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E - Cadherin Expression in Surface Epithelial Tumours of Ovary Using Immunohistochemistry

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ABSTRACT:
Background: Loss of E-cadherin(an epithelial marker) function during the development of most types of human epithelial cancers, including that of the breast, colon, prostate and lung, has been reported in different clinical and experimental studies. Only limited studies have been reported about the role of E cadherin in ovarian tumours, In this study we are going to analyse the expression of E- cadherin in histopathologically confirmed epithelial ovarian tumours and classify them to benign, borderline and malignant categories.
Materials and methods: This was a descriptive study .The study population were all hysterectomy specimens with unilateral or bilateral adnexa, salpingo-oophorectomy, oophorectomy and/or ovarian cystectomy specimens. After analysis of patient data and gross examination findings, cases were selected on the basis of inclusion and exclusion criteria. Histopathology of the sections were identified. Staining with E cadherin was done in 80 surface epithelial tumors of ovary.The study tools were proforma, propranolol, xylene, paraffin wax, coverslip, DPX mount, Hematoxylin & Eosin stain, glass slides,primary antibodies (E cadherin), EDTA buffer, triss buffer, chromogen (diaminobenzidine), blocking reagents, Biogenex IHC slides, and Olympus CH20i microscope. Pattern of staining, extent and intensity of E-cadherin and immunohistochemical composite score was calculated. Sensitivity, specificity, positive and negative predictive values and accuracy of E cadherin in surface epithelial tumors were evaluated.
Results: Out of 80 epithelial ovarian tumors studied,majority of the cases were benign serous tumors. 77% of benign epithelial ovarian tumors showed strong E cadherin expression whereas 12% of them showed moderate expression. 57% of borderline ovarian tumors showed moderate expression of E cadherin while 28 % showed low expression. Significant decrease in E cadherin expression was noted in malignant tumors as compared to benign tumors and also between borderline and benign tumors.
Conclusion: Strong and complete membranous expression of E cadherin can be considered as a useful measure to distinguish benign from malignant epithelial ovarian tumours apart from histopathology which is the gold standard.
Keyword: E cadherin; Immunohistochemistry; Surface epithelial tumor; Ovary

I. INTRODUCTION

Epithelial ovarian cancer is the fifth leading cancer in women and the leading cause of death from all gynaecological malignancies.Majority of the patients present withadvanced stage at the time of diagnosis[1]. Despite radical surgery and adjuvantplatinum based treatment, relapse occurs in more than half of the patients.Identification of new molecular markers could potentially lead to significantmodification of clinical management improving clinical outcome[2]. During tumour progression, cell-to-cell and cell-to-substrate interactions seem to be crucially altered . Cadherins represent the major component of adherent junction and mediate cell–cell adhesion through the calcium dependent homophylic interaction with the extracellular matrix(ECM)[2] . Epithelial cadherins, with E-cadherin as the prototype family member, play a crucial role in the formation and maintenance of epithelial structures.loss of cell- cell adhesion may play a relevant role in malignant transformation and the invasive behavior of malignant tumors[3]. Loss of E-cadherin function during the development of most types of human epithelialcancers, including that of the breast, colon, prostate and lung, has been reported in different clinical and experimental studies[2]. Only limited studies have been reported about the role of E cadherin in ovarian tumours.In this study we are going to analyse the expression of E- cadherin in histopathologically confirmed epithelial ovarian tumours and classify them to benign, borderline and malignant categories and also to study its expression levels associated with tumor stage and histological grade.

II.MATERIALS AND METHODS

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This prospective study was done in all patients with benign, borderline and malignant epithelial tumors of ovary diagnosed in the in the Department of Pathology, Govt Medical College Kannur,Pariyaram,during the period from January 2019 to January 2020.

Study Design: Descriptive study

Sample Design: Consecutive sampling

Study Location: Department of Pathology, Government Medical College, Kannur, Kerala

Study Duration: January 2019 to January 2020

Sample Size: 80patients

Sample Size Calculation: Sample size was calculated based on the results of the formulas, where p = prevalence of the disease, q = 100-p, d = allowable error, p = 83%,q = 17%, d = 5% (absolute precision).Based on the above formulas,sample size is obtained as 230 cases. All cases of epithelial ovarian tumours received in our department during the above mentioned period (January 2019-January 2020) were considered in my study.

