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Comparative Analysis of Serum and Salivary Uric Acid, Albumin and Lactate Dehydrogenase Levels in Smokeless Tobacco Consumers of Sriganganagar Population-A Case Control Study

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Keywords

Oral cancer, Albumin, uric acid, lactate dehydrogenase, oral precancerous lesions

ABSTRACT:

Introduction: Oral cancer (OC) is now considered one of the India's major public health challenges by the World Health Organization (WHO). Oral squamous cell carcinoma (OSCC) is the world's sixth most common malignancy. Saliva contains a number of biomarkers for the detection of oral diseases. These biomarkers are albumin, lactate dehydrogenase etc.

Aims and Objectives: To assess and compare the levels of serum and salivary uric acid, albumin and lactate dehydrogenase levels in smokeless tobacco consumers.

Materials and Methods: 25 healthy subjects without any deleterious habits of tobacco or betelnut consumption and without lesion on intraoral examination (Group 1), 25 patients with habit of smokeless tobacco consumption, but without any associated intraoral lesion/(s) clinically (Group 2), 25 patients with habit of smokeless tobacco consumption, and with associated intraoral lesion/(s) seen clinically (Group 3) were included in the study. Levels of serum and salivary uric acid, albumin and lactate dehydrogenase were evaluated using a semiautomatic autoanalyzer. The data obtained was analyzed using the SPSS version 22 software.

Results: LDH level in serum increases progressively from group 1 to group 2 and group 3. Salivary LDH value is also increases from group1 to group 2 and 3. Uric Acid level in serum decreases progressively from group 1 to group 2 and group 3. Salivary Uric Acid value is also decreases from group1 to group 2 and 3. Albumin level in serum decreases progressively from group 1 to group 2 and group 3. Salivary albumin value is increases from group1 to group 2 and 3.

Conclusion: Estimation of serum and salivary LDH and uric acid is only an auxiliary investigation which may act as an adjunct in diagnosis of OSCC and premalignant lesion

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and can only provide collaborative evidence.

INTRODUCTION

Oral squamous cell carcinoma (OSCC) is the sixth most unexceptional cancer globally. Oral squamous cell carcinoma (OSCC) is a multistage progression, from normal to dysplastic cells (precancerous lesions) and ultimately to squamous cell carcinoma.[1] Overdue diagnosis is the main explanation for high mortality rate of OSCC.[2] Saliva has been found to contain constituents that reflect the diseased or physiological state of the human body, and hence could be employed for diagnostic purposes.[3] Saliva contains various biomarkers for the detection of oral diseases and also for the precancerous conditions. These biomarkers are like albumin, lactate dehydrogenase, proteases, mucins, uric acid etc. LDH is a biomarker for cancer recognition, which is found in almost all cell types. Moreover, increased level of this tumor marker has been reported in malignant and premalignant oral lesions such as leukoplakia, submucosal fibrosis, and OLP, compared with normal tissue.[4] The roles of albumin in the body are understood well enough to allow it to be used as a consistent marker of oxidative stress; it could be beneficial for early diagnosis of various oral precancerous lesions and for determination of the propensity toward transformation into frank oral malignancy.[5,6] Uric acid is the end produce of purine catabolism and contributes to the antioxidant capacity of blood and saliva. Uric acid contributes approximately 70-85% of the total antioxidative capacity of resting and stimulated saliva from healthy as well as periodontally compromised subjects.[7,8] Very few studies are present in the literature about the serum and salivary expression of LDH, albumin and uric acid in conjunction with various OPMDs. Based on these assumptions present study is formulated to estimate LDH, Albumin and Uric acid level in serum and saliva of tobacco users with/without premalignant disorders.

MATERIALS AND METHODS

This case control study will include a total of randomly selected 75 subjects (male & female) with age range of 18 to 65 years, were divided into three groups, namely Group I (n=25): healthy subjects without any deleterious habits of tobacco or betelnut consumption and without lesion on intraoral examination, Group II (n=25): patients with habit of smokeless tobacco consumption, but without any associated intraoral lesion/(s) clinically, Group III (n=25): patients with habit of smokeless tobacco consumption, and with associated intraoral lesion/(s) seen clinically. Patients who will be using smokeless tobacco for a minimum of last 6 months will be included in Group II and III. An institutional Ethical committee clearance and a written informed consent were obtained from each participant.

