EVALUATION OF SERUM IRON AND FOLATE LEVELS IN PATIENTS WITH ORAL LEUKOPLAKIA

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Abstract

Objectives

- To assess the levels of serum folate and iron in patients with and without any visible Oral leukoplakia.
- To correlate serum folate and iron levels and clinical staging of Oral leukoplakia.
- To correlate serum folate and iron levels and degree of dysplasia

Materials & Methods: The study comprised of 102 patients (34 Oral leukoplakia patients with habit(s), 34 patients with habit(s) and no oral leukoplakia and 34 controls). The conditions were further clinically and histo-pathologically graded. Serum folate concentration was determined by chemi-luminescent immunoassay method from the serum (blood) sample without hemolysis and serum iron concentration was determined by photometric method.

Results: The mean folate level $\pm$ SD (ng/dl) was found to be 5.17 ± 2.71 ng/dl, 5.29 ± 2.10 ng/dl and 5.37 ± 4.77 ng/dl in Oral leukoplakia patients, patients with habits without Oral leukoplakia and controls respectively. The mean iron level $\pm$ SD (μ g/dl) was found to be 91.21 ± 28.73 μ g/dl, 105.74 ± 33.20 μ g/dl and 96.82 ± 38.92 μ g/dl in Oral leukoplakia patients, patients with habits without Oral leukoplakia and controls respectively.

Conclusion: In patients with habit and without leukoplakia and controls, there was a significant increase in the serum iron level than the oral leukoplakia patients. However, nosignificant differences seen in serum folate level in these three groups. Further studieswith larger sample size should be done to conclude serum folate and iron levels as adiagnostic marker in Oral leukoplakia.

Keywords: Diagnosis, Serum folate, serum iron, potentially malignant disorders, Oral leukoplakia, Oral squamous cell carcinoma, malignant transformation, Immuno-assay.

Introduction:

Oral squamous cell carcinoma is the sixth most common malignant neoplasm worldwide.¹ OSCC develops through a multistep process of genetic, epigenetic and metabolic changes resulting from exposure to carcinogens, with initially the presence of a precursor/pre-cancer lesion and the latter as a well-established one.² The most common oral mucosal diseases that have a high malignant transformation rate are oral leukoplakia, oral erythroplakia and oral submucous fibrosis with the most frequently reported etiological agents being tobacco, alcohol, chewing of betel quid containing areca nut.³ The importance of early identification, diagnosis and treatment of the potentially malignant disorders cannot be ignored as in later stages they might progress to severe dysplasia and even carcinoma in

situ and/or squamous cell carcinoma.³ Malignant transformation rate of oral leukoplakia varies between 0.13 – 17.5 %, with less transformation rates seen in homogenous leukoplakia than in non-homogenous leukoplakia.⁴ The malignant transformation rate in OSMF has been reported to be 7-13%, over a period of 10 years.⁵ Trace elements have been extensively studied in recent years to assess whether they have any modifying effects in the etiology of cancer. Copper, iron and selenium are essential for numerous enzymes and therefore it is reasonable to assume that variations in serum level of these biochemical markers maybe associated with the pathogenesis of oral cancer. The importance of these elements in cancer was reported by Schwartz⁶ which opened the door for new diagnostic and therapeutic endeavours in many areas of medicine and specifically in the areas of oncology.⁷

Immunological and biochemical alterations in the serum of such patients can help not only in the early diagnosis, appropriate treatment but also as indicators of prognosis, as the disease progresses.⁷ Metabolic alterations observed in trace elements like iron, can be a treasured approach to comprehend the biochemistry of such tumors. Further, the alterations in serum folic acid levels might ascend from the process of tumor development and consequential metabolic alterations may lead to the process of tumor progression. If hypofolatemia is a risk factor for head and neck carcinogenesis, then it may be advocated as a novel chemo-preventive agent in patients with premalignant lesions and patients with treated head and neck squamous cell carcinoma for loco-regional recurrence.⁸ Hence, the present study was done to determine the serum iron levels and folic acid as a potential biomarker in Oral Leukoplakia.

AIMS AND OBJECTIVES OF THE STUDY

1. To assess the levels of serum folate and iron in patients with and without any visible Oral leukoplakia.
2. To correlate serum folate and iron levels and clinical staging of Oral leukoplakia.
3. To correlate serum folate and iron levels and degree of dysplasia

MATERIALS AND METHODS

The study enrolled 34 Oral leukoplakia with habit(s) (Group 1), 34 patients with habit(s) and no Oral leukoplakia (Group 2), and 34 controls (Group 3), over 2 years. Further the conditions were graded clinically and histo-pathologically and Serum folate concentration was determined by chemi-luminescent immunoassay method from the serum (blood) sample without hemolysis and serum iron concentration was determined by photometric method.

