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Research Paper

Immunohistochemical Expression of EGFR and Ki67 in Adenocarcinoma of Stomach at Mandalay General Hospital

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ABSTRACT
Gastric cancer is one of the commonest malignant tumors worldwide. It is the fifth most common malignancy in the world. Epidermal growth factor receptor (EGFR) belongs to receptor tyrosine kinase and is the expression product of pro-oncogene ErbB1 (HER1). The nuclear protein Ki67 is an established prognostic and predictive indicator of cancer. The aim of this study is to study immunohistochemical (IHC) expression of EGFR and Ki67 in adenocarcinoma of stomach at Mandalay General Hospital. The hospital and laboratory-based, cross-sectional and descriptive study was conducted on 60 cases of adenocarcinoma of stomach from August 2018 to July 2019. There were 37 gastrectomy biopsies (61.7%) and 23 endoscopic biopsies (38.3%). For the histology types, 58 cases were of intestinal type and 2 cases of diffuse type. For the differentiation of adenocarcinoma, 7 (11.7%) were well-differentiated, 23 (38.3%) moderately differentiated and 30 (50%) poorly differentiated. In IHC expression of EGFR, there were 8 (13.3%), 17 (28.3%), 22 (36.7%) and 13 (21.7%) cases for score 0, 1+, 2+ and 3+ respectively. In IHC expression of Ki67, there were 9 (15%), 24 (40%) and 27 (45%) cases for <10%, 10-40% and >40% positivity respectively. According to the result, there was statistically significant association between differentiation of adenocarcinoma of stomach and IHC expressions of both EGFR and Ki 67 ($p < 0.05$). Therefore, the result from this study can be helpful in the selection of uses of anti-EGFR-targeted therapy in treatment options and disease management of adenocarcinoma of stomach.

KEY WORDS: *adenocarcinoma of stomach, EGFR, Ki 67, immunohistochemical expression*

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I. BACKGROUND INFORMATION (INCLUDING JUSTIFICATION):

Gastric cancer is one of the commonest malignant tumor worldwide.[1] It is the fifth most common malignancy in the world, after cancers of the lung, breast, colorectal and prostate. More than 70% of cases occur in developing countries and half of the world total occurs in Eastern Asia (mainly in China). Stomach cancer is the third leading cause of cancer death in both sexes worldwide. The highest estimated mortality rates are in Eastern Asia.[2]

Gastric cancer diagnosis often occurs in the advanced stages and thus the prognosis is usually poor.[3] Moreover, gastric cancer treatment remains a challenge.[4]

Epidermal growth factor receptor (EGFR) belongs to receptor tyrosine kinase and is the expression product of pro-oncogene ErbB1 (HER1). EGFR participates in the information control process in many cells, and its abnormal expression is closely related to many malignant tumors.[5] EGFR highly expresses in many malignant tumors and is related to the growth and invasion of tumors.[6]

Nowadays, targeted therapies are used on the evaluation and analysis of the status of target genes.[7] Epidermal growth factor receptor (EGFR) inhibitors have played a significant role in the management of solid malignancies including colorectal cancer. Currently, there are four EGFR inhibitors approved by the FDA including two small molecule tyrosine kinase inhibitors (erlotinib and gefitinib) and two monoclonal antibodies (cetuximab and panitumumab).[8-9]

The nuclear protein Ki67 (pKi67) is an established prognostic and predictive indicator for the assessment of biopsies from patients with cancer. Expression of the Ki67 protein (pKi67) is associated with the

proliferative activity of intrinsic cell populations in malignant tumors, allowing it to be used as a marker of tumor aggressiveness.[11]

According to annual statistical report of medical records department, Mandalay General Hospital (MGH), the number of patients with gastric cancer had increased from 2013 to 2017. Including follow up patients, there were 453, 449, 589, 755 and 906 in 2013, 2014, 2015, 2016 and 2017 respectively.[12]

In Myanmar, there were few studies on EGFR. In 2011, the significant of Ki 67, carcinoembryonic antigen and EGFR immmunoexpression in colorectal cancer was done.[13] And then, immmunohistochemical (IHC) expression and fluorescence in-situ hybridization (FISH) of EGFR in colorectal cancer was done in 2017.[14] Moreover, there was a study of p 53 immunoexpression in gastric cancer in 2015.[15]

Therefore, this study will detect the EGFR and Ki67 immunoexpression by using immunohistochemical method in adenocarcinoma of stomach at Mandalay General Hospital. It would be able to help in the treatment of patients with gastric cancer and provide more accurate prognosis in the future. Moreover, this study might contribute some useful basic data to further research in medical science in Myanmar.

