Immunoexpression of Epidermal Growth Factor Receptor (EGFR) in Malignant Surface Epithelial Tumours of Ovary

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Ovarian cancer is the deadliest gynaecological cancer and approximately 70% are diagnosed in an advanced stage with 5 years survival rate of only 35%. The aim of this study was to find out the distribution of epidermal growth factor receptor (EGFR) immunoexpression in different histological types and grades of malignant surface epithelial tumours of ovary. A total 54 cases of malignant surface epithelial tumours of ovary from North Okkalapa General and Teaching Hospital and Thingangyun Sanpya General Hospital from August 2015 to September 2016 were included. This study included 30 cases (56%) of serous tumour, 18 cases (33%) mucinous tumour, 5 cases (9%) clear cell tumour and one case (2%) of malignant Brenner tumour. Of the 54 cases, 11 cases (20%) were well differentiated, 33 cases (61%) moderately differentiated and 10 cases (19%) poorly differentiated tumours. Different histological types and grades of malignant surface epithelial ovarian tumours were determined for EGFR immunoexpression by peroxidase antiperoxidase method. Out of 54 cases, 31 cases (57.4%) were found to be positive for EGFR immunoexpression and 23 cases (42.6%) showed negative immunoexpression. Among 31 positive cases of EGFR, 54.8% were serous, 32.3% mucinous, 9.7% clear cell and 3.2% were malignant Brenner tumour. The highest EGFR immunoexpression was found in malignant serous, tumour (54.8%). Regarding the histological grades of the 31 positive cases, EGFR immunoexpression was found 9.7% in well differentiated, 64.5% moderately differentiated and 25.8% in poorly differentiated tumours. Increased EGFR immunoexpression was observed predominantly in higher histological grades of ovarian cancers. Since, high EGFR levels have a negative prognostic role in ovarian cancers, further studies with long-term follow-ups are required to determine the prognosis and management of patients with malignant surface epithelial ovarian tumours.

Keywords: Malignant surface epithelial tumours of ovary, Epidermal growth factor receptor (EGFR)

INTRODUCTION

Ovarian cancer is the deadliest gynaecologic cancer. It represents the most challenging of gynaecological malignancies: approximately 70% of ovarian cancers are diagnosed in an advanced stage and only 35% of patients survive at 5 years.[1] In Myanmar, ovarian cancer is the third commonest female genital tract malignancies and the incidence of malignant ovarian tumour has been increasing progressively. According to Globocan 2018, it ranked the 8th position in age standardized (world) incidence and mortality rates, top 10

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cancers.[2] According to the World Health organization (WHO) classification, ovarian tumours can be classified simply into surface epithelial stromal tumour, sex cord stromal tumours, germ cell tumours, malignant not otherwise specified and metastatic cancer from non-ovarian primary. Among the different types of ovarian tumours, surface epithelial tumours are numerically the most important, counting about 90% of all malignant neoplasms.[3]

Surface epithelial tumours are the most important group of neoplasms, derived from the epithelium that normally lines the outer aspect of the ovary. Neoplasms of surface epithelial origin account for the great majority of primary ovarian tumours and, in their malignant forms, account for almost 90% of ovarian cancers. Surface epithelial ovarian tumours are classified into serous tumours, mucinous tumours, endometroid, clear cell and malignant Brenner tumours. Among them, serous tumour is the most common and make up about one-fourth of all epithelial ovarian tumours.[4]

Epidermal growth factor receptor (EGFR) is generally known as plasma membrane tyrosine kinase receptor which sends signals to the nucleus via the mitogen activated protein kinase, the phospholipase C, and the phosphatidylinositol 3-kinase pathway. EGFR may enter the nucleus and directly act as transcriptional factor, by passing the protein phosphorylation cascades.[5] EGFR activation drives cellular processes linked to ovarian tumour development, tumour cells survival and metastasis. Findings indicate that EGFR axis is an important mechanism for supporting the autocrine growth of ovarian tumour.[6]

EGFR has been suggested as a promising target since up to 70% of ovarian cancers are EGFR-positive and EGFR overexpression is developed during cancer progression and correlated to poor prognosis.[7] The identification of EGFR as an oncogene has led to the development of anti-cancer therapeutics directed against EGFR (targeted therapy) and plays an important role in prognosis and survival. This study aims to find out the various distribution of EGFR immunoexpression in malignant surface epithelial tumours of ovary and levels of EGFR immunoexpression in different histological types and grades of malignant surface epithelial tumours of ovary. Therefore, the findings from this study will be clinically useful and highlight the different levels EGFR immunoexpression in different histological types and grades of malignant surface epithelial ovarian cancers.

