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Role of Trace Elements and Lipid Profile as Markers of Oral Submucous Fibrosis in Chennai Population

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Abstract: *Among various oral cavity carcinomas, oral squamous cell carcinoma (OSCC) is considered as the most common oral and maxillofacial malignancy. The morbidity and mortality rate of the disease are still high in most of the countries with a survival rate of 50-63%. Oral submucous fibrosis (OSMF) has been reported as the potentially malignant disorder (PMD) of the oral cavity that has high prevalence rate in India. Chennai is one of the metro cities located in the southern part of India. This study was carried out to evaluate the serum levels of various trace elements such as iron (Fe), zinc (Zn), selenium (Se), and copper (Cu) and serum lipid profile in OSMF patients who live in Chennai and belong to Hindu religion. The alterations in the levels were compared to age, gender- matched normal healthy individuals in order to identify a new non-invasive method for the prevention of its transformation to OSCC. Serum levels of trace elements were analyzed using inductively coupled plasma optical emission spectrophotometer (ICP-OES) and lipid profile was done using autoanalyzer. The results obtained in the present study revealed a significantly low level of iron, zinc and selenium and very high level of copper in the serum of patients with advanced stage of OSMF when compared to healthy individuals. Serum lipid profile of OSMF group also showed a decreasing tendency compared to healthy individuals. Thus, alterations in the levels of serum trace elements and lipid profile could be utilized as an important biomarker for the identification of oral mucosal pathologies.*

Key-words: *Oral submucous fibrosis, Trace elements, Lipid profile, Biomarker*

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I. Introduction

Oral mucosal diseases like oral submucous fibrosis (OSMF), the topic of interest, are increasing in an alarming frequency irrespective of metro cities in India. Chennai is one of the metro cities located in the southern part of India. Warnakulasuriya *et al*[1] reported OSMF as one of the potentially malignant disorders (PMD) of oral mucosa, which can lead to OSCC. Areca nut- associated oral squamous cell carcinoma is the third most common malignancy in the developing world and oral submucous fibrosis (OSMF) has been reported as the PMD of the oral cavity that has high prevalence rate in India [2]. According to Rajalalitha *et al* [3], the incidence rate of OSCC in patients with OSMF has been estimated to be 2.3-7.6%. The morbidity and mortality rate of the disease are still high in most of the countries with a survival rate of 50-63%[4,5].

In the present study, we intended to identify a new non-invasive method by which we can diagnose this disease at an early stage and thereby prevent its transformation to OSCC. Schwartz [6] reported that, assessment of various serum trace elements can be used as an index in oncology which can be further utilized for the early diagnosis and prognosis of precancerous and cancerous conditions. In this context, we designed our experiments to analyze the level of various trace elements and lipid profile in the serum samples of patients with OSMF.

II. Materials and Methods

Patients were selected from those who were attending the outpatient department of Oral Medicine and Radiology of Tagore Dental College and Hospital, Chennai. The protocol for the use of human serum samples was approved by the Institutional Ethical Committee.

The study comprised of 36 individuals with oral submucous fibrosis and 10 normal healthy age, gender- matched individuals devoid of any systemic disorders and other unhealthy habits. Written consent was obtained from each patient included in this study. We specifically utilized the Hindu community who live in Chennai region for our study. Personal history, duration of habit, type of product used, number of packets per day per sitting and duration per chew were collected from each patient.

Patients were divided into four groups by the method of Ranganathan [7] based on their mouth opening.
Group I (Control) - >40mm
Group II (Stage I) - 30-40mm
Group III (Stage II) - 20-30mm
Group IV (Stage III) - <20mm

Blood serum collection:

5.0 ml of blood was collected aseptically using coagulant coated vacutainer tubes with gel, from each patient and was allowed to clot at room temperature. It was then centrifuged at 3000 rpm for 10 minutes and the serum which was separated above the gel was collected in fresh eppendorf tubes and was used to carry out the following experiments.

Metal analysis using Inductively Coupled Plasma Optical Emission spectrophotometer

The levels of various trace elements such as Iron (Fe), Copper (Cu), Zinc (Zn) and Selenium (Se) in serum were determined using Perkin Elmer optima 5300 dv ICP-OES, available at the sophisticated analytical instrument facility, IIT Madras.