The following are the inclusion and exclusion criteria which are considered in the study.

Inclusion Criteria

Only healthy patients without any deleterious tobacco /betel nut will be included in Group I, Co-operative patients, patients with good oral compliance, patients free of any congenital diseases/disorders affecting salivary glands.

Exclusion Criteria

Patients who will be currently undergoing treatment for tobacco related diseases/lesions, patients who will be having medical problems that can cause alteration in the serum or salivary LDH, uric acid or albumin levels, patients undergoing treatment for OSCC, patients suffering from salivary gland disorders affecting salivary secretions, patients on medications affecting salivary secretion flow, pregnant females, patients with periodontal therapy 3 months prior to study, patients unwilling or unable to give informed consent.

METHODOLOGY

The whole unstimulated saliva will be collected with low force spitting method for 5 minutes at 1 minute interval by asking the patients to lean forward and spit saliva into sterile container Fig 1. The saliva collected will be centrifuged at 3000 revolutions /minute and will be stored in the ice box for LDH, Uric acid & Albumin analysis, and for the serum, 5 ml of blood will be withdrawn from the antecubital

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vein with minimal trauma under aseptic conditions, separately and will be centrifuged at 3000 revolutions/ minute and will be stored at - 200 Celsius for LDH, Uric acid & Albumin analysis a Fig 2 (Armamentarium for laboratory investigations).

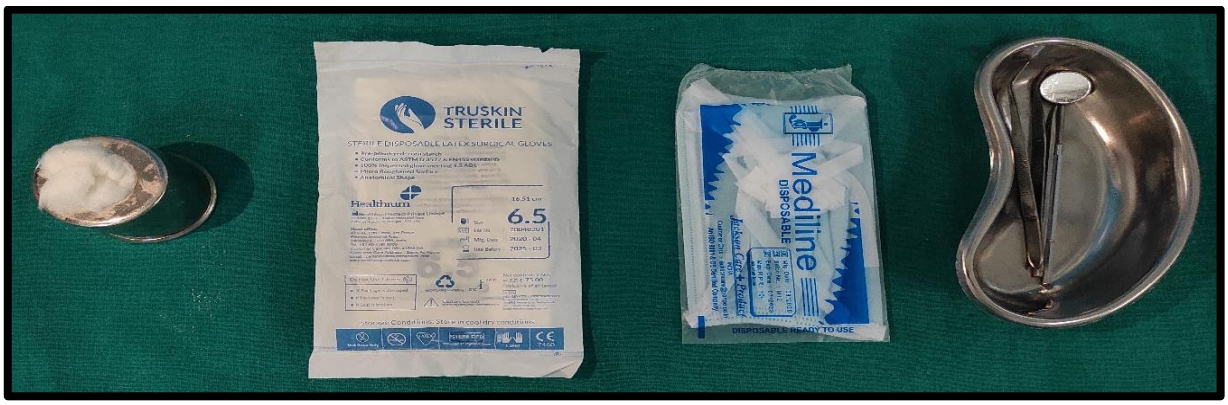

Figure 1: Armamentarium for clinical examination.

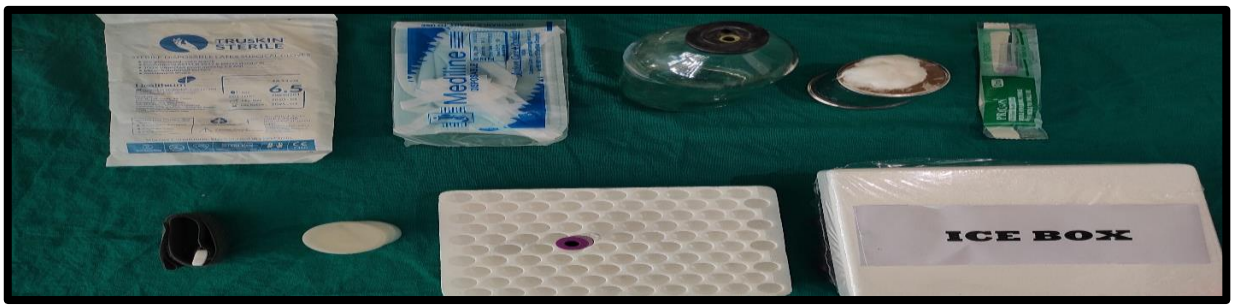

Figure 2: Armamentarium for serum and saliva sample collection.

