison to liquid chromatography: tandem mass spectrometry method. International Scholarly Research Notices. 2013;2013.

34] Kulie T, Groff A, Redmer J, Hounshell J, Schrager S. Vitamin D: an evidence-based review. The Journal of the American Board of Family Medicine. 2009 Nov 1;22(6):698-706.

35] Pathuri CK, Ramalakshmi NS. Enzyme linked immunosorbent assay-lab diagnosis: A review. Indian Journal of Microbiology Research.2021;8(1):10-14.

36] Youssef EMI, Ali HAA, Ewieda GH, EL-fatah WM, Khouly NE. Research in cancer and tumor. 2014;3(1)19-25.

37] Saarnio E, Pekkinen M, Itkonen ST, Kemi V, Karp H, Ivaska KK, Risteli J, Koivula MK, Kärkkäinen M, Mäkitie O, Sievänen H. Low free 25-hydroxyvitamin D and high vitamin D binding protein and parathyroid hormone in obese Caucasians. A complex association with bone. PLoS One. 2018 Feb 28;13(2):e0192596.

38] Elsafi SS, Nour BM, Abakar AD, Omer IH, Almugadam BS. Vitamin D level and it is association with the severity of pulmonary tuberculosis in patients

attended to Kosti Teaching Hospital, Sudan. AIMS microbiology. 2020;6(1):64.

39] Sajo EA, Okunade KS, Olorunfemi G, Rabiu KA, Anorlu RI. Serum vitamin D deficiency and risk of epithelial ovarian cancer in Lagos, Nigeria.cancer medical science.2020;14.

40] Arafat ES, Taha IM, Kattan SW, Babteen N, Fawzy I. Associations between Vitamin D and Type 2 Diabetes Mellitus: The Role of Vitamin D Receptor and Binding Protein. Journal of Diabetes Mellitus. 2020 Oct 20;10(04):222.

41] Fouda MS, Mekkawy SA, Ghabour MT, Othman RE, Ahmed NA, Habbib NA, Elkholey SA, Soliman RM, Fouad MC, Ayad EY, Shaqran MA. Evaluation of diagnostic performances of leptin and vitamin D for colorectal cancer diagnosis and follow-up. Journal of Bioscience and Applied Research. 2021 Sep 28;7(3):108-15.

42] Dehghani M, Khajeh A, Vojdani R, Sanei M, Keshavarz P, Namdari N. Serum Vitamin D3 Levels in Chronic Lymphocytic Leukemia and Its Relevance with Clinical Prognostic Factors. Shiraz E-Medical Journal. 2021 Jul 31;22(7).

43] Varikasuvu SR, Aloori S, Varshney S, Bhongir AV. Decreased circulatory levels of Vitamin D in Vitiligo: a meta-analysis. Anais Brasileiros de Dermatologia.2021 Jul 26;96:284-94.

44] Cho M, Peddi PF, Ding K, Chen L, Thomas D, Wang J, Lockhart AC, Tan B, Wang-Gillam A. Vitamin D deficiency and prognostics among patients with pancreatic adenocarcinoma. Journal of translational medicine. 2013 Dec;11(1):1-6.

45] Xie DD, Chen YH, Xu S, Zhang C, Wang DM, Wang H, Chen L, Zhang ZH, Xia MZ, Xu DX, Yu DX. Low vitamin D status is associated with inflammation in patients with prostate cancer. Oncotarget. 2017 Mar 28;8(13):22076.

46] Song GG, Lee YH. Vitamin D receptor FokI, BsmI, ApaI, and TaqI polymorphisms and susceptibility to ovarian cancer: a meta-analysis. Immunological investigations. 2013 Oct 1;42(7):661-72.

47] Shamsi U, Khan S, Azam I, Habib Khan A, Maqbool A, Hanif M, Gill T, Iqbal R, Callen D. A multicenter case control study of association of vitamin D with breast cancer among women in Karachi, Pakistan. PLoS One. 2020 Jan 22;15(1):e0225402.

48] Zhang L, Wang S, Che X, Li X. Vitamin D and lung cancer risk: a comprehensive review and meta-analysis. Cellular Physiology and Biochemistry. 2015;36(1):299-305.

49] Latacz M, Rozmus D, Fiedorowicz E, Snarska J, Jarmołowska B, Kordulewska N, Savelkoul H, Cieślińska A. Vitamin D receptor (VDR) gene polymorphism in patients diagnosed with colorectal cancer. Nutrients. 2021 Jan;13(1):200.