Lipid profiles

Levels of Total cholesterol (TC), Triglycerides (TG), High Density Lipoprotein (HDL), Low Density Lipoprotein (LDL) and Very Low Density Lipoprotein (VLDL) were determined by using automated analyser available at the biochemistry laboratory of VRR diagnostic centre, Chennai, Tamilnadu.

III. Statistical Analysis

Statistical analysis of serum levels of various trace elements and lipids among the four groups were done by the Analysis of variance (ANOVA) and intergroup relationships were assessed by utilizing post-hoc Tukey test.

IV. Results

The present study revealed that the disease mainly affected the individuals from poor socioeconomic background with male predominance. The study also reveals that the disease mainly affects the younger age groups, especially those who were in the age of 20- 35 years. The disease severity was clearly documented in figure 1 in terms of mouth opening.

Comparison of the mean serum iron, zinc, selenium and copper levels and lipid profiles of the four groups was done. The 'F' values observed in both multivariate analysis and in individual analysis of variance were significant ($p< 0.01$ or 0.05). The following inferences were obtained while corroborating the results of Tukey Test.

A significantly low level of serum iron (0.904) was observed in the advanced stage of OSMF (group IV) when compared to groups I,II and III (table 1, fig. 2a). There was no significant difference observed in case of group II and III when compared to that of healthy individual (group I). In case of serum zinc level (fig.2c), significant differences between all the groups ($p< 0.01$) were observed. The level decreased in the order 0.834> 0.657> 0.560 for group II, III, IV, respectively from that of the healthy individuals (group I, 0.896). The level of zinc in the serum was reduced during disease progression. Serum selenium level also showed a decreasing tendency similar to zinc (fig. 2d). The level was very much reduced (0.045) in the advanced stage of the disease (group IV) when compared to healthy individuals (group I). Unlike the levels of serum iron, zinc and selenium, the serum level of copper (fig. 2b) showed a significant increase (1.516; $p< 0.01$) at the advanced stage of the disease (group IV). The level was found to be reduced in group III (1.352) when compared to group IV while groups I and II exhibited no significant difference.

Lipids are the integral part of the cell membrane. The results obtained from the serum analysis of lipid profiles of OSMF patients and healthy individuals are represented in table 2 and figure 3. A significant decrease (139.5; $p< 0.05$) in the level of serum total cholesterol (fig. 3a) was found at the advanced stage of OSMF (group IV) when compared to the control group (group I), whereas, no significant changes were obtained in the other study groups (fig.3a). The mean level observed in group II (180.0) was quite comparable to that of group I (164.83). At the same time, a very low level of triglyceride and very low density lipoproteins (VLDL) (fig. 3b, fig. 3e) was noted at the advanced stage of the disease which was statistically significant ($p< 0.01$). The mean level of triglycerides obtained in group I, II, III and IV was in the order 142.83>142.00>122.50>97.00, while that of VLDL was 28.50>28.50>24.50>19.50. From this it is clear that, group I, II and III did not show any statistically significant change when compared with group IV which showed a drastic decrease in the mean level of both triglycerides and VLDL. The results here explain that the severity of the disease very much reduced serum triglyceride and VLDL level in group IV (advanced stage of OSMF). The level of serum high density

lipoprotein (HDL) (fig. 3c) noted in stage I (group II; 37.83) and II (group III; 36.50) of the disease, was quite similar to that of healthy individuals (42.83) and also the observed levels of HDL were not statistically significant, unlike at stage III (group IV), where a statistically significant decrease ($p< 0.01$) was found in the HDL level (32.67). A significantly higher level ($p< 0.05$) of LDL and the total cholesterol/HDL ratio (TC/HDL) (fig. 3f) was observed in the first stage of the disease (group II). The level rose to the maximum of 113.66 and 4.83 for LDL and TC/HDL ratio, respectively in this group. The level of both the parameters observed in group II and III was very much similar. But a significant difference ($p< 0.01$) was observed in the level of LDL when the comparisons were made between group III (100.00) and IV (87.50) unlike TC/HDL ratio, which showed a non significant change.

