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ORIGINAL ARTICLE **OPEN ACCESS**

To Estimate the Levels of Pyruvic Acid Level in Serum and Saliva of Patients with Oral Leukoplakia and Oral Squamous Cell Carcinoma

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ABSTRACT

The serum and salivary pyruvic acid levels could be a better adjuvant diagnostic marker along with the routine markers in patients with premalignant and malignant lesions. The pyruvic acid level measurement is simple, noninvasive and cost-effective screening, diagnostic and monitoring tool in cases of leukoplakia and oral squamous cell carcinoma. Oral cancer is most frequently encountered type of cancer in the developing countries. Oral dysplasia is detected in the form of potentially malignant lesion leukoplakia which appears as a whitish patch in oral cavity. There is a need to develop a screening method, with which malignant transformation from leukoplakia to oral cancer in these patients can be accurately predicted. To estimate serum and salivary pyruvic acid levels and correlate them with histopathological gradings of oral leukoplakia and oral squamous cell carcinoma. 90 patients were included in study. Out of theses 30 patients had or alleukoplakia, 30 with oral squamous cell carcinoma and 30 with control were included in the study. Patients were from OPD of SGT Dental College and SGT Medical College Budhera. Samples were taken from the patient as part of routine diagnostic investigation with their consent. The samples of Serum and saliva was collected and subjected to pyruvic acid level estimation using Digital colorimeter 112 by systronics. Univariate analysis, Pearson's chi square test was applied to find association between the qualitative variables. Student's t-test was applied to compare the mean values between the study groups. On biochemical analysis, serum Pyruvic acid levels were raised significantly (2.803g/l) in SCC group (p value 0.001) when compared with leukoplakia group (1.560g/l). TheSerum Pyruvic acid levels were raised with increasing histopathological grades of leukoplakiaas well as with the increasing histopathological grades of squamous cell carcinoma (SCC) Salivary Pyruvic acid levels were significantly increased in squamous cell carcinoma group (3.628 g/l) when compared with leukoplakia group (2.042g/l) and the levels were seen to be increased with increasing histopathological grades of leukoplakia. However the levels were seen to be decreased with increasing histopathological grades of squamous cell carcinoma (SCC). Levels of salivary pyruvic acid was increased when compared with levels of serum pyruvic acid.

KEYWORDS *:Serum pyruvic acid, salivary pyruvate, Leukoplakia, oral squamous cell carcinoma, Warburg's effect .*

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INTRODUCTION

Oral Cancer is one of the serious threats to public health in developing countries. However this risk has significantly increased in the developed countries. Recent epidemiologic data shows a sharp rise in the incidence of oral cancer all over the world [1]. In south-central Asia specifically in countries like Bangladesh, Sri Lanka, India and Pakistan oral cancer ranks third amongst commonest type of cancer. A remarkable increase in the incidence rate of oral and oropharyngeal cancers have additionally been reported in European countries and to a lesser extent in New Zealand, Japan, USA and Australia [2, 3].

Oral cancer is mainly related to lifestyle. Major lifestyle factors for this type of cancer have been related to different ways of use tobacco and alcohol consumption. It has been estimated to account for about 90% of cancers that occur in the oral cavity. In addition to smoking form, the use of smokeless form of tobacco has also been strongly associated with oral cancer [4].

About 40% of head and neck malignancies arising in the oral cavity are squamous cell carcinomas. The five-year survival rate of oral cancer varies from 42% for those with regional disease to 81% for patients with localized disease and 17% in cases when distant metastases are present. Hence, an early detection and prevent of spread can play a major role in improving the prognosis [5].

Generally, SCC is proceeded by potentially malignant lesions. Among the many potentially malignant lesions, leukoplakia has been found to be the most common. Hence, a timely clinical detection of leukoplakia can help in preventing the progression and malignant transformation[3].At present, the cancer screening techniques mainly include clinical examination, application of toluidine blue stain or use of vital stains, oral brush biopsy and chemiluminescence[6]. However, there are some limitations associated