50] Fedirko V, Duarte-Salles T, Bamia C, Trichopoulou A, Aleksandrova K, Trichopoulos D, Trepo E, Tjønneland A, Olsen A, Overvad K, Boutron-Ruault MC. Prediagnostic circulating vitamin D levels and risk of hepatocellular carcinoma in European populations: a nested case-control study. Hepatology. 2014 Oct;60(4):1222-30.

51] Liyanage UE, Law MH, Melanoma Meta-analysis Consortium, Barrett JH, Iles MM, MacGregor S. Is there a causal relationship between vitamin D and melanoma risk? A Mendelian randomization study. British Journal of Dermatology. 2020 Jan;182(1):97-103.

52] Gunduz M, Yikilmaz AS, Akinci S, Dilek I. Importance of vitamin d level in early-stage chronic lymphocytic leukemia and its comparison with healthy population. Medical Journal of Islamic World Academy of Sciences. 2018;26(1):6-9.

53] Deschasaux M, Souberbielle JC, Latino-Martel P, Sutton A, Charnaux N, Druesne-Pecollo N, Galan P, Hercberg S, Le Clerc S, Kesse-Guyot E, Ezzedine K. Prospective associations between vitamin D status, vitamin D–related gene polymorphisms, and risk of tobacco-related cancers. The American Journal of Clinical Nutrition. 2015 Nov 1;102(5):1207-15.

54] Mostafa BE, Abdelmageed HM, El-Begermy MM, Taha MS, Hamdy TA, Omran A, Lotfy N. Value of vitamin D assessment in patients with head and neck squamous cell cancer before treatment. The Egyptian Journal of Otolaryngology. 2016 Oct;32(4):279-86.

55] Udeabor SE, Albejadi AM, Al-Shehri WA, Onwuka CI, Al-Fathani SY, Al Nazeh AA, Aldhahri SF, Alshahrani FA. Serum levels of 25-hydroxy-vitamin D in patients with oral squamous cell carcinoma: Making a case for chemoprevention. Clinical and Experimental Dental Research. 2020 Aug;6(4):428-32.

56] Sufiawati I, Putra IN, Herawati DM, Indrati AR. Low Serum 25-hydroxyvitamin D Levels in Oral Cancer Patients. Journal of International Dental and Medical Research. 2021;14(1):216-20.

57] Pu Y, Zhu G, Xu Y, Zheng S, Tang B, Huang H, Wu IX, Huang D, Liu Y, Zhang X. Association between vitamin d exposure and head and neck cancer: a systematic review with meta-analysis. Frontiers in immunology. 2021:144.

58] Hiatt RA, Krieger N, Lobaugh B, Drezner MK, Vogelman JH, Orentreich N. Prediagnostic serum vitamin D and breast cancer. JNCI: Journal of the National Cancer Institute. 1998 Mar 18;90(6):461-3.

59] Wong YY, Hyde Z, McCaul KA, Yeap BB, Golledge J, Hankey GJ, Flicker L. In older men, lower plasma 25-hydroxyvitamin D is associated with reduced incidence of prostate, but not colorectal or lung cancer. PLoS One. 2014 Jun 20;9(6):e99954.

60] Bakan AB, Uzun N, Arli SK, Yıldız M. The relationship between the cotinine level in urine and vitamin D in the university students. Belitung Nursing Journal. 2020 Dec 21;6(6):214-9.

61] Manavi KR, Alston-Mills BP, Thompson MP, Allen JC. Effect of serum cotinine on vitamin D serum concentrations among American females with different ethnic backgrounds. Anticancer Research. 2015 Feb 1;35(2):1211-8.

62] Lipworth L, Rossi M, McLaughlin JK, Negri E, Talamini R, Levi F, Franceschi S, La Vecchia C. Dietary vitamin D and cancers of the oral cavity and esophagus. Annals of oncology. 2009 Sep 1;20(9):1576-81.

63] Arem H, Weinstein SJ, Horst RL, Virtamo J, Yu K, Albanes D, Abnet CC. Serum 25-hydroxyvitamin D and risk of oropharynx and larynx cancers in Finnish men. Cancer Epidemiology and Prevention Biomarkers. 2011 Jun 1;20(6):1178-84.

64] Dev R, Del Fabbro E, Schwartz GG, Hui D, Palla SL, Gutierrez N, Bruera E. Preliminary report: vitamin D deficiency in advanced cancer patients with symptoms of fatigue or anorexia. The oncologist. 2011 Nov;16(11):1637-41.