Figure 3: Collection of saliva sample (spit method)

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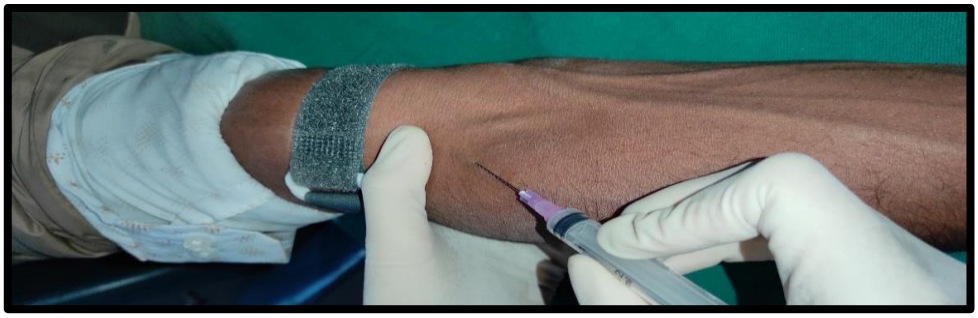

Figure 4: Collection of blood sample

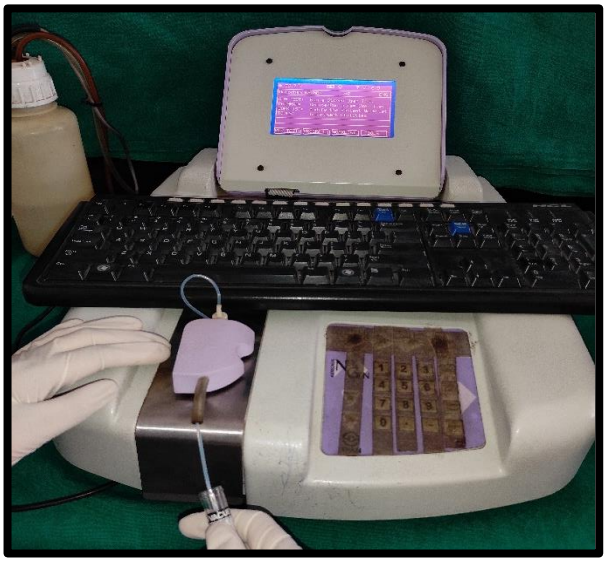

Figure 5: Armamentarium used for laboratory investigations

RESULTS

Present study includes 52 males and 23 females. Healthy controls incorporate 64% males and 36% females, study group 2 is having 72% males and 28% females. Group 3 of premalignant disorders have 72% males and 28% females.[Table1} {Graph1} The LDH level in serum of healthy control is 213.65 mg/dl and increases progressively in group 2 and group 3 as 337.48 mg/dl and 802.39 mg/dl respectively. Salivary LDH value is also increases from group1 to group 2 and 3 as shown in graph 2. The values are 140.39, 230.52 and 432.78 in study groups. {Table 2} {Graph 2}. Uric Acid level in serum of healthy control is 5.81 mg/dl and decreases progressively in group 2 and group 3 as 4.38 mg/dl and 2.60 mg/dl respectively. Salivary Uric Acid value is also decreases from group1 to group 2 and 3 as shown in graph 4. The values are 4.54, 3.53 and 2.345 in study groups. {Graph 3]. Albumin level in serum of healthy control is 4.59 mg/dl and decreases progressively in group 2 and group 3 as 3.56 mg/dl and 2.81 mg/dl respectively. Salivary albumin value is increases from group1 to group 2 and 3 as shown in graph 5. The values are 0.48, 0.65 and 0.85 in study groups.{Graph 4}. Anova test shows a significant mean difference in serum and salivary LDH, Albumin, Uric acid level of study groups.{Table 3}.{Table 4}{Table5}. T test between study group 2 (healthy control with tobacco habit and no lesions) and study group 3 (subjects with tobacco habit and PMDs) shows significant difference in Salivary Uric Acid level with p value .046, serum LDH level with value p value .000 and Salivary LDH with p value

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.000.{Table 6}. T test between study group 1 (healthy control with no tobacco habit) and study group 3 (subjects with tobacco habit and PMDs) shows significant difference in Serum Uric Acid level with p value .000, Salivary Uric acid level with p value .002, serum LDH level with value p value .002 and Salivary LDH with p value .000 {Table7}. T test between study group 1 (healthy control with no tobacco habit) and study group 2 (subjects with tobacco habit but no lesions) shows significant difference in salivary albumin level with p value .023, serum Uric acid level with p value .003, serum LDH level with value p value .000 and Salivary LDH with p value .000 {Table8}.

