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EXPRESSION OF EZRIN ON METASTATIC LYMPH NODES OF ORAL SQUAMOUS CELL CARCINOMA

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ABSTRACT:

Introduction: Ezrin is an immunohistochemistry biomarker of the ERM protein family. Ezrin has been expressed in many other cancers like osteosarcoma, breast cancer, head & neck cancer. Aim of the study was to assess the Ezrin expressibility in the OSCC metastatic & non metastatic cases & in lymph nodes of OSCC.

Material & methods: OSCC cases with metastatic lymph nodes (*n*=6) & non metastatic OSCC cases (*n*=3) were selected. IHC staining with Ezrin was performed. The staining propensity of Ezrin was scored. The scores were tabulated and interpreted in SPSS software. Comparison of scores between metastatic OSCC cores & lymph nodes and also between metastatic & non metastatic OSCC cores was done using Mann Whitney test. Association between metastatic OSCC cases & pTNM staging was done using Chi square test.

Results: Out of 3 non metastatic cores, all cases exhibited Ezrin (100%) & out of 6 metastatic cases, 83% (5 cases) of cases exhibited Ezrin positivity. In the metastatic cores, localization in cytoplasm was 60% & localization in combined (cytoplasmic & membrane) was 40%. In non metastatic cores, 100% of Ezrin was localized in the cytoplasm of the cell. All metastatic lymph node cases showed Ezrin expression. In metastatic lymph nodes, localization in cytoplasm was 66.6% & 33.3%.

Conclusion: Ezrin was expressed in both metastatic and non metastatic OSCC cases as well as in the metastatic lymph nodes. Localization of the lymph nodes were in the cytoplasm of the cell. Expression of Ezrin in metastatic lymph nodes can be an indicator of poor prognosis of OSCC cases.

Keywords: Ezrin, Metastatic lymph nodes, expression, intensity, proportionality, localization.

INTRODUCTION:

Oral squamous cell carcinoma (OSCC) is the most common malignancy of oral cavity, comprising 80%-90% of cancers in the oral cavity [1,2,3]. Oral cancer is an aggressive disease, leading to metastasis of adjoining structures. It is noted that more than 30% of OSCC cases have undergone lymph node metastasis [4,5] hence leading to poor prognosis [2]. There are various clinical pathological and histopathological methods to assess a tumor progression in oral squamous cell carcinoma, but by using a biological marker, true aggressive character of a cancer can be understood, hence affecting the prognosis of OSCC.

Ezrin is a member of the ezrin/radixin/moesin (ERM) protein family [6]. It plays a key role in tumorigenesis and metastasis. Ezrin expression has a significant influence on prognosis of cancer. Expression of Ezrin was performed on several different types of cancer to assess the tumorigenesis, including carcinoma of head and neck region, esophagus, breast, endometrium, cutaneous and uveal melanoma and soft tissue sarcoma [7]. Earlier Ezrin was thought to be a substrate of tyrosine kinase, which helped in the proliferation of intestinal microvilli [8]. Ezrin is grouped together with radixin and moesin as an ERM protein (ezrin/radixin/moesin) because of their high homology [8]. It belongs to the superfamily of band 4.1 proteins and shares a common ~300-amino-acid domain, named FERM (four-point one, ezrin/radixin/moesin) [8]. There are two main functions of the RRM proteins. First, they connect the plasma membrane proteins and F-actin filaments of the cytoskeleton, and secondly they help in signal transduction pathways (RhoA, Hedgehog, CD43/44 membrane receptor signaling) including cytoskeletal remodeling and transcriptional regulation [9]. Therefore ERM proteins are involved in cell-cell as well as cell-matrix interactions and hence play an important role in the modification of cell shape, cell adhesion, cell motility, cytokinesis [8,9] and phagocytosis as well as apoptosis [10]. In the N and C terminal ends of Ezrin, due to intermolecular or intramolecular interaction, active and inactive form is produced. The active form is localized in the membrane and the inactive form is localized in the cytoplasm [10,11].