Inclusion criteria:

- Patients with a habit history of smoking and chewing tobacco for more than a year, diagnosed with Oral Leukoplakia.
- Patients aged within 20-80 years.
- Patients with long standing habits of smoking and tobacco chewing for more than a year but without any visible oral lesions.

Exclusion criteria:

- Patients with nutritional disorder.
- Patients with autoimmune disorders.
- Patients undergoing chemotherapy.
- Patients with long term drug history for systemic diseases

Results

Tabulation of the results was carried out for patients with habits showing oral leukoplakia lesions and patients without oral leukoplakia and the control group. All the variables from the study were statistically analyzed by SPSS 22. Chi Square Test was used to compare the habits related characteristics between group 1 & 2 and Mann Whitney Test was used to compare the mean folate (ng/ml) and iron levels ($\mu\text{g/dl}$) between subjects with different types of tobacco habits in Group 1 & 2 and also to perform gender wise comparison of mean folate (ng/ml) and iron levels ($\mu\text{g/dl}$) in each group. Kruskal Wallis Test followed by Mann Whitney Post hoc Analysis was used to compare the mean folate (ng/ml) and iron levels ($\mu\text{g/dl}$) between 03 groups and also different age groups within each group. Similarly, comparison of mean folate (ng/ml) and iron levels ($\mu\text{g/dl}$) between varying grades of dysplasia in Group 1.

Table 1: Age and Gender distribution among study subjects in 03 groups

Variable	Category	Group 1		Group 2		Group 3	
		n	%	n	%	n	%
Age	20-30 yrs	9	26.5%	21	61.8%	24	70.6%
	31-40 yrs	8	23.5%	6	17.6%	4	11.8%
	41-50 yrs	8	23.5%	4	11.8%	2	5.9%
	51-60 yrs	6	17.6%	3	8.8%	4	11.8%
	>60 yrs	3	8.8%	0	0.0%	0	0.0%
	Mean	SD		Mean	SD	Mean	SD
	Mean&SD	41.9	12.9	33.1	10.3	30.5	10.5
Sex	Range	22 - 69		22 - 58		20 - 53	
	Males	28	82.4%	33	97.1%	12	35.3%
	Females	6	17.6%	64.7%	64.7%	64.7%	64.7%

Table 1: Age and Gender distribution among study subject

Table 1 shows the mean age of the three study groups.

Descriptive of cigarette and bidis smoking frequency and duration and smoking and tobacco chewing in group 1 and 2 is shown in graph 1

The mean folate level with SD (ng/ml) was found to be 5.17 ± 2.71 ng/ml, 5.29 ± 2.10 ng/ml and 5.37 ± 4.77 ng/ml respectively in Group 1, 2 and 3 and they were found to be statistically insignificant ($p < 0.05$). (Table 2 and figure 2)

Table 2: Comparison of mean Folate (ng/ml) and Iron Levels (µg/dl) between varying grades of Dysplasia in Group 1 using Mann Whitney Test							
Variables	Dysplasia Grades	N	Mean	SD	Min	Max	P-value
Folate (ng/ml)	No Dysplasia	26	4.98	2.49	1.4	10.4	0.90
	Mild Dysplasia	5	6.24	4.46	1	11.3	
	Mod. Dysplasia	2	5.50	0.57	5.1	5.9	
	Severe Dysplasia	1	4.20	.	4.2	4.2	
Iron (µg/dl)	No Dysplasia	26	93.65	30.16	60	189	0.90
	Mild Dysplasia	5	81.40	28.10	44	122	
	Mod. Dysplasia	2	91.00	22.63	75	107	
	Severe Dysplasia	1	77.00	.	77	77	

The mean iron level with SD (µg/dl) was found to be 91.21 ± 28.73 µg/dl, 105.74 ± 33.20 µg/dl and 96.82 ± 38.92 µg/dl in Group 1, Group 2 and Group 3 and they were found to be statistically significant ($p < 0.05$). (Table 2 and figure 3)

Table 2 shows the comparison of mean folate levels in histopathological subtypes i.e.- no dysplasia (4.98 ± 2.49 ng/ml), mild dysplasia (6.24 ± 4.46 ng/ml), moderate dysplasia (5.50 ± 0.57 ng/ml) and severe dysplasia (4.2 ± 0 ng/ml) was statistically insignificant ($p < 0.05$).

