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# A HOSPITAL BASED PROSPECTIVE STUDY TO EVALUATE THE COMPARISON OF RELATIONSHIP BETWEEN SERUM HOMOCYSTEINE AND VITAMIN B12 IN PATIENTS HAVING ORAL SUBMUCOUS FIBROSIS (OSMF) & ORAL LICHEN PLANUS WITH HEALTHY INDIVIDUALS AT NEWLY ESTABLISHED TERTIARY CARE CENTER

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## ABSTRACT

**Background:** Oral Submucous fibrosis (OSMF) and Lichen planus (LP) are chronic diseases of the oral mucosa that have different causes and manifestations. Serum homocysteine (Hcy), a nonessential amino acid, is considered as a helpful indicator of vitamin status for its strong correlation with Vitamin B12. Although Hcy levels in oral submucous fibrosis (OSMF) have been studied, the relationship between Hcy and Vitamin B12 has not been studied yet. This study is the first one to compare and correlate the levels of serum Hcy and Vitamin B12 in OSMF & LP patients.

**Materials and Methods:** This study is hospital based prospective study done on 60 participants and was divided into three groups. Group 1 comprised 20 patients, clinically diagnosed with OSMF, Group 2 comprised 20 patients with oral lichen planus and Group 3 comprised 20 healthy controls. The patients were selected from the Outpatient Department of Dentistry & Department of Skin & VD at RVRS Medical college, Bhilwara, Rajasthan, India during one-year period. Serum Hcy and Vitamin B12 estimation were done by chemiluminescence immunoassay. These levels in OSMF patients & LP patients were compared and correlated with corresponding levels in healthy controls.

**Results:** Hcy levels were elevated in OSMF & LP and were found to be statistically significant as compared to healthy controls. On the contrary, although Vitamin B12 levels were found to be higher in healthy controls, the difference was statistically nonsignificant. A significant correlation was found between Hcy and Vitamin B12 in between groups, i.e., decreased Vitamin B12 levels led to elevated Hcy levels but vice versa was not found.

**Conclusion:** Chronic inflammation in OSMF & LP leads to hyperhomocysteinemia, which may also be seen in cases of Vitamin B12 deficiency and certain systemic disorders. Thus, while serum Hcy could be used as biomarker for OSMF, Vitamin B12 deficiency and certain systemic disorders should be ruled out.

**Keywords:** OSMF, LP, Serum homocysteine, Vitamin B12.

## INTRODUCTION

Oral Submucous Fibrosis (OSMF) and Lichen Planus (LP) are two diseases that specifically affect the oral mucosa; even though both diseases are chronic and potentially severe they differ in their clinical manifestation as well as the aetiology.

Oral submucous fibrosis (OSMF), first described by Schwartz in 1952, is a collagen metabolic disorder and a chronic premalignant condition of the oral mucosa.[1] Pindborg and Sirsat defined OSMF as "an insidious, chronic disease affecting any part of the oral cavity and sometimes the pharynx. Although occasionally preceded by and/or associated with vesicle formation, it is always associated with juxtaepithelial inflammatory reaction followed by fibroelastic change of the lamina propria, with epithelial atrophy leading to stiffness of the oral mucosa and causing trismus and inability to eat."[2]

Another type is a site specific type and is commonly related to the practice of chewing betel quid and areca nut in South Asians and has a massive potential for malignant transformation which ranges from 7-13% of cases.[3] On the other hand, LP is a persistent inflammatory disease, whose etiology is thought to be autoimmune, involving the skin and mucosal membranes such as the oral tissue. It has been described with a broad clinical spectrum from normal-appearing white striations to painful erosive gingival pathology that adversely affects oral health and quality of life.[4,5]

