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To Evaluate the Role of Serum Sialic Acid as Non-Invasive Bio-marker of Leukoplakia and Oral Cancer

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ABSTRACT

OBJECTIVE: To evaluate the role of serum sialic acid as a non-invasive biomarker of Leukoplakia and oral cancer.

METHODOLOGY: This comparative cross-sectional study was conducted from December 2017 to November 2019 at the Institute of Biochemistry and Tertiary care hospital, Larkana. The 34 diagnosed cases of oral Leukoplakia and Erythroplakia were 20 to 72 years age. The controls with history of using tobacco, areca nut, alcohol, corticosteroid drugs and systemic diseases were excluded from this study. The dysplasia was graded histopathologically as mild, moderate and severe. The Blood samples were collected and centrifuged at 3000 rpm for 15 minutes; then, serum was separated and Sialic Acid was analyzed spectrophotometrically. Finally, Statistical analysis was done by SPSS version 20 and results were expressed with mean and standard deviation.

RESULTS: Out of 34 cases and controls15 (44.1%) were male, and 19(55.9%) were female. Their ages were from 20 to 72 years, and the mean age was 47.47±17.97. The typical sites of lesion were buccal mucosa 35.3%, tongue 26.5%, right cheek 14.70%, left cheek 8.8%, Juxta mandibular area 8.8% and lip 2.9%, respectively. Clinically Oral Leukoplakia was 58.8% and Erythroplakia 29.4%. The Sialic Acid levels were 78.7±16.6 mg/dl in Leukoplakia, 105.4±22mg/dl in Erythroplakia and was 60.2±4 mg/dl in controls. The serum sialic acid levels were increased in case group as compared to control group in both genders, with significant p-value 0.001.

CONCLUSION: The Sialic Acid levels were significantly increased in cases of Oral Leukoplakia and Erythroplakia as compared to healthy controls.

KEY WORDS: Sialic Acid, Oral Leukoplakia, Erythroplakia, Buccal mucosa, Juxta mandibular

INTRODUCTION

Worldwide, large populations are affected by cancer, including about 45% men and 20% of women; yearly, about 10 million people are newly diagnosed, whereas six million people die from cancer. Oral cancer is the most common among Asian populations, especially in the Indo-Pak subcontinent; 16-62% of oral cancers are caused by oral premalignant lesions like Leukoplakia and Erythroplakia. Oral Cancer is frequent due to the more common use of tobacco. All oral cancers are about 91%, but Leukoplakia and Erythroplakia are overall 80% responsible for oral cancers[1].

Oral Leukoplakia and Erythroplakia are premalignant lesions which may be defined as a predominantly white lesion/red lesion that cannot be characterized as any other definable lesion of the oral mucosa. It is one of the most common premalignant lesions with a strong male predilesction[21].

In the past, many studies have been conducted on different types of oral cancers, while a few research studies are available about Oral Leukoplakia and Erythroplakia. The oral mucosa's precancerous lesions are more common in males than females[3].

The etiological factors of oral premalignant lesions are tobacco, areca nut, alcohol, viruses including Ep-bar viruses, Human papilloma viruses, and a few nutritional deficiencies[4].

The diagnosis of oral premalignant lesions is made by histopathology, a time-consuming and invasive procedure. The histopathological examination reveals the degree of epithelial dysplasia, categorized as mild, moderate and severe dysplasia[5]. As dysplasia is a reversible condition, the early detection of premalignant lesions may be reversed by removing the causative or irritant agent, and the situation becomes normal.

Sialic Acid is a negatively charged nine carbons containing mono-saccharide, present as N-acetyl Neuraminic Acid (NANA) in the human body; which contributes to cell-to-cell recognition, cell-to-matrix interaction and as a receptor, it is found in serum, saliva, breast milk, gall bladder excretion, synovial fluid, gastric juices, sweat and urine[5,6].

