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Research Article

Serum LDH Level Estimation: an Adjunct in Screening, Diagnosis and Prognosis of Oscc.

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ABSTRACT: Tumor markers are the substances, which quantitatively change in serum, during tumor development. Malignant cells have a distinctive type of metabolism with altered biochemical parameters which are either increased or decreased. One such marker is serum LDH. The aim of this study was to evaluate serum LDH along with some other biochemical parameters like alkaline phosphatase, SGOT, SGPT in histopathologically confirmed cases of OSCC during various clinicopathological stages and also postoperatively for prognostic implication. The study group constituted of 60 randomly selected subjects from the outpatient clinic who were histopathologically confirmed cases of oral squamous cell carcinoma. Serum LDH level may serve as an adjunct biochemical tool to provide collaborative evidence in diagnosis of OSCC.

Key Words: Lactate Dehydrogenase (LDH), Oral Squamous Cell Carcinoma (OSCC), Enzyme, Pre Malignant lesion, Serum.

INTRODUCTION:

About 500000 new oral and pharyngeal cancers are diagnosed globally every year and three fourth of the are from developing countries(1).Around 90% reported cases of oral cancers are squamous cell carcinoma (OSCC)(2). The overall survival rate of OSCC has not significantly improved despite application of advanced therapeutic modalities through multidisciplinary approach. Early diagnosis and prompt treatment will avert mutilating surgery, improve patient's quality of life and can decrease morbidity and mortality associated with cancer(3). Quantative alteration in various biochemical substances in the serum during tumor development helps in evaluation of cancer(4). Also Malignant cells have a distinctive type of metabolism, leading to altered biochemical parameters(5).Research in to promising biomarkers can aid in early diagnosis and management of OSCC.

In the oral cavity, various tumor markers have been studied: These include oncofetal protein, (α-fetoprotein: Carcinoembryonic antigen), β−2 micro globulin and enzymes (lactate dehydrogenase [LDH]). One such marker is serum LDH(6).

LDH: Lactate dehydrogenase (LDH, L-lactate, NAD+ oxidoreductase, EC1.1.1.27) is a family of NAD+-dependent enzymes. There are five different clinically measurable LDH isoenzymes. They catalyze the reversible conversion of pyruvate to lactate with concomitant regeneration of NAD+, which is needed for the continuous generation of ATP to maintain glycolysis. LDH, a cytoplasmic enzyme is released into the peripheral blood after cell death caused by, e.g. ischemia, excess heat or cold, starvation, dehydration, injury, exposure to bacterial toxins, after ingestion of certain drugs, and from chemical poisonings(7). The three possible mechanisms responsible for the rise in the level of serum LDH in malignant subjects are: 1) Necrosis and cellular degeneration, 2) Induction process initiated by the tumor and involving normal tissue, 3) Muscle degeneration caused by protein deficit(6). Cancer cells preferentially utilize glycolysis instead of mitochondrial oxidative phosphorylation even in the presence of oxygen; this phenomenon is known as the Warburg effect(8). LDH-A has an important role in the final step of the Warburg effect by converting pyruvate to lactate. In esophageal squamous cell carcinoma, LDH- A is up-regulated in cancer tissues and promoted the survival of tumor cells. LDH-A is reported to enhance the growth and migration of gastric cancer cells(9).

The aim of this study was to evaluate serum LDH along with some other biochemical parameters like alkaline phosphatase, SGOT, SGPT in histopathologically confirmed cases of OSCC during various clinicopathological stages and also postoperatively for prognostic implication.