65] Grimm M, Alexander D, Munz A, Hoffmann J, Reinert S. Is 1, 25-dihydroxyvitamin D3 receptor expression a potential Achilles' heel of CD44+ oral squamous cell carcinoma cells?. Targeted oncology. 2013 Sep;8(3):189-201.

66] Fanidi A, Muller DC, Midttun Ø, Ueland PM, Vollset SE, Relton C, Vineis P, Weiderpass E, Skeie G, Brustad M, Palli D. Circulating vitamin D inrelation to cancer incidence and survival of the head and neck and oesophagus in the EPIC cohort. Scientific reports. 2016 Nov 4;6(1):1-1.

67] Adisa AO, Udeabor SE, Ogun GO, Orlowska A, Sader RA, Ghanaati S. Immunohistochemical expression of vitamin-D receptor in oral and skin squamous cell carcinoma of a black African subpopulation. Medical Journal of Zambia. 2017;44(2):64-8.

68] Laczmanski L, Laczmanska I, Lwow F. Association of select vitamin D receptor gene polymorphisms with the risk of tobacco-related cancers–a meta-analysis. Scientific reports. 2019 Nov 5;9(1):1-7.

69] Pandey A, Singh A, Singh S. Prevalence of Vitamin D deficiency in treatment-naive individual consecutive cancer patients. Cancer Research, Statistics, and Treatment. 2020 Jan 1;3(1):25.

70] Kumar S, Kumar S, Kumari A. Study of Serum Malondialdehyde and Calcitriol Level in Oral Submucus Fibrosis Patients. International journal of contemporary medical research. 2015;4(1):178-80.

71] Ogunkolade WB, Boucher BJ, Bustin SA, Burrin JM, Noonan K, Mannan N, Hitman GA. Vitamin D metabolism in peripheral blood mononuclear cells is influenced by chewing "betel nut"(Areca catechu) and vitamin D status. The Journal of Clinical Endocrinology & Metabolism. 2006 Jul 1;91(7):2612-7.

72] Grimnes G, Almaas B, Eggen AE, Emaus N, Figenschau Y, Hopstock LA, Hutchinson MS, Methlie P, Mihailova A, Sneve M, Torjesen P. Effect of smoking on the serum levels of 25-hydroxyvitamin D depends on the assay employed. European journal of endocrinology. 2010 Aug 1;163(2):339.

73] Hysi L, Rexha T, Mitre A. The effects of smoking on vitamin D status, PTH and calcium in postmenopausal women with problems of osteoporosis. The 1st International conference on "Research and education – Challenges towards the future". 2013.

74] Garg A, Chaturvedi P, Gupta PC. A review of the systemic adverse effects of areca nut or betel nut. Indian Journal of Medical and Paediatric Oncology. 2014 Jan;35(01):3-9.

75] Kassi EN, Stavropoulos S, Kokkoris P, Galanos A, Moutsatsou P, Dimas C, Papatheodorou A, Zafeiris C, Lyritis G. Smoking is a significant determinant

of low serum vitamin D in young and middle-aged healthy males. Hormones. 2015 April;14(2):241-50.

76] Shinkov A, Borissova AM, Dakovska L, Vlahov J, Kassabova L, Svinarov D. Winter 25-hydroxyvitamin D levels in young urban adults are affected by smoking, body mass index and educational level. European Journal of Clinical Nutrition. 2015 March;69(3):355-60.

77] Jiang CQ, Chan YH, Xu L, Jin YL, Zhu T, Zhang WS, Cheng KK, Lam TH. Smoking and serum vitamin D in older Chinese people: cross-sectional analysis based on the Guangzhou Biobank Cohort Study. BMJ open. 2016 Jun 1;6(6):e010946.

78] Khalid N, Ahmad MU, Farooq U, Asghar U. Prevalence of Vitamin D Deficiency in Young Smokers. Pakistan journal of medical and health sciences. 2018 Jan 1;12(1):183-6.

79] Kamboj P, Dwivedi S, Toteja GS. Prevalence of hypovitaminosis D in India & way forward. The Indian journal of medical research. 2018 Nov;148(5):548.

80] Paladugu L, PV A, Mureboina J, Tummala AC. Study of serum vitamin D2 and calcium in young and middle aged healthy male smokers in rural tertiary care center. International journal of advances in medicine.2022;7(2)308-313.

81] Jawad IH, Baiee HA. Cigarette smoking and serum level of Vitamin D among older adults. Medical Journal of Babylon. 2020 Jul 1;17(3):267.

82] Yang L, Zhao H, Liu K, Wang Y, Liu Q, Sun T, Chen S, Ren L. Smoking behavior and circulating vitamin D levels in adults: A meta-analysis. Food Science & Nutrition. 2021 Oct;9(10):5820-32.