Oral lichen planus (OLP), as a chronic immunologic mucocutaneous inflammatory oral mucosal disease, was associated with deficiencies of hemoglobin (Hb), iron, folic acid and vitamin B12 as well as increased blood homocysteine level.[6] Homocysteine is a sulfur-containing amino acid. It is a metabolite of methionine, another amino acid that is found in foods and transformed into homocysteine in the bloodstream. Vitamins B6 (pyridoxine), B12 and folate are essential cofactors in homocysteine metabolism. Being recycled back into methionine or converted to cysteine in the body are the two major metabolic pathways that lead to reduced homocysteine blood levels.[7,8] There are several factors that may lead to hyperhomocysteinemia, such as high methionine diets, vitamin (vitamin B12 and B6, folic acid) deficiencies, male gender, increased age, renal dysfunction and genetic abnormalities.7,8 Hyperhomocysteinemia (>15 umol/L) is also an independent cardiovascular risk factor that has been associated with atherosclerotic vascular diseases and ischemic heart attacks by a number of mechanisms of action, such as endothelial damage, promoting clot formation, decreasing the flexibility of blood vessels and reducing blood flow velocity. Homocysteine also increases levels of asymmetric dimethylarginine (ADMA), which is a natural inhibitor of nitric oxide (NO) synthase, which may increase production of superoxide. The oxidative reaction between superoxide and NO generates peroxynitrite and reduces NO which leading to endothelial dysfunction.[8,9]

Hcy is a valuable indicator for factors such as exercise, coffee drinking, smoking, vitamins and cholesterol. Only a handful of studies have shown an association of serum Hcy in PMDs of the oral cavity and elsewhere, with a few reports showing elevated Hcy levels.[10,11] Hence, the present study was undertaken to evaluate the serum levels of Hcy and Vitamin B12 in OSMF & lichen planus patients as compared to healthy controls.

## MATERIALS AND METHODS

This study is hospital based prospective study done on 60 participants and was divided into three groups. Group 1 comprised 20 patients, clinically diagnosed with OSMF, and grading was done according to the criteria given by Ranganathan et al,[12] Group 2 comprised 20 patients, clinically diagnosed with oral lichen planus and Group 3 comprised 20 healthy controls. The patients were selected from the Outpatient Department of Dentistry & Department of Skin & VD at RVRS Medical college, Bhilwara, Rajasthan, India during one year period.

Diagnostic Criteria Used for Selection of Cases

Inclusion criteria included patients between the age range of 20 and 60 years, documented clinically and histopathologically to have OSMF or LP and only those patients who gave consent to participate. Twenty healthy individuals without any habit of areca nut chewing and tobacco were included in the study. The exclusion criteria were other oral lesions, other pathological conditions which may affect the results of the study, previous history of cancer or precancerous conditions except OSMF or LP and patients on treatment of the diseases. Furthermore, patients undergoing any drug therapy capable of altering Hcy levels were not included.

**Methodology**

Data were collected through questionnaires and clinical assessment of the subjects. The demographic details included age and gender while clinical details consisted of the appearance of the lesion, its site and severity, while psychological effects elicited anxiety and levels of stress and functional oral impacts encompassed chewing, swallowing, and speaking. The clinical examination comprised an assessment of the oral cavity to capture lesion characteristics, and this was conducted by professional oral health practitioners. The degree of this lesion was determined following clinical standard severity ratings of OSMF and LP. In OSMF, according to the degree of fibrosis and restriction of mouth opening, we categorized the lesions as mildly, moderately or severely affected. In LP, the lesions were defined as reticular, erosive or ulcerative according to the clinical manifestation.

Venous blood samples were collected with aseptic precautions using spirit-soaked cotton. A volume of 5 ml of blood was drawn from the antecubital vein

(cephalic vein) using disposable syringe, and without any delay, the blood was transferred to the vacutainer. Cold centrifugation (15–17° C) at 4000 rpm was done immediately to separate the serum from the cells, and this separated serum was then subjected to chemiluminescence immunoassay (CLIA) for the estimation of Hcy and Vitamin B12.

