Research Article

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Serum Iron, Copper and Hb as Biochemical Markers in Early Diagnosis of OSF and Oral Cancer in Correlation with Normal Population

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ABSTRACT

Background: A randomized case control study was carried out on 150 patients, where the serum iron, copper and haemoglobin levels were evaluated in OSMF, OSCC patients and then compared with the normal population along with intra-stage comparison within OSMF group. Here the absorption spectrophotometry and Sahli's methods were used for the analysis of serum iron, copper and haemoglobin, respectively. There is a significant increase in serum iron concentration in stage I and stage II OSMF by 7.5% and 15%; a significant reduction in OSCC group. Similarly, an overall significant increase in serum copper levels was found in OSMF group along with the significant reduction in haemoglobin concentration in OSMF and OSCC group by 17% and 17.4% respectively with no intra-stage variation in OSMF group.

Keywords: Serum iron and copper, Haemoglobin, biochemical markers, prognosis, OSMF, OSCC.

INTRODUCTION

OSMF is a chronic disease of the oral cavity, which is characterized by an epithelial and sub epithelial inflammatory reaction, followed by fibro-elastic changes in the sub-mucosa (1). Around 7-13% of oral sub-mucous fibrosis cases undergo malignant transformation (2) and most of the times the carcinogenesis is an intricate complex mechanism where the multi-factorial causation makes it more difficult to find specific prognostic and therapeutic biomarkers (3). Though the diagnosis and prognosis of OSMF and OSCC can be established by biopsy, which is an invasive and time consuming procedure (4) biochemical investigations are of prime importance because of their advantages such as simplicity and minimal invasiveness, as well as being useful for monitoring the response to therapy (5). So, the development of newer diagnostic and predictive approaches that are less invasive, economical are amenable and imperative for early detection of these lesions to drastically improve the treatment outcomes and prognosis in such patients (3).

Thus, our study aims at evaluating the serum iron, serum copper and haemoglobin levels in OSMF, OSCC patients and comparing them with normal population and then to know whether we can use these parameters as biochemical markers in early diagnosis of OSMF, OSCC.

MATERIALS AND METHOD

Here, a cross sectional hospital based study was carried out between July 2015 to August 2016 in the OPD of a dental college and a cancer centre, which was designed to assess the serum iron, copper and haemoglobin levels in OSMF and oral cancer patients and to compare all the parameters with the normal subjects. The present study

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consists of 150 subjects: Group A control (age group of 21yrs to 40yrs with no accessible mucosal disease), Group B (50 OSCC patients between the age group of 31yrs to 70yrs) and Group C (50 OSMF patients between the age group of 16yrs to 70yrs) both were clinically and histopathologically proven.

Once the sampling was done permission of the ethical committee from the concerned hospitals were obtained followed by taking the informed consent from the patients, there clinical examination, recording of details in preformed proforma and performing phlebotomy procedure where 5ml of blood was drawn from ante-cubital vein. Here, the clinical and functional staging was given to OSMF based on Kerr et al. classification 2011 (6) and clinical diagnosis of oral cancer was done based on TNM staging (7).

The blood was transported immediately into EDTA tubes, sent to biochemical laboratory for centrifugation at 1000-1500 RPM for 15 minutes then 3ml of serum was collected and analyzed using Dade automatic analyzer for serum iron and by Biosystem BTS 340 semiautomatic analyzer for serum copper, respectively both of which works on the principle of absorption spectrophotometry while a drop of blood was taken into Sahli's haemoglobinometer for haemoglobin estimation (8). The obtained data was subjected to statistical analysis using one-way ANOVA test for the intra-group comparison among different stages in OSMF and unpaired t test for any two groups of OSMF, oral cancer and normal patients.

RESULTS

Mean comparison of Serum Iron among 3 groups: The mean serum Iron concentration in Group A, Group B, Group C was 81.76±21.40µg/dl, 21.13±27.41µg/dl and 81.94±35.32µg/dl respectively. There was no variation in Group C but a significant reduction in Group B was found compared to Group A and Group C (p=0.000) (Table1).