83] Yuan L, Ni J. National Trends in Tobacco Smoke Exposure and its Disruption on Vitamin D Levels Among US Population, 2001-2014.

84] More CB, Gupta S, Joshi J, Varma SN. Classification system for oral submucous fibrosis. Journal of Indian Academy of Oral Medicine and Radiology. 2012;24(1):24.

85] Glick M. Burket's oral medicine. People's Medical Publishing House—USA Shelton, Connecticut. 12th edition, 2015:182-83.

86] Zerwekh JE. Blood biomarkers of vitamin D status. The American journal of clinical nutrition. 2008 April 1;87(4):1087S-91S.

87] Duffy MJ, Murray A, Synnott NC, O'Donovan N, Crown J. Vitamin D analogues: Potential use in cancer treatment. Critical reviews in oncology/ hematology. 2017 April 1 ;112:190-7.

88] Tsuprykov O, Chen X, Hocher CF, Skoblo R, Yin L, Hocher B. Why should we measure free 25 (OH) vitamin D?. The Journal of steroid biochemistry and molecular biology. 2018 Jun 1;180:87-104.

89] Gunjaliya A, Patil R, Vaza J, Patel H, Maniyar A. Prevalence of vitamin D deficiency in higher socioeconomical class of Ahmedabad, Gujarat, India. International Journal of Medical Science and Public Health. 2015;4(5):617-20.

90] Chhetri N, Chhetri A, Bhattacharya GC, Mukherjee A, Sen S, Kumar A. Vitamin D status in outpatient department patients: a retrospective study. International Journal of Research in Medical Sciences. 2016 Jun;4(6):2276.

91] Bachhel R, Singh NR, Sidhu JS. Prevalence of vitamin D deficiency in north-west Punjab population: A cross-sectional study. International Journal of Applied and Basic Medical Research. 2015 Jan;5(1):7.

92] Suryanarayana P, Arlappa N, Sai Santhosh V, Balakrishna N, Lakshmi Rajkumar P, Prasad U, Raju BB, Shivakeseva K, Divya Shoshanni K, Seshacharyulu M, Geddam JB. Prevalence of vitamin D deficiency and its associated factors among the urban elderly population in Hyderabad metropolitan city, South India. Annals of human biology. 2018 Feb 17;45(2):133-9.

93] Rattan R, Sahoo D, Mahapatra S. Prevalence of Vitamin D deficiency in adults in the coastal regions of Odisha, India. IOSR J Pharm Biol Sci. 2016;11:49-52.

94] Villa A, Woo SB. Leukoplakia—a diagnostic and management algorithm. Journal of oral and maxillofacial surgery. 2017 April 1;75(4):723-34.

95] Lorini L, Bescós Atín C, Thavaraj S, Müller-Richter U, Alberola Ferranti M, Pamias Romero J, Sáez Barba M, de Pablo García-Cuenca A, Braña García I, Bossi P, Nuciforo P. Overview of oral potentially malignant disorders: from risk factors to specific therapies. Cancers. 2021;13(15):3696.

96] Hama T, Norizoe C, Suga H, Mimura T, Kato T, Moriyama H, Urashima M. Prognostic significance of vitamin D receptor polymorphisms in head and neck squamous cell carcinoma. PLoS One. 2011 Dec 29;6(12):e29634.

97] Bikle DD. Vitamin D and the skin. Journal of bone and mineral metabolism. 2010 March;28(2):117-30.

ANNEXURE I

CASE HISTORY PROFORMA

Registration No.: Date:

Name: Age/Sex:

Address:

Religion: Contact No.:

Education: Illiterate/ Literate

Martial status: Occupation:

Economic status: low/ moderate/ high

Chief Complaint:

History of present illness:

Medical history:

H/O major illness:

H/O allergy:

Current medical treatment if any:

Past dental history:

Family history:

Personal history:

Diet: vegetarian/ non- vegetarian/ mixed

Food habits:

Consumption of spicy food: occasional/ regular/ slight/ moderate/ severe

Green/ Red chillies: slight/ moderate/ severe

Fruits and Green vegetables: Regular/ occasional

Habits: Frequency Quantity Since Location

Per day per day if any

Arecanut chewing:

Kharra chewing:

Tobacco chewing:

Other, specify -

Smoking: Bidi/ Cigarette -

Alcohol -

Oral hygiene habits:

Cleaning teeth with: tobacco/ toothpaste/ coal/ snuff/ powder/ salt/ other

With tooth brush/ finger/ datoon

General Examination:

Built: Gait: Height: Weight:

Temperature: Pulse: Respiratory rate: Blood pressure:

Pallor: Icterus: Cyanosis:

Odema: Generalised lymphadenopathy:

Extraoral Examination:

Facial symmetry:

Eyes : Ears : Nose : Head :

TMJ :

Lymph nodes :

1) Region involved – submental/ submandibular/ parotid/ cervical

2) Side involved- ipsilateral/ contralateral/ bilateral

3) Size -

4) Number and condition - Solitary/ multiple (Discrete/ fused)

5) Consistency - Soft/ hard/ firm

6) Pain - Tender/ Non – tender

7) Relation with surrounding structure - Fixed/ freely movable.

Intraoral Examination:

Hard Tissue Examination:

1. Teeth:

2. Occlusion:

3. Attrition:

4. Erosion:

5. Abrasion:

6. Caries:

7. Tenderness:

8. Root piece:

9. Fracture:

10. Restored teeth:

11. Stains/ calculus:

12. Prosthesis:

LOCAL EXAMINATION FOR ORAL CANCER

Extraoral examination:

Nature: Growth/ ulcer/ ulcero-proliferative/ swelling

Inspection:

1) Site

2) Size

3) Shape

5) Colour

6) Overlying surface

7) Surrounding skin

Palpation:

1) Consistency

2) Tenderness

3) Induration of floor

4) Fixed to underlying structure

Intraoral examination –

Inspection –

Palpation –

Provisional Diagnosis :

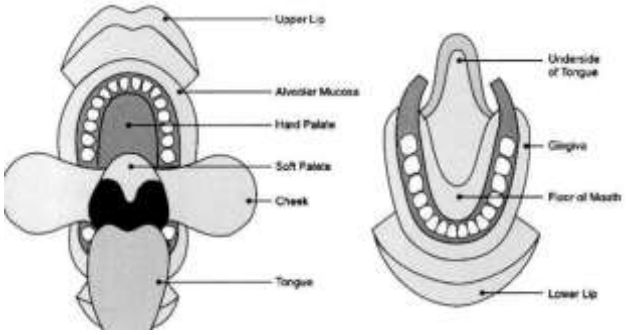
Investigations

Radiographic examination -

Hematological investigations –

Histopathological Diagnosis –

Final Diagnosis -



LOCAL EXAMINATION FOR ORAL LEUKOPLAKIA

Inspection –

1. Site
2. Size /Extent
3. Shape
4. Surrounding area

Palpation –

1. Texture
2. Consistency

Provisional Diagnosis -

Investigations

Hematological investigations -

Histopathological diagnosis -

Final Diagnosis -

LOCAL EXAMINATION FOR ORAL SUBMUCOUS FIBROSIS

Mouth opening – Interincisal distance - mm

Examination of Mucosa

1) Blanching
2) Pigmentation- increased/ decreased
3) Erythematous areas-
4) Ulcerations –
5) Fibrous bands – single/ multiple/ broad/ cirum oral/ vertical/ unilateral/ bilateral
6) Uvula – Normal/ atropic
7) Tongue protrusion – normal/ incisal tip/ up to vermilion border of lower lip/ beyond mucocutaneous junction.

Provisional diagnosis –

INVESTIGATIONS :

Haematological investigations -

Histopathological investigations -

Final Diagnosis -

Serum vitamin D level - ng/ml.

ANNEXURE II

INFORMED CONSENT FORM

Evaluation of serum Vitamin D levels in patients with oral leukoplakia, oral submucous fibrosis and oral squamous cell carcinoma.

Patients I.D.:

I, Mr./Master/Mrs./Miss._____

Resident of: _____aged _____ years, exercising my free will/choice, without any pressure/lure of incentive in any form, hereby give my consent/consent on behalf of patient named Mr./Master/Mrs./Miss._____

Resident of: _____aged _____ years,

as his/her _____.

I acknowledge that doctor has informed me about this research project suitably and sufficiently to my satisfaction. I agree to let my X-rays, photographs, impressions and other investigations to be taken as required. I agree to take part in this project and will not mix any other projects during the period of this trial. I shall report to the dental hospital or other place where called on given appointment dates and time. I shall inform the doctor on any adverse effects or unusual symptoms noticed by me. I shall co-operate with the doctors and paramedical staff, in all respects. I permit to publishing the results of my participation in this study. I shall not be given any reimbursement or compensation. I have been informed of my right to opt out of this research project at any time without giving any reason for doing so.

I hereby record my consent for participation in the said trial.

