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**ORIGINAL ARTICLE**

# Analysis of E-Cadherin Expression in Patients with Esophageal Squamous Cell Carcinoma

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## ABSTRACT

**Objectives:** Esophageal Squamous Cell Carcinoma is a common, deadly cancer. E-Cadherin expression in cancer was investigated along with its relation to parameters like age, gender, differentiation, and mortality.

**Methodology:** A 100-sample descriptive cross-sectional study utilising open EPI software with a 95% confidential interval was obtained. Data was evaluated by SPSS 23.0.

**Results:** E-Cadherin had a negative IRS (immune-reactive score) in 69% of samples, mild in 27%, and moderate in 4%. Chi-square test showed no association between E-cadherin immune-reactive score and the parameters considered. (>P=0.05)

**Conclusion:** This study found that patients with carcinoma express less E-Cadherin but its expression in Esophageal Squamous Cell Carcinoma patients is unrelated to the parameters considered.

**Keyword:** Esophageal Squamous Cell Carcinoma, Immuno-Histochemistry, Haematoxylin, Eosin, E-Cadherin.

## INTRODUCTION

Esophageal carcinoma ranks sixth in cancer mortality and ninth in prevalence worldwide. Majority are squamous in orign and common in men between 60s and 70s. Moderately Differentiated Esophageal Squamous Cell Carcinoma (ESCC) is the most common histologic form with a survival rate of 10–20%. Dysphagia and weight loss are common symptoms. [1-3]

Most epithelial cells produce calcium-dependent membrane glycoprotein E-Cadherin, which links cells. It has five extracellular repeats, a trans-membrane domain, and a highly conserved cytoplasmic tail.[4-5] Down-regulation decreases cell-cell adhesion and increases cell motility and metastasis.[6-9] Studies have linked lower E-Cadherin expression to poor prognosis in various other malignancies.[10-14] E-Cadherin helps diagnose and prognosticate Esophageal Squamous Cell Carcinoma and may be a promising biomarker for its immunohistochemistry.[15- 16]

Due to insufficient research, we wanted to determine the amount of cancer cells expressing the marker and their intensity in patients and link them with age, gender, differentiation, and survival status.

## METHODOLOGY

**Nature of Study:** The study followed ERC LUMHS standards and the proposal was approved in October 2019 as stated on order number LUMHS/RECC/139.The descriptive cross-sectional investigation used open EPI software and a confidential interval of 95% to calculate a sample size of 100 taking into account ESCC prevalence being 6.8% in Pakistan.[17]

**Specimen Collection:** The study's purpose was explained to all participants and June 2021–January 2022 was the trial period. A prospective database including patient demographics, procedure, histology, and long-term follow-up was gathered. LUMHS tumour repository delivered 100 tumour blocks of both genders included, while cases of patients that chemotherapy or radiation cases were excluded.

**Immuno-histo-chemical Staining and Evaluation:** The Leica microtome was used to cut a 3-5 µm thick piece. For basic histo-pathological characteristics, Haematoxylin and Eosin staining was used. Stained areas were graded according to World Health Organisation standards.

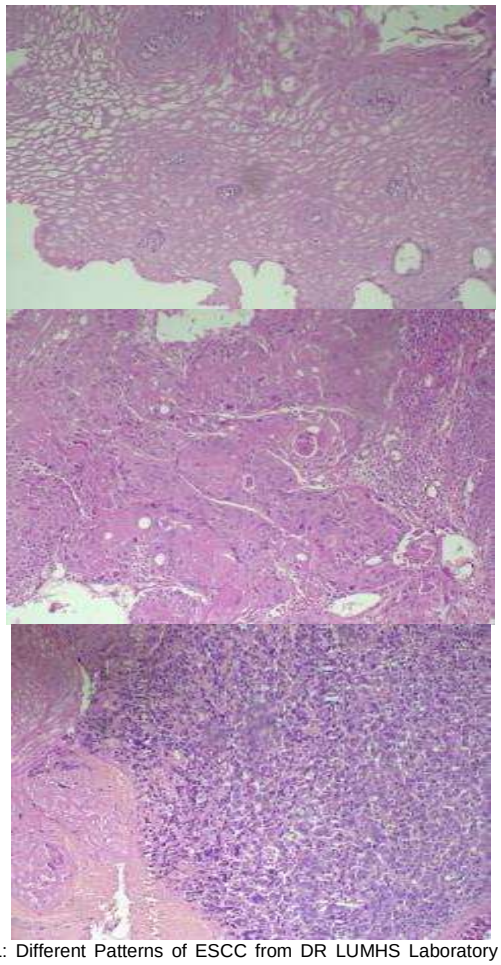


Figure.1: Different Patterns of ESCC from DR LUMHS Laboratory a) Well Differentiated b) Moderately Differentiated c) Poorly Differentiated Esophageal Squamous Cell Carcinoma