II. METHOD

The aim of this study is to study immunohistochemical (IHC) expression of EGFR and Ki67 in adenocarcinoma of stomach at Mandalay General Hospital. The hospital and laboratory-based, cross-sectional and descriptive study was conducted on 60 cases of adenocarcinoma of stomach from August 2018 to July 2019 at Mandalay General Hospital. Gastric cancer other than adenocarcinoma and metastatic carcinoma were excluded in this study. The endoscopic biopsy blocks or gastrectomy biopsy blocks already diagnosed as adenocarcinoma of stomach were collected. This study was done upon specimen of routine clinical management. All the biopsy tissues were processed for histological examination and stained with Haematoxylin and Eosin. All the histological slides were examined under the light microscope and determined types of adenocarcinoma. After that, wax blocks were cut approximately 3-5µm and made for two slides which were stained with monoclonal antibody to EGFR and Ki67 respectively by using IHC Biogenix kit. After that, stained slides were examined under the light microscope and determine histological types of adenocarcinoma and IHC expression of EGFR and Ki67.

Interpretation of the results

It was done by principal investigator and well experienced senior pathologist.

EGFR: Both membranous and cytoplasmic staining are considered for evaluation. The membranous positivity for EGFR is evaluated in the following:

0	no discernible staining or background type staining
1+	equivocal discontinuous membrane staining
2+	unequivocal membrane staining with moderate intensity
3+	strong and complete plasma membrane staining.

More than 10% of the cells are required to meet the criteria for EGFR analysis. Scores of 2+ and 3+ staining levels are considered to be EGFR overexpression.[16] For positive cytoplasmic EGFR staining, an intense homogenous staining of the cytoplasm is necessary. There is no EGFR without membrane staining.[17]

Ki67: Positive staining is defined as positive nuclear staining. Cytoplasmic staining was considered negative. The percentage of positive nuclei was expressed as a "Ki-67 labeling index" which is the percent of cells expressing Ki-67 determined by counting 1000 cells/slide. The percentage of positive cells was scored as follows.[18]

less than 10%	low proliferative activity
10% - 40%	moderate proliferative activity
more than 40%	high proliferative activity.

III. RESULTS

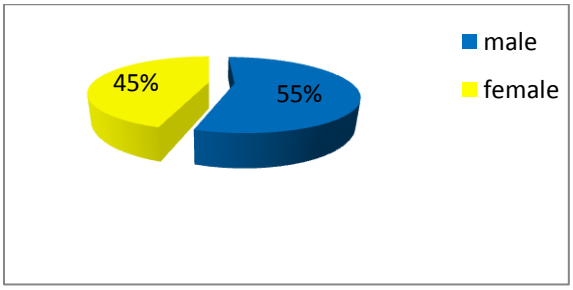

Figure 1. Frequency distribution of gastric adenocarcinoma patients according to sex

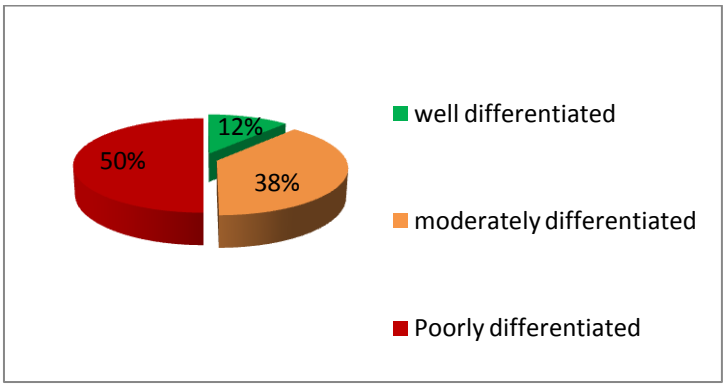

Figure 2. Frequency distribution of gastric adenocarcinoma patients according to differentiation

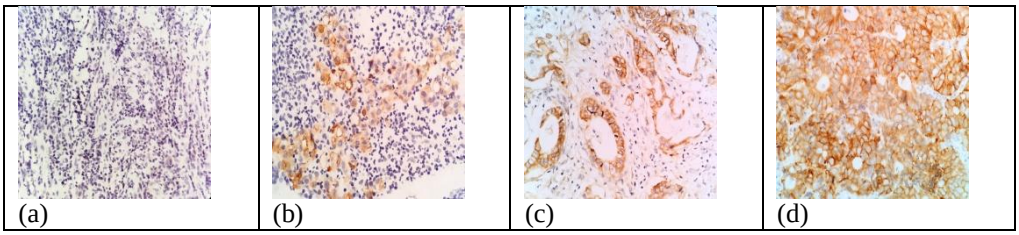

Figure (3). Immunohistochemical expression of EGFR (40x); (a) Score 0, (b) Score 1+, (c) Score 2+, (d) Score 3+