Material & Methods:

Seventy randomly selected subjects reporting at three different centers were divided into study group and control group. The study group constituted of 60 randomly selected subjects from the outpatient clinic who were histopathologically confirmed cases of oral squamous cell carcinoma. The control group consisted of 10 healthy volunteers with age and sex matching with the study group and visiting the outpatient clinic for routine dental checkup. Clinical inclusion criteria for control group were age within the range of 20-60 years, habit of tobacco use in any forms and without any clinically diagnosed oral lesion/condition. The exclusion criteria were any systemic diseases known to increase serum LDH levels such as MI, liver diseases, renal disease, and muscle dystrophy,oral conditions like periodontitis and patients having received dental treatment 48 hours prior to the study. Exclusion criteria in study group were similar conditions which could otherwise raise serum LDH level and subjects who were receiving radiation therapy, chemotherapy or chemo radiation.

Standard aseptic measures were adopted; tourniquet was applied 2 inches above the elbow on the upper arm. The site of puncture was cleaned using sterile gauze dipped in surgical spirit. Using 5ml syringe with a 22 gauge, 1 ½ inches needle, 4ml of blood was drawn from the antecubital vein. The blood was allowed to clot and the serum separated by centrifugation. The biochemical test was done along with routine blood investigation under single uniform standards.

RESULTS:

The mean age of the cases and controls was 48.45 (±11.72) and 45.30 (±13.23) years respectively (Table1, Fig. 1).

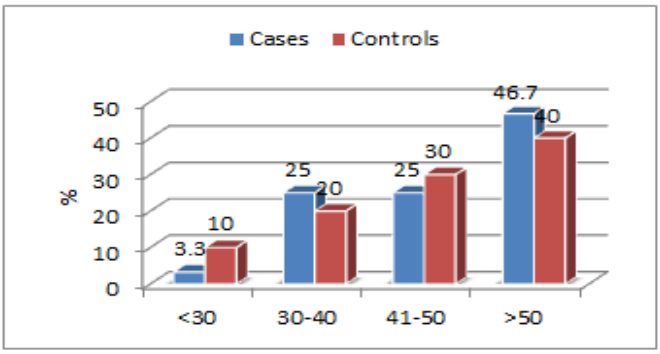
Table-1: Age distribution between cases and controls

Age in years	Cases (n=60)		Controls (n=10)		p-value[1]
	No.	%	No.	%	
<30	2	3.3	1	10.0	0.76
30-40	15	25.0	2	20.0	
41-50	15	25.0	3	30.0	
>50	28	46.7	4	40.0	
Mean±SD	48.45±11.72		45.30±13.23		

[1]Chi-square test

Table-1 & Fig. 1 shows the age distribution between cases and controls. More than one third of the cases (46.7%) and controls (40%) were above 50 years. The mean age of the cases and controls was 48.45 (±11.72) and 45.30 (±13.23) years respectively.

Fig. 1: Age distribution between cases and controls



Majority of the study subjects were male that was 75% of study group and 70% of control (Table 2, Fig. 2).

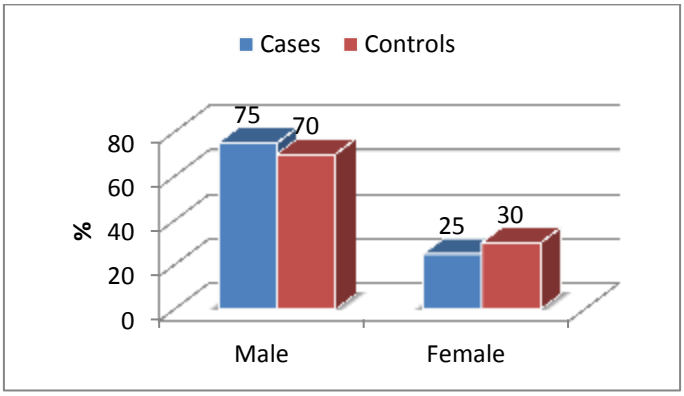
Table-2: Gender distribution between cases and controls

Gender	Cases (n=60)		Controls (n=10)		p-value[1]
	No.	%	No.	%	
Male	45	75.0	7	70.0	0.73
Female	15	25.0	3	30.0	

[1]Chi-square test

Table-2 & Fig. 2 shows the gender distribution between cases and controls. Majority of the both cases (75%) and controls (75%) were males (70%). There was no significant (p>0.05) difference in the gender between cases and controls showing comparability of the groups in terms of gender and age.

