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To determine the levels of serum pyruvic acid in oral squamous cell carcinoma

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Abstract
Aim: To determine the levels of serum pyruvic acid in oral squamous cell carcinoma.
Methods: The research comprised 50 individuals with clinically proved and histopathologically confirmed instances of OSCC. The control group consisted of 50 healthy adults. 5ml of venous blood was drawn from a big peripheral vein of the patients chosen. The samples were taken in sterile plastic tubes that had been treated with ethylenediaminetetraacetic acid (EDTA). By gently shaking the tubes, blood was mixed with EDTA. To stop the pace of biochemical and bacterial reactions, blood samples were immediately moved to a deep freeze at 4°C. The biochemical examination of the obtained serum was performed using the modified 2,4-dinitrophenylhydrazine technique, and the absorbance was measured using a spectrophotometer.
Results: T1 contained 11 (22% of the cases), T2 included 20 (40% of the cases), T3 included 11 (22% of the cases), and T4 included 3 (6% of the cases). OSCC patients were classified as well differentiated, moderately differentiated, or poorly differentiated based on histopathological grading. The well-differentiated grade comprised 20 (40%) examples, the moderately differentiated grade included 20 (40%) cases, and the badly differentiated grade included 10 (20%) cases. The levels of pyruvic acid in the blood were measured in both the study and control groups. The mean serum pyruvic acid levels with SD for the control group (0.78± 0.11) and OSCC (3.01± 0.45) are computed. The difference in mean serum pyruvic acid levels between groups was found to be statistically significant ($P < 0.05$).
Conclusion: Serum pyruvic acid levels steadily climbed from those who did not have OSCC to those who did. Higher serum pyruvic acid levels were seen with rising clinical stage, and mean serum pyruvic acid levels were revealed to be substantially increasing with advancing histological grades of OSCC.

Keywords: OSCC, Pyruvic acid

Introduction
Oral squamous cell carcinoma (OSCC) is the most prevalent kind of head and neck cancer, and its prevalence is rising in a number of nations [1]. Surgery is the mainstay of treatment, with radiation and/or chemotherapy used as supplements. Regrettably, medications have not considerably increased the survival rate of such individuals [2, 3] and the 5-year survival rate has maintained around 60% over the previous 20 years [4]. OSCC patients have a poor prognosis due in part to delayed diagnosis. Early detection and treatment may successfully halt the development of OSCC, boosting patient survival by up to 80% [5, 6]. Diagnosis of OSCC is based on clinical examination. However, some OSCC symptoms resemble those of oral ulcers or precancerous tumours, causing misunderstanding. This pattern shows that substantial expertise is required for early screening for OSCC. Pathological diagnosis, which is intrusive and causes pain and poor wound healing, is the gold standard for conclusive diagnosis of OSCC [7]. Aside from the time-consuming histopathology processes, sampling at various places may result in different pathological diagnoses, and the complicated pathological diagnostic techniques create a lag in getting clinical findings [8].
Uric acid is the end result of the enzymatic breakdown of purine nucleotides and free bases in the human body. Previous research has linked a decrease in blood uric acid levels to an increase in the risk of lung cancer, mouth cancer, and laryngeal cancer. Others, however, have discovered that uric acid acts as a prooxidant under oxidative stress circumstances through

interactions with nitric oxide, impairing vascular epithelial function and leading to the onset of systemic illnesses [9]. Uric acid seems to activate an antioxidant defence system against oxidative stress and the ageing processes induced by free radicals, which are also linked to DNA damage, tumour cell adhesion, migration, proliferation, and regulation, and mortality. On the other side, tumour cell degradation may raise blood uric acid levels, which boosts the immune system (particularly CD8+ T-lymphocytes) and improves cancer defence systems by triggering cytotoxic cell death and limiting tumour cell growth and migration. These findings back up the link between blood uric acid levels and survival in individuals with colon cancer and nasopharyngeal carcinoma [10]. Elevated serum uric acid levels, on the other hand, are inversely related to low adiponectin levels, and low adiponectin levels may overactivate the phosphorylation of PI3K/Akt (phosphoinositide 3-kinase/B kinase protein) and signalling pathways, eventually leading to an increase in tumour cell proliferation.

Material and methods

The research comprised 50 individuals with clinically proved and histopathologically confirmed instances of OSCC. The control group consisted of 50 healthy adults. This research excluded patients having a history of systemic ailments such as heart disease, diabetes, and other carbohydrate metabolic problems. The OSCC patients were clinically staged using tumor–node–metastasis (TNM) staging [11] and histopathologically graded using the modified Broder's categorization system [12].

