

## Estimation of Serum Homocysteine: As a Diagnostic Marker of Oral Sub Mucous Fibrosis

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### Abstract

Oral sub mucous fibrosis is indeed one of the classic "Diseases of civilization" with large differences being seen between races, geographic areas and individuals at different levels in both prevalence and degree to which it transforms into malignancy with continuation of habit, with increased frequency and duration. In majority of cases oral cancers develop from pre-existing lesions and conditions which are mainly a result of carcinogenic agents like tobacco, lime, alcohol, betel nut, spices etc. Several studies in the past have tried to evaluate serum levels of homocysteine in oral squamous cell carcinoma but till date there is no documentation on serum Homocysteine levels in Oral Pre cancers. The present research was carried to find out if serum homocysteine can be used for the diagnosis of O.S.M.F.

**Methodology:** The research was conducted on (n=50) patients suffering from the disease of O.S.M.F clinically and pathologically diagnosed not undergoing treatment.

**Results:** In our study serum homocysteine level was increased in all patients irrespective of gender and age. There was no statistically significant co-relation when comparing homocysteine level among clinical stages and pathological grading.

**Conclusion:** This is the first research to estimate serum homocysteine in OSMF which suggest that chronic inflammation in OSMF leads to hyperhomocysteinemia which could be used to assess the level of severity of disease and may be used as prognosticator for treatment of the disease.

**Keywords:** Oral submucous fibrosis; Homocysteine; Oxidative stress; Inflammation; NF-K $\beta$

### Introduction

Oral sub mucous fibrosis 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 a juxtra-epithelial inflammatory reaction followed by a fibro-elastic change of the lamina propriety, with epithelial inflammatory reaction followed by fibro-elastic change of lamina propria, with epithelial atrophy, leading to stiffness of oral mucosa and causing trismus and inability to eat [1]. Homocysteine found in humans is a sulphur containing amino-acid formed during methionine metabolism in the methionine cycle. It can dimerise to Homocysteine or form disulphide bonds with proteins to form "protein-bound" homocysteine. In plasma 80% of homocysteine is protein bound. Homocysteine is a four carbon amino acid [HS(CH<sub>2</sub>)<sub>2</sub>CHNH<sub>2</sub>COOH] resulting from the demethylation of methionine. Homocysteine is a dimer composed of two oxidized molecule of Homocysteine linked by a disulphide bond [2].

### Material and Methods

Chosen for evaluating homocysteine were (n=50) patients suffering from disease of oral sub mucous fibrosis. Patients suffering from Cardiovascular disease, Osteoporosis, Dementia, Alzheimer's disease, Lung cancer, Oral cancer, Breast cancer and any other malignancy were excluded from the study.

### Results

The mean value of serum Homocysteine found in males was 26.9  $\mu$ mol/lts ( $\pm$ 8.1) female was 21.4  $\mu$ mol/lts ( $\pm$ 3.4) & total 24.1  $\mu$ mol/lts ( $\pm$ 3.3) which showed that higher level of serum homocysteine found in OSMF patients and males had higher value than females (Table 1).

Gender	Mean Homocysteine ( $\mu$ mol/lit)
Males	26.9 ( $\pm$ 8.1)
Females	21.4 ( $\pm$ 3.4)
Total	24.2 ( $\pm$ 3.3)

**Table 1:** Mean serum homocysteine level in OSMF patients.

Mean Homocysteine level gradually increased from stage II to stage IV among males and females (p-value was 0.276 and 0.657 among males and females respectively which was found to be not significant)

indicating that clinical progression is directly related to level of Homocysteine as the clinical stage of disease advances (Table 2).

Gender	Stage I Mean ± (S.D)	Stage II Mean ± (S.D)	Stage III Mean ± (S.D)	Stage IV Mean ± (S.D)	F value	P Value
Males	-	25.88 (±7.84)	26.98 (±8.67)	32.93 (±5.41)	1.326	0.276 (N.S)
Females	-	20.35 (±4.17)	0.0±0.0	23.40 (±0.00)	0.356	0.657 (N.S)
Total	-	25.47 (±7.72)	26.98 (±8.67)	31.02 (±6.33)	1.059	0.355 (N.S)

**Table 2:** Comparison of mean values - Homocysteine (µmol/lit) with clinical staging of OSMF.

Gender	Grade 0 Mean ± (S.D)	Grade 1 Mean ± (S.D)	Grade II Mean ± (S.D)	Grade III Mean ± (S.D)	F value	P Value
Males	27.23 ± 8.40	25.14 ± 7.84	28.33 ± 6.43	-	0.323	0.725 (N.S)
Females	20.35 ± 4.17	23.40± 0.00	-	-	0.356	0.657 (N.S)
Total	26.86 ± 8.35	24.97± 7.41	28.33± 6.43	-	0.328	0.722 (N.S)

**Table 3:** Comparison of mean values of Homocysteine (µmol/lits) with pathological grading of OSMF.

The mean Serum Homocysteine level among males in grade 0 was 27.23 µmol/lits ± 8.40), in grade I 25.14 (±7.84), in grade II 28.33 (±6.43). Among females the mean value of homocysteine in grade 0 was 20.35 (±4.17), in grade I 23.40 µmol/lits ± 0.00) (Table 3).

Gender	Clinical Staging (Mean in µmol/lit)	Pathological Grading (Mean µmol/lit)	F-value	P-value
Males	28.6	26.9	49.34	0.09 (N.S)
Females	21.9	21.87		
F-value	1.07			
P-value	0.49 (N.S)			

**Table 4:** Comparison of homocysteine level between clinical staging and pathological grading of OSMF.

The mean value of Homocysteine was compared with different-pathological grades of Oral sub-mucous fibrosis was found to be statistically no significant difference exist between pathological grading of OSMF (Table 4).