V. Discussion

OSMF is a pathological lesion of the oral mucosa, which is characterized by chronic inflammation and epithelial atrophy along with loss of rete ridges leading to hyalinization and fibrosis of submucosal tissue. As the disease progresses, patients experience difficulty in opening their mouth due to excessive fibrosis of buccal mucosa. Areca nut chewing is the main factor that contributes to the pathogenesis of the disease. An experiment conducted in mice showed that, a long term topical application of aqueous extract of areca nut lead to the epithelial atrophy followed by the infiltration of immune cells into the subepithelial layer and the gradual deposition of collagenous matrix components [8]. This result highly supported the present study, where patients who developed OSMF only due to the habit of taking areca nut and its commercially available product were selected and showed similar clinical features and mouth opening.

The increase in serum copper (Cu) level observed in the present study is in agreement with the report of Gupta [9]. The high level of Cu in areca nut, which is available in both processed (areca nut + tobacco = guthka; areca nut + without tobacco = pan masala) and unprocessed forms, can be a contributing factor to the high serum Cu level. IARC highlighted that Cu content in the processed form of areca nut is 2.5 times higher than that of raw areca nut [10]. An increase in fibroblast proliferation was observed by Trivedy [11] on addition of Cu at various concentrations *in vitro.* She also reported that Cu induced mutagenesis through p53 aberrations in OSMF which may be critical in the progression of the disease to OSCC. The possible relationship behind the increased serum Cu level and the development of OSMF could be the initiation of fibrosis through the enhanced cross linking of collagen fibers by the Cu dependent enzyme lysyl oxidase (LOX) and inhibition of collagen degradation by the collagenase enzymes present in the human body [12].

Zn is an important trace element in different aspects. It plays a catalytic or structural role in many enzymes such as carbonic anhydrase, lactate dehydrogenase and more importantly superoxide dismutase which protects our body against free radical damage [13]. A very low level of serum Zn observed in the present study during the progression of the disease is supported by the report of Shatter [14] who highlighted a decreased serum zinc level and the disease in progression. Similarly, deficiency of Zn can also contribute to cancer initiation through the activation of NF- κB and the subsequent induction of other signaling pathways [15]. The level of Zn displays an inverse relationship with that of Cu, which is in line with the result of the current study, where a significant increase was seen in Cu level unlike Zn.

Being an integral part of hemoglobin, iron (Fe) plays a potent role in the nutritional status of an individual. Serum Fe is considered as an important biochemical index for nutritional assessment and also it participates in many energy producing reactions. Low levels of serum Fe observed in the present study could be due to the utilization of Fe by the enzymes involved in the hydroxylation of proline and lysine for the synthesis of collagen [16]. Similarly, Selenium (Se) acts as an antioxidant nutrient by becoming a part of various enzymes such as glutathione peroxidase, Type I iodothyronine, deiodinase, metalloprotein, fatty acid binding protein and Selenoprotein P. The low levels of serum Se observed in OSMF patients of the current study is in line with the report of Khanna [17]. The role of Se is very complex and can be attributed to its immune modulating and anti-proliferative properties which can influence immune response by making immune cells more resistant to oxidative stress [18]. Higher dietary intake of Se may provide protection, but the initial ulceration that occurs in the oral mucosa of OSMF patients limits the food intake which in turn leads to the nutritional depletion.

Lipids are the main components of cell membrane and it is very essential for various biological functions viz cell growth and division of normal and malignant tissues. More importantly, lipids are involved in the maintenance of structural and functional integrity of biological membranes [19]. An altered serum lipid profile has been previously reported by Gerber and Forones [20,21] in breast and colon carcinogenesis, respectively. The observed low levels of lipid profile in the present study could be due to the induction of lipid peroxidation by the ROS released during areca nut chewing and the increased membrane biogenesis by utilizing lipids including lipoproteins, total cholesterol and triglycerides by the system [22].

VI. Conclusion

Since trace elements such as Fe, Zn, Se and Cu play an essential role in various biochemical reactions, their right proportions are very much important for normal tissue homeostasis. Similarly, lipids play an important role by being as integral part of cell membranes. This study demonstrated that, there is an alteration in the serum level of trace elements and lipid profile of OSMF patients when compared to the healthy individuals and it also explains its direct relationship with the disease severity. Thus, it can be concluded that, serum trace elements and lipid profile play an important role in the pathogenesis of oral mucosa such as OSMF and can be utilized as a biomarker. Further studies are required to explore the molecular mechanism behind this by using a larger population.