Mean comparison of Serum Copper among 3 groups: The mean serum Copper concentration in Group A, Group B, Group C was 101.80±26.84µg/dl, 89.42±91.28µg/dl and 135.26±59.35 µg/dl respectively. There was no much variation in Group B but there was significant increase in serum copper levels in Group C in comparison with Group A (p=0.000) (Table 2).

Mean comparison of Haemoglobin among 3 groups: The mean serum Haemoglobin concentration in Group A, Group B, Group C was 86.68±6.93gm%, 69.32±15.06gm% and 69.73±10.24gm% respectively. There was significant reduction in Haemoglobin levels in Group B and Group C compared to Group A (P=0.000) (Table3).

Intra-stage comparision of Serum Iron, Copper and Haemoglobin in the OSMF group: There was reduction in mean serum Iron concentration in stage III, stage IV OSMF and increase in mean serum copper concentration in stage II, stage III, stage IV OSMF respectively which were statistically significant but there was no variation in mean Haemoglobin concentration (Graph 1)

DISCUSSION

Till today many pioneer researches has been carried out to evaluate serum iron and copper levels in OSMF, oral cancer patients where most of them has shown depletion in serum iron levels, increase in serum copper levels both in OSMF (9,10) and oral cancer patients (9,11) when compared to normal population. Further, these parameters has shown drastic variation with severity of the disease in OSMF (12-18). Apart from the above, many studies has shown a depletion in haemoglobin levels in OSMF (11,12,19) and oral cancer (16) patients compared to normal population which further depletes with the increased staging in OSMF (11,12).

All the results in our study were in consistent with the above studies, except that in our study there was no significant variation in serum iron in OSMF group and serum copper levels in OSCC group, respectively with the significant increase in serum copper levels only in OSMF group than OSCC group by 46% with no variation in serum iron and haemoglobin levels. While in the intra-stage comparison of OSMF, there was significant variation in serum iron and copper levels with no variation in haemoglobin. Thus, this minor variation of biochemical markers in our study when compared to other studies can be attributed to the small sample size, variation in male to female ratio

Table 1: Mean comparison of serum Iron between groups.

SERUM IRON	MEN µg/dlA	SD	DIFFERENCE MEAN±SD	t value	P value
NORMAL	81.76	21.40	60.63±6.01	12.327	0.000 Significant
OSCC	21.13	27.41			
NORMAL	81.76	21.40	0.18±13.92	0.030	0.976 Not significant
OSMF	81.94	35.32			
OSCC	21.13	27.41	60.81±7.91	9.582	0.000 Significant
OSMF	81.94	35.32			

Statistical Analysis: Unpaired t test. Statistically significant if P<0.05

Table 2: Mean comparison of serum Copper between groups.

SERUM COPPER	MEAN µg/dl	SD	DIFFERENCE MEAN±SD	t value	P value
NORMAL	101.80	26.84	12.38±64.44	1.054	0.295 Not Significant
OSCC	89.42	91.28			
NORMAL	101.80	26.84	33.46±32.51	3.626	0.000 Significant
OSMF	135.26	59.35			
OSCC	89.42	91.28	45.84±31.93	3.073	0.003 Significant
OSMF	135.26	59.35			

Statistical Analysis: Unpaired t test. Statistically significant if <0.05

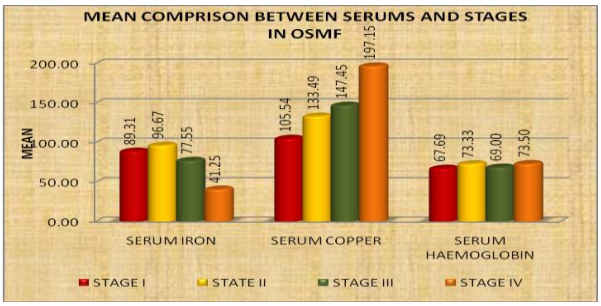
Table 3: Mean comparison of Haemoglobin between groups.