A study conducted by Patara et al, cytoplasmic expression of Ezrin was detected in aggressive colorectal carcinoma [12]. Another study by Tokunou et al, found cytoplasmic expression of Ezrin in lung adenocarcinomas [13,14]. Relating to Head and cancers, there are very few literatures on expression of Ezrin on OSCC. A study conducted by Safi et al, observed Ezrin expression in cytoplasm, membranous and in combination [13] in OSCC tissues. It was also inferred that high expression of Ezrin in the tissues suggested a decreased overall survival rate of the patient [13]. Little evidence is known on the ezrin expression in the metastatic node in OSCC. With this background, through the present study, we aim to evaluate the Ezrin expression in metastatic lymph nodes of OSCC.

MATERIALS AND METHODS:

Selection of tissue blocks (Sample Collection):

Paraffin embedded blocks of Oral Squamous Cell Carcinoma were selected from the archives of the Department of Oral and Maxillofacial Pathology between 2017 and 2019. Approval was received from the Institutional Review Board. Histopathological evaluation of lymph nodes and core slides of OSCC cases were performed. OSCC cases with metastatic lymph nodes (n=6) and non metastatic OSCC cases (n=3) were selected.

Preparation of OSCC Paraffin Embedded Blocks:

Lymph nodes and core blocks of OSCC cases were sectioned in 3mm thickness and fixed in gelatin coated slides. Sectioning of the slides were done on a LEICA Soft tissue microtome. The slides were incubated overnight and immunohistochemistry staining of OSCC slides using Ezrin as a biomarker was performed.

Immunohistochemistry staining using Ezrin:

The sections were retrieved from the incubator and were kept for deparaffinization in xylene, dehydrated in alcohol and then washed in distilled water. Antigen retrieval was performed under heat induced epitope retrieval in the citrate buffer (pH 6.0) for 10 minutes under pressure cooker. Endogenous Peroxidase was blocked for 10 minutes and protein block for 5 minutes. Sections were then incubated with Ezrin for 90 minutes in room temperature. Sections were then counterstained with Mayer's Hematoxylin. Slides were then dehydrated and mounted. Detection was performed using the Dako Polymer detection system. The sections were counterstained using Mayer's Hematoxylin and were then dehydrated and mounted using dibutyl phthalate in xylene mountant. Positive and negative controls were used in each run.

Slide Evaluation:

Brown coloured reaction at the site of target antigen is a sign of positive reactivity. Immunohistochemistry slide evaluation was made on assessing the staining characteristics. Scores were obtained after assessing the staining propensity based on certain parameters such as positivity of the stain, intensity of the stain, proportionality of the stain. Evaluation was made on the location of stain on cells of the core tissue.

Proportionality was scored under 4 scoring meters. Score 1: 0-25%, Score 2: 26-50%, Score 3: 51-75%, Score 4: 76-100%. Intensity was also scored under 4 scoring meters. Score 0: No Ezrin expression, Score 1 : Weak Ezrin Expression, Score 2: Moderate Ezrin Expression, Score 3: High Ezrin Expression. Location of Ezrin expression was assessed under 3 categories. Cytoplasmic, Membrane and Combination. Immunohistochemistry slides were assessed by a single blinded observer.

Statistical analysis:

Comparison of scores between metastatic OSCC cores and lymph nodes and also between metastatic and non metastatic cores was done using Mann Whitney test. Association between metastatic OSCC cases and pTNM staging was done using Chi square test. Value was set at p <0.05. 95% confidence levels were used. Statistical analysis was carried out using SPSS version 20.0 (IBM SPSS Statistics for Windows 10, IBM software group, Chicago, Illinois, USA).