This immunoassay is based on the specific binding of an antibody toward the Hcy enzyme conversion product, S-adenosyl-Hcy. The quantification thus achieved is through construction of a standard curve with multiple known concentrations of Hcy calibrators.

**Statistical Analysis**

Data collected were analyzed using Statistical Package for Social Science version 27. The differences between the groups were analysed with student paired 't' test.

## RESULTS

The study included a total of 60 patients. The mean age of participants in the OSMF group was 44.7 years (SD = 10.9), while the mean age in the LP group was 43.9 years (SD = 12.6) and mean age of healthy control was 45.45 years which was showing no significant difference between the groups (P>0.001). The gender distribution was similar as males comprised 60% of the OSMF group, 55% of the LP group and 50% in healthy control. [Table 1]

The duration of symptoms differed significantly between the two groups having OSMF patients reporting a mean duration of 24.3 months (SD = 7.6) as compared to 26.9 months (SD = 8.7) in LP patients (p<0.05*). Lesion characteristics also varied with 30% of OSMF patients presenting with white patches as compared to 50% of LP patients. Lesions located on the cheeks were more common in OSMF (60%) than in LP (30%). Also, severe lesions were more frequently observed in the OSMF group (40%) compared to the LP group (30%). [Table 1]

For adults, the biological reference range of serum Hcy is 5–15 µmol/L, whereas for Vitamin B12, the range is 200–900 pg/ml.

In our study, the mean Hcy levels were elevated in OSMF (46.3 ± 25.4 µmol/L), 43.5 ± 20.8 µmol/L in LP patients and were found to be statistically significant (P<0.0001*) as compared to healthy controls (31.9 ± 22.6 µmol/L). [Table 2]

The serum vitamin B12 level in the healthy control was significantly higher than in the OSMF patients & LP patients (P<0.0001). [Table 2]

**Table 1: Descriptive Statistics of Patient Demographics and Clinical Features**

| Variable | Group 1 (OSMF) (Mean±SD) (N=20) | Group 2 (LP) (Mean±SD) (N=20) | Group 3 (Healthy control) (Mean±SD) (N=20) |
|---|---|---|---|
| Age (years) | 44.7± 10.9 | 43.9 ± 12.6 | 45.45 ± 11.3 |
| Gender (Male, %) | 60% | 55% | 50% |
| Duration of Symptoms (months) | 24.3 ± 7.6 | 26.9 ± 8.7 | - |
| Lesion Appearance (White patches, %) | 30% | 50% | - |
| Lesion Location (Cheeks, %) | 60% | 30% | - |
| Lesion Severity (Severe, %) | 40% | 30% | - |

**Table 2: Difference between homocysteine levels in oral submucous fibrosis patients, Lichen planus patients and healthy controls**

| Variable | Group 1 (OSMF) (Mean±SD) (N=20) | Group 2 (LP) (Mean±SD) (N=20) | Group 3 (Healthy control) (Mean±SD) (N=20) | P-value |
|---|---|---|---|---|
| Mean Hcy levels (µmol/L) | 46.3 ± 25.4 | 43.5 ± 20.8 | 31.9 ± 22.6 | <0.0001* |
| Mean Vit. B12 levels (pg/ml) | 141.3 ± 89.6 | 138.5 ± 78.9 | 187.6 ± 145.5 | <0.0001* |

## DISCUSSION

OSMF, a PMD, has a multifactorial etiology although chewing of areca nut and tobacco are chiefly associated with this disorder in the Southeast Asian populations. It causes significant morbidity and has a malignant transformation rate of about 7%–13%.[13] The nutritional deficiencies such as iron, folates, Vitamin B12 and Vitamin B6 might not play a primary role in the etiopathogenesis, but it could synergize the symptomatology by contributing to epithelial atrophy.[14,15]

