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Original Research Article

Immunohistochemical expression of human epididymis 4 in ovarian malignancy

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ABSTRACT

Background: Ovarian malignancies has the highest mortality rate among all gynaecological malignancies. Surface epithelial tumors form two thirds of all ovarian neoplasm and 90% of all ovarian cancers are surface epithelial carcinomas. Mortality in case of ovarian malignancy is high due to late diagnosis. Early and accurate diagnosis can improve the case specific management. HE4 (Human Epididymis Protein 4) which is proved to be overexpressed in the ovarian cancer cells, is considered a new biomarker for ovarian cancer diagnosis which helps in early diagnosis and patient management. Aims and objectives of the study was to evaluate the immunohistochemical expression of HE4 in various ovarian malignancies.
Methods: It was a cross sectional, prospective, single institution-based study, conducted in the department of Pathology in collaboration with the Department of Gynaecology and Obstetrics, from December 2016 to January 2019 in institution. A total 74 ovarian malignancies were selected for this study.
Results: Serous carcinoma was the most common ovarian malignancy followed by endometrioid carcinoma. Highest percentage of expression of HE4 was seen in high grade serous cancer and malignant endometrioid tumor.
Conclusions: HE4 was highly expressed in malignant ovarian tumour especially serous and endometrioid carcinoma and can be used as an important biomarker for malignant ovarian neoplasm. Expression in high grade ovarian serous cancer support its prognostic value also.

Keywords: Biomarker, Immunohistochemical, Human epididymis protein 4, Ovarian malignancy

INTRODUCTION

Of all gynecological malignancies ovarian carcinoma has the highest mortality rate. It is the sixth most common cancer in women worldwide.[1] Surface epithelial tumors form two thirds of all ovarian neoplasm and 90% of all ovarian cancers are surface epithelial carcinomas.[2]

Serous carcinoma, which is of epithelial origin, makes up to 30-70% of all diagnosis. It is the most common type of ovarian carcinoma carrying the poorest prognosis.[3] Lack of symptoms until the advanced stages of tumor development results in an increased rate of metastasis at the time of diagnosis. Though ultrasound is a routinely performed imaging technique, high levels of expertise is required to differentiate between benign and malignant ovarian tissues. Hence there is a need to develop biomarkers for early diagnosis.

HE4 (Human Epididymis protein 4) is one of the most promising bio markers for early detection. HE4, was originally identified as a transcript exclusively expressed

in the epididymis.[4] HE4 is over expressed in ovarian cancer cells, especially in histologic subtypes of serous and endometrioid carcinoma and it has been suggested to be a serological marker also.[5] It serves as a valuable prognostic factor for the overall survival in patients with epithelial ovarian cancer and also an early indication of recurrence.[6]

The serologic detection of HE4 has most recently been shown to have increased sensitivity and specificity for the detection of ovarian cancer, compared with targeting CA125, the current gold standard serum bio-marker for screening metastatic ovarian carcinomas.[7,8] Several studies revealed a positive correlation between serum level and IHC expression of HE4. In this study author aim to evaluate the immunohistochemical expression of HE4 in different histopathological types of ovarian carcinoma.

METHODS

Study was conducted with oophorectomy specimen with or without uterus, fallopian tube and omentum obtained from the Gynaecology and Obstetrics Department in author's Institution after obtaining approval from Ethics Committee from December 2016 to June 2018.

Inclusion criteria

Clinically and radiologically suspected cases of ovarian malignancy were included in this study.

Exclusion criteria

Patients who were unfit and unwilling for undergoing surgery were excluded from this study.

All specimens received for histopathological sampling was fixed in 10% neutral buffered formalin solution and embedded in paraffin. The formalin fixed, paraffin embedded tissue blocks were sliced into 4-5 microns sections which were subsequently stained with hematoxylin and eosin.

HE4 antibody is a rabbit monoclonal antibody derived from cell culture supernatant that is concentrated, dialyzed filter sterilized and diluted in buffer pH 7.5, containing BSA and sodium azide as a preservative. Immunohistochemical staining with HE4 antibody done following antigen retrieval in microwave method. Human epididymis was taken as positive (with antibody) and negative (without antibody) control.