RESULTS:

In the present study, Ezrin was localized in the cytoplasm, a membrane with cytoplasm (combination) of the cells. Out of 3 non metastatic cores, all cases exhibited Ezrin (100%) and out of 6 metastatic cases, 83% (5 cases) of cases exhibited Ezrin positivity. Localization of cores in metastatic and non-metastatic cases were assessed. In the metastatic cores, localization in cytoplasm was 60% and localization in combined (cytoplasmic & membrane) was 40%. In non metastatic cores, 100% of Ezrin was localized in the cytoplasm of the cell. In metastatic lymph nodes, localization in cytoplasm was 66.6% and in combination (cytoplasmic & membrane) was 33.3%. (Table 1, Figure 3, 4)

	POSITIVITY	**INTENSITY SCORES**	**PROPORTIONALITYS CORES**	**LOCATION**
METASTATIC LYMPH NODES	100%	Score 0: 0% Score 1: 66.6% Score 2: 33.3% Score 3: 0%	0-25% : 16.7% 26-50% : 83.3% 51-75% : 0% 76-100% : 0%	Cytoplasm : 66.6% Membranous & cytoplasmic : 33.3%
METASTATIC CORES	POSITIVE: 83%	Score 0: 16.7% Score 1: 33.3% Score 2: 50% Score 3: 0%	0-25% : 16.7% 26-50% : 0% 51-75% : 33.3% 76-100% : 50%	Cytoplasm : 60% Membranous & cytoplasmic : 40%

NON METASTATIC CORES	POSITIVE: 100%	Score 0: 0% Score 1: 66.7% Score 2: 33/3% Score 3: 0%	0-25% : 0% 26-50%: 66.7% 51-75% : 33.3% 76-100% : 0%	Cytoplasm : 100% Membranous & cytoplasmic : 0%

Table 1: Ezrin scores in metastatic cores, non metastatic cores and metastatic cases. lymph node

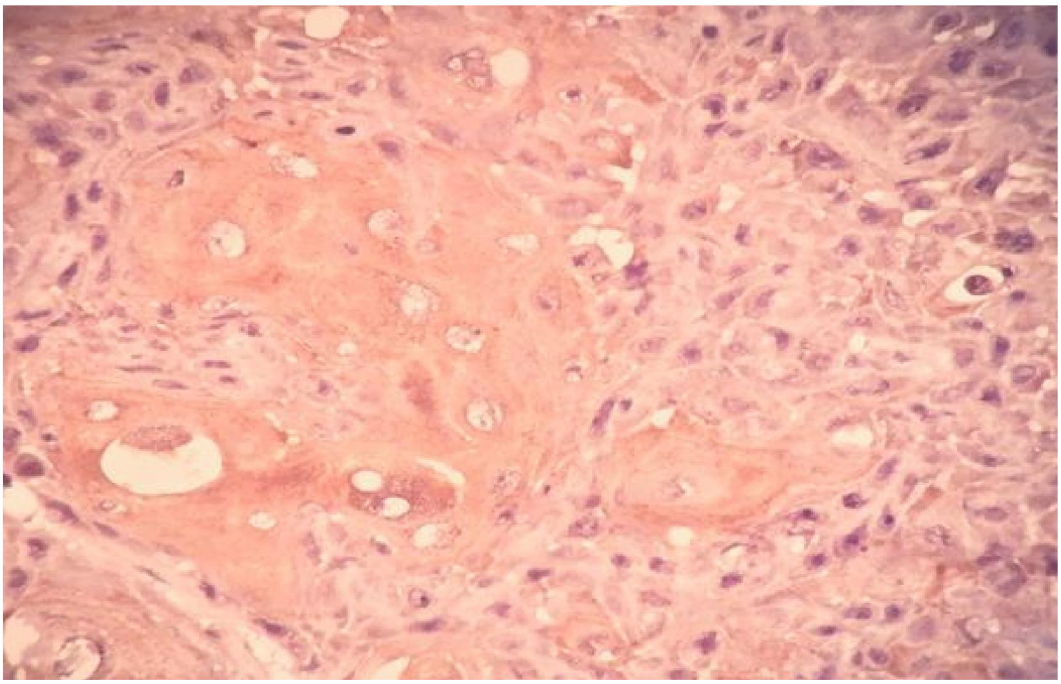

Figure 3: Ezrin stain on metastatic lymph nodes.