1.	_____	_____	_____	_____
	Patient's name	Signature/thumbprint	Date	Time
2.	Or _____	_____	_____	_____
	Person providing consent	Signature/thumbprint	Date	Time
3.	_____	_____	_____	_____
	Witness name	Signature	Date	Time
4.	_____	_____	_____	_____
	Investigator's name	Signature	Date	Time

INFORMED CONSENT FORM

Evaluation of serum Vitamin D levels in patients with oral leukoplakia, oral submucous fibrosis and oral squamous cell carcinoma.

वैयक्तिक जाणकारी

मरीज का नाम :

उमर / लिंग :

पत्ता :

मोबाईल नंबर :

मैं मानता हू कि चिकित्सक ने मुझे इस शोध परियोजना के बरे मी उपयुक्त और पर्याप्त रूप से मेरी संतुष्टी के बारे में बताया है. मै अपने एक्स रे, फोटो, इंप्रेशन और अन्य जांचो को जरुरी रूप में लेणे के लिये सहमत हू. मै इस परियोजना में भाग लेणे के लिये सहमत हूं और इस परीक्षण कि अवधिके दौरान किसी भी अन्य परियोजनाओ को मिला नही करेगा. मैं सभी मामलो में डॉक्टर और पेरामेडिकल स्टाफ के साथ मिलकर काम करुंगा. मैं इस अध्ययन में अपनी भागीदारी के परिणामोको प्रकाशित करणे कि अनुमती देता हूं. मुझे कोई प्रतीपूर्ती या क्षतीपूर्ती नही दि जायेगी. मुझे ऐसा करणे के लिये किसी भी कारण के बिना किसी भी समय इस शोधपरीयोजनासे ऑप्टआऊट करणे का मेरे अधिकार के बरे में सूचित किया गया है. मैं एतद्वारा परीक्षण में भाग लेणे के लिये मेरी सहमती रिकॉर्ड करता हूं.

_____	_____	_____	_____
मरीज का नाम	सही	तारीख	समय
_____	_____	_____	_____
साक्षीदार का नाम	सही	तारीख	समय
_____	_____	_____	_____
डॉक्टर का नाम	सही	तारीख	समय

INFORMED CONSENT FORM

Evaluation of serum Vitamin D levels in patients with oral leukoplakia, oral submucous fibrosis and oral squamous cell carcinoma

वैयक्तीक माहिती

रूग्णाचे नाव :
वय/लिंग :
पत्ता :

दिनांक :

मोबाईल नंबर :

मी कबूल करतो की डॉक्टरांनी मला या संशोधन प्रकल्पाबद्दल समाधानकारक माहिती दिली आहे. मी माझ्या एक्स-रे, छायाचित्रे, इंप्रेशन आणि आवश्यकतेनुसार अन्य तपासण्या करण्यास सहमत आहे. मी या प्रकल्पात भाग घेण्यास सहमती देतो आणि या चाचणीच्या कालावधीत कोणतेही अन्य प्रकल्प एकत्रित करणार नाही. मला डेन्टल हॉस्पिटल किंवा इतर ठिकाणी दिलेल्या भेटीची तारीख आणि वेळ सांगितली आहे. मी डॉक्टर आणि पॅरामेडिकल कर्मचा-यांना सर्व बाबतीत सहकार्य करेल. या अभ्यासात मी माझ्या सहभागाचे निकाल प्रकाशित करण्यास परवानगी देतो. मला कोणतीही नुकसान भरपाई दिली जाणार नाही. असे करण्यासाठी कोणतेही कारण न देता मला कोणत्याही वेळी या संशोधन प्रकल्पातून बाहेर पडण्याचा अधिकार मिळालेला आहे. मी या अन्वये केलेल्या चाचणीत सहभागासाठी माझी संमती नोंदवित आहे.

_____	_____	_____	_____
१) रूग्णाचे नाव	स्वाक्षरी	तारीख	वेळ
_____	_____	_____	_____
२) साक्षीदाराचे नाव	स्वाक्षरी	तारीख	वेळ
_____	_____	_____	_____
३) डॉक्टरचे नाव	स्वाक्षरी	तारीख	वेळ