Fig. 2: Gender distribution between cases and controls



There was no significant (p>0.05) difference in the age and gender between cases and controls showing comparability of the groups in terms of age and gender. The habit of tobacco chewing was significantly (p=0.006) higher among the cases (90%) compared to controls (50%). The serum LDH level was significantly (p=0.001) higher among the cases (519.44±196.24) compared to controls (147.40±24.21) before the surgery (Table 3, Fig. 3).

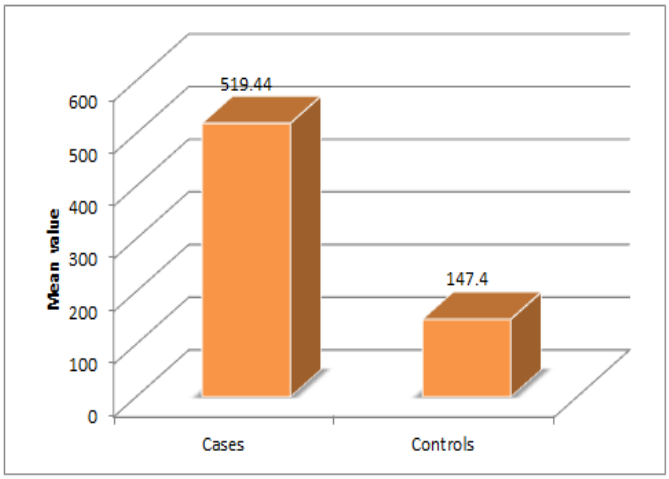
Table-3: Comparison of Pre-op serum LDH between cases and controls

Groups	Serum LDH (Mean±SD)
Cases	519.44±196.24
Controls	147.40±24.21
p-value[1]	0.0001*

[1]Unpaired t-test, *Significant

Table-3 & Fig. 3 shows the comparison of pre-op serum LDH level between cases and controls. The serum LDH level was significantly (p=0.001) higher among the cases (519.44±196.24) compared to controls (147.40±24.21) at pre-op.

Fig. 3: Comparison of Pre-op serum LDH between cases and controls



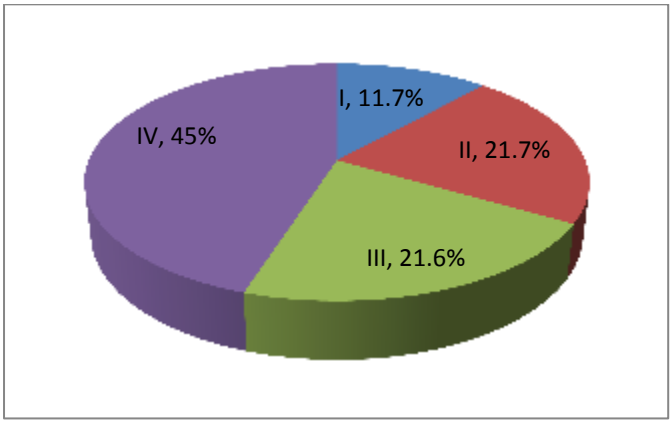
Among the studied subjects maximum reported in the advanced stage IV (45%) followed by stage II (21.7%), stage III (21.6%) and stage I (11.7%) (Table 4, Fig. 4).

Table-4: Distribution of clinical stage among the cases

Stage	No. (n=60)	%
I	7	11.7
II	13	21.7
III	13	21.6
IV	27	45.0

Table-4 & Fig. 4 shows the distribution of clinical stage among the cases. The stage IV (45%) was found among more than one third of the cases followed by stage II (21.7%), stage III (21.6%) and stage I (11.7%).

