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Research Article

Sarcomas

Correlation of clinical radiological features with histopathology and IHC to predict aggressive behaviour of soft tissue sarcomas

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Introduction: A study was taken to assess the pathological predictors of aggressive behaviour of soft tissue sarcomas (STSs) and also to the correlation of STSs with clinical, radiological of histopathological techniques. **Materials and methods:** This was a prospective conducted from January 2016 to June 2017. All those coming to this institute with oral and oropharyngeal squamous cell cancers (SCCs) included. Smokers, tobacco chewers were not considered. Punch biopsy and surgical biopsy were taken, respectively from inoperable and operable cases, paraffin blocks were prepared. Tissue microarray (TMA) were constructed using these blocks. Tissue inhibitor of metalloproteinases 1 (TIMP 1) stain-ing was assessed on the cell membrane and cytoplasm of the tumour cells (TCs). Two areas that showed high-density stained cells were selected, several stained TCs was counted. The intensity of staining was classified as negative, weak, moderate and strong. AXL kinase staining was scored as 0, 1+, 2+, and 3+. Eps8 staining was ranged from negative (0), to strong and diffuse (3+). P less than 0.05 was considered statistically significant. **Results:** A total of 30 (100%) partic-ipants were included. With TIMP 1 expression, in normal mucosa, 63.3% (19) specimens did not stain; statistically, there was a significant difference. Around 53.3% (16) of normal mucosa did not stain with EPS8; statistically, there was a significant difference. In the samples of SCC, 60% (18) showed 3+ sting and 40% (12) showed 2+. Statistically, there was a significant difference. **Conclusion:** The expression of TIMP1, EPS8 and AXL establish their role in the pathogenesis of oral and oropharyngeal SCCs.

Keywords: Cancer, Histopathology, Staining, Tumor

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Introduction

Sarcomas are a rare heterogeneous group of malignant tumors of mesenchyme origin. These account for less than 1% of all adult malignancies and 12% of pediatric cancers. [1, 2]. Nearly 80% of sarcomas originate from soft tissue. [2]. Approximately 50% of sarcomas arise in extremities, with a threefold higher rate noted in lower extremities. [3]. The soft tissue sarcomas (STSs) are a highly heterogeneous group of tumors classified on a histogenesis basis according to adult tissue and all-around about 70 different variants. The correct clinical staging is critical for the selection of treatment and based on imaging modality and histopathogensis and grading of the tumour with FNAC. [4]. Sarcomas are described as being localized regional or metastatic. [6]. About 58% of sarcomas are localized and the 5-year survival rate was 80%. Nearly 18% were in the regional stage, the 5-year survival rate was 54%. The remaining were in the metastatic stage and the 5-year survival rate was 16%. [6]. There is a need for a more precise and objective grading system. Techniques such as Ki67 IHC staining [5, 6]. and correlation with other modalities such as imaging and grading systems can help in bridging the gap. This also gives us a route to find the aggressive behavior of the STSs. With this information, a study was taken to assess the pathological predictors of aggressive behaviour of STSs and also to the correlation of STSs with clinical, radiological with that of histopathological techniques.

Materials and methods

Settings: The study was conducted in the Department of Surgical Oncology, Vydehi Institute of Medical Sciences, Bangalore.

Duration and type of study: This was a prospective conducted from January 2016 to June 2017, 18 months.

Sampling method: Random sampling was considered.

Sample size calculation: All the eligible members who satisfy the inclusion criteria were considered in this study.

Inclusion criteria: All those coming to this institute irrespective of age, gender and stage of disease with oral and oropharyngeal squamous Cell cancers with a history of chewing tobacco were included in this research.

Exclusion criteria: Smokers, tobacco chewers cum smokers, individuals on chemotherapy, previous irradiation for head and neck tumours and non-cooperative were not considered in this research.

