BIOCHEMICAL AND MOLECULAR BIOMARKERS IN ORAL SQUAMOUS CELL CARCINOMA

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Abstract

Aim: The aim of the study was to evaluate the biochemical and molecular variables in the epiglottis, tongue, and hard palate and compare with healthy controls in oral squamous cell carcinoma (OSCC).

Methods: This was a prospective and cross-sectional study conducted at Malla Reddy Medical college and tertiary care hospitals in Hyderabad with individuals having oral malignancies (OM). 60 adults aged ≥ 18 years, diagnosed with OM (20 each of tongue, epiglottis, and hard palate) were included in the study. Those who had cancer at more than one area were excluded from the study. Blood samples were collected from each participant and used for testing the expression of biochemical and molecular markers in oral squamous cell carcinoma. They were analysed by the genomic sequencing method. Student 't' test and one-way ANOVA with Bonferroni's t-test was used for statistical analysis.

Results: Biomarkers and proteins such as Schiff bases, diene conjugate, triene conjugate, haptoglobin (Hapto), transferrin (Transf), β2-microglobulin, ceruloplasmin, complement 4 components (C4c), complement 3 component (C3c), Transthyretin (TTR), lipid bound sialic acid (LBSA) and total sialic acid (TSA) in HPV positive (+ve) and negative (-ve) samples were expressed significantly compared to control ($p < 0.001$)**.** The difference between them was not significant even though most of the studied markers were highly expressed in all three types of malignancy compared to the control

Conclusion: The levels of oxidative parameters, protein metabolites, biochemical markers and the conjugate were elevated in tongue, hard palate and epiglottis compared to the normal control. However, the above parameters did not change in tongue hard palate and epiglottis showing cancer can cause similar type of effect. The assessment of these parameters may help in early detection of oral malignancies.

Keywords: Diene conjugate, triene Conjugate, Ceruloplasmin, sialic acid, HNSCC, LBSA, C3c, C4c, Cp, OSCC.

Introduction

Cancers of the head and neck, also known as head and neck squamous cell carcinomas (HNSCC), are a diverse group of cancer which includes malignancies of the oral cavity, nasopharynx, oropharynx, hypopharynx, and larynx. High rates of these malignancies can be

seen in the Mediterranean region of Europe and in South America, making them the seventh most common malignancy diagnosed worldwide [1-3] The prognosis is quite dismal, with a 5-year survival. There is a danger of synchronous or metachronous tumour development, although tumours can arise from anywhere in a population where appropriate genetic mutations have occurred.

In order to develop biomarkers or successful treatment approaches, perhaps targeting not only tumours but also the early alterations, it is necessary to have a thorough understanding of the processes in the evolution of these diseases [4, 5]. According to the initial multi-step model for carcinogenesis in HNSCC, dysplasia reflects an earlier stage of cancer progression from normal mucosa to invasive carcinoma. While carcinogens and viruses account for vast majority of occurrences of HNSCC, some cases can be traced back to a single family [6]. Fanconi anaemia, an autosomal recessive genomic-instability condition that causes bone marrow failure, leukaemia, congenital abnormalities, and susceptibility to cross-linking agents, is the syndromic aetiology linked to an elevated risk of HNSCC. [7] Patients with Fanconi anaemia have an extremely high risk of HNSCC, as shown by cohort studies, with the observed number of HNSCC cases being higher than 700 times the predicted number of cases [8].

In order to better understand molecular anomalies in HNSCC and to create new therapies, genomic profiling studies are essential. Therefore, the purpose of this research study was to analyse the biochemical and molecular markers and proteins such as Schiff bases, dine conjugate, triene conjugate, Haptoglobin (Hapto), Transferrin (Transf), β2-Microglobulin, Ceruloplasmin (Cp), Complement 4 component (C4c), Complement 3 component (C3c), Transthyretin (TTR), Lipid based sialic acid (LBSA) and Total sialic acid (TSA).