Fig. 4: Distribution of clinical stage among the cases



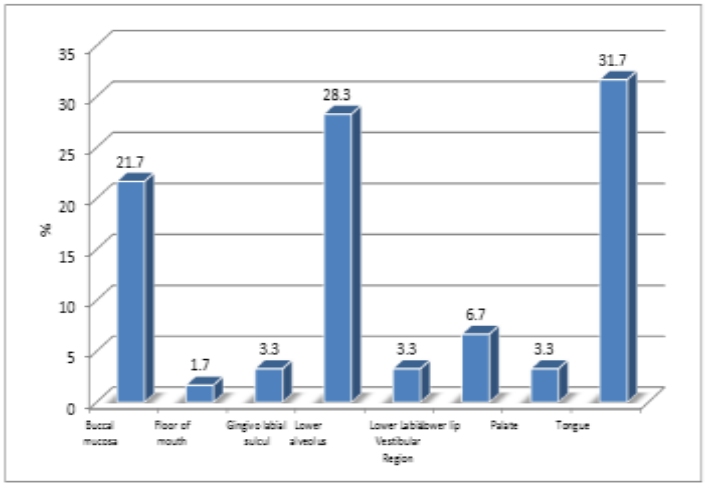
Among the different oral subsites tongue was the most affected site (31.7 %) followed by lower alveolus (28.3%) and buccal mucosa (21.7%). (Table 5, fig 5).

Table-5: Distribution of area of lesions among the cases

Lesions	No. (n=60)	%
Buccal mucosa	13	21.7
Floor of mouth	1	1.7
Gingivo labial sulcul	2	3.3
Lower alveolus	17	28.3
Lower Labial Vestibular Region	2	3.3
Lower lip	4	6.7
Palate	2	3.3
Tongue	19	31.7

Table-5 & Fig. 5 shows the distribution of area of lesions among the cases. Tongue (31.7%) was found among about one third of the cases followed by lower alveolus (28.3%) and buccal mucosa (21.7%). The percentage of other lesions was less than 10%.

Fig. 5: Distribution of area of lesions among the cases



The serum LDH level was 568.48±221.40 at pre-op which decreased to 530.71±255.81 at post-op. The mean change (37.77±358.90) in the serum LDH from pre-op to post-op was statistically insignificant (p>0.05) (Table 6, Fig 6).

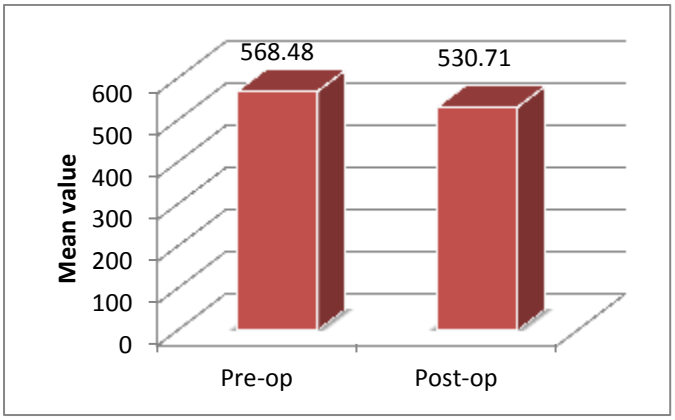
Table-6: Comparison of change in serum LDH level from pre-op to post-op among the cases

Time period	Serum LDH (Mean±SD) (n=30)
Pre-op	568.48±221.40
Post-op	530.71±255.81
Mean change	37.77±358.90
p-value[1]	0.56

Table-6 & Fig. 6 shows the comparison of serum LDH level from pre-op to post-op among the cases. The serum LDH

level was 568.48±221.40 at pre-op which decreased to 530.71±255.81 at post-op. The mean change (37.77±358.90) in the serum LDH from pre-op to post-op was statistically insignificant (p>0.05).

Fig. 6: Comparison of change in serum LDH level from pre-op to post-op among the cases



There was positive correlation of biochemical parameters with gender among the cases, as serum LDH was raised in males compared to female. For the correlation of biochemical parameters in the cases; only SGPT and SGOT was found to be positively significantly correlated (r=i0.82, p=0.0001) among the cases (Table 7, Fig 7).