Sialic Acid is altered in oral cancer patients due to dysplastic epithelial changes as it plays a biologically crucial role in glyco-conjugation. The elevation in serum Sialic Acid levels provides the early detection of malignant changes, which indicates cell transformation[8]. Changes in Sialic Acid levels may vary at the beginning of the tumour and development of carcinoma[9]. The regular monitoring of SA may detect malignancy at its early stage[10]. The raised levels of serum Sialic Acid were observed in proportion to different grades of epithelial dysplasia[11]. This study showed the raised levels of serum Sialic Acid in patients of oral cancer and Leukoplakia compared to healthy controls[2].

Thus, the present study showed markedly increased serum Sialic Acid levels in Oral Leukoplakia patients at different grades of epithelial dysplasia compared to healthy controls[12]. This finding is consistent with the studies that show an elevation in the levels of serum Sialic Acid, showing early indication of a malignancy progression[13].

Irrespective of this study's findings, the potential benefits of sialic acid screening are enormous. The clinical diagnosis supplemented with sialic acid levels can gain diagnostic importance in the near future. Moreover, sialic acid levels can be used to monitor the prognosis of oral leukoplakia[22].

Hence, this study was proposed to evaluate levels of serum Sialic Acid in diagnosed patients of Oral Leukoplakia and Erythroplakia with different grades of dysplasia. The results were compared with healthy individuals.

METHODOLOGY

This cross-sectional case and control study with random sampling was conducted at the Institute of Biochemistry and Tertiary care hospital, Larkana, from December 2017 to November 2019. Consent was obtained from 34 healthy controls and 34 diagnosed patients of Oral Leukoplakia and Erythroplakia. The age ranges of cases and controls were from 20-72 years. In comparison, the healthy controls with a history of using tobacco, areca nut, alcohol, or on corticosteroid medications, chronic diseases like Type-II Diabetes Mellitus, Chronic Obstructive Pulmonary Disease and Cardio Vascular Disease were excluded from this study. Histopathologically, epithelial dysplasia grades were divided into mild, moderate and severe. The Blood samples of all participants were collected by vein puncture using a standardized technique. Each sample was centrifuged at 3000 RPM for 15 minutes, and serum was transferred to a proper container and stored at -70^{0}C. Then, serum sialic Acid was determined using a spectrophotometer, and levels were measured using the formula below.

$$SA = \frac{\text{Optical Density of Sample}}{\text{Optical Density of standard}} \times \frac{\text{Consent: of standard}}{\text{volume of test}} \times 100$$

Data analysis was done by SPSS version 20, and the results were represented in Mean with standard deviation. Kruskal Wallis Test was applied to compare the results between the cases and healthy controls.

RESULTS

The tumour biomarkers may help in the early detection, prevention and prognosis of oral premalignant lesions. In the healthy individuals, the levels of serum Sialic Acid were not changed, but a slight increase was noted with increasing age, hypoproteinemia, anaemia and rich carbohydrate diet consumption. The serum Sialic Acid levels may increase in the blood due to shedding off from premalignant cells.

Table I, shows the male and female gender-wise distribution of cases and controls. In the control group, out of 34 individuals, 15 (44.1%) were males, and 19 (55.9%) were females. In the case group, out of 34 cases, oral Leukoplakia was in 10(43.47%) males, and 13 (65.52%) were female out of 23(67.6%) cases. Erythroplakia was seen in 11(32.3%) cases, out of which 5(45.45%) cases were male, and 6 (54.54%) were females. The p-value more than ($p>0.05$) shows no significant difference between the study groups.

TABLE I: GENDER-WISE DISTRIBUTION OF CASES AND CONTROLS

Gender	**Controls** (n=34)	**Leukoplakia** (n=23) (67.6%)	**Erythroplakia** (n=11) (32.3%)	**P-value**
Male	15 (44.1%)	10 (43.47%)	5 (45.45%)	0.128
Female	19 (55.9%)	13 (65.52%)	6 (54.54%)	

Table II shows the mean serum Sialic Acid levels in mg/dl among patients of Leukoplakia, Erythroplakia and healthy controls. In healthy controls, mean SA levels were 60.2±4.27, whereas mean SA increased to 78.7±16.62 in the cases of Leukoplakia. Likewise, the levels were raised to 105.4±22.03 in Erythroplakia; it was noted that the levels of mean sialic Acid were significantly increased in cases of Leukoplakia and erythroplakia as compared with healthy controls with p-value >0.001. While the comparison between Leukoplakia and erythroplakia again shows that the mean serum sialic acid levels were significantly increased in Erythroplakia with a p-value >0.001.