Data collection, procedure: From the diseased mucosa of the study participants, punch biopsy and surgical biopsy were taken, respectively from inoperable and operable cases. Simultaneously, punch biopsies were taken from the normal-looking mucosa as control. Paraffin blocks were prepared with these biopsy specimens. Tissue microarray (TMA) were constructed using these blocks. From each paraffin block, 2 mm cores were embedded into a recipient paraffin block. The paraffin-embedded tissue was deparaffinised followed by antigen retrieval using the heat-induced epitope retrieval (HIER) technique. Immunohistochemistry (IHC) was carried out with this TMA. The endogenous peroxidase was quenched using hydrogen peroxide. The tissue section was incubated with primary antibodies specific for the target protein overnight, followed by HRP conjugated secondary antibody. The signal will be developed using DAB chromogen. The expression and cellular location of the molecular markers were examined in diseased and normal oral mucosa by IHC. Smears were screened by two pathologists without knowledge of the clinical impression. Occasional disagreements were discussed to reach a consensus. In cases of persistent differences between them, the sections were studied by a third independent observer and the majority decision was considered. Tissue inhibitor of metalloproteinases 1 (TIMP 1) staining was assessed on the cell membrane and cytoplasm of the tumour cells (TCs) under the light microscope. Two areas that showed high-density stained cells were selected under 40x, then the number of stained TCs out of 200 was counted carefully. The intensity of staining was classified as negative, weak, moderate and strong. [7, 8] AXL kinase staining was scored as 0, 1+, 2+, and 3+ in the TC cytoplasm. [9, 10] For Eps8, the staining was assessed in TC cytoplasm and ranged from negative (0), focal and weak (1+), moderate (2+), strong and diffuse (3+). [11].

Statistical analysis: Data were analyzed using Microsoft XL. The quantitative data comparison was carried out using the Chi-Square

Test. P less than or equal to 0.05 was considered statistically significant.

Results

A total of 30 (100%) participants were included in this research. Gender wise, 11 (36.6%) were male and 19 (63.4%) were female participants; the male-female ratio was 0.57. The age of the study participants was ranged between 28 – 85 years and 55.42 years was the mean age. Site wise, the oral cavity is the major contributor (26; 86%) followed by the oropharyngeal (14%; 4). Histopathological grading wise, the majority (60%; 18) of the participants were moderately differentiated (G2) followed by well-differentiated (G1) grading (40%; 12).

When the TIMP 1 expression was analysed, in normal mucosa, 63.3% (19) specimens did not stain and just 3.3% (1) smears were graded to be 3+. Whereas among the SCC, 66.7% (20) smears were graded 3+. Statistically, there was a significant difference (Table 1).

Table 1: Comparison of scores between TIMP1 and TIMP1 normal in the study participants; n (%).

Variable	Score				
	0	1+	2+	3+	Total
TIMP 1	0	0	10 (35.5)	20 (66.7)	30 (100)
TIMP 1 normal	19 (63.3)	2 (6.7)	8 (26.6)	1 (3.3)	30 (100)
Statistical analysis	Ψ2 = 39.664; P = 0.000				
	Statically significant				

In the normal mucosa, 63.3% (19) specimens did not stain and just 3.3% were graded to be 3+.

It was found in this research that 53.3% (16) normal mucosa did not stain with EPS8 and just 3.3% (1) were scored to be 3+. Whereas in SCC cases, 56.6% (17) were scored to be 2+ and 40% (12) were 3+. Statistically, there was a significant difference (Table 2).

Table 2: Comparison of scores between EPS8 and EPS8 normal in the study participants; n (%).

Variable	Score				
	0	1+	2+	3+	Total
EPS8	0	1 (3.3)	17 (56.6)	12 (40)	30 (100)
EPS8 normal	16 (53.3)	8 (26.6)	5 (16.6)	1 (3.3)	30 (100)
Statistical analysis	Ψ2 = 39.056; P = 0.000				
	Statically significant				

In SCC, 56.6% (17) were scored to be 2+ and 40% (12) were 3+.

In this, 53.3% (16) of normal mucosa did not show any string and just 3.3% (1) showed 3+. Whereas, in the samples of SCC, 60% (18) showed 3+ sting and 40% (12) showed 2+. Statistically, there was a significant difference (Table 3).

Table 3: Comparison of scores between AXL and AXL normal in the study participants; n (%).

Variable	Score				
	0	1+	2+	3+	Total
AXL	0	0	18 (60)	12 (40)	30 (100)
AXL normal	16 (53.3)	7 (23.3)	6 (20)	1 (3.3)	30 (100)
Statistical analysis	Ψ2 = 40.286; P = 0.000				
	Statically significant				

Just 3.3% (1) of normal mucosa showed 3+.