Interpretation of immunostain

Scoring system cytoplasmic staining was graded for intensity (0- negative,1- weak positive, 2-moderate, and 3- strong) and percentage of positive cell [0, 1(1-24%), 2 (25-49%), and 3(50-100%). The grades were multiplied to determine an H score. Protein expression was then defined as negative (H score=0), weak (H score=1-3), or strong (H score>4).[9]

Statistical analysis

Data has been analyzed by appropriate statistical tests using statistical software SPSS 20.0 (IBM, Armonk, New York, USA). Microsoft Excel worksheet has been used to record the relevant demographic, clinical, laboratory data. Records has been kept confidential and available only to the authorized staff.

RESULTS

Author found 74 malignant ovarian tumor cases of which 36 cases were serous adenocarcinoma, the incidence being highest (48%) followed by endometrioid tumor, 10 cases in number, 13% (10/74) incidence. Mucinous adenoca and dysgerminoma, each being 4 in number i.e. each having 5% incidence. (4/74). Clear cell tumor, mixed epithelial and mesenchymal tumor, yolk sac tumor, mixed germ cell tumor, immature teratoma and small cell carcinoma each being 2 in number constituting 3% incidence (2/74) each Metastatic ovarian tumor i.e. Krukenberg tumor was 8 in number (8/74) the incidence being 10% (Table 1).

Table 1: Distribution of malignant ovarian tumor into different types (n=74).

Malignant tumor	No of cases
Serous tumor	36
Mucinous tumor	4
Endometrioid tumor	10
Clear cell tumor	2
Mixed epithelial and mesenchymal tumor	2
Dysgerminoma	4
Yolk sac tumor	2
Mixed germ cell tumor	2
Immature teratoma	2
Small cell carcinoma	2
Krukenberg tumor	8
Total	74

Author got 36 cases of serous carcinoma, 26 cases were High Grade (HGSC) and 10 cases were Low Grade (LGSC). All high-grade serous carcinoma shows strong HE4 positivity. i.e.100% positivity (26/26). Out of10 cases of LGSC 6 show strong positivity. i.e., 60%, 2 cases showing weak positivity (20%) and 2 cases showing negative expression (20%).

Out of four cases of mucinous tumors two show weak (50%) and two show negative expression (50%). The figure 1 shows a serous carcinoma where more than 90% of tumor cells has taken cytoplasmic staining with strong intensity on, HE4 IHC staining. The H score being 3x3 i.e. 9. (strong positive) (Table 2) In contrast, the (Figure 2) shows a malignant mucinous tumor where 24% of

tumor cells have weak cytoplasmic staining on HE4 IHC staining. H score being 1x1 i.e. 1 (weak positive.) Among 10 malignant endometrioid tumor 8 shows (80%) strong positivity. (Table 2)

All of clear cell tumor (2 cases) and mixed epithelial and mesenchymal tumors (2 cases) show weak expression.

Table 2: HE4 score of epithelial ovarian cancer.

Histological type	Total no.	Weak positive (H score=1-3)	Strong positive (H score>4)	Negative (H score=0)
Malignant serous tumor a) low grade (LGSC)	10	2(20%)	06(60%)	02(20%)
B) high grade (HGSC)	26	0	26 (100%)	0
Malignant mucinous tumor	4	2(50%)	0	2(50%)
Endometrioid tumors- malignant	10	0	8(80%)	2(20%)
Clear cell tumor- malignant	2	2(100%)	0	0
Mixed epithelial and mesenchymal tumor	2	2(100%)	0	0

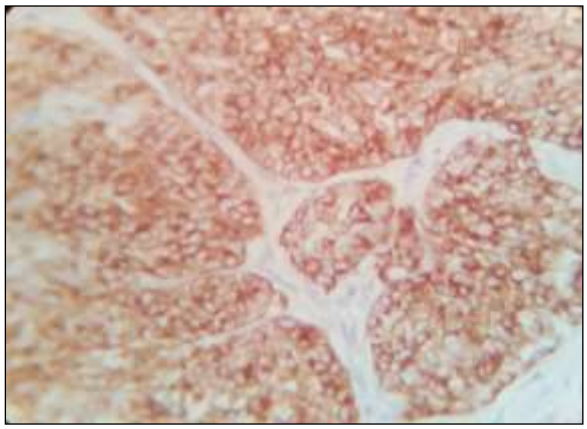

Figure 1: Strong cytoplasmic positivity in high grade serous carcinoma (400x) HE4 IHC stain. (H score=9).

Among the 20 cases of nonepithelial tumors strong positivity with high H scores is seen in 50% cases of dysgerminoma (2 out of 4 cases) and 100% cases of mixed germ cell tumor (2 out of 2 cases) followed by Krukenberg tumor which in 50% cases show strong positive expression (4 out of 8) Yolk sac tumor, immature teratoma, small cell carcinoma show weak expression with low H score in all the cases (Table 3).