MATERIALS AND METHODS

A cross-sectional, descriptive study was carried out on 54 cases of primary malignant surface epithelial tumours of ovary admitted to Gynaecology Ward of North Okkalapa General and Teaching Hospital (NOGTH) and Thingangyun Sanpya General and Teaching Hospital (TSGTH) from August 2015 to December 2016. The biopsied samples sent to the section of Pathology Department, NOGTH and TSGTH were fixed with 10% buffered paraformaldehyde. After finishing the adequate fixation, tissue processing and proper paraffin wax embedding, all the tissue sections were stained with haematoxylin and eosin.

Histological types and grades of ovarian tumours were examined under light microscope. Only malignant surface epithelial ovarian tumours were stained for EGFR immunoexpression by PAP method. For immunohistochemical staining, primary antibodies (Anti-EGFR-PAN EP 38 Y) Code-NU473-UCE 1:20 dilution (BioGenex, USA) and antigen detection kit N-Histofine simple stain MAX PO (MULTI), universal immunoperoxidase polymer, anti-mouse and anti-rabbit were used. Immunohistochemical staining was done at Common Research Laboratory, University of Medicine 2, Yangon.

Immunohistochemistry method

Sections with 5 µm thickness were placed on slides coated with silane. The sections were dried at 37°C overnight and 55°C for 1 hour

in incubator. Then, the sections were deparaffinised through xylenoethanol series (100%, 95%, 85%) (each change for 3 minutes) and washed in distilled water for 5 minutes. Antigen sites were retrieved by heat, using microwave antigen retrieval method. The sections were cooled down to room temperature and immersed in phosphate buffered saline (PBS). The sections were incubated with 30% hydrogen peroxide and methanol for 10 minutes to block endogenous peroxidase activity because it interferes with interpretation. The sections were then washed with PBS pH 7.6 for 3 times (each time for 5 minutes) and incubated in a moist chamber overnight at 4°C with the primary antibodies: (Anti-EGFR-PAN EP 38 Y) 1:20 dilution.

After incubation was completed, the sections were washed individually with PBS (pH 7.6) for 3 times (each time for 5 minutes). After washing with PBS, the sections were covered with peroxidase conjugated anti-human antibody (N- Histofine Simple Stain MAX PO) for one hour at room temperature. The diaminoazobenzadine substrate solution was freshly prepared and covered on the sections and incubated for 5-20 minutes. The slides were then observed for the desired signal under the ordinary light microscope which was the appearance of a brown tinge. Then, the slides were washed in distilled water for 5 minutes. After that, the sections were counterstained with hematoxylin for 5-10 seconds and rinsed in water and proceeded in ethanol 85%, 95%, 100%, xylene and mounted in Distyrene Plasticizer Xylene (DPX) mountant. EGFR immuno-reactivity was estimated based on number of positive cells expressed as the percentage of tumour cells. EGFR positive staining was regarded as complete or incomplete circumferential brown membrane staining in tumour cells.[8]

The quantity of staining was graded as:

<5% positive tumour cells	= negative
5-25% positive tumour cells	= +
26-50% positive tumour cells	= ++
51-75% positive tumour cells	= +++
76-100% positive tumour cells	= ++++[9]

Data analysis

The collected data were analyzed by using the Statistical Package for Social Science (SPSS) Study version 23. The association between EGFR immunoexpression and different types of malignant surface epithelial tumours of ovary was tested using Chi square test. Categorical variables such as histo-logical types and grades were summarized as frequency tables.

Ethical consideration

This study was reviewed and approved by Academic and Ethical Board of University of Medicine 2, Yangon, Myanmar.

RESULTS

Age distribution of malignant surface epithelial tumours of ovary

Among the 54 cases of malignant surface epithelial tumours of ovary, the youngest age was 15 years and the oldest age was 93 years. The majority of cases belonged to age group of 41-60 years (65%) followed by age group of 61-80 years (16%). The mean age was 53 years (+/-2SD) and the commonest age encountered was 54 years.

Distribution of malignant surface epithelial tumours of ovary by different histological types and grades

Among 54 cases of malignant surface epithelial tumours of ovary, 30 cases (56%) were serous, 18 cases (33%) were mucinous, 5 cases (9%) were clear cell and one case (2%) was malignant Brenner tumour. Of the 54 cases, 11 cases (20%) were well differentiated, 33 cases (61%) moderately differentiated and 10 cases (19%) poorly differentiated (Table 1).

Of the 30 cases of serous tumours, 13(43%) were negative, 15(51%) + positivity, 1(3%) ++ positivity and 1(3%) +++ positivity of EGFR immunoexpression. As for 18 cases of mucinous tumours, 8(44%) were negative, 6(33%) + positivity, 3(17%) ++ positivity and 1(6%) +++ positivity of EGFR immunoexpression.