VII. Acknowledgment

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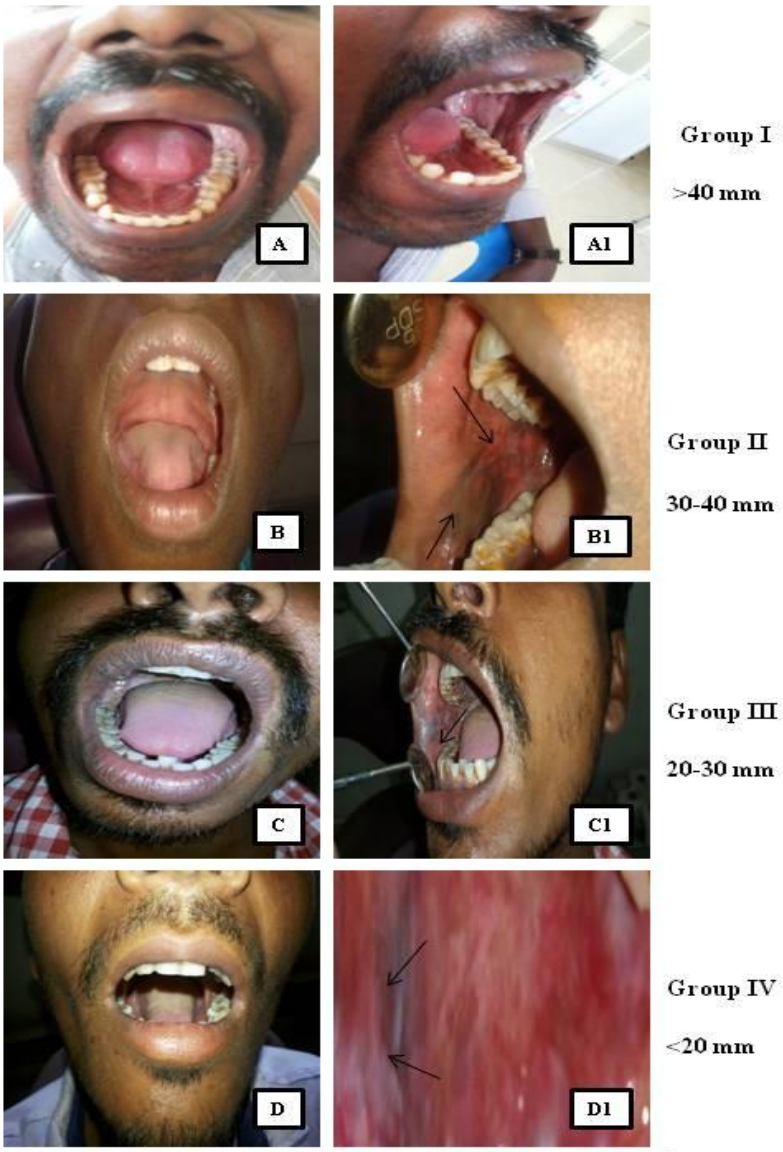
Fig. 1 STUDY GROUPS BASED ON MOUTH OPENING



Figures. A-D show the photographs of extra oral and mouth opening of patients.
Figures. A1- D1 are the intra oral photographs of patients showing lesions of OSMF, indicated using black arrows.