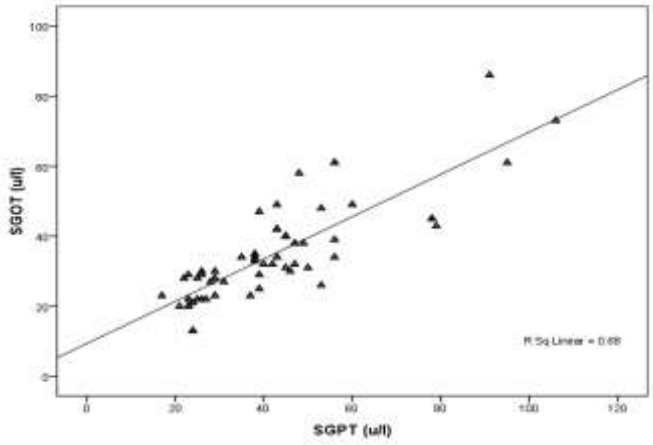
Table-7: Correlation among the biochemical parameters in cases

		Pre OP level of Serum LDH	Alkaline phosphatase	SGOT	SGPT	RBS
Pre OP level of Serum LDH (u/l)	r	1				
	p-value					
Alkaline phosphatase (u/l)	r	0.16	1			
	p-value	0.21				
SGOT (u/l)	r	0.15	-0.06	1		
	p-value	0.23	0.65			
SGPT (u/l)	r	0.17	0.02	0.82**	1	
	p-value	0.18	0.84	0.0001		
RBS (mg%)	r	0.12	0.33*	0.05	0.17	1
	p-value	0.36	0.01	0.66	0.18	

r: Correlation coefficient, *. Correlation is significant at the 0.05 level (2-tailed), **. Correlation is significant at the 0.01 level (2-tailed).

Table-7 shows the correlation of among biochemical parameters in the cases. Only SGPT and SGOT was found to be positively significantly correlated (r=0.82, p=0.0001) among the cases.

Fig. 7: Scatter diagram showing correlation between SGOT and SGPT among the cases



There was positive correlation of serum LDH with clinical stages of OSCC among the cases; however, it was statistically not significant (Table 8, Fig 8).

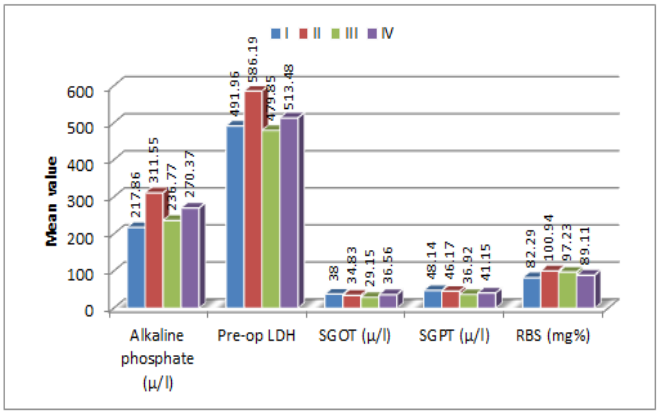
Table-8: Correlation of biochemical parameters with clinical stage among the cases

Biochemical parameters	Clinical stage				p-value[1]
	I	II	III	IV	
Alkaline phosphate (µ/l)	217.86±90.64	311.55±167.38	236.77±68.94	270.37±157.34	0.45
Pre-op LDH	491.96±146.55	586.19±281.90	479.85±96.46	513.48±195.24	0.54
SGOT (µ/l)	38.00±16.78	34.83±12.40	29.15±9.25	36.56±16.00	0.42
SGPT (µ/l)	48.14±26.80	46.17±24.99	36.92±15.81	41.15±16.01	0.54
RBS (mg %)	82.29±7.04	100.94±31.05	97.23±18.85	89.11±19.50	0.12

[1]ANOVA test

Table-8 & Fig. 8 shows the correlation of among biochemical parameters according to clinical stage in the cases. There was no significant (p>0.05) difference in the biochemical parameters among the clinical stage in the cases.