Inclusion criteria

1. Female patients with epithelial ovarian tumors either primary or metastatic
2. Age: 17 to 78 years

Exclusion criteria

1. Other ovarian and extraovarian lesions
2. Non consenting patients

Procedure methodology

Informed consents in both mother tongue and English were taken from the patients before performing the study. Specimens were received at the department in 10% formalin. From the study population, after analysis of patient data and gross examination findings, cases were selected on the basis of inclusion and exclusion criteria. Histopathology of the sections were studied. Histopathology was taken as the gold standard in the diagnosis of surface epithelial ovarian tumors. E cadherin staining was done in surface epithelial ovarian tumors and the results were observed. All consecutive sections were done staining until the sample size was attained.. The results of E cadherin staining in different epithelial ovarian tumors were observed.

Evaluation and quantification of immunostaining

The following patterns of immunostaining were considered positive: E cadherin-membranous, cytoplasmic and membranous.

Pattern of immunostaining, extent and intensity of E cadherin was evaluated by a semiquantitative system.This scoring system considers the percentage of positively stained cells (0%=0, <10%=1, 10-50%=2, 51-80%=3, >80%=4) and the intensity of the staining (negative=0, low=1, moderate=2, strong=3). The level of expression was classified into four groups: 0 (no staining), 1-4 (low), 5-8 (moderate) and 9-12 (strong staining).

For the staining pattern, we used the system of Giurgea L et al[4]. which they applied to evaluate the expression of p53 and Ki-67, but we considered it suitable for evaluation of E-cadherin in order to detect areas of the tumor with different intensity of IHC expression:

1. A focus pattern with small number of tumor expressing cells;
2. A heterogeneous pattern with islands of strong or moderate positive expression alternating with regions of weak positive expression;
3.A diffuse pattern representing diffuse positivity.

To correlate extent and intensity, these values in positive cases were converted into immunohistochemical composite scores by multiplying the individual scores of extent by intensity.

Statistical analysis

Descriptive statistics like frequency and percentage were used. Sensitivity, specificity, positive and negative predictive values and accuracy of E cadherin were calculated. Tables and other graphical representations were also used. Statistical analysis was done using SPSS version-13 software.

III.RESULTS

Out of the 80 cases studied, majority of the cases were benign serous cystadenomas.58.7% of patients were in the age group 51-78 years.The most common clinical presentation of benign tumors were menstrual disturbances in the form of menorrhagia.Most common clinical presentation of borderline and malignant were postmenopausal bleeding. Predominant gross appearance of tumors were cystic (65%). Predominant histological subtype in benign tumors were serous cystadenomas (32.5%)followed by mucinous.Predominant histological subtype in malignant tumors were serous carcinomas(19/80 cases).Bilaterality was noted in 3.7% of benign, 42% of borderline and 75% of malignant cases. There is a significant relationship between

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bilaterality and aggressiveness of tumor. Out of 19 malignant epithelial tumors, 13 cases (68.4%) were in FIGO stage III.