OSMF patients either present with the complaint of reduced mouth opening or with burning sensation, which results in difficulty in consumption of normal diet leading to poor nutrition. Deficiency of iron and Vitamin B complex, other trace elements and lipids, could possibly initiate anemia, alter the cell-mediated immunity and generate free radicals and reactive oxygen species from the peroxidation of lipids and induce DNA damage.[16,17] The psychological stress due to burning, pain and reduced mouth opening may lead to reduced intake of food, leading to the nutritional deficiency, consequently leading to an increase in Hcy levels.[18]

Elevated Hcy concentrations are also associated with specific pathological conditions, including cancer development, autoimmune diseases, vascular dysfunction and neurodegenerative disease. Moreover, Vitamin B12, folate and B6 are needed in the Hcy remethylation pathway and transsulfuration pathway.[19]

In earlier studies conducted by Goel et al. in 2014,[20] and Jaganath et al. in 2016,[21] Hcy was determined

using high-performance liquid chromatography; but in the present research, CLIA was used, which is much more advanced and sophisticated method used nowadays.

It can be observed that the lesion site differs based on the two diseases where the cheeks are more affected in OSMF patients than the tongue and the gums in LP patients. This finding corroborates earlier research that suggested that OSMF mainly affect the buccal mucosa because of the disease's fibrotic tendencies, while LP, being an immunologically mediated disease, commonly involves non-keratinized mucosa.[3] Given the anatomy pathological distribution of the lesions, fibrosis is considered pivotal to OSMF and it typically manifests in the oral cavity on the buccal mucosa and occasionally in the palate or the retromolar areas.[4] The present study revealed the fact that the duration of the symptoms in the LP patients was significantly higher than in the patients having OSMF. This is in line with the knowledge that LP can be a chronic and recurrent disease that has times of worsening and improvement.5 OSMF, on the other hand, presents frequently with a more progressive course, going from asymptomatic to notably worsening functional status owing to the degree of fibrosis.[22,23]

In our study, the mean Hcy levels were elevated in OSMF (46.3 ± 25.4 µmol/L), 43.5 ± 20.8 µmol/L in LP patients and were found to be statistically significant (P<0.0001*) as compared to healthy controls (31.9 ± 22.6 µmol/L). This is in accordance with the studies conducted by Bais et al. in 2013,[24] and Narang et al. in 2014,[10] on OSMF patients. In these studies, no healthy controls were taken, and it was observed that serum Hcy level was increased in all the patients irrespective of gender and age.

The serum vitamin B12 level in the healthy control was significantly higher than in the OSMF patients & LP patients (P<0.0001) in our study. These results obtained are in congruence with the study done by Chen et al. in 2015,[11] in which the Hcy concentrations and Vitamin B12 levels of oral lichen planus (OLP) patients were measured and compared with the corresponding levels in healthy controls. OLP patients showed a significantly higher mean Hcy level than healthy controls and a lower mean Vitamin B12 level when compared to healthy controls. It was found in their research that OLP patients had a significantly higher frequency of Vitamin B12 deficiency and had an abnormally elevated Hcy level than the control group.

Rasool et al. in 2012,[25] did a study and demonstrated an inverse relationship between serum levels of Vitamin B12 and Hcy in patients with functional dyspepsia. Likewise, Sun et al. in 2012,[18] and Lin et al. in 2013,[26] evaluated an intimate association of deficiency of Vitamin B12 and high blood Hcy level in patients with atrophic glossitis and burning mouth syndrome, respectively.

According to the current literature search, the relationship between serum Hcy and Vitamin B12 in OSMF has not been studied till now; therefore, this study was undertaken to compare and correlate the levels of serum Hcy and Vitamin B12 in OSMF.

## CONCLUSION

Chronic inflammation in OSMF & LP leads to hyperhomocysteinemia, which may also be seen in cases of Vitamin B12 deficiency and certain systemic disorders. Thus, while serum Hcy could be used as biomarker for OSMF, Vitamin B12 deficiency and certain systemic disorders should be ruled out

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