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Research Article

The Diagnostic Role of Salivary Lactate Dehydrogenase in Oral Squamous Cell Carcinoma

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Abstract

Background and Objectives: Oral cancers considered one of the most common head and neck cancers; they are associated with high rates of morality especially during advanced stages. Early detection and proper management obviously improve survival rates and decrease mortality. On one-hand cancers depends on anaerobic glycolysis to produce the important energy needed for mitosis and biogenesis, on the other hand lactate dehydrogenase enzyme plays a major role in anaerobic glycolysis, for these reasons this research aims to study the effectiveness of using the saliva as a diagnostic tool for oral cancers by estimation of lactate dehydrogenase.

Methods: This prospective study involved 60 cases admitted to Al-Mowassat university hospital and AlBayrouni hospital subdivided into three groups (Oral cancers, oral benign lesions, and control group). The saliva samples collected in the morning and the patients prohibited from consuming water or food for at least 12 hours before sampling to prevent food and water interference in salivary enzyme levels.

Results: An elevation in salivary LDH noticed in oral cancers group compared to benign and control groups, it also noticed between the three grades groups but no correlation has improved between salivary LDH and tumors size.

Conclusion: The increase in salivary LDH levels could be used as a diagnostic tool, furthermore, the levels significantly correlated with the histological grade of OSCC.

Keywords: Lactate Dehydrogenase Enzyme; Saliva; Oral Cancers; Anaerobic Glycolysis

Introduction

Oral squamous cell carcinoma is the most common cancer in the head and neck.

In 2020, more than 377 thousand new cases diagnosed all around the world and this number has increased significantly since 2018.

The most common preventable risk factors are smoking and alcohol consumption, their synergistic effect associated with the development of oral SCC, although resent reviews showed increasing rates of oral tongue cancers of unknown etiology in young patients. Unfortunately, in last year, 177 thousand of oral cancer patients died, this high mortality rate associated with late diagnosis and lack of proper treatment. Thus, cancer diagnosis at early stages improve survival rate and contributes in decreasing mortalities.

As known, Tumors are uncontrolled proliferation of cancer cells. In order to meet the increased energy needed for cell growth and division, cancer cells use multiple mechanisms, which are different from those of normal cells because of the metabolic reprogramming.

Normal cells in well-oxygenated conditions depend on oxidative phosphorylation to produce energy, only in absence or limited oxygen it resort to anaerobic glycolysis. In contrast, cancer cells metabolize glucose to lactate even in the presence of oxygen (aerobic glycolysis), and generate large amounts of lactate despite the abundance presence of oxygen. This concept established by Otto Warburg in 1924 (Basics of cancer).

The final step of glycolysis is conversion of pyruvate to lactate with regeneration of NADH to NAD+. Lactate dehydrogenase enzyme plays a major role in catalyzing his reversible reaction in hypoxic environment.

LDH enzyme present in almost all body tissues, but at highly concentration in muscles, liver, and kidney. It exhibits five isomers forms, each one have differential expression in different tissues. Many conditions increases LDH levels in blood like tissue injury, necrosis, anemia, heart attack, infections, and cancers.

Several researches have reported the overexpression of serum LDH in various cancers for instance, breast, gastric and pancreatic cancers. Only few studies have been conducted in evaluation of LDH in cancer patients' saliva.

For these reasons, our research aims to study the effectiveness of salivary LDH in order to detect oral squamous cell carcinoma at early stages, considering that saliva is an easy accessible, noninvasive method, which make it a good choice for screening purposes in the future.

Materials and Methods

Study population

This prospective study involved 60 patients admitted to al-Mowasat university hospital and alBairouny hospital between June 2019 and March 2021.

Patients with recurrent cancer and who undergone surgery, chemotherapy or radiotherapy we excluded from the study, in addition to every oral disease that elevates salivary LDH levels like periodontitis.

Saliva collection and LDH estimation in saliva

In order to prevent biochemical changes in saliva during the day, samples of unstimulated saliva were collected in the morning after 12 hours of fasting. Patients were asked to spit in sterilized package (approximately 2ml) and samples were transfer immediately to the laboratory, to be centrifuged for 5 minutes at 2000 rpm. Then LDH measured via LDH DyaSis kits, using the IFCC method standardized to 30 C as recommended by the German society of clinical chemistry (GSKC) which allows LDH activity to be evaluated by monitoring the NAD reduction at 340 nm.

Statistical methods

Data were analyzed using SPSS v22. One way ANOVA and PostHoc tests used to test the significant of differences between the three groups and different histological grades.

Results

The research included 60 individuals subdivided into three groups 20 individual in each group: (Oral SCC patients, oral benign lesion patients and control group). The OSCC patients were from different stages and histological grades.

As it shown in table1, the mean age of oral SCC patients was higher than the other groups, also were the male/female range and smoking habits (Table 1).

	Oral SCC	Oral Benign lesions	Control
Age	61.2 ± 12.8	45.2 ± 17.5	47.4 ± 21.7
Male/female	13/7	9/11	10/10
Smokers/non smokerd	14/6	4/16	10/10

Table 1: Age, gender and smoking of participants in study groups.

The most common site for both OSCC and benign lesions was oral tongue (55% and 40%) respectively (Figure 1).