Discussion

Earlier studies have indicated that chronic exposure to cigarette smoke provides a better model in vitro than acute exposure to cigarette smoke. [11]. Several cellular models have been developed and employed to understand the mechanisms of cellular transformation from normal to tumorigenic phenotype in response to chronic exposure to cigarette smoke. [11 – 16]. Studies showed that chronic cigarette smoke exposure/treatment induces the selection of clones that incorporated the molecular level changes necessary for cancer progression and resistance to apoptosis. Smokeless tobacco or chewing tobacco is a known risk factor in the development of oral cancer. Although the tumour-inducing property of chewing tobacco was proven in the year 1964, the molecular events that lead to tumor growth and progression upon its consumption are not known. [17]. It is important to understand the molecular mechanisms and pathobiology of oral cancer resulting from chewing tobacco as it differs significantly from cigarette smoking because of differences in composition. Chewing tobacco contains several compounds such as nicotine, tobacco-specific N-nitrosamines and polycyclic aromatic hydrocarbons which are known to be carcinogenic.

Studies have been done at the molecular level to know the expression of different molecules in oral cancers. Though studies have shown the

Role of TIMP1, EPS8 and AXL in oral and oropharyngeal cancers, the role of these proteins in tobacco-induced cancers is not known especially in the Indian population where the prevalence of tobacco use is high. In a study done by Nanjappa et al. [4]. an in vitro cell line model was developed by treating the normal oral keratinocytes, OKF6/TERT1 chronically with chewing tobacco. They found a 2.7 fold overexpression of TIMP1 in the cell line treated with tobacco. In a study by Singh R et al. tissue analysis showed 2.4-fold and 1.3-fold increase in TIMP-1 and TIMP-2 mRNA expression levels in comparison to histologically adjacent normal mucosa respectively. [18]. In a study of 68 oral squamous cell carcinoma by Vincent et al. [8]. expression of TIMP-1 by immunohistochemistry was detected in 45 cases (66.2%). In all of these TIMP-1 was expressed in tumoral tissue, and in 19 of them also in the surrounding stroma. In this study, When the TIMP 1 expression was analysed, in normal mucosa, 63.3% (19) specimens did not stain and just 3.3% (1) smears were graded to be 3+. Whereas among the SCC, 66.7% (20) smears were graded 3+. Statistically, there was a significant difference (Table 1). Nanjappa et al. [4]. Demonstrated a 2.0 fold increase in the expression of EPS8 in their cell line treated with tobacco. In another study by Jenei V et al. [19]. EPS 8 expression was enhanced by >5-fold in the OSCC cell lines relative to normal keratinocytes. Eps8 expression by IHC was detected in 19 of 59 (32%) of the tumours examined. The staining was cytoplasmic and ranged from weak and focal to strong and diffuse. In this research 53.3% (16) normal mucosa did not stain with EPS8 and just 3.3% (1) were scored to be 3+. Whereas in SCC cases, 56.6% (17) were scored to be 2+ and 40% (12) were 3+. Statistically, there was a significant difference (Table 2). In a study by Jenei V et al. [19]. Eps8 expression was identified in 186 of the 205 cases of OSCC (91%) and the aberrance occurred primarily in the cytoplasm of OSCC cells. As far as AXL kinase is concerned, Nangappa et al. [4]. Demonstrated a 1.6 fold increase. Lee CH et al. [20]. reported that AXL was found to be low in normal epithelium and a progressively increased positive percentage was noted from normal/hyperplastic epithelium (10.9%) to dysplasia (30.8%) to cancer tissue (54.5%). AXL expression correlated with lymph node status (P = 0.001) and clinical stage (P = .014) of OSCC. Patients with high expression of AXL showed poor prognosis compared with those with low AXL expression patients (P < .001). Studies with non-oral cancer as control and studies with a larger sample size are needed to further establish the fact.

Conclusion

The expression of TIMP1, EPS8 and AXL establish their role in the pathogenesis of oral and oropharyngeal SCCs. Novel targeted therapies may then be researched that can detect and target these molecules at an earlier stage of the pathogenesis of these tumours.

Limitations of the research: Small sample size is the major limitation of this research.

What does this study add to the existing knowledge? Markers such as TIMP1, EPS8 and AXL are highly useful in the diagnosis of SCCs.

Author's contribution: Mandava Sumanth: Sample collection. Goginenu Tarun Chowdary: Literature search, proofreading. T Jaya Chandra: Article writing and Sriram Burugupalli: Literature search, Article writing.

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