

Original Research

Estimation of Serum Folate and Homocysteine Levels in Oral Submucous Fibrosis – A Prospective Study

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ABSTRACT

Aim & Objective: The aim of the study was to evaluate the levels of serum folate and homocysteine in clinical stages of Oral submucous fibrosis (OSF) patients. **Methodology:** The study sample comprised of 40 clinically and histopathologically diagnosed OSF patients those who are not undergoing treatment and 10 healthy controls. OSF was categorised by clinical staging. Serum folate and homocysteine levels estimation was done by reverse phase high performance liquid chromatography analysis unit and chemiluminescence immuno assay, respectively. Results were statistically analysed using one-way ANOVA [Analysis of variance]. **Results:** Irrespective of age and gender, serum folate deficiency and high levels of homocysteine were noted in OSF patients when compared with healthy controls. **Conclusion:** Serum hyper homocysteinemia and folate deficiency were noted with the progression of the disease. Folate supplements can be given to decrease the levels of homocysteine, to arrest the progression of the disease. However, further longitudinal studies are needed to establish an association between serum homocysteine and serum folate.

KEYWORDS: Oral submucous fibrosis, Serum folate, Serum homocysteine Histo pathology, Anova, HPLC, Oxidative DNA, Carcinogenesis

INTRODUCTION

Oral submucous fibrosis (OSF), most prevalent potentially malignant disorder (PMD) in South Asian population, is a chronic insidious debilitating disease associated with areca nut chewing, an ingredient of betel quid. Alongside the role of local irritants, an equally significant aspect which needs to be weighed is the pre-conditioning of the oral mucosa following sustained, chronic iron and/or vitamin B complex deficiency.

Homocysteine is a sulphur containing non-protein amino acid with free thiol group and formed from methionine

through *S*-adenosyl methionine. It can be converted back to methionine with vitamins like folate and vitamin B12 [1].

Altered levels of serum homocysteine play a vital role in etiopathogenesis of OSF by initiating carcinogenesis through oxidative DNA damage. It can be considered sensitive functional marker of inadequate cellular folate and vitamin B12 concentration for its strong correlation with these vitamins in the metabolism of homocysteine[2].

Insufficient folic acid, secondary to iron deficiency, may cause oral mucosal alterations making it more

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susceptible for the action of local irritants and the reduction of which also causes collagenase enzyme inactivation leading to fibrosis [3].

Hence, this study was conducted to assess the levels of serum folate and homocysteine in clinical stages of OSF patients when compared with healthy controls.

MATERIALS AND METHODS

This prospective study was conducted in the Department of Oral Medicine and Radiology Government Dental College and Hospital, Hyderabad. Study comprised a total of 50 patients out of which 40 were OSF cases and 10 were healthy controls.

Inclusion Criteria

- Patients of age groups 20 to 40 years.
- Clinically and histopathologically diagnosed OSF cases before the start of treatment.
- Patients with no habit history were included as healthy subjects.

Exclusion Criteria

- Patients suffering from known systemic diseases, infections like HIV, AIDS and known malignancies were excluded.
- Patients with known nutritional deficiencies, bleeding and clotting disorders were excluded.

All the participants of the study were explained of the nature of the study, and informed consent was obtained. Detailed case history was noted and OSF patients were categorised into four subgroups (10 each) according to clinical staging given by Ranganathan *et al.*

Five millilitres of venous blood was collected in EDTA (Ethylenediaminetetraacetic acid) test tubes from case group and controls under aseptic conditions using spirit and cotton. The samples were centrifused at 2,500 rotations per minute (rpm) for 15 min. The separated sera were subjected to reverse phase high performance

liquid chromatography analysis unit and chemiluminiscence immuno assay to determine homocysteine levels and folate levels.

The results obtained were statistically analysed using one-way ANOVA and unpaired *t*-test.

RESULTS

In control group, 8 were males and 2 were females. Study group comprised of 28 males and 12 females. The mean serum homocysteine level found in males and females in OSF patients were 38.667 ± 16.64 and 39.700 ± 20.40 , indicating a slight higher value in females (Table 1).

Table 1: Comparison of mean homocysteine level in males and females of OSF patients

Gender	No. of Cases	Mean Homocysteine ($\mu\text{Mol/l}$)	<i>p</i> -Value
Males	28	38.66 ± 16.64	0.686
Females	12	39.70 ± 20.40	
Total	40	39.18 ± 18.52	

The mean homocysteine level increased gradually from stage-I to stage-IV OSF patients (stage I – 34.97 ± 17.72 ; stage II – 37.69 ± 20.07 ; stage III – 37.69 ± 20.07 ; stage IV – 43.28 ± 18.35) with a *p*-value of 0.767 indicating statistically significant difference between disease progression and mean serum homocysteine concentration (Table 2 and Figure 1).