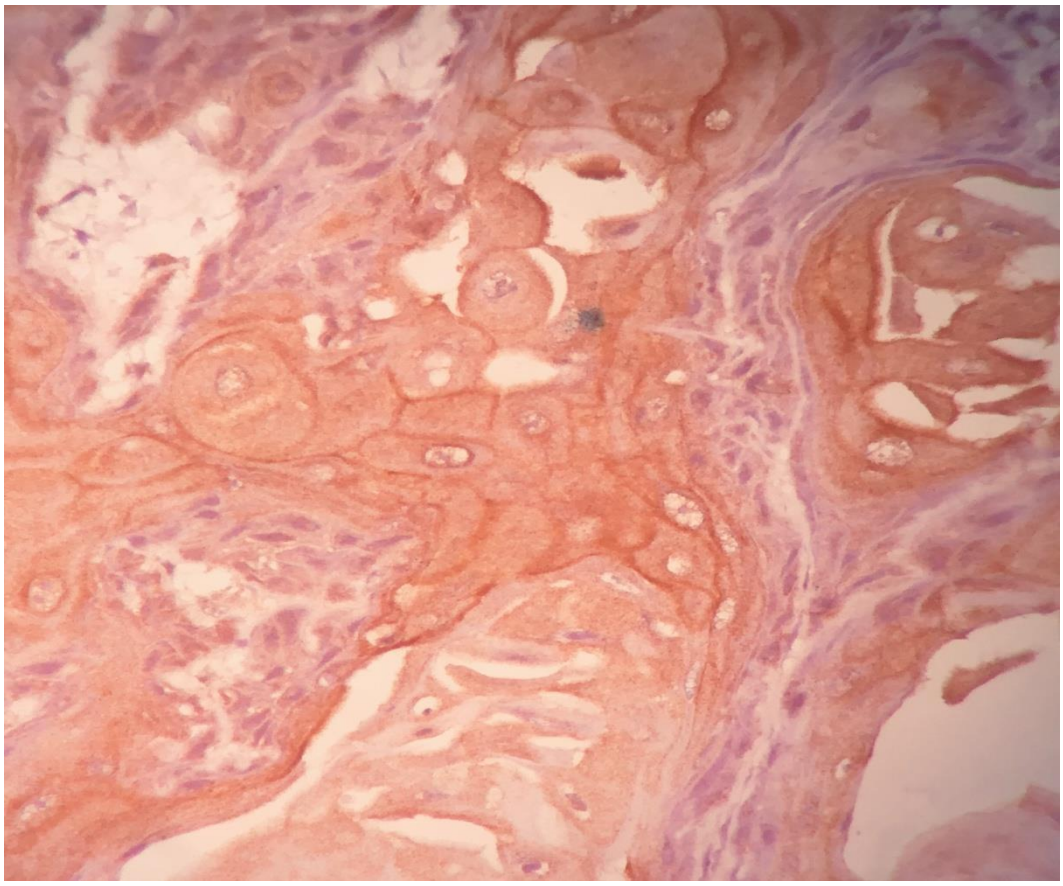

Figure 4: Ezrin on metastatic cores in OSCC

Expressions in non-metastatic cores were assessed. Ezrin was expressed in 100% cases. Intensity of Ezrin was assessed in the non metastatic cores. Weak expression was observed in 66.7% and moderate expression was observed in 33.3%, Ezrin was expressed as 26-50% in proportionality in 66.7% of non metastatic cores.

Out of 6 metastatic core cases, 5 cases showed Ezrin positivity 83%. Weak expression was expressed in 33.3% and moderate expression was expressed in 50%. Ezrin was expressed as 76-100% in proportionality in 50% of metastatic core cases. Location of Ezrin in cytoplasm is 60% and in membranes and cytoplasm is 40%.

Out of 6 metastatic lymph node cases, 100% cases showed Ezrin positivity. Weak expression was expressed in 66.6% of metastatic lymph node cases, 33.3% had moderate expression of Ezrin. Ezrin was expressed as 26-50% in proportionality to 83% in metastatic lymph node cases. Location of Ezrin in cytoplasm is 66.6% and in membranous and cytoplasm was 33.3%.