Fig. 8: Correlation of biochemical parameters with clinical stage among the cases



In the study there was positive co relation of serum LDH with habit of tobacco chewing as pre-op serum LDH was raised in tobacco chewers however, it was statistically not significant (Table 9, Fig 9).

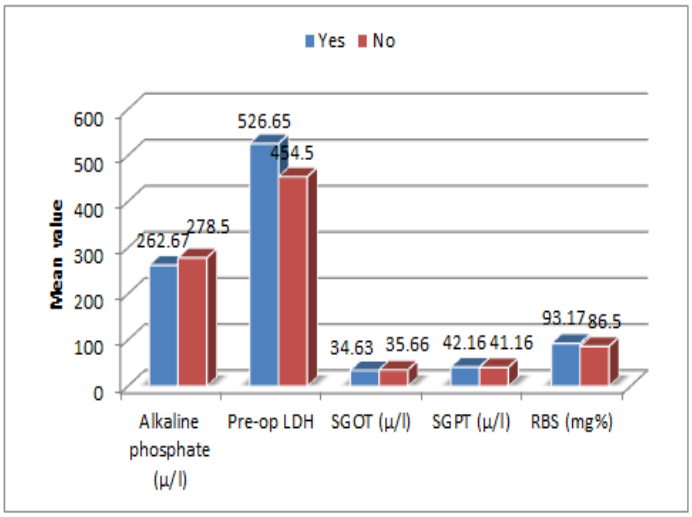
Table-9: Correlation of biochemical parameters with tobacco chewing among the cases

Biochemical parameters	Tobacco chewing		p-value[1]
	Yes	No	
Alkaline phosphate (µ/l)	262.67±141.70	278.50±105.14	0.79
Pre-op LDH	526.65±200.84	454.50±145.59	0.39
SGOT (µ/l)	34.63±14.80	35.66±6.80	0.86
SGPT (µ/l)	42.16±20.24	41.16±8.28	0.90
RBS (mg%)	93.17±20.37	86.50±6.50	0.43

[1]Unpaired t-test

There was no significant (p>0.05) in the biochemical parameters between tobacco chewers and non-chewers among the cases (Table-9 & Fig.9).

Fig. 9: Correlation of biochemical parameters with tobacco chewing among the cases



There was positive co relation of serum LDH with the area of lesion as the level of serum LDH was raised in order to lower labial vestibule > palate > gingivo labial sulcus > buccal mucosa > tongue > lower alveolus > floor of the mouth > lower lip (Table 10).

Table-10: Correlation of biochemical parameters with area of lesions among the cases

Biochemical parameters	Histopathological grading								p-value[1]
	Buccal mucosa	Floor of mouth	Gingivo labial sulcul	Lower alveolus	Lower Labial Vestibular Region	Lower lip	Palate	Tongue	
Alkaline phosphate (µ/l)	307.92 ±213.15	386.00 ±0.00	154.50 ±23.33	287.06 ±153.64	269.00 ±26.87	180.50 ±41.33	265.50 ±19.09	237.17 ±70.51	0.55
Pre-op LDH	520.54 ±235.68	482.00 ±0.00	540.00±226.27	484.71 ±177.33	936.50 ±372.65	424.18 ±55.43	599.50 ±156.27	517.29 ±159.67	0.08
SGOT (µ/l)	34.83 ±8.14	27.00 ±0.00	35.50 ±9.19	32.94 ±13.37	26.50 ±4.95	22.75 ±10.69	30.00 ±11.31	40.53 ±18.19	0.31
SGPT (µ/l)	46.50 ±18.44	31.00 ±0.00	41.00 ±2.83	35.94 ±12.21	33.00 ±5.66	29.25 ±13.23	36.00 ±15.56	49.74 ±25.53	0.26
RBS (mg%)	97.36 ±15.10	86.00 ±0.00	77.00 ±4.24	96.82 ±30.09	87.00 ±2.83	87.75 ±16.60	90.50 ±9.19	89.32 ±12.11	0.73

[1]ANOVA test (except for floor of mouth)

DISCUSSION:

The study was carried out to evaluate serum level of LDH along with few other diagnostic enzymes like alkaline phosphatase, SGOT, SGPT among the study and control groups in OSCC.