Table 2 also shows the comparison of mean iron levels in histopathological subtypes i.e.- no dysplasia (93.65 ± 30.16 µg/dl), mild dysplasia (81.40 ± 28.10 µg/dl), moderate dysplasia (91.0 ± 22.63 µg/dl) and severe dysplasia (77 ± 0 µg/dl) was statistically insignificant ($p < 0.05$).

Figure 4 shows the distribution of Group 1 patients under Vander Waal's classification into L2P0 and L2P1 groups with 91.2% and 8.8% respectively.

Figure 1: Descriptive of cigarette and bidis smoking frequency and duration and smoking and tobacco chewing in group 1 and 2

Figure 2: Comparison of mean Folate across the 3 groups (ng/ml)

Figure 3: Comparison of mean Iron levels across the 3 groups(µg/ml)

Figure 4: Distribution of Lesion Grades based on Vander Waal's Classification in Group 1

DISCUSSION

The term cancer is highly stressful to the patient, the physician and the relatives of the patient. Over the years there is enormous amount of research that has

been performed, whose data is still inconclusive and cancer still remains a challenge. The development of cancer is a multi-stage process, with initially the presence of a precursor/pre-cancer lesion and the latter as a well-established one.⁹ The assumption that malignancy in a patient with a potentially malignant lesion would arise at the site of the lesion and that malignancy could arise in any anatomical site, in potentially malignant conditions was the distinction that was made between potentially malignant lesions and potentially malignant conditions in the past.⁹ The presence of dysplasia in a distinct separate anatomic site is suggestive of a pathway to malignant transformation. Hence the recommendation was made to refer to all clinical presentations that carry a risk of cancer as potentially malignant disorders.⁹ As the saying goes "Prevention is better than cure", over the years there has been a lot of research to treasure and find out some useful markers, which not only helps in early diagnosis, but also helps in prevention of further disease progression and the ultimate result being a better prognosis and treatment outcome. In our study we evaluated the serum folic acid and iron levels as they are involved in many metabolic purposes in the body. Hence, a change in the levels of the same are highly important and the evaluation of the same may be highly beneficial and is worth all the hard work that research signifies. It was also found that about 82.4% patients with oral leukoplakia and 97.1% patients with habits (>1 year) without oral leukoplakia were males. Similarly, in a study done by Pooja et al in 1999 to assess the role of serum iron, ferritin as well as total iron binding capacity (TIBC) in the pathogenesis and treatment planning of OSMF and Leukoplakia, showed that about 88% of patients with oral leukoplakia and OSMF (22) were males and 12% (03) were females.¹⁰ The mean folate level with SD (ng/dl) was found to be 5.17 ± 2.71 ng/dl, 5.29 ± 2.10 ng/dl and 5.37 ± 4.77 ng/dl in oral leukoplakia patients, patients with habits (>1 year) without oral leukoplakia and controls, was found to be statistically insignificant ($p < 0.05$). A study was done by Raval N et al in 2002 to assess the Vitamin B12 and Folate Status in Head and Neck Cancer showed that as there was a significant positive correlation between vitamin B12 and folate levels in the subjects consuming tobacco, and more so in patients with oropharyngeal cancer.¹¹ Also, that there was a decrease in the plasma vitamin B12 and folate levels with respect to tobacco habits which was in accordance with the present study. In another study by Heimbürger et al., 1988 and