Materials and Methods

This was a prospective, cross-sectional study of OSCC. 60 individuals with OM (20 of tongue; 20 of the epiglottises; 20 of the hard palate) from Malla Reddy Cancer Hospital and Research Institute and Tertiary Care Hospitals (Hyderabad, India) were the+ participants. Adults aged ≥ 18 years both male and female diagnosed with oral squamous cell carcinoma, histologically confirmed were included. Individuals with parotid tumours**,** previous radiotherapy, chemotherapy, and heavy tumour bleeding from the mouth were excluded. The present study was approved by the MRMCW Institute Ethics committee (Project No. MRMCWIEC/AP/80/2022). Information about the research study was given in English and Telugu (regional language) and signed consent was obtained from the participants. Confidentiality of the information was maintained. 17 normal individuals were also participated in the study as control.

Methodology:

5mL of blood sample was collected from each participant and plasma was separated. DNA was extracted using genomic DNA isolation kit (Thermofisher scientific K0512 Genomic DNA Purification Kit). DNA quality was checked by UV absorption at 260 and 280 nm by agarose gel electrophoresis. The isolated DNA was stored at $4^{o}C$ for genetic analysis. Analysis of parameters such as Schiff bases, Diene conjugate, Triene conjugate, Haptoglobin (Hapto mg/dl), Transferrin (Transf mg/dl), β2-Microglobulin (mg/dl), Ceruloplasmin (Cp mg/dl), Complement 4 component (C4c mg/dl), Complement 3 component (C3c mg/dl), Transthyretin (TTR mg/dl), Lipid bounded sialic acid (LBSA mg/dl) and Total sialic acid

(TSA mg/dl) was done by using enzyme linked immunosorbent assays using commercial kits from Assay pro (3400 Harry S Trumen Blvd, St. Charles USA) using ELISA reader (Transasia Bio-medicals Ltd, Andheri (EAST) Mumbai, India) and ELISA Washer (Erba Diagnostics Man-nheim, Germany).

Statistical analysis:

The data were expressed as mean ± and standard error. The means were compared by one-way analysis of variance with Bonferroni's t-test for multiple comparison. SigmaPlot 14.5 version (Systat Software Inc., San Jose, USA) was used for the statistical analysis and graph plotting. A p-value of less than 0.05 was taken as statistically significant.

Results:

The biochemical and molecular markers expression can be analysed by a variety of biomarkers and proteins such as Schiff bases, dine conjugate, triene conjugate, Haptoglobin (Hapto mg/dl), Transferrin (Transf mg/dl), β_2-Microglobulin (mg/dl), Ceruloplasmin (Cp mg/dl), Complement 4 component (C4c mg/dl), Complement 3 component (C3c mg/dl), Transthyretin (TTR mg/dl), Lipid bounded sialic acid (LBSA mg/dl) and Total sialic acid (TSA mg/dl) in HPV +ve and -ve samples . In the present study these markers were evaluated and expressed as (mean ± SE).

The Schiff base was 0.49 ± 0.0 similar in both HPV +ve and -ve participants. Diene conjugate and triene conjugate with HPV +ve and -ve was 2.6 ± 0.07, 2.7 ± 0.1 and 0.92 ± 0.01, 0.94 ± 0.01 respectively. The increase in expression was significant compared to control ($p < 0.001$). (Figure.1) OM of tongue, hard palate and epiglottis were showing higher value but there was no difference between the 3 OM.

The Hapto (mg/dl) was 83.0 ±1.0, 82.17±1.0 and β_2-microglobulin (mg/dl) was 2.83 ± 0.03, 2.78 ± 0.04 respectively in HPV +ve and -ve. This was statistically significant compared to control ($P < 0.001$). Transf was 258.5 ± 2.61, 259.1 ± 3.23 (mg/dl) and was not statistically significant ($P < 0.973$). OM of tongue, hard palate and epiglottis were showing higher value but there was no difference between them. (Figure.2).

The expression of Cp (mg/dl) was 40.16 ± 1.19, 37.73 ± 1.4, C4c (mg/dl) was 23.48 ± 0.16, 23.26 ± 0.23 and C3c (mg/dl) was 113.16 ± 1.08, 114.91 ± 1.34 respectively in HPV +ve and -ve participants and was statistically significant compared to the control ($p < 0.001$). OM of tongue, hard palate and epiglottis were showing higher value and there was no difference between them (Figure.3). The expression of Cp, C4c and C3c were considered as an elevated marker in proteins.