MASTER SHEET

SR.NO	AGE	GENDER	HABITS	DIAGNOSIS	VITAMIN D LEVELS
1	22	male	no	normal	39.0 ng/ml
2	35	female	no	normal	36.8 ng/ml
3	45	female	no	normal	21.5 ng/ml
4	37	female	no	normal	25.5 ng/ml
5	51	female	no	normal	19.5 ng/ml
6	29	female	no	normal	45.5 ng/ml
7	27	female	no	normal	37.0 ng/ml
8	32	male	no	normal	30.0 ng/ml
9	28	male	no	normal	46.0 ng/ml
10	30	male	no	normal	42.5 ng/ml
11	32	male	no	normal	30.5 ng/ml
12	55	female	no	normal	21.6 ng/ml
13	35	male	no	normal	32.5 ng/ml
14	40	male	no	normal	37.0 ng/ml
15	26	female	no	normal	46.6 ng/ml
16	35	male	no	normal	45.5 ng/ml
17	29	male	no	normal	42.6 ng/ml
18	35	female	no	normal	37.0 ng/ml
19	36	male	no	normal	35.5 ng/ml
20	42	male	no	normal	22.6 ng/ml
21	45	female	no	normal	20.0 ng/ml
22	45	male	no	normal	26.5 ng/ml
23	40	male	no	normal	25.1 ng/ml
24	39	female	no	normal	33.6 ng/ml
25	30	male	no	normal	36.0 ng/ml
26	53	male	no	normal	18.5 ng/ml
27	52	female	no	normal	20.0 ng/ml
28	49	female	no	normal	21.0 ng/ml
29	50	male	no	normal	22.6 ng/ml
30	34	female	no	normal	35.5 ng/ml

SR.NO	AGE	GENDER	HABITS	DIAGNOSIS	VITAMIN D LEVELS
31	25	male	K	Stains +	28.1 ng/ml
32	21	male	T	Stains++, calculus +	30.5 ng/ml
33	48	female	A	Stains +++	18.0 ng/ml
34	60	male	T	Stains +,calculus ++	06.5 ng/ml
35	24	male	K	Stains++	33.5 ng/ml
36	34	male	A	Stains ++,calculus +	15.0 ng/ml
37	21	male	K	Stains +, calculus+	28.5 ng/ml
38	21	male	K	Stains ++	25.6 ng/ml
39	29	male	K	Stains +++	31.5 ng/ml
40	25	male	S	Stains +++	22.0 ng/ml
41	25	male	A	Stains +,calculus ++	15.0 ng/ml
42	27	male	A,S	Stains++,calculus+	22.2 ng/ml
43	50	female	K	Stains ++,calculus+	18.0 ng/ml
44	24	male	K	Stains++	25.5 ng/ml
45	42	female	T	Stains+, calculus+	16.0 ng/ml
46	27	male	K	Stains+,calculus++	30.5 ng/ml
47	50	male	T,A	Stains++, calculus+	18.0 ng/ml
48	56	male	K	Stains++	12.0 ng/ml
49	40	female	A	Stains ++	20.0 ng/ml
50	29	male	S	Stains+++	06.5 ng/ml
51	35	female	A	Stains+,calculus+	27.3 ng/ml
52	26	female	K	Stains++,calculus+	16.7 ng/ml
53	30	female	T	Stains+,calculus+	13.1 ng/ml
54	40	female	A	Stains++	09.5 ng/ml
55	35	female	K	Stains++,calculus+	18.0 ng/ml
56	40	female	T	Stains+,calculus++	16.9 ng/ml
57	33	female	K	Stains+++	09.3 ng/ml
58	34	female	A	Stains++,calculus+	27.1 ng/ml
59	30	male	K	Stains+,calculus+	24.7 ng/ml
60	41	male	K,A	Stains+++	20.5 ng/ml
61	55	female	A	OL	09.0 ng/ml

SR.NO	AGE	GENDER	HABITS	DIAGNOSIS	VITAMIN D LEVELS
62	46	male	K	OL	10.0 ng/ml
63	57	male	K	OL	05.5 ng/ml
64	40	male	T,S	OL	19.1 ng/ml
65	52	male	T,A	OL	10.0 ng/ml
66	45	female	K	OL	15.5 ng/ml
67	40	male	T	OL	12.6 ng/ml
68	50	female	T	OL	10.0 ng/ml
69	49	male	A	OL	18.0 ng/ml
70	39	female	K	OL	12.0 ng/ml
71	51	male	K	OL	15.5 ng/ml
72	51	male	T,S	OL	10.0 ng/ml
73	42	female	K	OL	22.2 ng/ml
74	35	male	K	OL	18.0 ng/ml
75	49	male	T	OL	17.0 ng/ml
76	50	female	T,A	OL	10.1 ng/ml
77	33	male	K	OL	21.0 ng/ml
78	42	male	T,A	OL	16.5 ng/ml
79	35	male	T	OL	15.0 ng/ml
80	42	male	A,K	OL	10.0 ng/ml
81	52	male	T	OL	12.5 ng/ml
82	45	male	K	OL	21.0 ng/ml
83	44	female	K	OL	15.0 ng/ml
84	36	male	K	OL	18.5 ng/ml
85	32	male	T	OL	13.6 ng/ml
86	45	male	K	OL	20.0 ng/ml
87	55	female	T	OL	11.5 ng/ml
88	45	male	K	OL	15.0 ng/ml
89	40	male	T,K	OL	11.2 ng/ml
90	41	male	T	OL	15.5 ng/ml
91	25	male	K	OSMF group II	18.0 ng/ml
92	55	male	K	OSMF group II	10.5 ng/ml