Methodology

5ml of venous blood was drawn from a big peripheral vein of the patients chosen. The samples were taken in sterile plastic tubes that had been treated with ethylenediaminetetraacetic acid (EDTA). By gently shaking the tubes, blood was mixed with EDTA. To stop the pace of biochemical and bacterial reactions, blood samples were immediately moved to a deep freeze at 4 °C. The biochemical examination of the obtained serum was performed using the modified 2,4-dinitrophenylhydrazine technique, and the absorbance was measured using a spectrophotometer. 2 ml of heparinized blood was combined with 4 ml of 0.6M per chloric acid and placed in an ice bath for 10 minutes before centrifugation at 3000 rpm for 5 minutes and supernatant fluid was recovered. 3 mL of supernatant fluid was combined with 1 mL of dipotassium phosphate solution, centrifuged at 3000 rpm for 10 minutes, and the supernatant fluid was collected again. This supernatant fluid was a protein-free blood sample filtrate. After 10 minutes, 1 ml of dinitrophenylhydrazine was added to each solution and maintained at 37 °C for 10 minutes, followed by 10 ml of newly made 0.4M sodium hydroxide, which was measured using a spectrophotometer with a wavelength of 540 nm.

Results

Table 1 shows the demographics of the study and control groups. Table 2 shows that the clinical phases of OSCC patients were clinically categorised into T1, T2, T3, and T4. T1 contained 11 (22% of the cases), T2 included 20 (40% of the cases), T3 included 11 (22% of the cases), and T4 included 3 (6% of the cases). OSCC patients were classified as well differentiated, moderately differentiated, or poorly differentiated based on histopathological grading. The well-differentiated grade comprised 20 (40%) examples, the moderately differentiated grade included 20 (40%) cases, and the badly differentiated grade included 10 (20%) cases. The levels of pyruvic acid in the blood were measured in both the study and control groups. The mean serum pyruvic acid levels with SD for the control group (0.78± 0.11) and OSCC (3.01± 0.45) are computed. The difference in mean serum pyruvic acid levels between groups was found to be statistically significant ($P< 0.05$). The mean values for T1, T2, T3, and T4 clinical stages were 1.71, 2.62, 3.33, and 3.97, respectively. The rise in mean blood pyruvic acid levels between any two groups was shown to be statistically significant ($P< 0.05$) in a pair-wise comparison of clinical stages of OSCC using Tukey's multiple post hoc technique. The mean values for well differentiated, moderately differentiated, and poorly differentiated squamous cell carcinoma, respectively, were 1.82, 2.71, and 3.55. The rise in mean blood pyruvic acid levels between any two distinct grades of OSCC was shown to be statistically significant ($P< 0.05$) in a pair-wise comparison of various histological grades of OSCC using Tukey's multiple post hoc technique.

Table 1: Demographic profile

Gender	Study group	%	Control group	%
Male	26	52	25	50
Female	24	48	25	50
Age				
Below 20	6	12	5	10
20-30	12	24	10	20
30-40	22	44	20	40
Above 40	10	20	15	30

Table 2: Stages distribution of OSCC patients

Stages	N	%
I	11	22%
II	20	40%
III	11	22%
IV	3	6%

Table 3: Serum pyruvic acid levels

	Study group	Control group	P- value
Serum pyruvic acid levels	3.01±0.45	0.78±11	0.0001

Discussion

In a normal physiologic state, the pyruvic acid generated by the glycolysis cycle is used by the Kreb's Cycle in the mitochondria to make more ATP. When compared to glycolysis, ATP is created in mitochondria through oxidative phosphorylation, which is a more efficient metabolic pathway that creates more ATP molecules from a given quantity of glucose. However, in cancer cells, this process of ATP generation is hindered. As a result, cells might adopt alternate metabolic pathways, such as enhanced glycolysis, to preserve their energy supply. Cancer cells exhibit changes not only in the glycolytic pathway, but also in the Krebs cycle, b-oxidation, and anabolic metabolism in general, which are reoriented to respond to the cell's new primary function (i.e. uncontrolled proliferation) by providing not only energy, but also nucleotide, amino acid, and fatty acid synthesis [13]. Pyruvic acid plays an important role in glucose metabolism. The final result of the physiologic process of glycolysis was pyruvic acid and lactic acid. This produces two ATPs from a single glucose molecule. This energy generation cascade is continued by oxidative phosphorylation of the glycolysis end product (pyruvate) in the Krebs cycle in mitochondria. Cancer cells exhibit changes not only in the glycolytic pathway, but

also in the Krebs cycle, -oxidation, and anabolic metabolism, all of which are reoriented to respond to the new primary function of uncontrolled cell proliferation by providing not only energy, but also nucleotide, amino acid, and fatty acid synthesis [14].