The expression of TTR (mg/dl) was 25.51 ± 0.33, 25.43 ± 0.4 in HPV +ve and -ve and was not statistically significant ($P < 0.608$). LBSA (mg/dl) was 13.63 ± 0.61, 12.41 ± 0.51. This was statistically significant ($p < 0.001$). TSA (mg/dl) was 78.55 ± 1.68, 78.64 ± 2.2. This also was statistically significant ($p < 0.001$). OM of tongue, hard palate and epiglottis were showing higher value without difference between but was significant compared to control. (Figure.4).

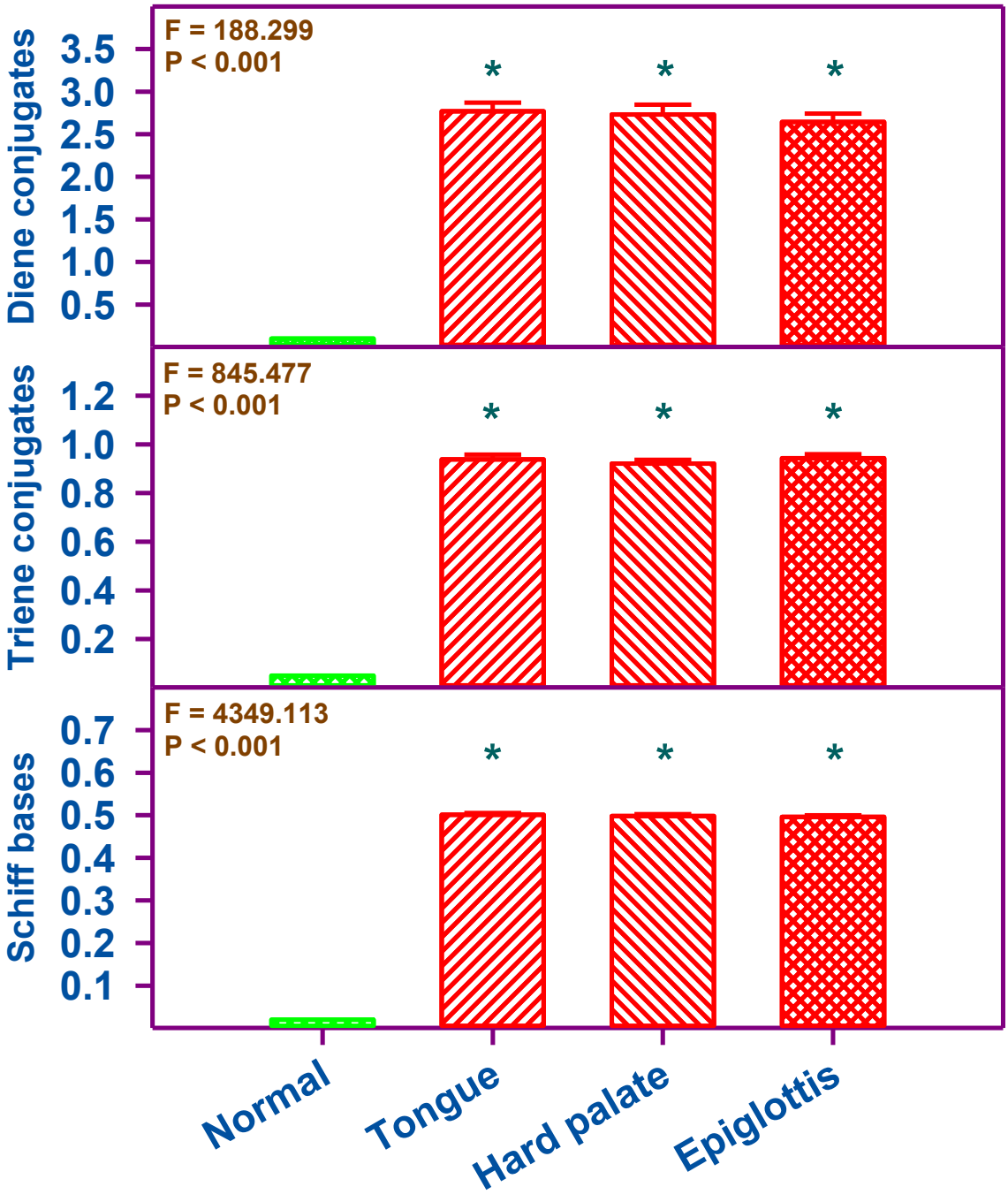

Figure 1: Parameters of Schiff bases, Diene conjugate and Triene conjugate of normal, and cancer of the tongue, hard palate, and epiglottis.
n = Normal = 17; Tongue, Hard palate, and Epiglottis n= 20.
The 'F' and 'P' values were by one-way ANOVA with Bonferroni's t-test.
*Significantly different from normal.