Mann-Whitney U test Comparison of scores was made between intensity of metastatic OSCC cores and non metastatic scores was done using Mann Whitney test. Mann-Whitney U test was statistically not significant. Mann Whitney U test value was 7.00, with *p* value 0.371, indicating statistically not significant. Mann Whitney U test of proportionality was 3.00, and *p* value was 0.102, indicating statistically not significant.

Mann-Whitney U test was made between intensity of ezrin expression of metastatic cores and lymph nodes. Mann Whitney U test value was 12 with *p* value 0.269, indicating statistically not significant. Mann Whitney U test of proportionality was 2.50 with *p* value 0.007, indicating statistically significant.

All the 6 metastatic cases have stage IV pTNM staging. Association graphs of the intensity and proportionality of Ezrin expression with pTNM staging was done in metastatic lymph node cases using Chi square test. Proportionality of Ezrin expression showed association with pTNM staging but was not statistically significant. As observed in the present study, Ezrin was expressed in the lymph node metastatic OSCC cases having pTNM staging IV, hence depicting a poor prognostic impact (figure 1, 2). It is then safe to conclude that expression of Ezrin in the metastatic lymph nodes of OSCC cases may depict a poorer prognosis of the OSCC case.

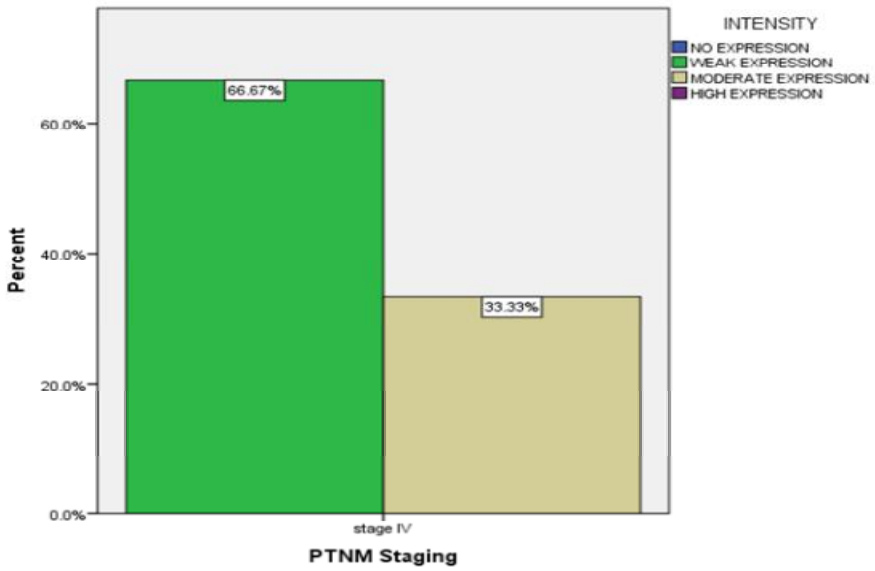

Figure 1: Bar graph depicts association between intensity and pTNM staging.