TABLE II: MEAN SERUM SIALIC ACID LEVELS IN CASES AND CONTROLS

Serum Biomarker	**Healthy Controls** (n=34)	**Oral Leukoplakia** (n=23)	**Oral Erythroplakia** (n=11)	**P-value**
	(Mean ± S.D)	(Mean ± S.D)	(Mean ± S.D)	
SA (mg/dl)	60.2 ± 4.27	78.7 ± 16.6	105.4 ± 22	0.001

DISCUSSION

The most frequent lesions of the oral mucosa are Leukoplakia and Erythroplakia, found more commonly in females than males. Leukoplakia and Erythroplakia are precancerous lesions which may be defined as a white/red lesion of the oral mucosa that cannot be characterized as any other definable lesion[23].

The incidence and death rate of cancer is increasing multi-fold; hence early diagnosis and timely treatment may save lives from the curse of oral Leukoplakia and erythroplakia due to their malignant changes. So many researchers are searching for reliable and readily available non-invasive biomarkers, which may help in the early diagnosis of the disease in its pre-malignancy[24].

Nowadays, researchers are targeting the cell surface glycoproteins, which play an essential role during the malignant transformation of the cells; such glycol-conjugates contain up to 30% Sialic acid[25].

The benefits of evaluating serum Sialic Acid levels include diagnosing oral cancer and precancerous lesion in their initial stages; it also helps assess the treatment planning and prognosis of disease[18]. Clinically, shortly the serum sialic acid levels may be used as a more reliable and accurate tool for the diagnosis as well as for prognosis[19]

In the present study, the serum sialic acid levels were evaluated and compared among Leukoplakia, Erythroplakia and healthy control groups. It was significantly increased in Leukoplakia and erythroplakia groups compared to a healthy control group. Our findings are consistent with Taqi et al., Raval et al., Joshi et al., Kinnari et al., and many other previous studies[7].

Other findings of the present study show that in cases of Oral Leukoplakia, the serum sialic acid levels increased with the degree of dysplasia. Therefore, when compared to healthy controls, an increase in serum sialic acid levels was noted in cases of mild, moderate and severe dysplasia. Thus this finding is also inconsistent with the studies by Hementh SC et al. and Anand K et al. [26].

The increase in serum sialic Acid levels may be due to the release of glycoproteins in the cases of precancerous lesions as compared to healthy individuals. The elevation in serum sialic Acid levels may provide an early indication of a premalignant change [27].

The laboratory method for detecting serum sialic Acid is by using the spectrophotometer, which may detect any slight change of colour, as it is used to measure the range of light from 350 to 800 nm in coloured compounds[14,15].

As per our study, the most accurate and susceptible method for the determination of colour changes of light in chemical compounds[16]. Hence it is more frequently used in laboratory procedures than other laboratory methods because of its easy, simple and non-expensive method[17].

CONCLUSION

The study has concluded that serum sialic acid (SA) levels are manageable, non-expensive and non-invasive biomarkers of oral premalignant lesions.

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AUTHOR CONTRIBUTIONS

Tagar MR:	Conception, design, collection and data analysis
Khuhawar SR:	Drafting of article, analysis and interpretation of data
Shah YA:	Critical revision of article, final approval
Shaikh MA:	Analysis and interpretation of data, drafting of article
Shaikh AA:	Critical revision of article,, data analysis
Bux A:	Statistical analysis, data analysis
Hafeez MA:	Drafting of article, statistical analysis

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