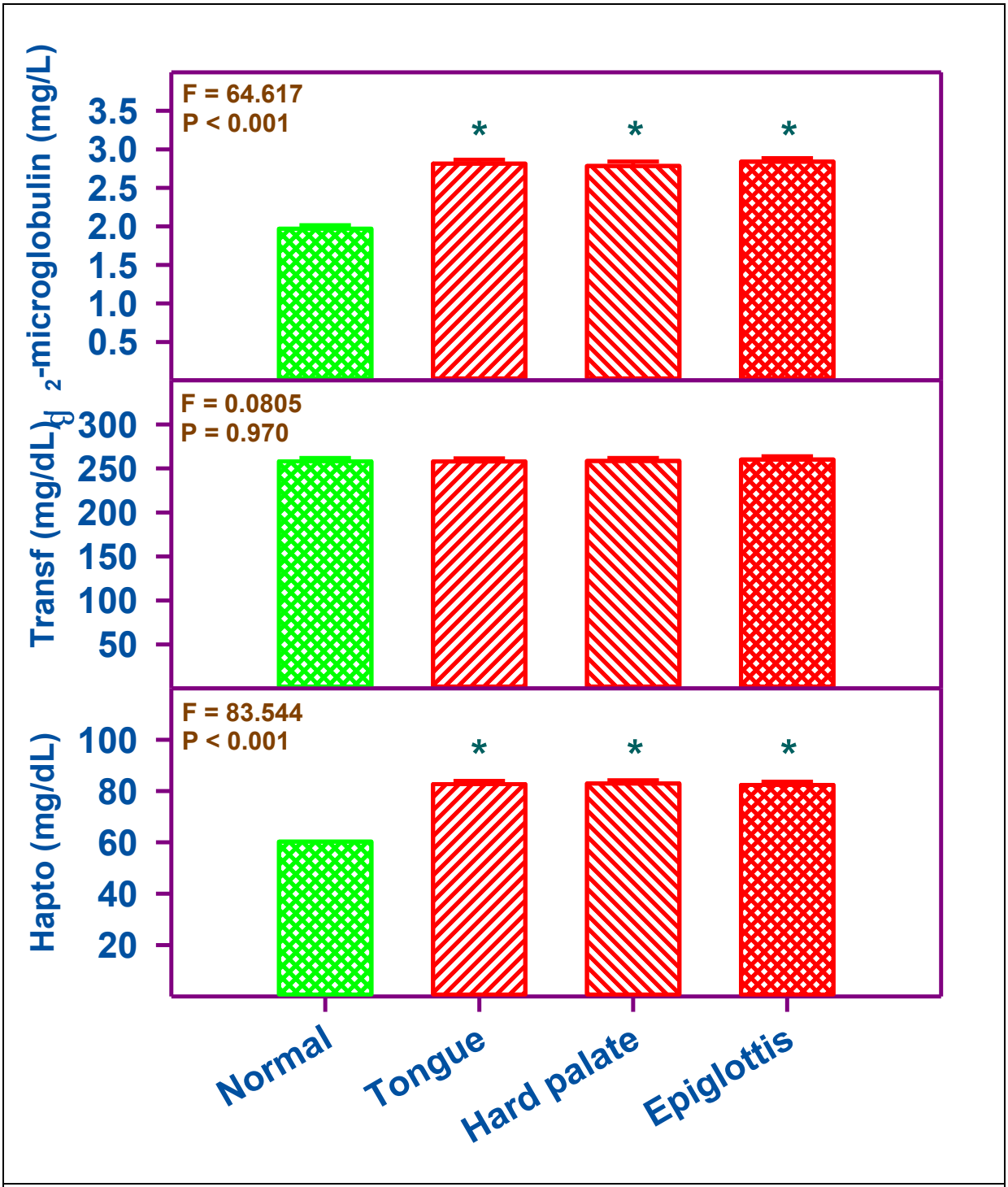

Figure 2: Parameters of Hapto (mg/dl), Transf (mg/dl) and β_2 microglobulin (mg/dl) of normal, and cancer of the tongue, hard palate, and epiglottis.
n = Normal = 17; Tongue, Hard palate, and Epiglottis n=20.
The 'F' and 'P' values were by one-way ANOVA with Bonferroni's t-test.
*Significantly different from normal.