IHC staining of a Krukenberg tumor where 50% of tumor cells has taken cytoplasmic staining with weak intensity, (H score being 2x1=2), i.e. weak positive. (Figure 3)

Normal human epididymis with IHC staining taken as positive control. As a whole, 48 out of 74 cases i.e. 65% of malignant tumors show strong positivity(Figure 4). Only 5% show negative expression (Table 4).

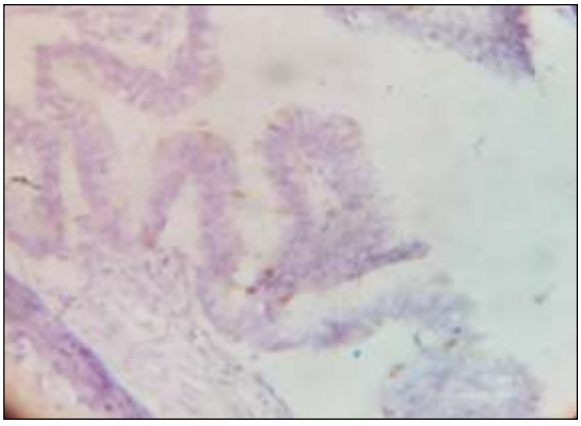

Figure 2: Weak cytoplasmic positivity in mucinous carcinoma HE4 IHC stain (H score=1).

Table 3: HE4 expression pattern of different non-epithelial ovarian cancer (N-20).

Tumor type	Total	Strong positive (H score>4)	Weak positive (H score=1-3)	Negative (H score=0)
Dysgerminoma	4	2(50%)	2(50%)	0
Yolk sac tumor	2	0	2(100%)	0
Mixed gem cell tumor	2	2(100%)	0	0
Immature teratoma	2	0	2(100%)	0
Small cell carcinoma	2	0	2(100%)	0
Krukenberg tumor	8	4(50%)	4(50%)	0

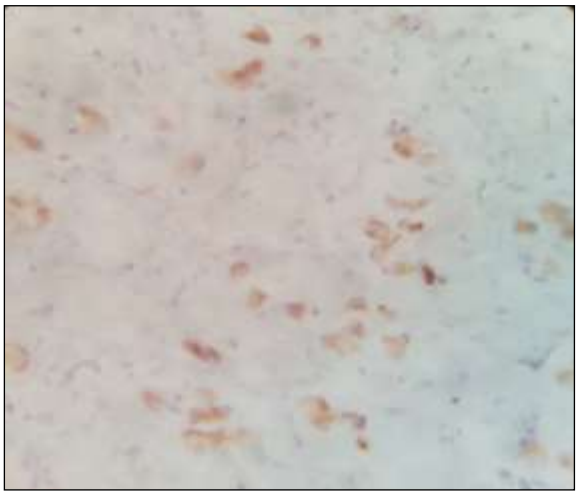

Figure 3: Krukenberg tumour 400x (weak positive).

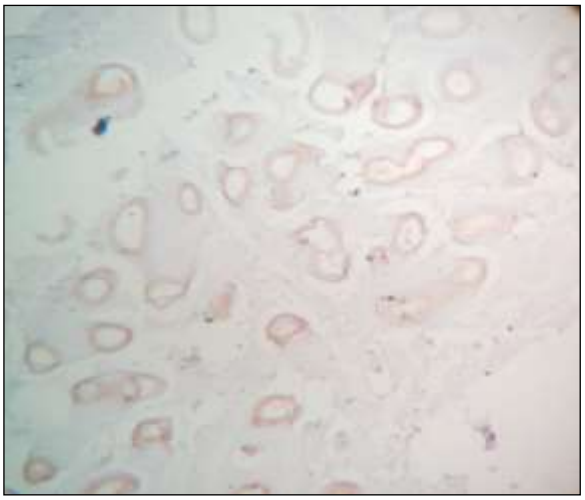

Figure 4: Positive control of human epididymis (HE4 IHC stain).

Table 4: expression pattern of malignant ovarian tumor (n=74).

Tumor type	Strong positive (H score>4)	Weak positive (H score=1-3)	Negative (H score= 0)	Total
Malignant	48(65%)	22(30%)	4(5%)	74

DISCUSSION

The serologic detection of HE4 has most recently been shown to have increased sensitivity and specificity for the detection of ovarian cancer, compared with targeting CA125, the current gold standard serum bio-marker for screening ovarian carcinomas.[7,8] Several studies revealed a positive correlation between serum level and IHC expression of HE4.