Table: 1- Description of gender distribution in each study group

Study Group	Male	Male %	Female	Female %	Total
Healthy Controls	16	64	9	36	25
Tobacco No Lesion	18	72	7	28	25
PMDS	18	72	7	28	25
Total	52	-	23	-	75

Graph: 1- Comparison of gender distribution in each study group

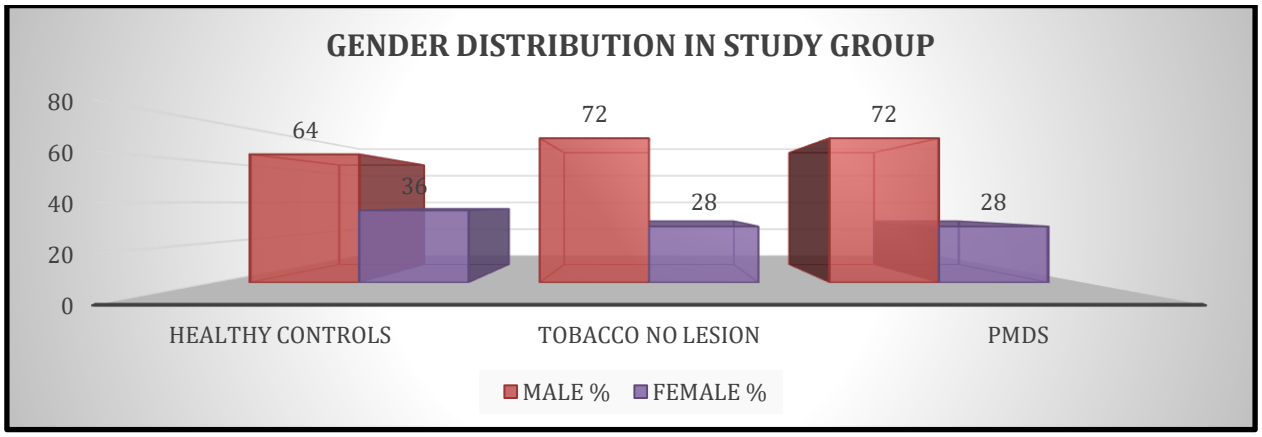

Table: 2- Description of level of LDH, Albumin and Uric acid in saliva and serum with respect to study groups

Study Group	Serum LDH	Saliv LDH	Serum ALB	Saliv ALB	Serum UA	Saliv UA
Healthy Controls	213.65	140.39	4.59	0.48	5.81	4.54
Tobacco No Lesion	337.48	230.52	3.56	0.65	4.38	3.53
PMDS	802.39	432.78	2.81	0.85	2.60	2.34