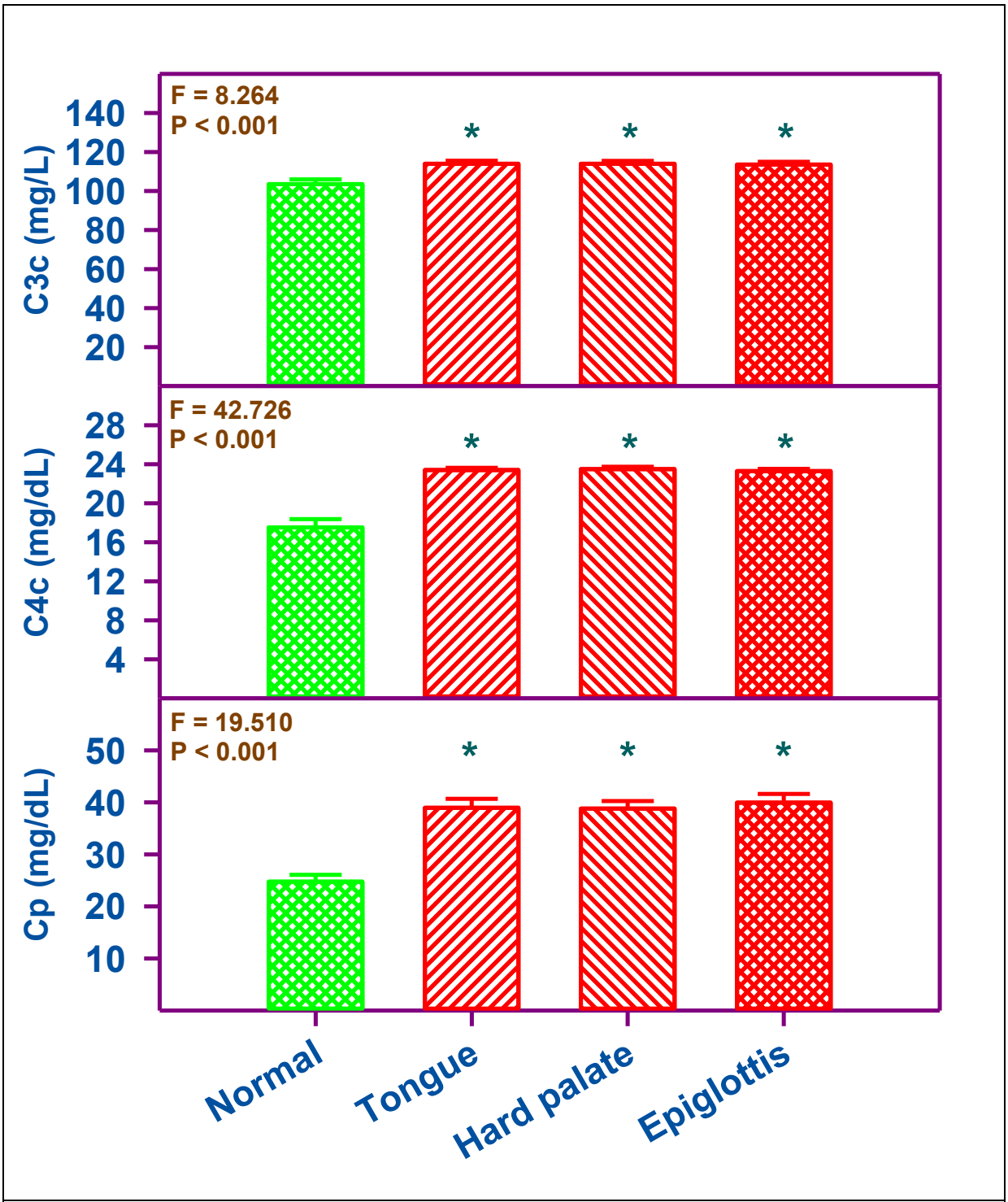

Figure 3: Parameters of Cp (mg/dl), C4c (mg/dl) and C3c (mg/dl) of normal, and cancer of the tongue, hard palate, and epiglottis.
n = Normal = 17; Tongue, Hard palate, and Epiglottis n =20.
The 'F' and 'P' values were by one-way ANOVA with Bonferroni's t-test.
*Significantly different from normal.