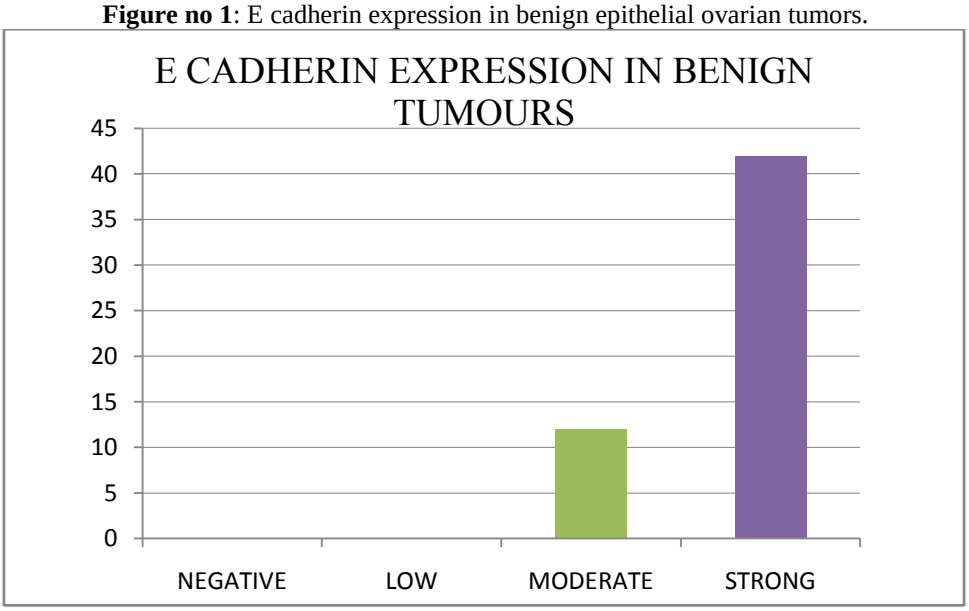
Immunohistochemical findings

E- cadherin immunostaining : Majority of benign tumors showed strong membranous expression whereas majority of borderline and malignant showed both membranous and cytoplasmic expression.77% of benign tumors showed strong E cadherin expression and 22% of them showed moderate expression .while none of them showed weak expression. 52% of malignant tumors showed moderate expression and 42% showed low expression and 5% showed strong expression.54% of T3 tumors showed moderate expression, 46% showed low expressison.while none of T3 tumors showed strong expression. Significant decrease in the level of E cadherin expression was noted in malignant tumors when compared to benign tumors (highly significant) and also between benign and borderline tumors.No significant difference in E cadherin expression was noted between borderline and malignant tumors

Table no 1:E cadherin expression in benign epithelial ovarian tumors.

DIAGNOSIS	NEGATIVE	LOW	MODERATE	STRONG
BENIGN OVARIAN EPITHELIAL TUMORS(n=54)	0	0	12(22%)	42(77%)

Figure no 1: E cadherin expression in benign epithelial ovarian tumors.



77% of benign epithelial ovarian tumors showed strong E cadherin expression whereas 12% of them showed moderate expression

Table no 2: E cadherin expression in borderline epithelial ovarian tumors

DIAGNOSIS	NEGATIVE	LOW	MODERATE	STRONG
BORDERLINE EPITHELIAL TUMORS(n=7)	0	2(28%)	4(57%)	1(14%)

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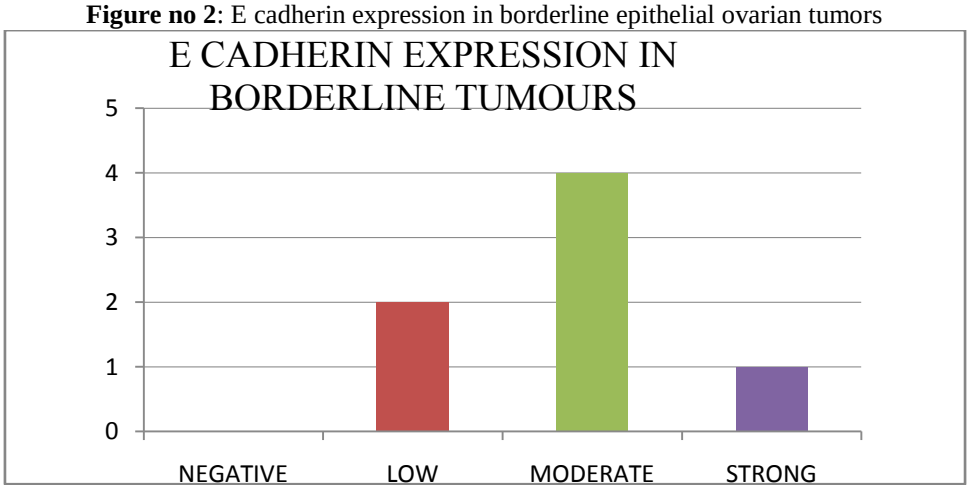
Figure no 2: E cadherin expression in borderline epithelial ovarian tumors