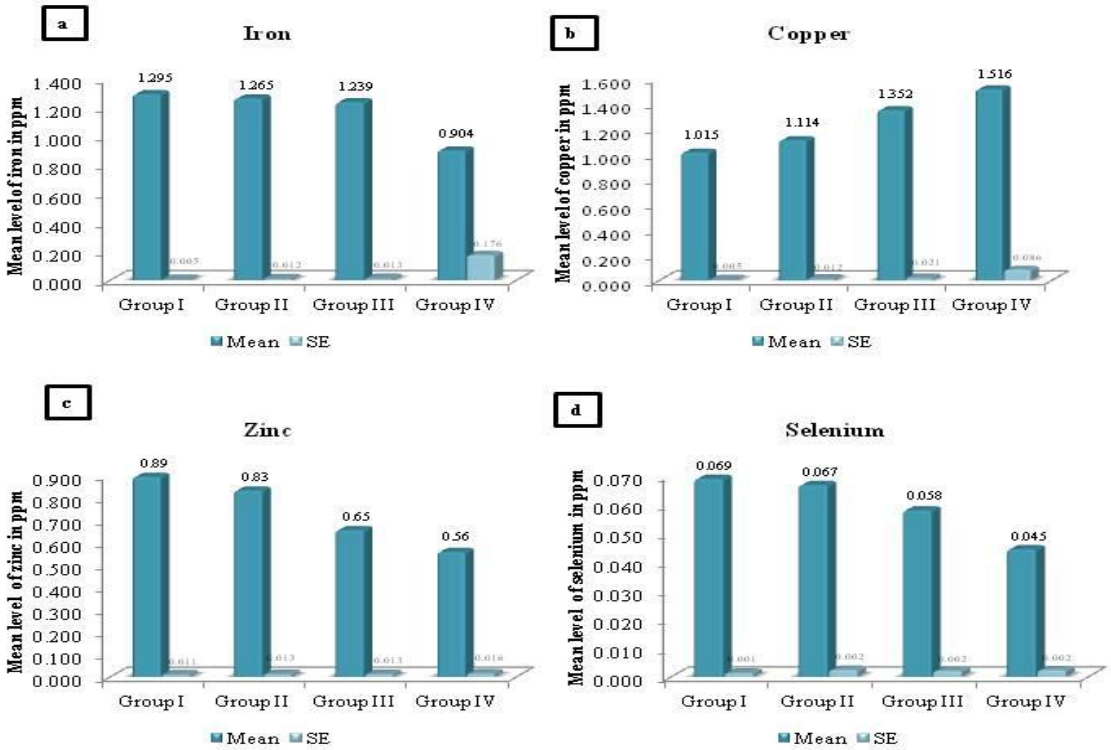
Table 1. Results of ANOVA and Tukey Test for multiple comparisons of Mean values for trace elements

Trace elements	Subgrouping of means			
	Set 1	**Set 2**	**Set 3**	**Set 4**
Iron F = 4.28*	Group I [1.295] Group III [1.24]	Group II [1.2646] Group III [1.24] Group IV [0.9036]		
Zinc F = 132.16**	Group I [0.896]	Group II [0.834]	Group III [0.657]	Group III [0.560]
Selenium F = 27.38**	Group I [0.069] Group II [0.067]	Group III [0.058]	Group IV [0.045]	
Copper F= 25.85**	Group I [1.015] Group II [1.114]	Group III [1.352]	Group IV [1.516]	

Mean values of serum iron, zinc, selenium and copper levels obtained in each group is expressed as parts per million (ppm).

* Significant $p < 0.05$- Mean values within the sets are almost similar, ** Significant $p < 0.01$- Mean values between different sets are significantly different.