Ramaswamy et al., 1996 reported that the mean plasma levels of vitamin B12 and folate were lower in cancer patients in comparison to the healthy individuals.¹¹ The mean iron level with SD ($\mu\text{g/dl}$) was found to be $91.21 \pm 28.73 \mu\text{g/dl}$, $105.74 \pm 33.20 \mu\text{g/dl}$ and $96.82 \pm 38.92 \mu\text{g/dl}$ in oral leukoplakia patients, patients with habits (>1 year) without oral leukoplakia and controls, was found to be statistically significant ($p < 0.05$). A study done for the assessment of serum copper, iron and immune complexes in potentially malignant disorders and oral cancer by Tiwari R et al showed that the serum levels of iron showed a significant decrease in the PMD group ($110.9 \pm 10.54 \mu\text{g}/100\text{ml}$) and the oral cancer group ($114.29 \pm 25.83 \mu\text{g}/100\text{ml}$) as compared with the control group ($136.85 \pm 14.48 \mu\text{g}/100\text{ml}$).¹² Similarly, another study done by Guruprasad R et al to assess the serum vitamin C and iron levels in oral submucous fibrosis with 35 patients showed that iron levels was significantly decreased in OSMF patients than in controls.¹³ Another study done Jayadeep A et al to assess the levels of copper, zinc, iron and ceruloplasmin in oral leukoplakia and squamous cell carcinoma showed that iron levels was significantly reduced in carcinoma patients and slightly decreased in oral leukoplakia patients than in controls.¹⁴ Patients with leukoplakia showed a mean frequency of 8 cigarettes/day for 11 years while patients with habits (>1 year) without oral leukoplakia showed a mean frequency of 5 cigarettes/day for 8 years. The mean folate level with SD (ng/dl) in patients without and with cigarette smoking habits in oral leukoplakia patients was found to be $5.24 \pm 2.42 \text{ ng/dl}$, and $5.06 \pm 3.23 \text{ ng/dl}$ respectively whereas higher levels were seen in patients with habits (>1 year) without oral leukoplakia ($5.29 \pm 2.43 \text{ ng/dl}$, and $5.29 \pm 2.01 \text{ ng/dl}$). Both were found to be statistically insignificant ($p < 0.05$). The mean iron level with SD ($\mu\text{g/dl}$) in patients without and with cigarette smoking habits in oral leukoplakia patients was found to be $93.67 \pm 32.06 \mu\text{g/dl}$, and $87.23 \pm 23.01 \mu\text{g/dl}$ respectively, whereas higher levels were seen in patients with habits (>1 year) without oral leukoplakia ($96 \pm 33.91 \mu\text{g/dl}$, and $109.79 \pm 32.76 \mu\text{g/dl}$). Both were found to be statistically insignificant ($p < 0.05$). Patients with leukoplakia showed a mean frequency and duration of 17 bidi's/ day for 16 years whereas patients with habits (>1 year) without oral leukoplakia showed a mean frequency of 10 bidi's/day in the last 3 years. The mean folate level with SD (ng/dl) in patients without and with Bidi's smoking habits in oral

leukoplakia patients was found to be $4.89 \pm 2.84 \text{ ng/dl}$, and $6.48 \pm 1.52 \text{ ng/dl}$ respectively whereas higher levels were seen in patients with habits (>1 year) without oral leukoplakia ($5.33 \pm 2.12 \text{ ng/dl}$, and $4.10 \pm 0 \text{ ng/dl}$). Both were found to be statistically insignificant ($p < 0.05$). The mean iron level with SD (ng/dl) in patients without and with Bidi's smoking habits in oral leukoplakia patients was found to be $91.21 \pm 30.63 \mu\text{g/dl}$, and $91.17 \pm 19.53 \mu\text{g/dl}$ respectively whereas higher levels were seen in patients with habits (>1 year) without oral leukoplakia ($104.03 \pm 32.17 \mu\text{g/dl}$, and $162 \pm 0 \mu\text{g/dl}$). Both were found to be statistically insignificant ($p < 0.05$). In a study done by Khanna SS and Karjodkar FR, to evaluate the Circulating Immune Complexes and trace elements (Copper, Iron and Selenium) as markers in oral pre-cancer and cancer found statistically significant reduction in serum iron levels in the pre-cancer group. A decrease in the iron levels in the cancer group, but higher than that of pre-cancer groups was found to be significant.¹⁵ Recently, hematological abnormalities in oral leukoplakia was reported by Chellacombe.¹⁶ It was further reported that poor correlation between iron indices, tumor parameters, serum iron and hemoglobin was probably due to utilization of iron by bone marrow and tumours.¹⁵ The lowered level of iron appears to be the effect of the disease process rather than its cause. Serum iron levels are considered biochemical indicators for nutritional assessment. The cancer cells also have essentially the same qualitative iron requirements like the normal cells. They express their own transferrin receptors to obtain iron which may explain depletion of iron in such states. Hence, hypoferremia can be seen in association with carcinogenesis and iron levels can serve as prognostic indicator. Iron overload on the other hand has been known to aid carcinogenesis by provoking DNA damage. Iron induced oxidative stress causes redox regulation failure leading to lipid peroxidation and DNA and protein damage. Iron binding sites on macromolecules serve as Centre for repeated production of hydroxyl radicals generated via the Fenton reaction. It is considered that iron and oxygen together constitute a biologically damaging mixture due to increased formation of free radicals.¹² The mean folate level with SD (ng/dl) in patients without and with tobacco chewing habits in oral leukoplakia patients was found to be $5.84 \pm 2.82 \text{ ng/dl}$, and $4.51 \pm 2.50 \text{ ng/dl}$ respectively whereas higher levels were seen in patients with habits (>1 year) without oral