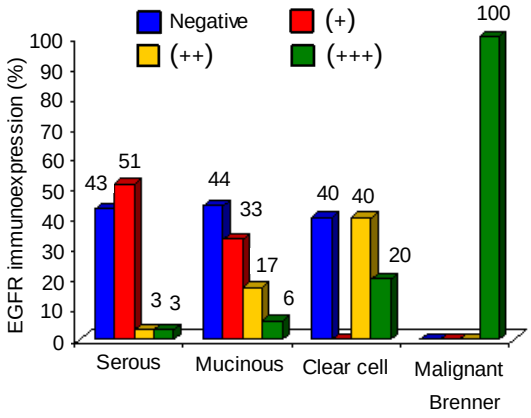
Table 1. Age distribution, histological types and grades of patients with malignant surface epithelial tumours of ovary

	Number of cases (%)
Age (Completed years)	
≤20	1(2)
2 - 40	7(13)
41- 60	35(65)
61- 80	9(16)
>80	2(4)
Histological types	
Serous	30(56)
Mucinous	18(33)
Clear cell	5(9)
Malignant Brenner	1(2)
Histological grades	
Well differentiated	11(20)
Moderately differentiated	33(61)
Poorly differentiated	10(19)

Table 2. EGFR immunoexpression in different histological types and grades

Cases	EGFR (+) n(%)	EGFR (-) n(%)	Total
Histological Types			
Serous	17(57)	13(43)	30
Mucinous	10(56)	8(44)	18
Clear cell	3(60)	2(40)	5
Malignant Brenner	1(100%)	-	1
Histological grades			
Well differentiated	3(27)	8(73)	11
Moderately differentiated	20(61)	13(39)	33
Poorly differentiated	8(80)	2(20)	10

EGFR=Epidermal growth factor receptor

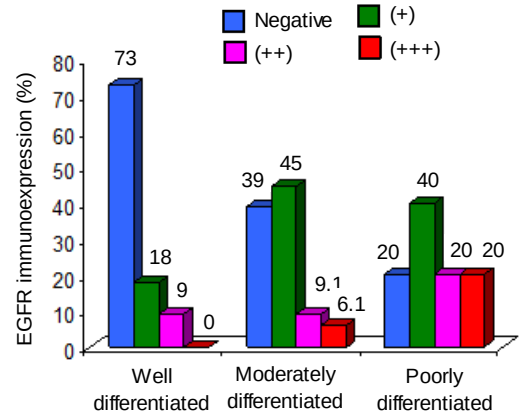


Types of malignant surface epithelial tumours of ovary

Fig. 1. Distribution of EGFR immunoexpression on different histological types of malignant surface epithelial tumours of ovary

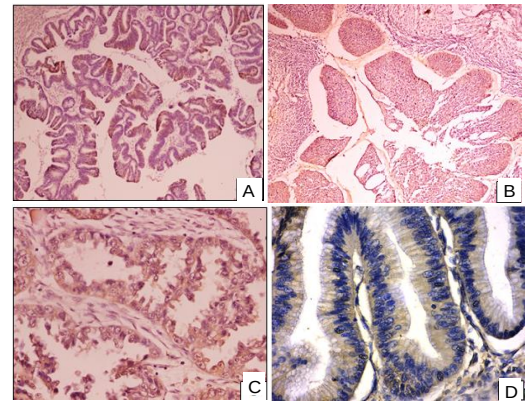
Table 2 shows EGFR immunoexpression in different histological types and grades.

As for 5 cases of clear cell tumours, 2(40%) were negative, 2(40%) ++ positivity and 1(20%) +++ positivity. There was only one case of malignant Brenner tumour which showed (100%) +++ positivity of EGFR immunoexpression (Fig. 1).



Different grades of malignant surface ovarian tumours

Fig. 2. Distribution of EGFR immunoexpression on different histological grades of malignant surface epithelial tumours of ovary



A = Papillary serous cyst adenocarcinoma of ovary (well differentiated) x 100 (+)
B = Malignant Brenner tumour x 100 (+++)
C = Papillary clear cell carcinoma of ovary (moderately differentiated) x 400 (+++)
D = Mucinous cystadenocarcinoma of ovary (well differentiated) x 400 (++)

Fig. 3. IHC staining of EGFR immunoexpression in different histological types of malignant surface ovarian tumours

Of the 11 cases of well differentiated tumours, 8(73%) showed negative, 2(18%) + positivity and 1(9%) ++ positivity of EGFR

immunoexpression. As for 33 cases of moderately differentiated tumours, 13(39%) showed negative, 15(45.4%) + positivity, 3(9.1%) ++ positivity and 2(6.1%) +++ positivity. As for 10 cases of poorly differentiated tumours, 2(20%) showed negative, 4(40%) + positivity, 2(20%) ++ positivity and 2(20%) +++ positivity of EGFR immunoexpression. The strongest (+++) EGFR immunoreactivity was found in moderately and poorly differentiated ovarian tumours and not in well-differentiated ones (Fig. 2 & 3). According to these data, the increased EGFR immunoexpression was observed predominantly in higher histological grade of malignant surface epithelial tumours of ovary.