The mean serum folate level found in males and females in OSF patients were 4.12 ± 2.59 and 3.74 ± 3.02 (*p*-value = 0.689), indicating a slight higher value in males (Table 3).

The mean serum folate level decreased gradually from stage I to stage IV OSF patients compared with healthy controls (controls – 7.87 ± 2.87 ; stage I – 5.40 ± 2.84 ; stage II – 4.22 ± 2.86 ; stage III – 3.53 ± 1.957 ; stage IV – 2.89 ± 2.75) with a *p*-value of 0.19. A statistically significant difference was noted as disease progressed (Table 4 and Figure 2).

Table 2: Comparison of mean homocysteine levels in controls and clinical stages of OSF patients

Clinical Stage	No. of Cases	Mean Homocysteine (µMol/l)	F-value	p-value
Controls	10	17.23±4.56	0.147	0.653
Stage I	10	34.97±17.72	0.382	0.767
Stage II	10	37.69±20.07		
Stage III	10	39.97±15.67		
Stage IV	10	43.28±18.35		
Total	50	38.97±17.59		

Table 4: Comparison of mean folate levels in controls and stages of OSF patients

Clinical Stage	No. of Cases	Mean Folate (ng/ml)	F-value	p-value
Controls	10	7.87±2.87	2.65	0.23
Stage I	10	5.40±2.84	1.66	0.19
Stage II	10	4.22±2.86		
Stage III	10	3.53±1.957		
Stage IV	10	2.89±2.75		
Total	50	4.01±2.69		

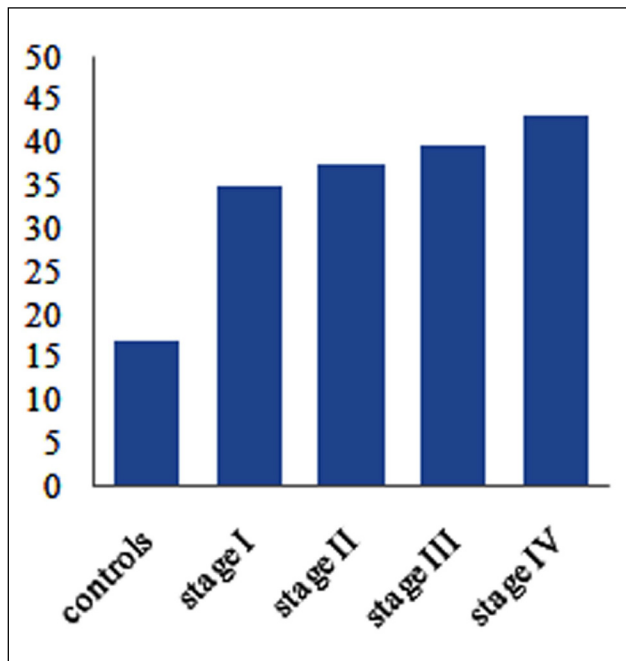


Figure 1: Comparison of serum homocysteine with clinical stages of OSF

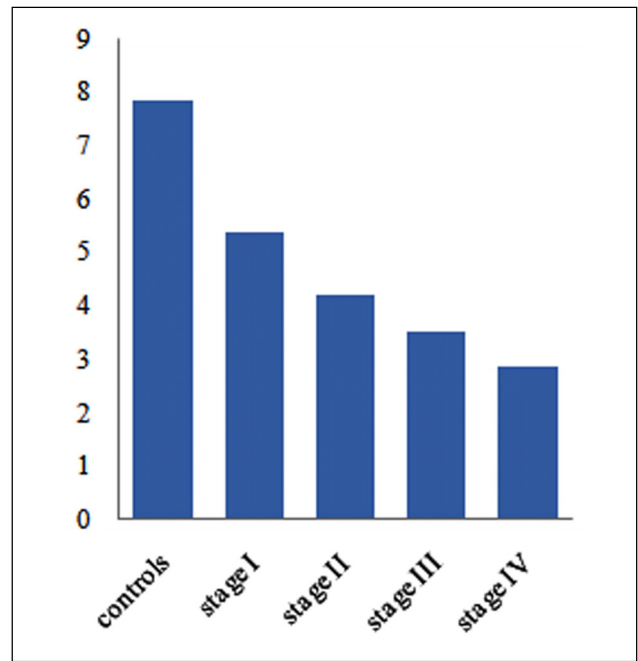


Figure 2: Comparison of serum folate values with various clinical stages of OSF

Table 3: Comparison of mean folate levels in males and females of OSF patients

Gender	No. of cases	Mean Folate (ng/ml)	p-value
Males	28	4.12±2.59	0.689
Females	12	3.74±3.02	
Total	40	3.93±2.80	

DISCUSSION

OSF is an insidious chronic disease affecting any part of 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 a fibroblastic change of the lamina propria with epithelial atrophy leading to

stiffness of mucosa and causing trismus and inability to eat [4]. It has a high rate of morbidity with a malignant transformation of 7 to 13% [5].