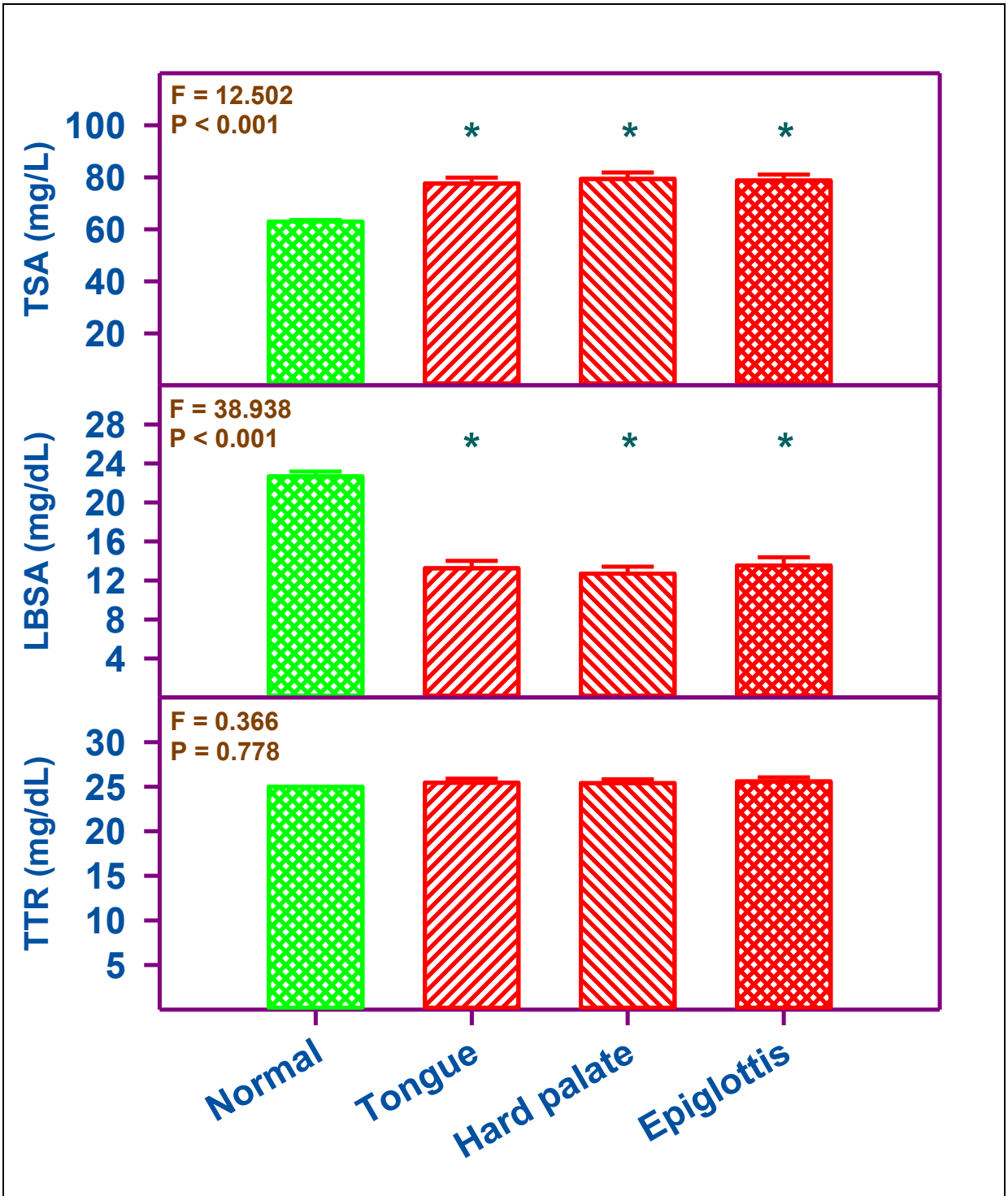

Figure 4: Parameters of TTR (mg/dl), LBSA (mg/dl) and TSA (mg/dl) of normal, and cancer of the tongue, hard palate, and epiglottis.
n = Normal = 17; Tongue, Hard palate, and Epiglottis n = 20.
The 'F' and 'P' values were by one-way ANOVA with Bonferroni's t-test.
*Significantly different from normal.