Slides were prepared in PT-Link and stained with a DAKO auto-stainer for immune-histochemistry (IHC). Tissues were cut into 3 to 4 µm on DAKO IHC microscopic slides and fixed in the oven at 58 to 60 º C for an hour or 80 º C for 20 to 25 minutes. Later, tissue was hydrated by de-waxing in xylene and washing in tap water. For antigen retrieval, the slide was rinsed with concentrated Phosphate Buffered Saline (PBS) IHC Wash Buffer 20X concentration 200ml with pH 9 and diluted in de-iodinized water at 1:20. Two drops of CELL MARQUE 15 ml peroxidase blocking solution were added to the tissue and incubated at room temperature for 10 minutes. Washing buffer was used to wash the slides twice for 5 minutes. A 12ml bottle of monoclonal mouse-derived anti-human E-Cadherin antibody, clone NCH 38, from DAKO was utilised to cover the slide's tissue. After 20–30 minutes in a humidity chamber at ambient temperature, it was rinsed twice for 5 minutes with washing buffer. High pH secondary antibody followed. The slides received 3,3′-Diaminobenzidine (DAB), haematoxylin, and eosin. Hundred cases in 10 batches of 10 were completed with a negative and positive control. Invasive ductal cell carcinoma of breast was a positive and invasive lobular carcinoma a negative control. The slide was dehydrated in rising alcohol, cleaned in xylene, mounted with Dibutylphthalate Polystyrene Xylene (DPX), and cover-slipped with DAKO cover-glass.

**Scoring:** Brown cell membrane immune-staining indicated E-cadherin immune-positivity. The formulae of IRS (immune-reactive score) = proportion of immunopositive cells (A) times intensity of immunostaining (B) was applied.[18]

**The percentage of E-cadherin immunopositive cells (A) was estimated and rated on a scale of 0-4 in five random fields as follows:**

- 0 points: There are no immune-positive cells.
- 1 point equals 10% immune-positive cells.
- 2 points for immune-positive cells ranging from 10% to 29%.
- 3 points: immune-positive cells ranging from 30% to 59%
- 4 points: immune-positive cells ranging from 60% to 100%

**E-cadherin immunostaining (B) intensity was rated on a scale of 0-3 points as follows:**

- 0 = no staining;
- 1 = mild staining;
- 2 = strong staining;
- 3 = intense staining

**IRS = immunopositive cell percentage (A) x intensity of immunostaining (B):**

- 0-1: Negative
- 2-3: Mild
- 4-8: Moderate
- 9-12: Extremely Positive

**Statistical Analysis**: SPSS 23.0 was utilized for analysing the data. Significant p-values were considered below 0.05. Age, gender, degree of differentiation, and survival status frequencies were determined, followed by mean and standard deviation for continuous variables like age and tumour size.

## RESULTS

This study included 100 patients—67 females and 33 males. Mean presenting age was 44.63 with a standard deviation of 13.945. Forty seven percent of patients were between 41-60 years age group, 41% patients were 21-40, 10% were 61-80, and 2% were under 20. Tumours averaged 0.675 cm with a standard deviation of 0.366. Ninety Four of patients had moderately differentiated carcinoma, while 4% and 2% had poorly and well-differentiated cancer (TABLE 1). Patients had a mortality rate of 77% (TABLE 1).

Among the samples that had been observed with E-Cadherin expression of immune-positive cells, 62% had less than 10% expression in their carcinoma reacted cells, 29% had 10-29%, 24% had 30-59%, and 2% had 60-100% (TABLE 3). While, 59% had negative E-Cadherin staining, 36% mild intense, and 5% moderate intense (TABLE 3). After computation, 69 percent of patients had negative IRS (immune-reactive score) for E-Cadherin, 27% had mild, and 4% had moderate (TABLE 3).

Table 1:

| Variables: | Frequency | Percentage |
|---|---|---|
| Gender | | |
| Male | 67 | 67% |
| Female | 33 | 33% |
| Age Group | | |
| <20 | 2 | 2% |
| 21-40 | 41 | 41% |
| 41-60 | 47 | 47% |
| 61-80 | 10 | 10% |
| Tumour Diffrentiation | | |
| Poorly Diffrentited Carcinoma | 4 | 4% |
| Moderrtely Diffrentiated Keratinizing Carcinoma | 94<br>2 | 94 %<br>2 % |
| Well Diffrentiated Keratinizing Carcinoma | | |
| Survival Status | | |
| Dead | 77 | 77 |
| Alive | 23 | 23 |
| Total | 100 | 100% |

Table 2:

| Descriptive Statistics | Mean | STD. Deviation |
|---|---|---|
| Age | 44.63 | 13.495 |
| Size (CMS) | 0.6750 | 0.36608 |