leukoplakia (5.29 ± 2.01 ng/dl, and 5.29 ± 2.43 ng/dl). Both were found to be statistically insignificant ($p < 0.05$). In a study done by Jaber MA to estimate the serum levels of folate and vitamin B12 in oral epithelial dysplasia, a decrease in the plasma folate levels was observed in the patients consuming tobacco as compared to the nonsmokers.¹⁷ A similar observation was made by Almadoriet al. from Italy in 2005 who found that serum folate levels were significantly lower in patients with head and neck carcinoma and in patients with laryngeal leukoplakia compared with serum folate levels in both the smoker and non-smoker control group.¹⁷ A study done in 1996 by Ramaswamy et al. reported low levels of vitamin B12 and folate in a group of Indian patients with oral leukoplakia.⁹ Several investigators have suggested that deficiency of folate enhances development of pre-neoplastic and neoplastic lesions, which are suppressed by folate supplementation.¹⁷ Low folate level probably does not have an independent role as an initiating factor. Instead, presumably, acts synergistically with other genetic and environmental factors, such as tobacco carcinogens, making cells more susceptible to mutagens and increasing the rate of tumor progression.¹⁸ Some of the carcinogenic substances present in tobacco smoke 'primarily organic nitrites, cyanates, and isocyanates', have been shown to interact with folate and vitamin B12 coenzymes, transforming them into biologically inactive compounds. These chemical interactions may have physiological significance is supported by reports of lower circulating folate and B12 levels in smokers and the buccal mucosal cells of tobacco smokers were shown to have a decreased concentration of folate.¹⁷ The mean iron level with SD (ng/dl) in patients without and with tobacco chewing habits in oral leukoplakia patients was found to be 90.47 ± 21.53 µg/dl, and 91.94 ± 35.19 µg/dl respectively whereas higher levels were seen in patients with habits (>1 year) without oral leukoplakia (113.04 ± 33.93 µg/dl, and 88.20 ± 24.78 µg/dl). It was statistically significant ($p < 0.05$) in patients with habits (>1 year) without oral leukoplakia ($p < 0.05$). A study done by Apeksha et al in 2010 also showed a decrease in serum iron levels in patient with OSMF, oral leukoplakia and oral cancer compared to control group and they stated that all patient included were of same socioeconomic status with lower serum iron levels appear to be the effect of disease process rather than its cause. Lack of consumption of normal

diet results in anemia which is further perpetuated by progression of disease.^{7,18} Oral leukoplakia group were divided into patients with no dysplasia, mild dysplasia, moderate dysplasia and severe dysplasia which is about 76.5%, 14.7%, 5.9%, 2.9% respectively. According to Vander Waal's classification, the distribution of oral leukoplakia patients into L2P0 and L2P1 was 91.2% and 8.8% respectively.

A comparison of mean folate levels in histopathological subtypes i.e.- no dysplasia (4.98 ± 2.49 ng/dl), mild dysplasia (6.24 ± 4.46 ng/dl), moderate dysplasia (5.50 ± 0.57 ng/dl) and severe dysplasia (4.2 ± 0 ng/dl) was statistically insignificant ($P < 0.05$).

A comparison of mean iron levels in histopathological subtypes i.e.- no dysplasia (93.65 ± 30.16 ng/dl), mild dysplasia (81.40 ± 28.10 ng/dl), moderate dysplasia (91.0 ± 22.63 ng/dl) and severe dysplasia (77 ± 0 ng/dl) was statistically insignificant ($P < 0.05$).

A study done by Shettar S et al in 2016 for estimation of serum iron levels in patients with oral cancer showed that there was no significant change in serum iron levels among clinical and histological grades in oral cancer patients.¹⁹

A study done by Keerthika et al in 2016 for the Estimation of Serum Copper, Zinc and Iron in Patients with Oral Cancer concluded that utilization of iron in collagen synthesis by the hydroxylation of proline and lysine leads to decrease serum iron levels in patients with oral cancer.²⁰ Serum iron and folate levels in the test groups were lower than the control group. There appears to be an association between the serum iron content and oral carcinogenesis. More detailed studies on a large data base should be instituted to elucidate the exact role of iron. The limitation of our current study is that only serum iron and folate as a potential biomarker of Oral leukoplakia is studied. Further research involving a large number of biomarkers in a larger study sample of Oral leukoplakia patients, may explain the role of these biomarkers in the development of Oral leukoplakia and their malignant transformation.

CONCLUSION

We found that the serum iron levels were reduced in Oral leukoplakia and control patients and were elevated in patients with habits and without Oral leukoplakia. Also, there were no significant differences in serum folate levels across Oral leukoplakia, patients with habits and without Oral

leukoplakia and control patients. There were no significant differences in the serum folate and iron levels across the clinical and histopathological stages of Oral leukoplakia. Hence, further studies are the need of the hour for a better understanding of the relation between folate and iron with the deleterious habits and its correlation with Oral leukoplakia.

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