It is the most common PMD prevailing in south Asian decent, associated with areca nut chewing. Other associated factors like nutritional deficiencies may have synergistic action on symptoms of OSF, thus contributing to etiopathogenesis of OSF [6].

Homocysteine is a non-essential sulphur containing amino acid. Various hormonal, genetic and nutritional factors are characterised by elevations in circulating serum homocysteine levels. Homocysteine may also associate with specific pathologies including carcinogenesis, autoimmune diseases, vascular dysfunction and neurodegenerative disease [7].

In the metabolism of homocysteine, water-soluble vitamins (vitamin B12, vitamin B6, folate) play a vital role by donating one-carbon moieties for maintaining DNA stability. The deficiency of micronutrients such as folate leading to hyperhomocysteinemia causes impaired methyl transfer in the methionine cycle, disrupting the stability of normal structure of DNA, inducing chromosomal mutability, DNA strand deterioration and malignant transformation [8]. In addition, hyperhomocysteinemia promotes chronic inflammation through via oxidative stress. Increased cell adhesion molecules, elevated cytokines and chemokines secondary to increased homocysteine levels, contribute to carcinogenesis. Narang *et al.* carried out a study in OSF patients to determine if serum homocysteine could aid in the diagnosis of OSF. The mean homocysteine levels increased when compared with controls [9]. An association between serum homocysteine and clinical staging of OSF was found by Prabakar *et al.* [10]. Hallikerimath *et al.* concluded significant reduction in the folate levels in study group of OSF patients as compared with controls [3].

In the present study, it was found that as the disease progressed, serum homocysteine levels were elevated irrespective of age and gender. Conversely with the

disease progression, folate deficiency was noted indicating an association between homocysteine and folate levels. These findings were in accordance with that found in the literature. Folate supplementation may be considered to decrease serum level of homocysteine and repair DNA synthesis & methylation for preventing carcinogenesis.

CONCLUSION

The mean serum homocysteine level increased from stage I to stage IV when compared with controls, with slight higher female preponderance. The mean serum folate levels were decreased with disease progression from stage I to stage IV, with slight higher value in males. Folate supplements can be given to OSF patients to lower the homocysteine levels, thus arresting the progression of disease. However, further longitudinal studies are to be carried out to confirm an association between serum homocysteine and serum folate.

REFERENCES

- [1] Ramakrishnan KN, Sulochana S, Lakshmi R, Selvi, Angayarkanni N. Biochemistry of homocysteine in health and diseases. *Indian Journal of Biochemistry & Biophysics* 2006;43:275–83.
- [2] Wollina U, Verma SB, Ali FM, Patil K. Oral submucous fibrosis: an update. *Clinical, Cosmetic and Investigational Dermatology* 2015;8(Apr):193–204.
- [3] Hallikerimath S, Kale A. Estimation of folic acid levels in oral submucous fibrosis and its implications in the etiopathogenesis. *Journal of Oral Oncology* 2011;6(181):28–73. <http://dx.doi.org/10.1016/j.oraloncology.2011.06.181>.
- [4] Pindborg JJ, Sirsat SM. Oral submucous fibrosis. *Oral Surgery, Oral Medicine, and Oral Pathology* 1966;22:764–79.
- [5] Lee CH, Ko YC, Huang HL, Chao YY, Tsai CC, Shieh TY, Lin LM. The precancer risk of betel quid chewing, tobacco use and alcohol consumption in oral leukoplakia and oral submucous fibrosis in southern Taiwan. *British Journal of Cancer* 2003;88:366–72.

- [6] Dyavanagoudar SN. Oral submucous fibrosis: review on etiopathogenesis. *Journal of Cancer Science and Therapy* 2009;1(2):72–7.
- [7] Pindborg JJ, Sirsat SM. Oral submucous fibrosis. *Oral Surgery, Oral Medicine, and Oral Pathology* 1966;22:764–79.
- [8] Ulrich CM, Neuhouser M, Liu AY, Boynton A, Gregory JR, Shane B, James SJ, Reed MC, Nijhout HF. Mathematical modeling of folate metabolism: predicted effects of genetic polymorphisms on mechanisms and biomarkers relevant to carcinogenesis. *Cancer Epidemiology, Biomarkers and Prevention* 2008;17:1822–31.
- [9] Narang D, Shishodiya S, Sur J, Khan NF. Estimation of serum homocysteine: as a diagnostic marker of oral sub mucous fibrosis. *Journal of Carcinogenesis and Mutagenesis* 2014;5:187.
- [10] Bais DS, Chauhan P, Mohan S. An evaluation of serum homocysteine level as a biological marker in Oral sub mucous fibrosis. *Journal of Dental and Medical Sciences* 2013;9(5):66–9.