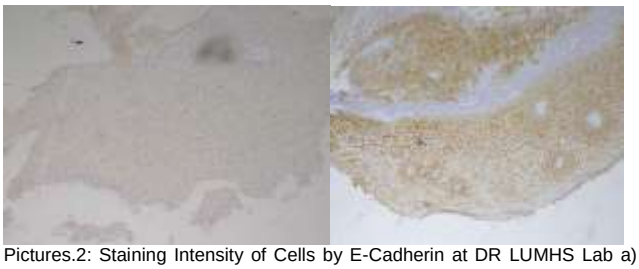
Table 3:

| Expression of Immune-Positive Cells | Frequency | Percentage |
|---|---|---|
| Immune-positivity: | | |
| <10% | 62 | 62% |
| 10-29% | 12 | 12% |
| 30-59% | 24 | 24% |
| >60-100% | 2 | 2% |
| E-Cadherin Immunostaining: | | |
| • Negative Staining | | |
| • Mild Staining | 59 | 59% |
| • Moderate Staining | 36 | 36% |
| | 5 | 5% |
| Ecadherin IRS (Immune-Reactive Score): | | |
| 1-2(Negative) | 69 | 69% |
| • 2-3(Mild) | 27 | 27% |
| 4-8(Moderate) | 4 | 4% |
| Total | 100 | 100% |

After chi-square test, E-Cadherin immune-reactive score did not correlate with gender, age group, tumour differentiation, or fatality (>P=0.05) (TABLE 4):

Table 4:

| Ecadherin IRS | 1-2 (Negative)% | 2-3 (Mild)% | 4-8 (Moderate)% | P - Value |
|---|---|---|---|---|
| Gender<br>Female<br>Male | 46%<br>23% | 18%<br>9% | 3%<br>1% | 0.941 |
| AGE GROUP | | | | 0.946 |
| <20 | 2% | 0% | 0% | |
| 21-40 | 28% | 11% | 2% | |
| 41-60 | 32% | 13% | 2% | |
| 61-80 | 7% | 3% | 0% | |
| Tumour diffrentiation<br>Poorly diffrentited carcinoma<br>Moderrtely diffrentiated keratinizing carcinoma<br>Well diffrentiated keratinizing carcinoma | 4 %<br>64%<br>1 % | 0%<br>26%<br>1% | 0%<br>4%<br>1% | 0.660 |
| Survival status | | | | 0.221 |
| Dead | 50% | 23% | 4% | |
| Alive | 19% | 4% | 0% | |
| Total | 100% | 100% | 100% | |



Pictures.2: Staining Intensity of Cells by E-Cadherin at DR LUMHS Lab a) Mild Intense Staining b) Moderate Intense Staining

## DISCUSSION

Women (N=67) outnumbered men (N=33) with carcinoma. Various studies found male prevalence.[19-20] Islami F et al. showed hot tea increased women's Esophageal Squamous Cell Carcinoma risk.[21] Despite our data, most studies show men being supreme victims, so feminine proclivity cannot be explained.

With a mean age of presentation of 44.6 years for both genders, 41–60-year-olds were most vulnerable. Our mean age of presentation was lower than Then EO et al.'s 66.3.[19] Our investigation indicated younger locals had higher cancer rates.

Moderately differentiated carcinoma (94%) was the most prevalent histopathological variation of ESCC next to poorly differentiated (4%), and well differentiated (2%). Then EO et al. identified 39.51% moderately differentiated carcinoma, 35.07% poorly differentiated, and 4.68% highly differentiated.[19] It was a major contrast to our findings.

After follow-up, 77% of tumour patients died. Studies confirm the low patient survival rate. [22-23]

In our research, 62% of cells had less than 10% E-Cadherin expression, 59% have negative marker staining, and 69% have negative immunoreactive score. Low E-Cadherin levels restrict tumor growth in many cancers. Cheng L et al. discovered 40% of Esophageal squamous cell carcinoma patients had severely decreased E-Cadherin expression compared to controls.[24] Qin Y et al. found that ESCC patients with less E Cadherin had a worse prognosis.[16] Zhu S et al. found significant lack of E-Cadherin expression in ESCC compared to non-cancerous tissues.[25] These studies are much in link to our findings.

The chi-square test showed no significant association between parameters and E-Cadherin expression. A multivariate analysis by Qin Y et al. demonstrated that above-mentioned characteristics were significantly associated with reduced E-Cadherin expression in ESCC, contradicting our findings.[16] Ma L et al. found that age and gender did not affect low E-Cadherin expression.[15] Cheng L et al. discovered no correlation between tumour differentiation and low E-Cadherin expression, validating our claim.[11]

## CONCLUSION

E-Cadherin is negatively linked with the presence of carcinoma in patients. Further, no significant co-relation was found between E-Cadherin expression and parameters stated. Hence the hypothesis is nullified that the E-Cadherin expression in the ESCC patients is co-related to the parameters like age, gender, degree of differentiation of tumour and fatality.

**Limitations**

- The study's limited sample size may affect statistical power and generalizability.
- The study only examined E-Cadherin expression, not other important markers or the type of growth or location of the tumour in the esophageal lumen, and the samples received for blocking were minute pieces of the tumour with their reports having no information related to TNM staging, location and type that could potentially influence the progression of carcinoma.
- Finally, the study was conducted in one region and may not apply to other populations or places with different genetic and environmental characteristics.

**Recommendations**

- E-Cadherin expression and ESCC might be studied with TNM staging, tumour type, and location.
- Increased sample size may improve accuracy.
- Long-term follow-up could examine E-Cadherin expression affects on survival and recurrence rates.
- Molecular profiling might reveal more about E-Cadherin and other markers in ESCC.

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