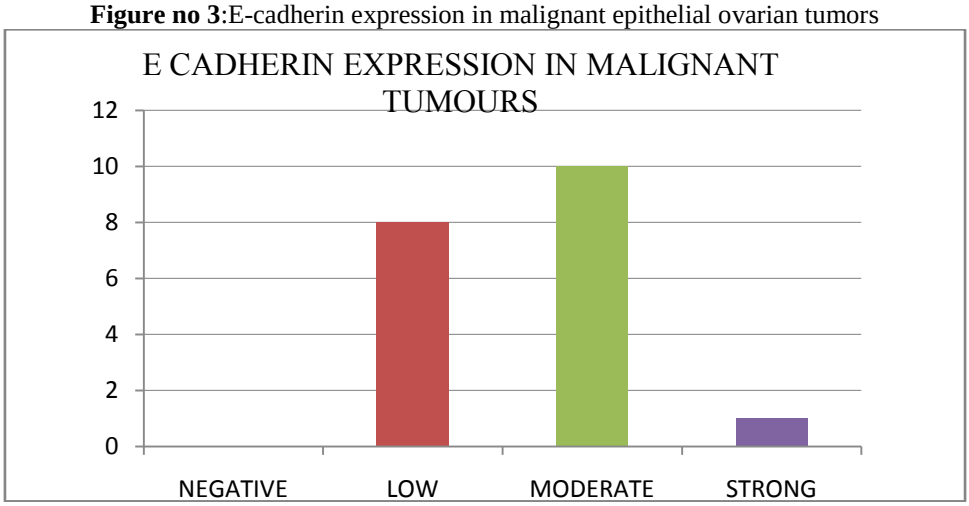


57% of borderline ovarian tumors showed moderate expression of E cadherin while 28 % showed low expression. One of the cases showed strong expression.

Table no 3 : E-cadherin expression in malignant epithelial ovarian tumors

DIAGNOSIS	NEGATIVE	LOW	MODERATE	STRONG
MALIGNANT EPITHELIAL OVARIAN TUMORS(n=19)	0	8(42%)	10(52%)	1(5.26%)

Figure no 3:E-cadherin expression in malignant epithelial ovarian tumors



52% of malignant ovarian tumors showed moderate expression and 42% showed low expression of E cadherin.

Table no 4:To Distinguish benign tumor from borderline and malignant tumor

E cadherin expression	Borderline and malignant	Benign	Total
Low	24	12	36
Strong	2	42	44
Total	26	54	80

Measrement of agreement Kappa = 0.637 p<0.001

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IV. DISCUSSION

Epithelial Ovarian cancer is the fifth most common female cancer and the 4th leading cause of death due to cancers in women[1].Majority of patients present at an advanced stage at the time of diagnosis.Despite radical surgery and chemotherapy, relapse occurs in more than half of the patients.Identification of new molecular markers could lead to significant modification of clinical management and improveclinical outcome.

During tumor progression, cell-to-cell and cell-to-substrate interactions seem to be crucially altered. Cadherins represent the major component of adherent junctions and mediate cell–cell adhesion through the calcium dependent homophylic interaction with the extracellular matrix(ECM). Epithelial cadherins, with E-cadherin as the prototype family member, play a crucial role in the formation and maintenance of epithelial structures. Furthermore, loss of cell- cell adhesion may play a relevant role in malignant transformation and the invasive behavior of malignant tumors.

In this study E cadherin expression was analysed using immunohistochemistry in benign, borderline and malignant surface epithelial ovarian tumors and the results were compared with those obtained from other studies.A total of 80cases of surface epithelial ovarian tumor which satisfied the inclusion criteria were studied.We analysed the staining pattern, intensity and the level of expression of E cadherin in the epithelial cells of benign, borderline and malignant epithelilal ovarian tumors.We also analysed the level of expression of E cadherin in relation to tumor stage and histological grade. Staining was strong and membranous in benign ovarian epithelium, but gradually decreased in intensity and percentage in the borderline tumors and the pattern was changed to cytoplasmic expression in carcinoma and metastatic lesions.

Among the 80 cases of ovarian surface epithelial tumors that we studied between an age range of 17-78 years, majority of the lesions were benign(67%).This was in contrast to the study of Dominque et al[2] and Dr Mohammed[5] from Cairo,Egypt who studied 36 cases and 50 cases respectively and found that malignant epithelial tumors constituted the major share.While Darai et al[6] studied equal number of benign, borderline and malignant tumors (i.e20 cases each) from a total of 60 cases.