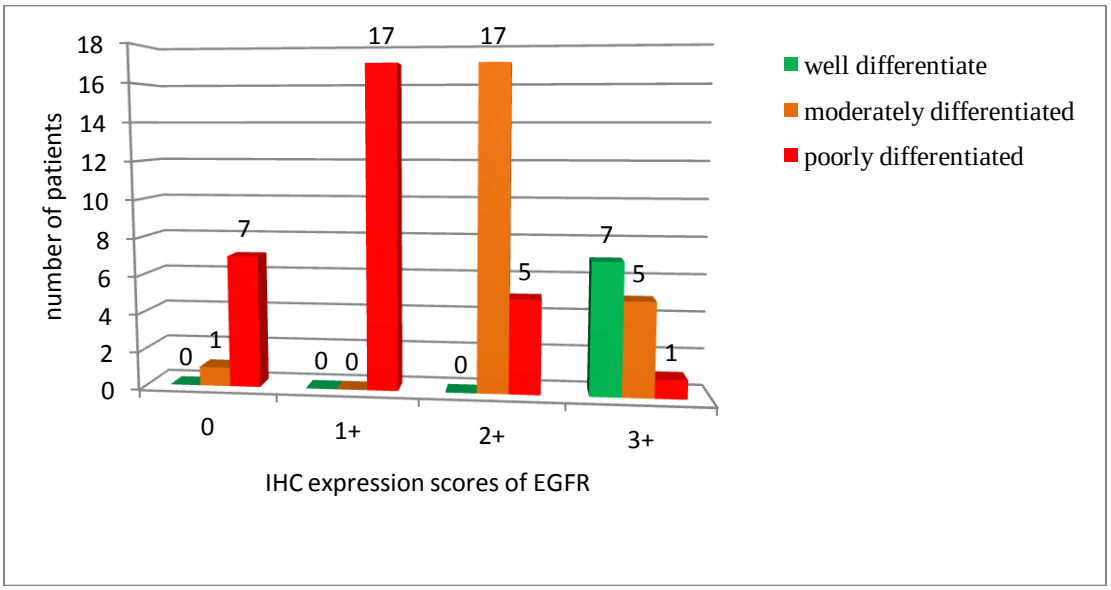

Figure (4). Association between differentiation of gastric adenocarcinoma and different IHC expression scores of EGFR

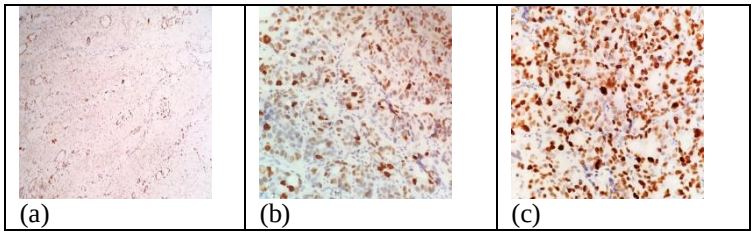

Figure (5). Immunohistochemical expression of Ki67; (a) <10% positivity (10x), (b) 10-40% positivity (40x), (c) > 40% positivity (40x)