with these methods and thus cannot be practiced every time thus being a reason for a likely delay in diagnosis. Even though there has been a huge progress in the field of molecular biology, still a search for a marker that can reliably predict malignant transformation in patients keeps going [3].
Cancer develops because of the ability of the mutated cell in the body to rapidly divide and undergo an uncontrolled proliferation. The cancer cells use an alternative method to obtain energy from the environment in order to maintain their unchecked growth pace[7]. Even though there is plenty of oxygen available, the majority of cancer cells rely on glycolysis for generation a sufficient amount of ATP.[8]The tendency of cancer cells to anaerobic glycolysis under a well-oxygenated (aerobic) conditions is known as the Warburg effect [7]. One of the earliest documented biochemical characteristic of cancer cells is the metabolic change. There is a possibility that Warburg's effect may start with hypoxia. This could be due to the fact that the rate of growth of tumour is more than that of rate of angiogenesis seen in the tumour tissue. This results in creation of oxygen depleted environmental condition in the region of the tumour area and this will induce glycolysis pathway, an alternative respiratory method, for survival and energy production. Thus, Glycolysis becomes one important metabolic pathway. This very carefully regulatesthe cell proliferation which is achieved by adaptation of the cancer cell's metabolism to the conditions of its current selective situation. Lactic acid and Pyruvic acid are formed as end products due to increased glycolysis. Because of unstable nature of Lactic acid, the compound is converted back to pyruvate. The total amounts of pyruvic acid are eventually increased. This extra amount of pyruvic acid which is formed, either reaches into blood or into the local area (oral cavity). Therefore, assessing the levels of pyruvic acid may give an idea about the relative degree of PMD severity[7].Thus, estimating the pyruvic acid levels in serum and saliva might possibly represent the range oral cancer. Therefore, the study aimed at evaluating and comparing the pyruvic acid levels in saliva and serum in normal, leukoplakia and oral squamous cell carcinoma subjects.

MATERIAL AND METHODS

90 patients were included in study. out of these 90 patients , 30 had oral leukoplakia, 30 had oral squamous cell carcinoma and 30 were normal individuals . The patients that were included were from the OPD of SGT Dental College and SGT Medical College Budhera and from camps. The samples of Serum and saliva were takenand pyruvic acid levels were estimated by biochemical analysis.. Samples were taken from the patient's as part of routine diagnostic investigation with their consent. The study was further conducted in Biochemistry department in SGT Medical College and in Department of Oral pathology SGT Dental College.The necessary ethical clearance was taken from the ethical committee, Sri Guru Gobind Tricentenary Dental College, Budhera, Gurugram.
For the study, 30 subjects with Oral leukoplakia (OL) and 30 subjects with Oral Squamous cell carcinoma (OSCC) diagnosed by history, clinical examination and laboratory findings were included. Exclusion criteria included subjects with any other systemic disease or co-morbid condition and subjects on radiotherapy/chemotherapy or with any other lesion in oral cavity. 3ml of saliva and 5ml of venous blood was collected from the subjects that were included in study. The saliva was collected in sterile cups and was stored at -20° c until analysed. The serum was separated by centrifugation andwas stored at -20° c till the analysis could be carried out. Biopsies were taken from the patient with OL and OSSC and evaluated histopathologically.
(1) 4 ml of 0.6M per chloric acid and 5 ml of heparinized blood were mixed. This was placedin an ice bath for 10 minutes . This was then centrifuged at 3000 rpm for 5 minutes. After this the supernatant fluid was collected.
(2) 3 ml of supernatant fluid was combined with 1 ml of di-potassium phosphate solution. After that, it was centrifuged for 10 minutes at 3000rpm. This supernatant fluid which was obtained was protein free filtrate of blood sample.
(3) In each solution 1 ml of dinitro phenyl hydrazine was added andwas kept at 37°c centigrade for 10 min.
(4)10 ml 0.4M sodium hydroxide was freshly prepared was added to the solution . It was further quantified with colorimetry at wavelength 540 nm after 10 min.
(5)Blank solutions were analysed simultaneously.
Step 1 and 2 were bypassed in saliva samples. This was done as protein concentration in saliva are lower than what is present in serum. The rest of the steps were then followed.[3]
The data were collected and entered in excel sheet and was analysed using SPSS software version 20. Univariate analysis was done to calculate mean, median, and standard deviation. Pearson's chi square test was used to find association between qualitative variables and was considered as significant at $p<0.05$. Student's t-test was applied to compare the mean values between the study groups. Correlation test was

applied to find a correlation between serum and salivary levels of pyruvic acid with histopathological findings.

RESULT

In the present study gender distribution was assessed in cases of leukoplakia and SCC. A male predilection was observed in cases of leukoplakia and in OSCC. On histopathological evaluation 50% of cases were of mild dysplasia , 43.33% were of moderate dysplasia and 6.66% were of severe dysplasia. In cases of patients with OSCC, well differentiated Squamous cell carcinoma (WSCC) accounted for 60% cases , 30% cases were of moderately differentiated Squamous cell carcinoma(MDSCC) and 10% cases were of poorly differentiated Squamous cell carcinoma(PDSCC).

On biochemical analysis, level of Serum Pyruvic acid was observed to be significantly increased (p value 0.001)in leukoplakia and Squamous Cell Carcinoma group when compared with control group. (Table No 1)

While, on further comparison of the two groups with each other, it was found that the Serum Pyruvic acid levels were higher (2.803g/l) in SCC group (p value 0.001) as compared with leukoplakia group (1.560g/l) significantly.