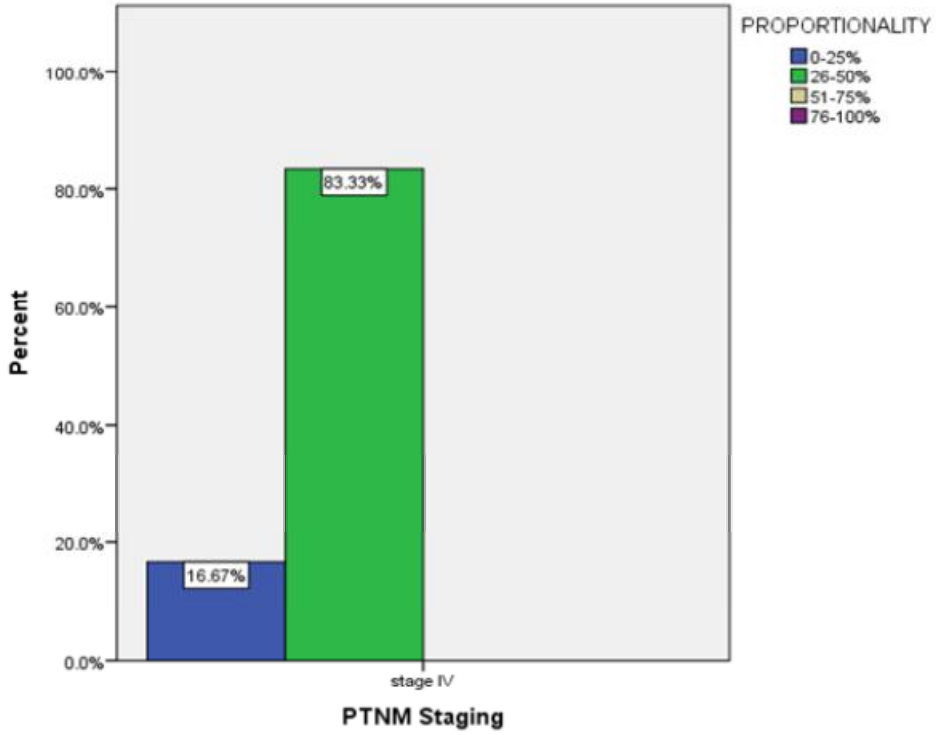

Figure 2: Bar graph depicts the association between proportionality and pTNM staging.

DISCUSSION:

OSCC is the major cause of mortality in many developing countries. OSCC is an aggressive condition. It has a high rate of metastasis and recurrence. Clinicians and pathologists have tried finding newer and better methods of assessing tumor proliferation. IHC staining methods help in identifying tumor cells. It helps in identifying the tumor cell proliferation, and is useful in identification of a tissue of unknown origin. Ezrin was used to assess the staining capability of metastatic lymph nodes of OSCC cases.

In our study, 83% of metastatic core cases and 100% of non metastatic core cases took the Ezrin stain. 50% of metastatic core cases displayed moderate Ezrin expression and 33.3% of metastatic core cases expressed weak Ezrin expression. 66.6% of metastatic lymph node cases displayed weak Ezrin Expression and 33.3% had moderate expression of Ezrin. Study conducted by Safi et al, observed similar findings. In his study, 86% of the OSCC cases with loco-regional metastasis displayed strong or moderate Ezrin expression and only 16% displayed weak Ezrin expression [13]. None of the cases displayed negative Ezrin expression in the study conducted by Safi et al [13]. In our study, out of 9 OSCC cases, 1 case did not display any Ezrin expression. Study conducted by Saito et al in 2013 observed a strong Ezrin expression in

the metastatic lymph nodes cases of tongue carcinoma (62%) [7].

In the present study, out of 9 cases, the majority of cases expressed Ezrin in the cytoplasm. In metastatic core cases, 50% of the cases had expression of Ezrin in cytoplasm, according to the results procured from Safi et al, localizations were mostly in the membrane. A study conducted by Ferrari et al, observed that Ezrin is mostly expressed in cytoplasm (47%) and cytoplasm and in membrane (53%) in cases of Osteosarcoma [15]. Kobayashi in 2003 observed that higher expression of Ezrin in cytoplasm leads to higher metastatic rates [16].

It is well discussed that Ezrin plays a prominent role in tumor proliferation. Studies have claimed that high expression of Ezrin leads to a lesser survival rate. In a study conducted by Raghini et al, Ezrin plays a major role in conversion of oral premalignant disorders into OSCC [17].

This study is an attempt to study the expression of Ezrin in lymph nodes of OSCC. All metastatic lymph node cases showed expression and particularly it was observed that metastatic lymph nodes showed expression of Ezrin in poor prognosis cases, hence concluding the fact that expression of Ezrin in metastatic lymph node cases leads to poor prognosis of the OSCC case. Ezrin can be used as an efficient prognostic tool for detection of tumor progression and prognosis of OSCC cases. More studies with higher sample size are needed in future.

CONCLUSION:

Ezrin was expressed in both metastatic and non metastatic OSCC cases as well as in the metastatic lymph nodes. Localization of the lymph nodes were in the cytoplasm of the cell. Expression of Ezrin in metastatic lymph nodes can be an indicator of poor prognosis of OSCC cases.

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CONFLICT OF INTERESTS:

There was no conflict of interest.

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