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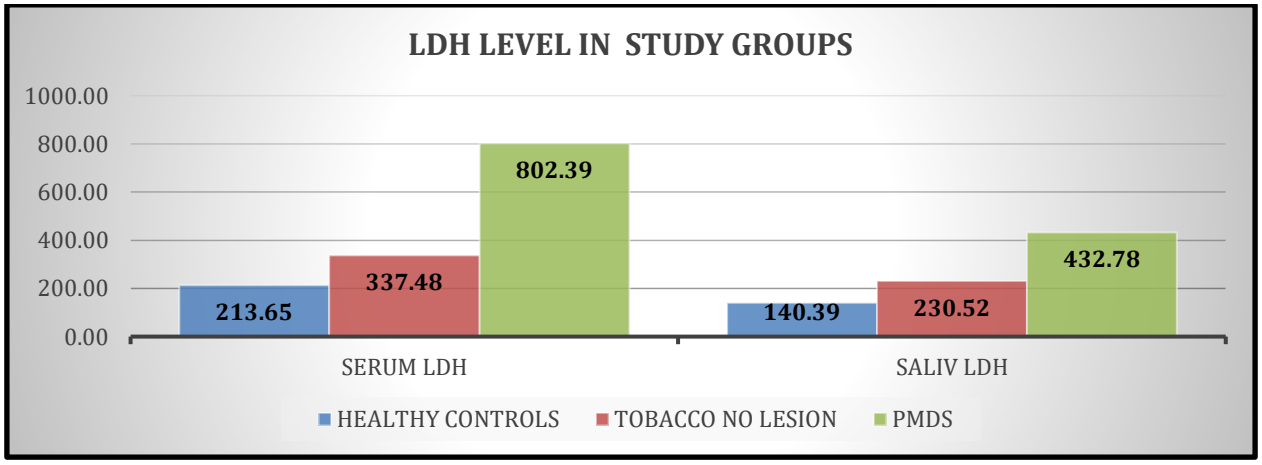
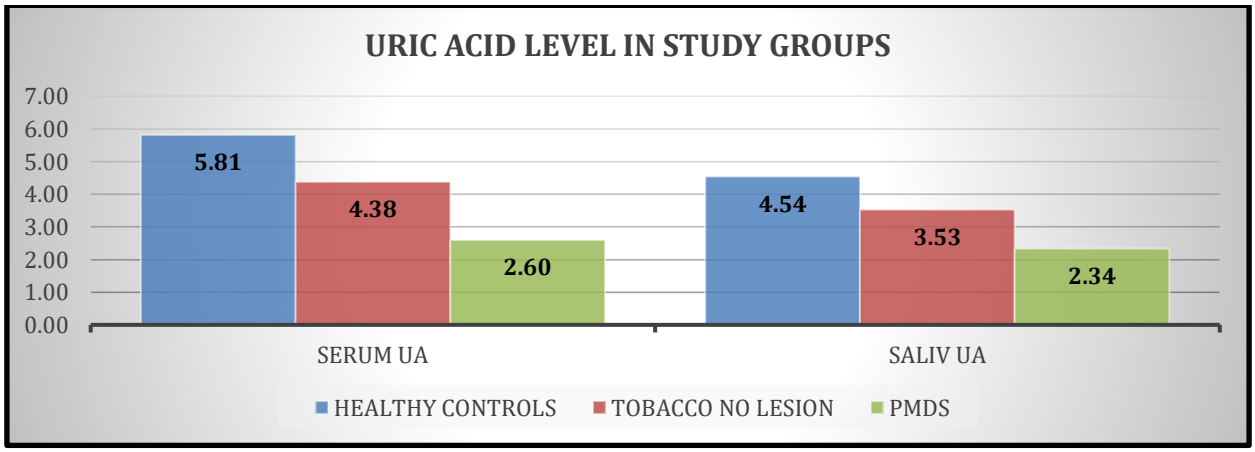
Graph: 2- Comparison of level of LDH, in saliva and serum with respect to study groups



Graph: 3-Comparison of level of Uric Acid, in saliva and serum with respect to study groups.



Graph: 4- Comparison of level of Albumin, in saliva and serum with respect to study groups.

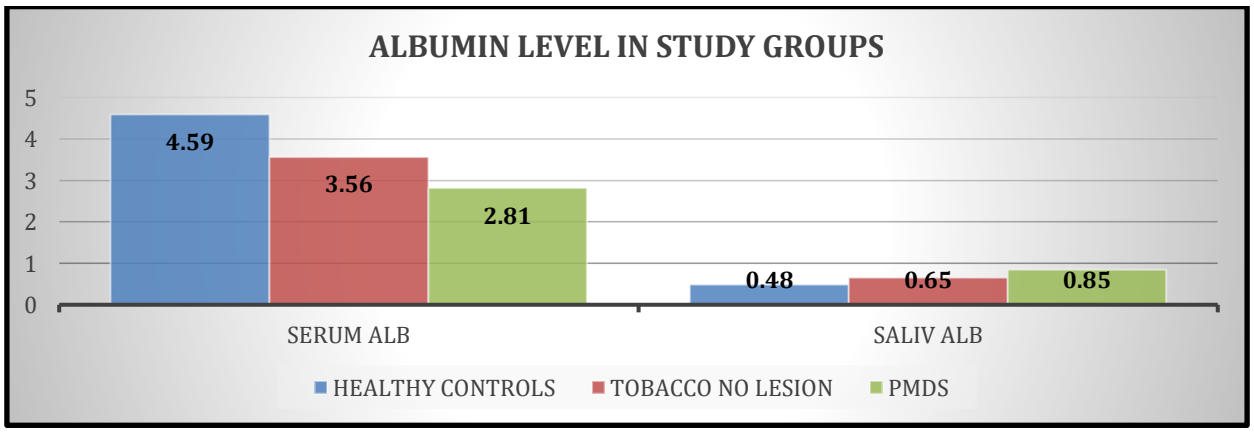


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Table 3: - Analysis of LDH level in serum and saliva between study groups (**ANOVA)**