Hence, it was found that Levels were increased in leukoplakia and squamous cell carcinoma. On analysing the levels of serum Pyruvic acid in the various histopathological grades of Leukoplakia, it was observed that the levels of serum pyruvic acid were estimated to be 1.504g/l in mild dysplasia, 1.583 g/l in moderate dysplasia and 1.79 g/l in severe dysplasia. Thus, the levelsof serum pyruvic acid was seen to be increased with increasing histopathological grades of Leukoplakia. (Table No 3)

Similarly when serum pyruvic acid levels were assessed invarious grades of squamous cell carcinoma, a mean value of 2.71g/l was observed in WDSCC,, 2.83 g/l in MDSCC and 2.92 g/l in PDSCC. Serum Pyruvic acid levels were found to be increased with increasing histopathological grades of Squamous Cell Carcinoma. (Table No 3)

A similar evaluation was also done on salivary pyruvic acid levels. When compared with control group, it was observed that the salivary pyruvic acid levels were increased in leukoplakia and OSCC group. This was statistically significant with the p value 0.001. (Table No 2)

On comparison salivary pyruvic acid levels between leukoplakia group and OSCC group, the group with squamous cell carcinoma had a considerably higher amount of Salivary Pyruvic acid levels (3.628 g/l) when compared with leukoplakia group (2.042g/l), which was statistically significant with p value 0.001.

On evaluation of Salivary Pyruvic acid levels in the different grades of Leukoplakia, mean values was found to be 1.97g/l in Mild Dysplasia, 2.135g/l in Moderate Dysplasia and 2.55g/l in Severe Dysplasia. Salivary Pyruvic acid levels were seen to be increased with the increasing histopathological grades of leukoplakia. (Table No 3)

On evaluation of Salivary Pyruvic acid levels in the different grades of squamous cell carcinoma, it was observed that salivary Pyruvic acid levels were 3.72 g/l in WDSCC, 3.61g/l in MDSCC and 3.49g/l in PDSCC.The levels were decreased with increasing histopathological grades of Squamous Cell Carcinoma. (Table No 3)

When salivary and serum pyruvic acid were compared, it was observed that levels of salivary pyruvic acid were increased when compared with the levels of serum pyruvic acid.

Table 1 Comparing serum pyruvic acid level in control, leukoplakia and squamous cell carcinoma groups.

GROUP	N	Average(g/l)
Control	30	1.12
Leukoplakia	30	1.56
SCC	30	2.80

Table 2 Comparing salivary pyruvic acid level in control leukoplakia and squamous cell carcinoma groups.

GROUP	N	Average (g/l)
Control	30	1.52
Leukoplakia	30	2.04
SCC	30	3.63

Table 3 . Comparing of serum pyruvic and salivary pyruvic acid level acid level according to histopathology in leukoplakia

	No of cases	Average (g/l)	
		Serum pyruvic	Salivary pyruvic
Mild dysplasia	15	1.50	1.90
Moderate dysplasia	13	1.58	2.14
Severe dysplasia	2	1.79	2.55

Table 4 Comparing of serum pyruvic acid level and salivary pyruvic acid level according to histopathology in squamous cell carcinoma

	No of cases	Average (g/l)	
		Serum pyruvic	Salivary pyruvic
Well differentiated	9	2.71	3.72
Moderately differentiated	18	2.83	3.61
Poorly differentiated	3	2.92	3.49

Table 5. Comparing of serum and salivary pyruvic acid level in all three groups

	Mean Serum PA level	Mean Salivary PA level
Control	1.12 ± 0.09	1.52 ± 0.09
Leukoplakia	1.56 ± 0.18	2.04 ± 0.43
SCC	2.80 ± 0.36	3.63 ± 0.25

DISCUSSION

Early identification of oral cancer offers better chances for long-term survival and may enhance the treatment outcomes, thereby making healthcare affordable[9]. However there is an urgent need to devise critical diagnostic tools, which can be utilized for an early detection of oral potentially premalignant and malignant lesions. Also such tools need to be practical, non-invasive and should be easy to carry out.Many technologies have been developed for an early diagnosis of oral cancer such use of vital stains, brush biopsies, salivary diagnostics and the use of optical imaging systems like ViziLite, VELscope. But, unfortunately the practical application of these non- invasive tools in a community setup has not been fruitful.. Patients are still being diagnosed in advanced stages of oral cancer [10]. Molecular biomarkers that are efficient in identification of the subset of lesions which are most likely to convert into cancer are being widely explored including genetic and epigenetic alterations observed in oral precancerous lesions [11].