SR.NO	AGE	GENDER	HABITS	DIAGNOSIS	VITAMIN D LEVELS
93	46	male	A	OSMF group II	08.2 ng/ml
94	25	male	T,A	OSMF group II	26.1 ng/ml
95	52	male	T	OSMF group II	07.9 ng/ml
96	45	male	A	OSMF group II	15.5 ng/ml
97	38	male	K,S	OSMF group II	20.0 ng/ml
98	32	female	K,A	OSMF group II	18.0 ng/ml
99	33	female	K	OSMF group II	18.5 ng/ml
100	52	female	A	OSMF group II	12.2 ng/ml
101	30	male	K	OSMF group III	10.2 ng/ml
102	40	male	A	OSMF group III	05.8 ng/ml
103	54	female	A	OSMF group III	06.6 ng/ml
104	29	male	K	OSMF group III	26.8 ng/ml
105	23	male	K	OSMF group III	16.1 ng/ml
106	27	male	K	OSMF group III	23.0 ng/ml
107	45	female	A	OSMF group III	21.5 ng/ml
108	39	female	K	OSMF group III	15.0 ng/ml
109	30	female	A	OSMF group III	08.7 ng/ml
110	27	male	A	OSMF group III	18.5 ng/ml
111	45	female	A	OSMF group IV	10.0 ng/ml
112	23	male	A,K	OSMF group IV	18.0 ng/ml
113	29	male	A,S	OSMF group IV	28.0 ng/ml
114	44	female	K	OSMF group IV	11.5 ng/ml
115	50	female	A	OSMF group IV	10.5 ng/ml
116	39	male	K,A	OSMF group IV	19.5 ng/ml
117	52	female	K	OSMF group IV	10.0 ng/ml
118	44	male	A	OSMF group IV	09.7 ng/ml
119	40	female	A	OSMF group IV	13.9 ng/ml
120	35	female	A	OSMF group IV	12.0 ng/ml
121	43	male	K	OSCC	09.1 ng/ml
122	38	male	T	OSCC	10.0 ng/ml
123	40	male	K	OSCC	15.1 ng/ml

SR.NO	AGE	GENDER	HABITS	DIAGNOSIS	VITAMIN D LEVELS
124	42	male	K	OSCC	19.7 ng/ml
125	52	female	K	OSCC	09.4 ng/ml
126	40	male	K	OSCC	05.0 ng/ml
127	37	male	K	OSCC	18.0 ng/ml
128	35	male	K	OSCC	15.5 ng/ml
129	52	female	T	OSCC	04.5 ng/ml
130	49	female	T	OSCC	12.5 ng/ml
131	48	male	K	OSCC	10.0 ng/ml
132	56	female	K	OSCC	08.0 ng/ml
133	40	male	T	OSCC	12.0 ng/ml
134	56	male	T	OSCC	05.4 ng/ml
135	42	female	T	OSCC	18.0 ng/ml
136	35	male	K	OSCC	12.5 ng/ml
137	45	male	T	OSCC	18.0 ng/ml
138	51	female	K	OSCC	12.5 ng/ml
139	38	male	K	OSCC	18.0 ng/ml
140	32	male	T	OSCC	08.0 ng/ml
141	49	female	T,A	OSCC	18.0 ng/ml
142	60	male	T	OSCC	06.0 ng/ml
143	50	female	K	OSCC	10.0 ng/ml
144	36	male	T	OSCC	10.0 ng/ml
145	46	female	K	OSCC	09.1 ng/ml
146	52	male	T	OSCC	08.0 ng/ml
147	50	male	T	OSCC	10.0 ng/ml
148	45	female	K	OSCC	16.5 ng/ml
149	56	male	T	OSCC	11.1 ng/ml
150	48	male	T,S	OSCC	19.5 ng/ml

K – Kharra, T- Tobacco, A – Arecanut, S – Smoking.