Discussion:

Head and neck cancer is a broad term that includes epithelial malignancies which are squamous cell carcinoma of the head and neck (SCCHN). They can invade adjoining parts of the body and spread to other organs [9]. HNSCC constitute a heterogeneous group of cancers, which include cancers arising in the oral cavity, nasopharynx, oropharynx, hypopharynx, and larynx [10].

Many recent studies have reported genetic and epigenetic changes in HNSCC [11-13]. Genetic changes to the stages of evolution of HNSCCs from the earliest stages to transition to malignancy and progression to metastases. Schiff bases and their complexes are very functional components in medicinal and pharmaceutical fields because of their widespread range of biological activities like anticancer [14-16]. Tadele et. al., has reported the increased activity of Schiff bases [17]. In the present study Schiff base was elevated in all three types of OM, may be an internal prevention against cancerous growth. Gargouri et. al., has confirmed in the plasma by the high levels of conjugated dienes ($p < 0.001$) [18]. Therefore, it was an important both diene and triene conjugate will rise as a result of lipid peroxidase. In the present study identified diene and triene conjugates levels elevated.

Correlation between cellular haptoglobin and clinical characteristics of HNSCC was analysed to assess the prognostic value of cellular haptoglobin level. Data presented show that cellular expression of haptoglobin is closely related to recurrence rate in HNSCC patients. The cellular expression of haptoglobin may be a prognostic factor in HNSCC [19]. According to Lee et al., the Hp genotype may be a prognostic factor in patients with HNSCC and has significantly increased Haptoglobulin in multivariate analysis [20]. The present study can be additional evidence for elevated Hapto. Mauting Lin et. al., and ShanL, et. al., has explained the overexpression transferrin in HNSCC. Transferrin biochemical parameter always increases in the plasma with tumour size [21, 22]. In the present study also agreement with the previous study identified as transferrin role is overexpressed. According to Niki G, et. al., Chen CH, et. al., Ogino T, et. al., Meissner M, et. al., [23-26] β_2 microglobulin levels was elevated and present study also in agreement with the above studies supporting increased β_2 microglobulin in HNSCC.

The present study result ceruloplasmin was additional evidence to support with Senra et al., the in Cp was significantly ($P < 0.001$) elevated in advanced stages of malignant tumours of HNSCC [27]. Finally, the results of Complement component (C3c, C4c) levels of HNSCC patients were significantly ($P < 0.001$) higher than those of the healthy individuals. In agreement with the same study of Yuan, et. al., suggest the high C3c, C4c deposition observed to correlate with OM [28].

According to Ivica K et. al., the concentration of lipid sialic acid was significantly fell to somewhat lower levels [29]. Present study confirmed that low LBSA was an important predictor for somatic copy number alterations (SCNAs) of overall survival. Xin Zhou et. al., diagnose the increased levels of TTR in nasopharyngeal carcinoma (NPC) [30]. However in the present study, there was no considerable change in TTR. Joshi, et. al., stated that the mean serum total sialic acid (TSA) level in oral precancer and oral cancer group was statistically significant ($P<0.05$). Serum total sialic acid levels can be used as an adjunctive diagnostic marker in head and neck cancer [31]. In present study also identified TSA was elevated in HNSCC.

Conclusion:

HNSCC is the most emerging serious disease throughout the world which need early detection. The oxidative parameters, protein metabolites, biochemical markers and the conjugate were elevated with OM of tongue, hard palate and epiglottis. Estimation of these parameters may benefit in early detection of such cancer.

Conflict of Interest: Nil

Funding Source: Nil

Acknowledgement: The authors thank the participants for giving consent to make this study possible. Authors also express gratitude to the management of both Saveetha Institute of Medical and Technical Sciences, Chennai and Malla Reddy Medical College for Women, Hyderabad.

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