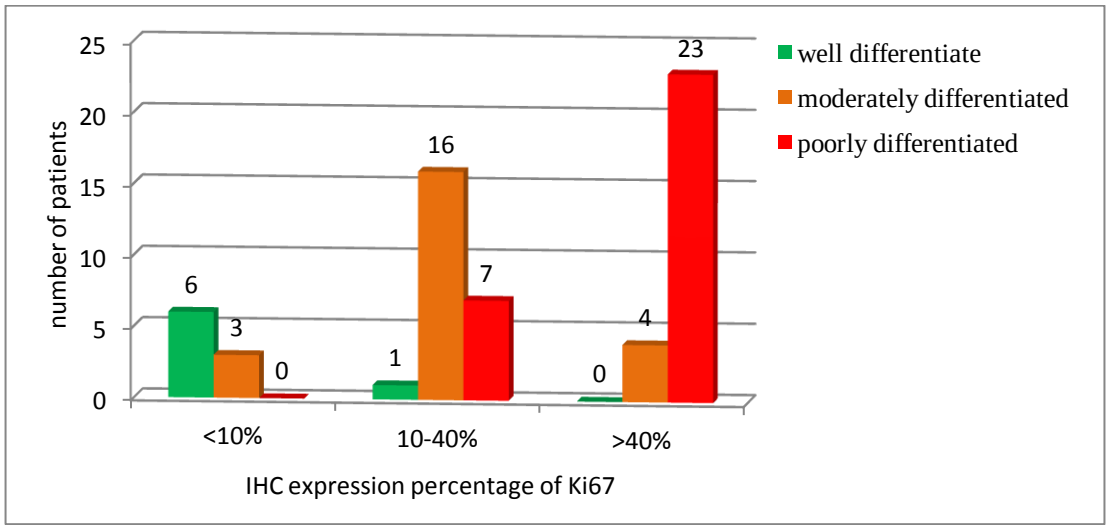

Figure (6). Association between differentiation of gastric adenocarcinoma and different IHC expression percentages of Ki67

IV. DISCUSSION

Gastric cancer is a malignant epithelial tumor, overwhelmingly adenocarcinoma (90%). originating from glandular epithelium of the gastric mucosa. Around 5% of gastric malignancies are lymphoma (MALT lymphoma). Carcinoid and stromal tumors may occur.[19] Post diagnostic mean survival rate of advanced gastric cancer is approximately 10 to 11 months.[4]

This study detected the IHC expression of EGFR and Ki67 in gastric adenocarcinoma. In this study, the mean age of participants was 56.9 ± 13.5 years, with 33 males (55%) and 27 females (45%) (Figure-1). There were 37 gastrectomy biopsies (61.7%) and 23 endoscopic biopsies (38.3%). For the histology types, 58 cases were of intestinal type and 2 cases of diffuse type. For the differentiation of adenocarcinoma, 7 (11.7%) were well-differentiated, 23 (38.3%) moderately differentiated and 30 (50%) poorly differentiated (Figure-2).

In this study, in IHC expression of EGFR, there were 8 (13.3%), 17 (28.3%), 22 (36.7%) and 13 (21.7%) cases for score 0, 1+, 2+ and 3+ respectively. There is no EGFR IHC expression (score 0) in one cases of moderately differentiated adenocarcinoma and seven cases of poorly differentiated adenocarcinoma. In this study, all cases of low EGFR IHC expression (score 1+) are detected in poorly differentiated adenocarcinoma. In addition, all cases of well differentiated adenocarcinoma were high EGFR IHC expression (score 3+) (Figure-4). So, the association between differentiation of gastric adenocarcinoma and different IHC expression scores of EGFR is statistically significant ($p < 0.05$).

In China study, EGFR positive expression rate in the 78 cases of gastric cancer tissue was 57.7 %(45/78). The high EGFR expression was positively correlated with the position of gastric cancer, tumor size, cell differentiation, invasive depth, lymph node metastasis and TNM staging, but having no obvious relation with gender or age.[20] These finding were relatively similar with this study. The clinical history such as position, size and invasion of tumor was not noted in this study.

In Ki67 IHC expression, there were 9 (15%), 24 (40%) and 27 (45%) cases for <10%, 10-40% and >40% positivity respectively. In IHC expression of Ki67, the high Ki67 expression is mostly detected in moderately and poorly differentiated adenocarcinoma of stomach and low expression was mostly found in well differentiated adenocarcinoma (Figure-6). According to the results, there was statistically significant association between differentiation of adenocarcinoma of stomach and IHC expressions of Ki 67 ($p < 0.05$). Clinically, Ki67 has been shown to correlate with metastasis and the clinical stage of tumors. In addition, it has been shown that Ki67 expression is significantly higher malignant tissues with poorly differentiated tumor cells, as

compared with normal tissue.[10] It has been also found that high Ki 67 expression rate was associated with poor recurrence- free survival, poor progression-free survival, poor disease-specific survival and worsen overall survival.[21]

V. CONCLUSION

Therefore, immunohistochemical expressions of EGFR and Ki 67 are highly associated with the differentiation of gastric adenocarcinoma. The result from this study can support pathologically in treatment and prognosis of adenocarcinoma of stomach.

VI. RECOMMENDATIONS

The relationship between the EGFR expression and clinical prognosis of gastric cancer should be done on further study. Moreover, it is needed to do the advanced researches related to EGFR gene expression in gastric cancer.

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