The comparison of mean value of Homocysteine between clinical stages in males was 28.6 µmol/lit and in females was 21.9 µmol/lit & was found to be not statistically significant suggestive of homocysteine level remains constant irrespective of clinical stages & pathological grades and gender.

## Discussion

### Mean serum homocysteine level in OSMF patients

The mean value of serum Homocysteine found in males was 26.9 µmol/lits (±8.1) female was 21.4 µmol/lits (±3.4) & total 24.1 µmol/lits (±3.3) which showed that higher level of serum homocysteine found in OSMF patients and males had higher value than females.

Chronic inflammation produces oxidative stress which will lead to hyperhomocysteinemia via regulation of nuclear factor kappa light chain enhancer of activated B-cells transcription factor. Increased levels of hyperhomocysteinemia exert its detrimental effects through induction of acute and chronic inflammation pathway such as endothelial adhesion, leukocyte adhesion.

### Comparison of mean values of homocysteine (µmol/lits) with clinical staging of OSMF

The mean value of Homocysteine in stage II among males was 25.88 µmol/lits (±7.84), in stage III 26.98 (±8.67), in stage IV 32.93 ± (5.41). Among females the mean value of homocysteine in stage II was 20.35 (±4.17), in stage IV 23.40 µmol/lits (±0.00).

The mean serum Homocysteine was compared with different clinical stages of Oral submucous fibrosis found to be statistically no significant difference exist among clinical stages of OSMF.

Serum homocysteine level does not vary with the clinical stages of laryngeal pre-cancers and Oral cancer patients; this is in accordance with Anna Eleftheriadou et al (2006) [3].

### Comparison of mean values of homocysteine (µmol/lits) with pathological grading of OSMF

The mean Serum Homocysteine level among males in grade 0 was 27.23 µmol/lits (±8.40), in grade I 25.14 (±7.84), in grade II 28.33 (±6.43). Among females the mean value of homocysteine in grade 0 was 20.35 (±4.17), in grade I 23.40µmol/lits (±0.00).

The mean value of Homocysteine was compared with different-pathological grades of Oral sub-mucous fibrosis was found to be statistically no significant difference exist between pathological grading of OSMF.

Serum homocysteine level does not vary with pathological grades of OSMF and it will remain constant throughout the disease. This in accordance with Eleftheriadou et al (2006) [3] who carried out a research in laryngeal pre-cancer and oral cancer indicative of

pathological grading is not a diagnostic marker to assess the level of Homocysteine among different grades.

The mean homocysteine level among different clinical stages was 28.6  $\mu\text{mol/lts}$  in males and 21.9  $\mu\text{mol/lts}$  in females and pathological grading was 26.9  $\mu\text{mol/lts}$  and 21.87  $\mu\text{mol/lts}$  in males & females respectively. Comparing the mean Homocysteine level among all the clinical stages and pathological grades in males and females was found to be statistically no significant difference exist. The Serum Homocysteine level remained constant throughout the course of the OSMF disease. The level does not vary as the clinical staging and pathological grading advances. This is in accordance with Eleftheriadou et al (2006) [3] who carried out a research in laryngeal pre-cancer and oral cancer.

## Conclusion

In our study the serum Homocysteine levels in OSMF was increased than the normal threshold level. (5.32-16.50  $\mu\text{mol/lts}$ .)

The mean value of serum Homocysteine found in males was 26.9  $\mu\text{mol/lts}$  ( $\pm 8.1$ ) female was 21.4  $\mu\text{mol/lts}$  ( $\pm 3.4$ ) & total 24.1  $\mu\text{mol/lts}$  ( $\pm 3.3$ ) which showed that higher level of serum homocysteine found in OSMF patients and males had higher value than females.

Mean Homocysteine level gradually increased from stage II to stage IV among males and females (p-value was 0.276 and 0.657 among males and females respectively which was found to be not significant) indicating that clinical progression is directly related to level of Homocysteine as the clinical stage of disease advances.

The mean Serum Homocysteine level among males in grade 0 was 27.23  $\mu\text{mol/lts}$  ( $\pm 8.40$ ), in grade I 25.14 ( $\pm 7.84$ ), in grade II 28.33 ( $\pm 6.43$ ). Among females the mean value of homocysteine in grade 0 was 20.35 ( $\pm 4.17$ ), in grade I 23.40  $\mu\text{mol/lts}$  ( $\pm 0.00$ ).

The mean value of Homocysteine was compared with different-pathological grades of Oral sub-mucous fibrosis was found to be statistically no significant difference exist between pathological grading of OSMF.

Oral sub-mucous fibrosis “an high-risk potentially malignant condition of the oral cavity carries risk of transition to oral cancer and cause for mortality and morbidity” is a chronic inflammatory disease which is caused by micro trauma produced by friction of coarse fibers of areca nut facilitating diffusion of alkaloids into the sub epithelial connective tissue resulting in juxtraepithelial inflammatory cell infiltration (causes muscle spasm leading to fibrosis and reduced mouth opening) resulting in oxidative stress which leads to hyperhomocysteinemia and exerts its detrimental effects through induction of acute and chronic inflammation pathway such as endothelial adhesion and leukocyte adhesion, via the regulation of nuclear factor kappa light chain enhancer of activated B-cells transcription factor.

We conclude that serum homocysteine can be used as a therapeutic prognosticator marker for evaluating the outcome of treatment in the OSMF patients.

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