HAEMOGLOBIN	MEAN gm%	SD	DIFFERENCE MEAN±SD	t value	P value
NORMAL	86.68	6.93	17.36±8.13	7.405	0.000 Significant
OSCC	69.32	15.06			
NORMAL	86.68	6.93	16.95±3.31	9.659	0.000 Significant
OSMF	69.73	10.24			
OSCC	69.32	15.06	0.41±4.82	0.160	0.873 Not significant
OSMF	69.73	10.24			

included in our sample, difference in the techniques for analyzing serum iron, copper and haemoglobin.

The role of serum iron in pathogenesis of OSMF can be attributed to the cytochrome oxidase, an iron dependent enzyme which is required for the normal maturation of the epithelium whose deficiency leads to epithelial atrophy making the oral mucosa vulnerable to the soluble irritants (17).

Furthermore, lack of iron in the tissues results in decreased vascularity facilitating percolation of arecoline causing increased fibroblastic proliferation and collagen formation which is a hallmark of OSMF (18)due to the utilization of iron for the hydroxylation of proline and lysine of an amino acid hydroxyproline found only in collagen, as ferrous iron along with ascorbic acid (15) increasing the production of highly cross linked



Graph 1: Intra-staging comparison of serum Iron, Copper and Haemoglobin in OSMF.

insoluble collagen Type I coupled with loss of more soluble procollagen Type III and Type VI collagen with upregulation of lysyl oxidase (20) (increases with the disease progression from stage I to stage IV). Further the burning sensation, vesiculation and ulceration of the oral mucosa render a phase for difficulty in consumption of the normal diet leading to poor nutrition leading to further deficiency of iron and further progression of the disease (21).

In OSCC, the pathogensis can be attributed to impaired cell mediated immunity where iron as a transition metal generates the reactive oxygen species including hydroxyl radicals which attacks DNA causing mutation (22). Further iron as a component of the enzyme system catalyzes the electron-transfer and respiration reactions which are essential for cell viability, thus deficiency of iron leads to loss of cell viability making it prone to OSCC (23).

Now, the pathogenesis of increased levels of serum copper in OSMF can be attributed to an upregulation of the copper-dependent extracellular enzyme lysyl oxidase by fibroblasts leading to an excessive cross linkage and accumulation of collagen (24). Further copper is a required cofactor for the function of many key mediators of angiogenesis, such as basic fibroblast growth factor (25). Apart from these, the high copper content of arecanut causes significant increase in serum copper on chewing for 5-30minutes, further activating lysyl oxidase action (26),its collagen synthesis and its post-transitional modification of collagen fibers rendering them resistant to the action of collagenases (27,28).

In OSCC group, the pathogenesis can be attributed to biological damage caused by superoxide radicals or other reducing agents such as ascorbate, which reduce the copper complex to react with hydrogen peroxide to form hydroxyl radicals causing irreparable damage to protein, RNA, DNA, polyunsaturated fatty acids of cell membrane, thus initiating the malignant process (29) and further excessive use of areca nut may cause fibrosis due to increased synthesis of collagen still worsening the tumor genesis (30). Apart from this, the hidden fact of the increased production of copper-containing ceruloplasmin by liver as an inflammatory response and its decreased catabolism has found to be contributing to the pathogenesis (31).

Currently, the role of the haemoglobin in pathogenesis of OSMF and OSCC can be attributed to the fact that the synthesis of haemoglobin requires iron as it's structural component. So, when there is a significant reduction in serum iron concentrations in OSMF group it leads to significant reduction in haemoglobin. Further after a frank establishment of the lesion, anemia may further perpetuate by inadequate intake of food due to fibrosis and trismus resulting in significant reduction in haemoglobin levels (32). If this anemia is severe enough, it promotes intratumoral hypoxia promoting molecular and cellular changes that favour malignant progression and formation of metastases (33).

CONCLUSION

Finally, we conclude by emphasizing the role of serum iron, copper and hemoglobin in the etiopathogenesis of OSF and oral cancer where they can be used as biochemical markers in early diagnosis, however further research perspectives on large population are required.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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