Though there has been a, incredible progress in the field of molecular biology, still we do not have a single marker that is reliable and enables us to predict chances of development of malignancy in a patient. Therefore, there is a need to develop screening techniques that can mask the limitations of the screening methods that are presently available [3]. Lactic acid and pyruvic acid are formed as the byproducts of the physiologic process of glycolysis [12]. Due to excess, uncontrolled proliferation of cancer cells, there is an increase need for energy .This is provided by an compensatory increase in the rate of glycolysis. As a result of this, production of lactic acid and pyruvic acid rises. Hypoxia due to uncontrolled proliferation of cells initiates the Warburg's effect, which induces glycolytic pathway for the energy production and its survival [13], in an oxygen depleted environment in the tumor tissue. Therefore it has been suggested that glycolysis is the main metabolic pathway which is finely regulating cell proliferation and this is done by adjusting the metabolism of cancer cell to the available environment of its current specific situation [15, 16]. Excess glycolysis produces excess end products in the form of lactate and pyruvate. As, lactate is unstable at room temperature, it is converted into pyruvic acid which is either exuded into blood or saliva. Therefore, estimation of pyruvic acid may be able to reflect the spectrum of oral cancer. Hence, the study was aimed to quantify pyruvic acid level in serum and saliva of patients with leukoplakia and oral squamous cell carcinoma [3].

In the study, on comparison serum pyruvic acid levels in Leukoplakia and SCC, the levels were seen to be significantly increased in SCC as compared to leukoplakia. Similar observations were made by Anithraj Bhat et al [3]and Manohara A Bhat et al [16] who also showed significantly raised serum pyruvic acid level in leukoplakia and oral cancer patients. In our present study it was seen that in patients with leukoplakia, the mean value of serum pyruvic acid was highest in case of severe dysplasia as compared to moderate and mild dysplasia. As per our knowledge, studies have not been done to correlate the levels of serum pyruvic acid level according to histopathological grading of leukoplakia.

No previous studies have not been done to correlate the serum pyruvic acid level with the histopathological grades of squamous cell carcinoma. In thisstudy, the analysis was carried out and a significant direct correlation was established between the levels of serum pyruvic acid with the histopathological grades of squamous cell carcinoma ie the mean value of serum pyruvic acid level was raised in PDSCC as compared to that observed in WDSCC .

A similar analysis was also done on Salivary pyruvic acid levels in cases of leukoplakia and squamous cell carcinoma. It was observed that there was a significant increase in levels of Salivary pyruvic acid in patients with squamous cell carcinoma as compared to levels seen in cases of leukoplakia. Similar observations were made by Anithraj Bhat et al [3] and Manohara A Bhat et al [16] who showed significantly raised pyruvic acid levels in squamous cell carcinoma as compared to leukoplakia.

Studies have not been done yet to correlate the levels of salivary pyruvic acid level with histopathological grades of epithelial dysplasia those of SCC.In the study, it was also observed that the mean value of salivary pyruvic acid was highest in case of severe dysplasia as compared to moderate and mild dysplasia. In addition to this, it was also seen that salivary pyruvic acid level was highest in case of WDSCCas compared to that observed in MDSCC and in PDSCC.

This rise in pyruvic acid as the disease progress from leukoplakia to oral squamous cell carcinoma may be due to adaption of cancer cell metabolism in which glycolysis becomes a central metabolic pathway which regulates cell proliferation. Cancer cells, for their energy requirement, exhibit increased glycolysis rate . As a result of this there is an additional formation of by-products such as lactic acid and pyruvic acidIn the study, it was observed that the serum and salivary pyruvic acid levels were raised in leukoplakia and squamous cell carcinoma patients as compared to that seen in control group.

CONCLUSION

The present study aimed at evaluating the serum and salivary pyruvic acid level in three groups : control group, oral leukoplakia and oral squamous cell carcinoma. The patients with leukoplakia and squamous cell carcinoma were categorized histopathologically. Histopathological grading of the lesion was done after staining the biopsied tissues with Hematoxylin and Eosin staining. The serum and salivary pyruvic acid levels were estimated.

In the study, the mean level of serum pyruvic acid was seen to increase from control group to the leukoplakia group and this was found to be statistically significant. The levels were found to be elevated squamous cell carcinoma patients when compared with patients having leukoplakia and this was also seen to be statistically significant.

In the study, it was observed that the mean salivary pyruvic acid levels were found to be increased from control group to the patients with leukoplakia and was statistically significant. The levels were found to be elevated in the patients with squamous cell carcinoma when compared with patients with leukoplakia and this was also statistically significant.

In conclusion, the finding from this study suggest that the serum and salivary pyruvic acid levels could be a better adjuvant diagnostic marker along with the routine markers in patients with premalignant and malignant lesions. The pyruvic acid level measurement is simple, noninvasive and cost effective screening, diagnostic and monitoring tool in cases of leukoplakia and squamous cell carcinoma.

Thus, the time calls for further studies with a larger sample size to establish the diagnostic efficacy of Serum and Salivary pyruvic acid levels in the early diagnosis of premalignant and malignant lesions of the oral cavity.

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