IMMUNOEXPRESSION OF MATRIX METALLOPROTEINASE-9 IN CARCINOMA STOMACH

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Carcinoma stomach is one of the most lethal cancers with high mortality and morbidity usually diagnosed in late stage. Prognosis of carcinoma stomach is determined by tumor invasion and metastasis so that the molecules participating in these steps become popular as potential prognostic markers. Matrix metalloproteinase-9 is a gelatinase B which breaks down the basement membrane and degrades the extracellular matrix resulting in tumor local invasion and metastasis. A cross-sectional descriptive study was done at Yangon General Hospital and University of Medicine 1 in which 37 gastrectomy specimens of gastric adenocarcinoma were included. MMP-9 immunoexpression and its association with different histological types and grades of carcinoma stomach were determined. The mean age in the carcinoma stomach was 60 ± 9.77 years with male: female ratio of 2.7:1. Out of 37 cases of gastric adenocarcinoma, 11% (4/37) were exophytic, 38% (14/37) were ulcerative, 19% (7/37) were ulceroinfiltrative and 32% (12/37) were diffuse infiltrative type. Histologically, 64.9% (24/37) were intestinal type and 35.1% (13/37) were diffuse type. Regarding histological grades of gastric adenocarcinoma, 18.9% (7/37) were well differentiated and 29.7% (11/37) and 51.4% (19/37) were moderately and poorly differentiated gastric adenocarcinoma respectively. MMP-9 immunoexpression was determined by immunohistochemical technique. All the cases have shown 100% positive immunoexpression. Out of 24 cases of intestinal type gastric adenocarcinoma, 50% (12/24) showed weakly positive and the remaining 50% (12/24) showed strongly positive MMP-9 immunoexpression. In the diffuse type gastric adenocarcinoma, 38.5% (5/13) showed weakly positive and 61.5% (8/13) showed strongly positive MMP-9 immunoexpression (p=0.73). Among 18 cases of well and moderately differentiated adenocarcinoma, 66.7% (12/18) showed weakly positive immunoexpression and 33.3% (6/18) were strongly positive immunoexpression. Out of 19 cases of poorly differentiated adenocarcinoma, 26.3% (5/19) showed weakly positive immunoexpression and 73.7% (14/19) showed strongly positive immunoexpression (p=0.02). The findings of this study has pointed out that MMP-9 immunoexpression was positively associated with histological grades of gastric adenocarcinoma. It is hoped that the results of the study may be useful as baseline data for further research, for predicting prognosis and helpful in better management of carcinoma stomach by introducing targeted therapy against MMP-9.

Keywords: matrix metalloproteinase-9, carcinoma stomach

INTRODUCTION

Carcinoma stomach is the fifth most common cancer worldwide, with an estimated 952,000 new cases (7% of total cancer incidence) and 723,000 deaths (9% of total cancer mortality) in 2012. Almost three quarters of the new cases occurred in Asia, and more than two fifths occurred in China. There is an international variation in stomach cancer incidence, rates in men twice than those in women[1]. According to the data from the Yangon Cancer Registry, the total number of new carcinoma stomach patients admitted to Yangon General Hospital were 355 cases out of 9108 in 2015 (3.9%)[2]. Carcinoma stomach is a curable cancer if it is detected in early stages. However, if diagnosed late, mortality rate rises significantly[1]. The gold standard for diagnosis of gastric carcinoma is histopathological examination of tissue specimens. It is further augmented by advanced methods such as immuno-histochemistry, cytogenetic and molecular techniques in order to detect disease progression, choice of therapeutic agents and for prognosis[3]. Nearly all gastric carcinomas are of the adenocarcinoma. Two major categories are classified by Lauren, intestinal (53%) and diffuse (33%).[3]

Gastric cancer mortality is determined by tumor invasion and metastasis and molecules participating in these steps are regarded as potential prognostic factors. These include growth factors such as epidermal growth factor (EGF), vascular endothelial growth factor (VEGF) and HER-2, cell adhesion molecules such as E-cadherin and dysadherin and matrix metalloproteinases (MMPs) [4].