		Sum of Squares	df	Mean Square	F	Sig.
SERUM_LDH	Between Groups	4790633.307	2	2395316.653	1008.174	.000
	Within Groups	171064.480	72	2375.896		
	Total	4961697.787	74			
SAL_LDH	Between Groups	1123403.840	2	561701.920	390.801	.000
	Within Groups	103486.160	72	1437.308		
	Total	1226890.000	74			
Anova test shows a significant mean difference in serum and salivary LDH level of study groups						

Table 4: - Descriptive analysis of Albumin level in serum and saliva of study groups (**ANOVA)**.

		Sum of Squares	df	Mean Square	F	Sig.
SERUM_ALB	Between Groups	38.739	2	19.369	52.976	**.000**
	Within Groups	26.325	72	.366		
	Total	65.064	74			
SAL_ALB	Between Groups	1.653	2	.826	26.561	**.000**
	Within Groups	2.240	72	.031		
	Total	3.893	74			
Anova test shows a significant mean difference in serum and salivary Albumin level of study groups						

Table 5: - Descriptive analysis of Uric Acid level in serum and saliva of study groups (**ANOVA)**

		Sum of Squares	df	Mean Square	F	Sig.
SERUM_UA	Between Groups	124.923	2	62.461	208.471	.000
	Within Groups	21.572	72	.300		
	Total	146.495	74			
SAL_UA	Between Groups	62.223	2	31.112	22.705	.000

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	Within Groups	98.658	72	1.370		
	Total	160.881	74			
Anova test shows a significant mean difference in serum and salivary Uric Acid level of study groups						

Table 6- T test between Study Group 2 and group 3

	Levene's Test for Equality of Variances		t-test for Equality of Means	
	F	Sig.	t	df
SERUM_ALB	.485	.490	4.159	48
SAL_ALB	.227	.636	-4.392	48
SERUM_UA	1.250	.269	9.931	48
SAL_UA	4.178	**.046**	4.409	48
SERUM_LDH	30.510	**.000**	-30.643	48
SAL_LDH	32.041	**.000**	-15.464	48

Table 7- T test between Study Group 1 and group 3

	Levene's Test for Equality of Variances		t-test for Equality of Means	
	F	Sig.	t	df
SERUM_ALB	.035	.852	10.721	48
SAL_ALB	3.115	.084	-6.826	48
SERUM_UA	24.495	**.000**	21.407	48
SAL_UA	10.436	**.002**	6.746	48
SERUM_LDH	10.452	**.002**	-35.795	48

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SAL_LDH	68.375	**.000**	-23.558	48

Table 8- T test between Study Group 1 and 2

	Levene's Test for Equality of Variances		t-test for Equality of Means	
	F	Sig.	t	df
SERUM_ALB	.772	.384	5.883	48
SAL_ALB	5.528	**.023**	-3.243	48
SERUM_UA	10.066	**.003**	10.171	48
SAL_UA	1.528	.222	2.670	48
SERUM_LDH	15.194	**.000**	-14.746	48
SAL_LDH	37.312	**.000**	-20.585	48

DISCUSSION

Oral squamous cell carcinoma is the sixth most common malignancy, and is a major cause of cancer mortality worldwide. Globally, about 500,000 new oral and pharyngeal cancers are diagnosed annually, and three quarters of these are from the developing world.[9,10] In India, oral cancer is highly prevalent, due to habit of tobacco chewing.[11] Diagnosis of OSCC and oral premalignant lesions/conditions is based on the findings obtained from the history, clinical examination and diagnostic aids such as radiological and histopathological investigations. Recently, tumor markers are receiving more attention in the early detection of the lesion. Various biological markers have been ushered in the field of dentistry. The use of saliva as a diagnostic tool has many advantages: it is easy to collect using a noninvasive technique that can be performed at home, no special equipment is required for collection, and it has fewer complications than many alternatives.[12] In our Study Serum LDH level in healthy controls were 213.65, in tobacco without lesion were 337.48 and potentially malignant lesion was 802.39. Serum LDH levels showed exponential rise in group with potentially malignant lesions. Visjna and Turshijan in 2008 found elevated serum LDH levels in patients with breast cancer and they have opined that elevated level of LDH might be a prognostic sign of disease progression.[13] Basically, there are three mechanisms responsible for the rise in the level of serum LDH in malignant subjects. Necrosis and cellular degeneration, Induction process initiated by the tumor and involving normal tissue, Muscle degeneration caused by protein deficit. The significant increased serum LDH level correlates with the increased cellular activity. The growth rate of cancer cells can thus be correlated with glycolysis, with a more malignant cell showing higher rates of aerobic and anaerobic glycolysis than a less malignant cell of similar origin.[3] Salivary LDH level in healthy controls were 140.39,in tobacco without lesion were 230.52 and potentially malignant lesion was 432.78.Salivary LDH levels also showed exponential rise in group with potentially malignant lesions. Shipter et al. in studies conducted in 2007