The first report mentioning HE4 as a potential serum biomarker for ovarian cancer was published in 2003.[10]

ROMA (Risk of Malignancy Algorithm) based on CA125 and the novel HE4 marker has recently emerged as a promising approach to the preoperative categorization of malignancy risk the diagnostic performance of ROMA was advocated for the first time by Moore et al. Moore et al, (2008, 2009) published a series of papers that use a combination of CA125, HE4 and menopausal status to predict the presence of a malignant ovarian tumor (ROMA).[11] Recently, a study showed HE4 is more specific than CA125 in benign and malignant condition.[12] HE4 serum level may be abnormal mainly in patient with renal failure or effusion in patient with lung cancer or with chronic liver disease.

HE4 protein is frequently over expressed in ovarian cancer, especially in serous and endometrioid histology.[13] However HE4 is not a specific of ovarian cancer & some expression has also been found in other malignancy mainly pulmonary and endometrial carcinoma.[12]

Author found 74 malignant cases of which 48% (36/74) are serous, 5% (4/74) mucinous, 13% (10/74) endometrioid and dysgerminoma 5% (4/74). Clear cell tumor, mixed epithelial and mesenchymal tumor, yolk sac tumor, mixed germ cell tumor (YSTand dysgerminoma), immature teratoma and small cell carcinoma constitute 3% (2/74) each.

Of all the malignant cases, serous cyst adenocarcinomas constituted the largest group with 48% (36/74) cases in this study This finding is similar with Jha R et al, who find serous adenocarcinoma as commonest malignant tumor (46.2%).[14] This finding was however dissimilar with Maheshwari et al, who reported that mucinous carcinomas constituted the largest group among malignant neoplasms.[15]

Study by Husaini AL, Soudy H et al, found Dysgerminoma is the most common malignant primitive germ cell tumor of the ovary and comprises only 1-2 % of all malignant ovarian tumors.[16]

This study also find dysgerminoma as most common primitive germ cell tumor and comprises 5% of all malignant ovarian tumor.

Sovona M Devan's study also found that significant expression of HE4 in malignant epithelial ovarian tumor compared to benign epithelial ovarian tumor.[17]

Statistically 100% HGSC, 80% LGSC, 80% endometrioid carcinoma, 50% mucinous carcinoma 100% clear cell carcinoma and 100% mixed malignant epithelial and mesenchymal tumor shows positive expression in this study.

Study by F Rahmat and Hairuszah Ithnin (2017) on Immunohistochemical Expression of HE4 in Ovarian Serous Carcinoma, found 100% positive staining in women <40 yrs.[18] old, among women above 40 yrs. 98% show positive staining and only one negative staining to HE4 in ovarian serous carcinoma, which support this study where author found only 2 negative staining to HE4 in ovarian serous carcinoma out of 36.

Study by Penelope Georgakopoulos et al, showed HE4 protein expression was 100% in serous cancer, intensity of HE4 staining was strong in majority of serous carcinoma.[19] Expression of high-grade serous carcinoma correlate with this study which also show 100% positive expression.

HE4 Expression of serous carcinoma of this study also correlate with study of T Bulut et al, who found 90.32% HE4 tissue expression among ovarian serous carcinoma.[9]

Study by Li-e Zheng et al, found HE4 expression in serous ovarian carcinoma is 100% (30/30), mucinous ovarian tumor 66.67% (8/12), clear cell carcinomas 66.7% (2/3), endometrioid carcinoma 100% (6/6), yolk sac tumor 25% (1/4) and dysgerminoma 75% (3/4).[20]

Though serum value of HE4 is not taken in this study but considering the intensity of immune expression this study corroborates with this.

Limitations of this study was that serum HE4 level estimation before surgery would help us to correlate with the tissue expression of HE4 which is lacking in this study. Little is known about the specific biological processes that are involved in the regulation of HE4 expression. Literature search reveals no study in this field in this region. According to study design and short duration of study period, follow up of cases was not possible.

CONCLUSION

Serous carcinoma is the most common epithelial ovarian carcinoma. HE4 is highly expressed in certain types of ovarian cancer and can be used as biomarker for ovarian cancer particularly of serous (the most common ovarian cancer) and endometrial variety. Low level of expression is seen in mucinous cancer, clear cell cancer in this study, but because of the limited cases of these subtype of tumor, the conclusion may be different in general and needs further study.

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Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

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