DISCUSSION

Ovarian cancers are the fifth leading cause of cancer-related death among women and the deadliest of all gynaecological cancers. In spite of improvement in surgical management and advances in cytotoxic therapy, the overall 5 years survival rate for women with ovarian cancer remains still low.[10] Malignant ovarian tumours are common in older women, between the ages of 45 and 65 years. In this study, the commonest age encountered was 41-60 years and these findings were consistent with the previous studies in which the commonest age was between 43-72 years.[11] The most common histological type found in this study was serous tumour 30/54 cases (56%) followed by mucinous tumour 18/54 cases (33%), clear cell tumour 5/54 cases (9%) and malignant Brenner tumour 1/54 case (2%). The majority of cases found in this study were moderately differentiated 33/54 cases (61%).

Psyrri *et al.*[12] stated that increased EGFR expression was observed in approximately 70% of ovarian cancer and its expression was inversely correlated with outcome of epithelial ovarian cancer. In 2009, Hudson *et al.*[13] stated that EGFR expression was found in 10-70% of surface epithelial ovarian cancer. In the present study, total 54 cases of malignant surface epithelial ovarian tumours were observed and 31 cases (57.4%) were found to be positive for EGFR immunoexpression. The finding in this study was in accordance with the above study.

Among 31 positive cases of EGFR, 54.8% were serous (17/31 cases), 32.3% mucinous (10/31 cases), 9.7% clear cell (3/31 cases) and 3.2% were malignant Brenner tumour (1/31 case). The highest EGFR immunoexpression was found in malignant serous tumour. In a study of Romania,[14] EGFR cytoplasmic expression was observed in 87.5% of serous carcinomas and 48.2% of mucinous carcinoma. The present study also showed higher EGFR expression in serous (54.8%) than mucinous carcinoma (32.3%). However, there is no statistically significant association between EGFR mmunoexpression and histological types of malignant surface epithelial ovarian tumours.

The study done by Fujiwara *et al.*[15] reported that 39.3% of serous carcinomas showed cytoplasmic EGFR expression. Also, Brustmann *et al*.[11] reported EGFR negativity in benign and borderline serous tumours compared with the positivity (64%) in malignant serous carcinoma. In the present study, EGFR immunoexpression was found in 54.8% of malignant serous tumour. So, findings of the recent study agree well with them.

Several studies have concluded that EGFR immunoexpression was related to the grade of tumour. High EGFR expression was positively associated with high histological grade of ovarian tumour.[16] The study of Mohamed Y stated that statistically significant increase of EGFR expression was observed only in higher tumour grades.[17] In this study, of the 31 positive cases, EGFR immunoexpression showed 9.7% in well differentiated (3/31 cases), 64.5% in moderately differentiated (20/31 cases) and 25.8% in poorly differentiated tumours (8/31 cases). The strongest (+++) EGFR immunoexpression was also found in moderately and poorly differentiated ovarian tumours and not in well differentiated one. According to

these data, EGFR immuno-expression was commonly seen in higher histological grades of malignant surface epithelial ovarian tumours.

Some studies report little or no relationship between EGFR expression and a variety of clinic pathological characteristics such as disease stage, tumour grade, histological subtype, response to therapy.[13] In recent study, it can be concluded that EGFR expression is not associated with clinico-pathological indicators like age, parity, family history, types and grades of ovarian tumours. Although, the current study could not give a statistically significant association between EGFR immunoexpression and histological types and grades of malignant surface epithelial ovarian tumours, strong EGFR immunoexpression (++, +++) was observed predominantly in higher histological grades of malignant ovarian tumours.

Several lines of evidence support EGFR as a molecular target for therapy in epithelial ovarian cancer. The study of Psyrri *et al.* was concluded that EGFR immunoreactivity might be used as predictive marker for poor prognosis. High EGFR levels have a negative prognostic role in ovarian cancer patients.[12] However, in the present study, time frame did not allow to evaluate the association between survival analysis and EGFR immunoexpression in malignant surface epithelial ovarian cancers. It still needs further evaluation and long-term follow-ups to clarify the role of EGFR in prognosis and management of malignant surface epithelial ovarian cancers.

Conclusion

EGFR immunoexpression was observed in 31 cases (57.4%) of malignant surface epithelial ovarian tumours. The results from this study highlighted that the highest values of immunostaining score were observed only in higher histological grades of tumours. Therefore, EGFR immunoexpression may be considered as a potential immunohisto-chemical marker for assessing the disease progression and further management of the patients with malignant surface epithelial tumours of ovary.

Competing interests

The authors declare that they have no competing interests.

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