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and 2009 demonstrated a complete change in the composition of saliva in oral cancer patients. They reported alterations in parameters such as matrix metalloproteinases 2 and 9, IGF-1, and sIgA in the saliva and demonstrated a significant increase in salivary LDH level of oral cancer patients, which was in agreement with the current results[6,8]. Serum Albumin level in healthy controls were 4.59,in tobacco without lesion were 3.56 and potentially malignant lesion was 2.81.Serum albumin levels were low in groups with potentially malignant lesions.In addition to this Salivary Albumin levels were also analysed that showed 0.48 in healthy control.0.65 in in tobacco habit without lesion and 0.85 in potentially malignant lesion. Here the values were opposite to serum albumin levels. Salivary albumin levels were high in patient with lesions. R Metgud et al found Serum albumin levels decreased in oral pre-malignancy and oral malignancy cases compared to healthy individuals. Salivary albumin levels increased in oral pre-malignancy and oral malignancy cases compared to healthy individuals. Albumin is the most abundant plasma protein in humans. It accounts for 55 – 60% of measured serum proteins. It is a single polypeptide chain of 585 amino acids with a molecular weight of 66,500 Da. The total body albumin pool is about 3.5 – 5.0 g/kg body weight. The molecule is flexible and changes shape readily with variations in environmental conditions and with binding of ligands.[14] Albumin is thought to have antioxidant potential, which may be related to the abundance of sulfhydryl (-SH) and free thiol groups in the albumin molecule[15,16]. Because of this, albumin can provide ten times more antioxidant protection against various ROS than other plasma proteins. Therefore, it is involved in scavenging oxygen free radicals that are thought to be involved in the pathogenesis of oral cancer. Our observations are consistent with reports by Yasunori Iwao et al. (2006), Lawal et al. (2010) and Nayyar et al. (2011).[17,18] In our study Serum uric acid level in healthy controls were 5.18,in tobacco without lesion were 4.38 and potentially malignant lesion was 2.60. Serum uric acids levels were low in groups with potentially malignant lesions.In addition to this Salivary uric acid levels were also analysed that showed 4.54 in healthy control.3.53 in in tobacco habit without lesion and 2.34 in potentially malignant lesion. This was corroborated by Bozkir et al[7] who reported a significantly lower Serum uric acid in lung cancer patients compared to healthy controls.[19] The low Serum uric acid in oral cancer patients in this study may be due to nutritional compromise of the patients due to Tumour necrosis Factor (TNF) and Interleukin 6 (IL-6) produced in cancer patients, which cause loss of appetite.[20] Serum uric acid level is also affected by alcohol consumption, fructose containing sugars, and defects in purine metabolism, impaired renal function, hyperinsulinemia, drugs such as diuretics and genetic factors.[21.22]

CONCLUSION

Estimation of serum and salivary LDH and uric acid is only an auxiliary investigation which may act as an adjunct in diagnosis of OSCC and premalignant lesion and can only provide collaborative evidence. Serum LDH estimation can prove, to be a valuable biochemical marker; as it is a simple procedure and may be easily accepted by the patient. Toxicity by oxygen radicals has been suggested as an important cause of cancer. Uric acid has been demonstrated to be an important antioxidant and a free radical scavenger in humans. It is one of the major radical-trapping antioxidants in plasma and is reported to protect the erythrocyte membrane against lipid peroxidation. This study showed that serum uric acid was lower in oral premalignant patients when compared with healthy volunteers and low serum uric acid was associated with increased risk of oral cancer development.

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