The rationale for increased pyruvic acid synthesis stems from the fact that most cancer cells make energy by glycolysis rather than oxidative phosphorylation via the tricarboxylic acid cycle, even in the presence of a sufficient oxygen supply, a process known as the "Warburg effect" [15]. Cancer cells, unlike normal cells, primarily employ glycolysis in their cytoplasm to make ATP, which provides the energy necessary for cell multiplication. The Warburg effect, which is a hallmark of cancer cell metabolism, is related to this occurrence of so-called aerobic glycolysis. Glycolysis is upregulated in tumour cells, which is accompanied with mitochondrial dysfunction and reduced oxidative phosphorylation [16]. Many studies have shown a link between tumour growth and an increase in mtDNA mutations [17]. Excessive glycation of mitochondrial proteins, lipids, and mtDNA caused by carbonyl stress has been linked to mitochondrial malfunction and mutations [18]. Thus, increased glycolysis in tumour cells may lead to greater mitochondrial damage, resulting in a vicious loop that amplifies the Warburg effect. Because greater glycolysis leads in higher concentrations of glycolytic intermediates, pyruvic acid levels are expected to be higher in established malignancies [19]. Warburg's effect may also be triggered by hypoxia since the pace of angiogenesis is lower than the rate of tumour development; this results in a depleted oxygen environment in the neoplastic region and stimulates an alternate respiratory route for energy generation and survival, i.e., glycolysis pathway. As a result, glycolysis is a major metabolic route that finely controls cell proliferation by adjusting the metabolism of the cancer cell to the parameters of its present selective scenario. Another cause for a faster rate of glycolysis in malignant cells is data from immunohistochemistry investigations that demonstrated a higher expression of glucose transporter proteins (GLUT) like GLUT 1 in increasing grade of tumours, suggesting a larger quantity of glucose consumption by malignant cells. It has also been demonstrated that epigenetic and/or mutagenic changes in cancer cells can cause: (1) overexpression of type 2 hexokinase; (2) activation of normally insulin-regulated glucose membrane receptors, particularly GLUT1, GLUT3, and GLUT5, allowing extracellular glucose to easily penetrate cancer cells; and (3) overexpression of all glycolytic enzymes in aerobic and anaerobic conditions [20-22].

Because the glycolytic route is the primary metabolic pathway in cancer cells, quantitative measurements of its metabolic products give more sensitive indicators than enzymes in cancer patients. As a result, the present research was conducted to examine the levels of serum pyruvic acid, an end product of glycolysis, in patients with OSCC and compare these values to those of healthy persons. Several studies have been presented by various researchers, with the conclusion that elevated blood pyruvic acid levels above normal levels suggest biological anomalies in cancer tissues.

Diers *et al.* [23] and Thangaraju *et al.* [24] found a substantial increase in serum pyruvic acid levels in breast cancer and colon cancer, respectively. Hur *et al.* [25] also conducted a research to assess the amounts of organic acids in stomach cancer patients. They observed that when comparing cancer patients to the general population, there was a considerable rise in the levels of organic acids such as pyruvic acid, lactate, succinic acid, malic acid, and -ketoglutaric acid. Similarly, Bhat *et al.* [26] calculated serum pyruvic acid levels in healthy and potentially malignant disorder (PMD) subjects and concluded that there was a significant increase in serum pyruvic acid levels in PMD subjects compared to healthy individuals, implying that serum pyruvic acid estimation can be used as a screening tool for PMD and malignant diseases.

In the current investigation, there is a substantial rise in blood pyruvic acid levels in participants with OSCC when compared to controls ($P > 0.001$). These findings are consistent with the findings of Bhat *et al.* [26] who discovered an increase in blood pyruvic acid levels in individuals with OSCC. Tukey's multiple post hoc test was used in our work to compare blood pyruvic acid levels to clinical staging and histological grading. The findings revealed a statistically significant rise in levels as the clinical stage of OSCC progressed ($P < 0.005$). Similarly, when the histopathological grade of OSCC progressed from good to bad, serum pyruvic acid levels increased statistically significantly ($P < 0.005$). This might be attributed to tumour differentiation and enhanced malignant cell shedding into the blood as a consequence of metastasis. This was the first research of its sort to examine the levels of pyruvic acid in OSCC patients based on clinical staging and histological grading.

Conclusion

Serum pyruvic acid levels steadily climbed from those who did not have OSCC to those who did. Higher serum pyruvic acid levels were seen with rising clinical stage, and mean serum pyruvic acid levels were revealed to be substantially increasing with advancing histological grades of OSCC.

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