In conjunction with **Warburg** effect**,** Kim JW et al described mechanisms underlying this fundamental alterations in metabolism during carcinogenesis include mutations in the mitochondrial DNA resulting in functional impairment, oncogenic transformation linked with upregulation of glycolysis, enhanced expression of metabolic enzymes and adaptation to the hypoxic tumour micro-milieu in case of solid tumours (10).

According to Ludwig JA et al diagnostic and prognostic biomarkers are quantifiable traits that help clinical oncologists at the first interaction with the suspected patients. These particularly aid in (i) identifying who is at risk, (ii) diagnose at an early stage, (iii) select the best treatment modality, and (iv) monitor response to treatment(11).

Majority of the subjects with OSCC were found to be male above the age of 50 years. Oral SCC more frequently affects men than women (M: F = 1.5:1) most probably because more men than women indulge in high-risk habits. The probability of developing oral SCC increases with the period of exposure to risk factors, and increasing age adds the further dimension of age-related mutagenic and epigenetic changes. In the USA the median age of diagnosis of oral SCC is 62 years. However, the incidence of oral SCC in persons under the age of 45 is increasing(3).

Among the OSCC patients use of smokeless form of tobacco was significantly higher (p=0.006). Snuff and chewing tobacco have also been associated with an increased risk for oral cancer(12). A significant number of oral cancers in smokeless tobacco users develop cancer at the site of tobacco placement. However, the use of smokeless tobacco appears to be associated with a much lower cancer risk than that associated with smoked tobacco(13). This association was in contrast to the findings in our present study.

There was positive co relation of pre-op serum LDH level with cases (OSCC) compared to controls, and the statistical data was also significant. Hariharan et al. studied serum LDH and its isoenzymes in buccal mucosa cancer(14). Muralidhar et al. in 1988 also reported a definite rise of serum LDH levels from normal in premalignant and malignant cases(15). Görögh et al. studied LDH isoenzymes in human epithelial cells from squamous cell carcinomas and healthy tissues of the oral cavity(16).

The distribution of area of lesions among the cases, Tongue (31.7%) was found among about one third of the cases followed by lower alveolus (28.3%) and buccal mucosa (21.7%). The percentage of other lesions was less than 10%. In Western countries oral SCC affects the tongue in 20% - 40% of cases and the floor of the mouth in 15% - 20% of the cases, and together these sites account for about 50% of all cases of OSCC(17). The most common site for intraoral carcinoma is the tongue, which accounts for around 40 percent of all cases in the oral cavity proper. The floor of the mouth is the second most common intraoral location. Less- common sites include the gingiva, buccal mucosa, labial mucosa, and hard palate(3).

There was positive correlation of serum LDH with clinical stages of OSCC among the cases, however, it was statistically not significant. Liaw CC et al observed increased levels of serum LDH in patients with OSCC and the levels correlated positively with the clinical stage of the disease(18). As stated by Pereira T et al, serum LDH level correlates with the histological grade of OSCC (6).There was positive correlation of serum LDH with histopathological grading among the cases, however, the statistical data was not significant. Subramanian et al. in 2009 on studies of cervical carcinoma has concluded that LDH isoenzyme is an important biochemical marker in assessing the grade of malignancy(19).

Estimation of serum LDH is only an auxiliary investigation which may act as an adjunct in diagnosis of OSCC and can only provide collaborative evidence.

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