Fig. 2 SERUM TRACE ELEMENTS ANALYSIS



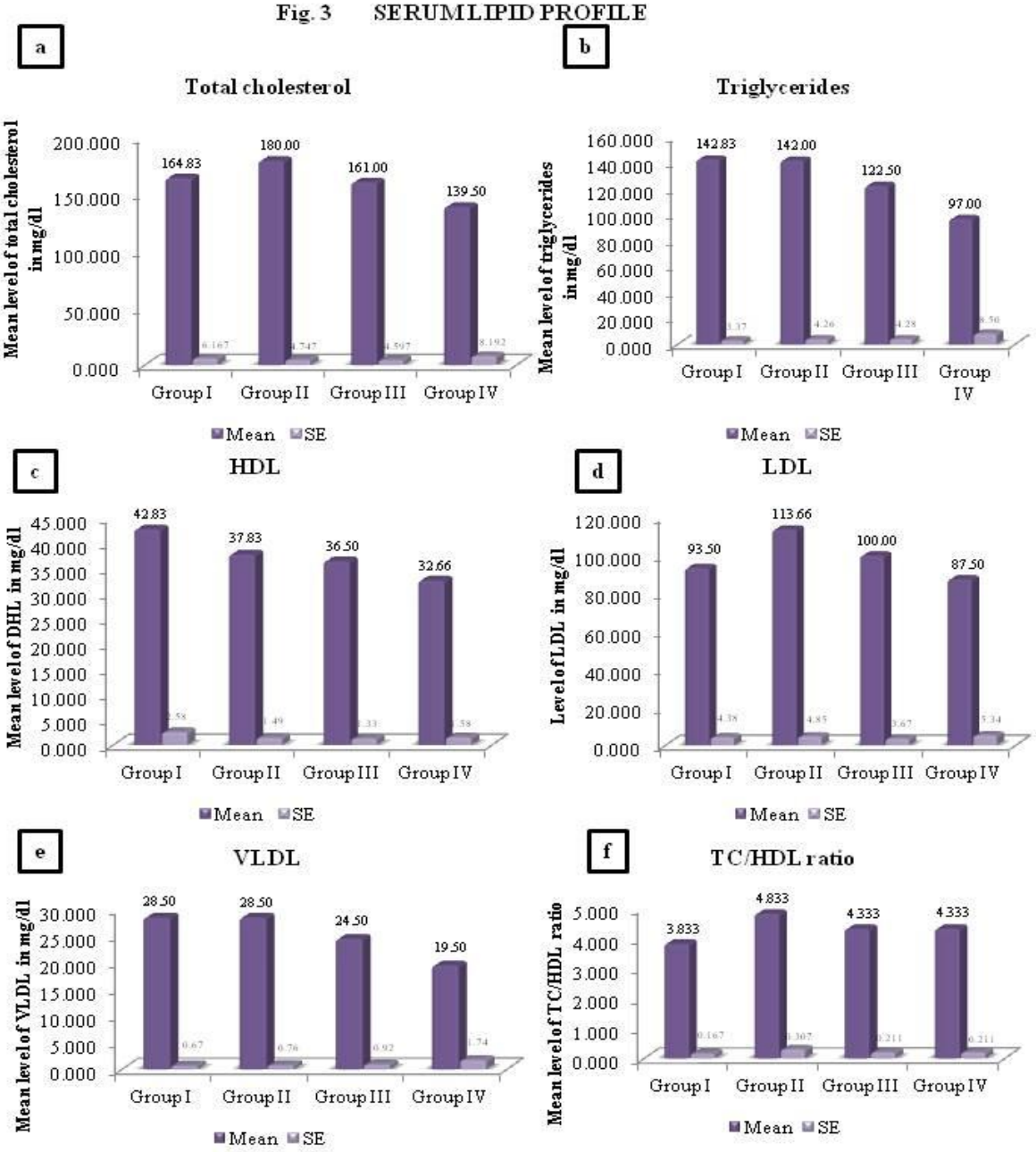
Bar diagrams showing serum levels of (a) Iron, (b) Copper, (c) Zinc and (d) Selenium of the four studied groups. SE → Standard error.

Table 2. Results of ANOVA and Tukey Test for multiple comparisons of mean values for lipid profile

Lipid profiles	Subgrouping of means	
	Set 1	Set 2
TC	Group I [164.833]	Group III [161.04]
F = 7.504**	Group II [180.0]	Group IV [139.5]
	Group III [161.0]	
TG	Group I [142.833]	Group IV [97.0]
F = 15.437**	Group II [142.0]	
	Group III [122.5]	
HDL	Group I [42.833]	Group II [37.833]
F = 5.338**	Group II [37.833]	Group III [36.5]
	Group III [36.5]	Group IV [32.677]
LDL	Group I [93.5]	Group II [113.667]
F = 5.941**	Group III [100.0]	Group III [100.0]
	Group IV [87.5]	
TC/HDL	Group I [3.833]	Group II [4.833]
F = 3.158*	Group III [4.333]	Group III [4.333]
	Group IV [4.333]	Group IV [4.333]

Mean values of serum total cholesterol (TC), triglycerides (TG), high density lipoprotein (HDL), low density lipoprotein (LDL) and very low density lipoprotein (VLDL) expressed in µg/dl. * Significant $p < 0.05$- Mean values within the sets are almost similar, ** Significant $p < 0.01$- Mean values between different sets are significantly different.

Fig. 3 SERUM LIPID PROFILE



Bar diagram showing serum lipid profiles of various study groups. (a) Total cholesterol (TC), (b) Triglycerides (TG), (c) High density lipoprotein (HDL), (d) Low density lipoprotein (LDL), (e) Very low density lipoprotein (VLDL), (f)Total cholesterol/ High density lipoprotein (TC/HDL) ratio. SE → Standard error.

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