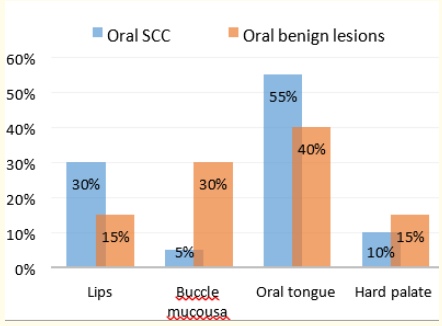

Figure 1: Distribution of OSCC and benign lesion sites.

The mean and standard deviation values of salivary LDH level in OSCC, oral benign lesions and control groups were 678.65 ± 123, 253.7 ± 102 and 281.95 ± 160 respectively. One way ANOVA and PostHoc tests revealed a significant difference between OSCC and oral benign lesions with a P value 0.001. The difference between OSCC and control group was also significant with a P value 0.001. oral benign lesions and control group revealed non-significant difference with a P value 0.575 as shown in table 2.

	Category	% (N)	Mean salivary LDH ± SD	Correlation P value
Tumor size	T1	%02 (4)	718.8 ± 106.4	0.501
	T2	%44 (9)	702.67 ± 119.7	
	T3	%02 (4)	598.5 ± 119.2	
	T4	%54 (3)	660 ± 169.8	
Histological Grade	Grade I	%04 (4)	566.8 ± 48.1	0.007
	Grade II	%44 (55)	683.1 ± 96.4	
	Grade III	%02 (4)	806.3 ± 137.9	

Table 2

Table 3 shows the OSCC group classification according to tumor size and histological grade. The highest salivary LDH.

Comparing Group pairs	P value
OSCC – oral benign leasions	0.001
OSCC - controls	0.001
Oral benign leasions - controls	0.575
Grade I – Grade II	0.04
Grade II – Grade III	0.44
Grade I – Grade III	0.002

Table 3

The tests revealed no significant differentiation among the four subgroups of tumor size with a P value 0.501, whereas it revealed significant differentiation between Grade I and Grade II, Grade II and Grade III, and between grade I and Grade III with P value 0.04, 0.44, 0.002 respectively (Table 2-3).

Discussion

Oral SCC is the most common cancer in head and neck cancers. Mortality rates in low-income countries have increased in the last ten years especially due to late diagnosis. Despite the sensitivity and specificity of CT scan, MRI and biopsies, they are expensive and invasive diagnostic methods.

Thus, the need for new reliable cost effective diagnostic tool made saliva a good choice even better than plasma, because collecting it is easier, none-invasive and cheaper than blood collection.

Saliva contains 99% water and the last 1% is micro and macromolecules such as DNA, RNAm, proteins, and enzymes including lactate dehydrogenase, hence it is a valuable substance for research.

LDH is an important cytoplasmic enzyme that catalysis the reversible conversion of Lactate to Pyruvate during aerobic glycolysis (Warburg effect). In 2001, Nagler., *et al*. [1] conclude that the higher salivary LDH main resource in oral cancer patients is not from salivary glands but from the oral mucosa, so the damage and necrosis in mucosal cells releases LDH into saliva.

In this research, we focused on the effectiveness of salivary LDH in Oral SCC detection, and noticed that levels of salivary LDH were significantly higher in oral SCC compared to benign oral lesions and control groups. Because of increasing demand of oxygen, cancer cells switch the metabolism to aerobic glycolysis increasing the need of Lactate dehydrogenase enzyme. Other researches in India and Iran corresponding with our results, and found an elevation of salivary LDH level in oral SCC patients.

Hornamand., *et al*. [2] reported an increase of salivary LDH level in oral lichen planus compared to OSCC. Also reported Kallalli., *et al*. an elevation of salivary LDH levels in oral submucosal fibrosis subjects. Thus, it may provide a predictive tool for malignant changes in premalignant lesions.

Tumor size in the TNM staging system used to describe the primary tumor size and its invasion into adjacent tissues. Our research results showed that there was no statistical correlation between tumor size and salivary LDH levels.

Mean salivary LDH	Patel. S., *et al.*	Lokesh. K., *et al.*	Our study
OSCC	686.42	1225 ± 221.79	678.65 ± 123
Controls	065.56	497 ± 51.57	281.95 ± 76.78
Grade I	744.43	1049.07 ± 46.89	466.8 ± 48.1
Grade II	799.509	1309.50 ± 68.79	683.5 ± 96.4
Grade III	808.04	1586.2 ± 203.20	826.3 ± 137.9

Table 4

Acharya., *et al*. [3] results agreed with us, their research included 90 subjects and assessed LDH levels in saliva and serum, the results revealed no significant difference between tumor size groups.

Histological grade is a description of the microscopic appearance of the cancer's cell and tissue.it reflects the aggressiveness of the tumor.

Main reason for increased LDH levels is mainly due to increased mitotic index and tumor cells also produce more amount of lactic acid.

Our results showed that salivary LDH levels increased from grade I to grade II and from grade II to grade III. Several researches also reported an increase in salivary LDH correlated with advanced grades of OSCC as shown in Table 4. This results support Nagler's prospective theory about the possibility of defining the aggressiveness of oral cancer in dependence on LDH levels.

Salivary LDH could be a promising diagnostic tool for OSCC. Further studies are recommended on larger sample size comparing LDH level between saliva and serum [4-12].

Conclusion

Patients with OSCC had higher salivary LDH levels compared to benign and control groups.

In addition, salivary LDH levels were significantly higher according to histological grades.

The estimation of salivary LDH can be a useful, noninvasive diagnostic tool. Also a predictive tool for the aggressiveness of OSCC.

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