In our study, predominant gross appearance of the tumors were cystic (65%).The most common clinical presentation of benign tumors were mentrual disturbances in the form of menorrhagia and the most common clinical presentation of malignant epithelial tumors in our study was postmenopausal bleeding.So this suggest that cases of postmenopausal bleeding should be screened for carcinoma ovary.

In our study the predominant histological subtype in benign tumors was serous cysadenoma (32.5%) followed by mucinous cystadenomas(27.5%)which was similar to study of Desislava et al[7]. In our study major histological subtype in malignant epithelial ovarian tumor was Serous subtype which constitute 23.7% cases followed by endometriod. Clear cell and mucinous carcinoma constituted only 1 case each.In the study of Desislava et al[7] major histological subtype among malignant tumors was serous carcinomas followed by mucinous carcinoma and the most uncommon subtype was clear cell type.

In our study,bilaterality was noted in 3.7% of benign cases, 42% of borderline and 75% of malignant lesions. In the study of Dr Mohammed, bilateral benign tumors were seen in 16.7% cases,borderline and malignant cases showed 25% and 83% respectively.We noted a statistically significant relationship between incidence of bilaterality and aggressiveness of the tumor(p value is 0.0001).

Out of the 19 malignant epithelial tumors, 13cases (68.4%) were in FIGO stage III which was similar to the study of Dr Mohammed and Dominique et al who obtained 60% cases in stage III/IV and 68.3% in stage III respectively. In our study the expression of E-cadherin in benign ovarian lesions was localized in the intercellular border and pericellular region of benign surface epithelial cells. E-Cadherin was expressed over the whole surface of polarized epithelial cells and no nuclear or cytoplasmic expression was detected in benign ovarian tumors. E-Cadherin showed reduced expression in epithelial ovarian carcinoma and was localized irregularly in the intercellular border and pericellular region of epithelial ovarian cancer cells which was similar to study done by Koensegen et al.

Among the serous tumors E cadherin membranous expression was noted in all benign tumors with a level of expression score of 3. Majority of borderline and malignant epithelial tumors showed both membranous and cytoplasmic positivity with level of expression score of 2.Two cases of serous carcinoma and two2 metastatic serous carcinoma and showed focal cytoplasmic positivity with low level of expression (score 1)..

Among the malignant ovarian tumors 52% of malignant ovarian tumors showed moderate expression and 42% showed low expression of E

cadherin and 5% showed strong expression. Desislava et al[7] who studied 33 cases of ovarian carcinoma found moderate and low E cadherin expression in 33% and 39% cases whereas strong expression was found in 18% cases.

In our study 94% of benign epithelial tumors, 28 % of borderline tumors and only 5% of malignant ovarian tumors showed complete membranous positivity.5% of benign , 71% of borderline and 68% of malignant tumors showed heterogenous positivityi.e a heterogeneous pattern with islands of strong or moderate positive expression alternating with regions of weak positive expression.26% of malignant epithelial tumor showed focal cytoplasmic positivity.Similar results were also obtained by Desilslava et al[7].

We found a statistically significant decrease in E cadherin expression in malignant epithelial ovarian tumors when compared to benign tumors(i.e p value<0.001).This finding is similar to the study done by Dominique et al[2] , Dr Mohammed ,Wang et al[8] and Darai et al[6] .

But in our study no significant difference in E cadherin expression were noted between borderline and malignant tumors which is similar to the study done by Dr. Mohammed .Whereas a significant difference was seen in studies of Darai et al[6] and Wang et al[8].We were not able to find significant relationship between E cadherin expression with tumor stage and histological grade which is similar to study done by Dominique et al.This finding is contrast to Dr Mohammed who found a significant association between E cadherin expression and tumor stage.

V. CONCLUSION

A decrease in the expression levels of E-cadherin was found in epithelial ovarian carcinomas when compared to borderline and benign categories. There was a significant difference in the expression levels of E cadherin between benign and malignant epithelial tumors and also between benign and borderline tumors. But there was no significant difference noted between borderline and malignant epithelial ovarian tumors. We could not find any significant decrease in expression levels of E cadherin with increase in the stage and grade of tumor**.**

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