Matrix metalloproteinases are a family of enzymes involved in the degradation of extracellular matrix and individual enzyme has its own role in tumor invasion and spread [5]. One of the most widely associated pathogenesis of gelatinase, matrix metallo-proteinase-9 (MMP-9), is its main role in extracellular matrix remodeling and angiogenesis. Matrix metalloproteinase-9 breaks down the basement membrane and degrades the extracellular matrix that allow cancer cells to migrate resulting in local invasion and metastasis. Matrix metallo-proteinase-9 is important in initial stage of tumor invasion as they degrade components of basement membrane [6]. Clonal expansion of transformed cells is also an essential step in tumour progression and is facilitated by inflammation and involves a change in equilibrium between proliferation, apoptosis and angiogenesis. MMP-9 plays a key role in these steps through MMP-9-mediated release and activation of non-matrix cytokines, such as TNFα and IL-1β and matrix-associated growth factors, such as VEGF, TGFβ and FGFs and the degradation of growth inhibitors [7]. MMP-9 also triggers the angiogenic switch by mobilising and activating angiogenic mitogens from matrix stores at the onset of tumour-associated angiogenesis [8]. Matrix metalloproteinase-9 expression can be determined by immunohistochemical method, enzyme-linked immunosorbent assay (ELISA) and molecular techniques. In this study, the immunohistochemical expression of matrix metalloproteinase-9 were studied in relation to different histological types and grades of gastric cancer. It provided the information of immunoexpression of matrix metallo-proteinase-9 in gastric cancer which can be helpful in prognosis and better management of the disease.

MATERIALS AND METHOD

A cross-sectional descriptive study was done on 37 gastrectomy specimens of clinically diagnosed carcinoma stomach. The resected specimens of stomach tissue were sent to the Histopathological section of Pathology Department, Yangon General Hospital after proper fixation with 10% buffered formalin. After adequate fixation, tissue processing was done by automatic tissue processor for histological examination by Haematoxylin and Eosin. Histological diagnosis and histological grading according to Lauren classification were done. Then the paraffin wax block was proceeded for immuno-histochemical staining with MMP-9 monoclonal antibody (Rabbit Antihuman MMP9 antigen, CloneEP1255Y: Code: AN504-10M BioGenex, Emergo Europe) by using the peroxidase-antiperoxidase method. Appropriate positive and negative controls were added in parallel with each batch of immunostaining. MMP-9 immunoexpression was observed as brownish staining in the cytoplasm of the cells. MMP-9 immunoexpression was scored by multiplying the percentage of immunopositive cells and the staining intensity [9]. Score 0 was taken as negative immunoexpression, 1-4 was weakly positive immunoexpression and score 6-9 was taken as strongly positive immunoexpression. Data was entered in Microsoft Excel Spread Sheet and was summarized in frequency tables. The proportion and percentage were calculated. Statistical analysis of the data was done by using the SPSS software, version 20 and Fisher's exact test.

RESULTS

During one year study period, 37 cases of gastric adenocarcinoma cases attending the Yangon General Hospital were studied. The mean age in this study was 60 ± 9.77 years with M:F ratio of 2.7:1. The largest number of cases were seen in 50-69 year age group. There was a male preponderance with male to female ratio of 2.7:1.

The location, 56.8% (21/37) were localized at the antrum, 32.4% (12/37) were localized at the body of stomach and the remaining 10.8% (4/37) were localized at the

cardia of the stomach showing that antrum is the most frequent site for gastric adenocarcinoma. Macroscopically, 11% (4/37) were exophytic, 38% (14/37) were ulcerative, 19% (7/37) were ulceroinfiltrative and 32% (12/37) were diffuse infiltrative. These findings showed that ulcerative type is the most prevalent type. Histologically, 64.9% (24/37) were intestinal type and 35.1% (13/37) were diffuse type in which 18.9% (7/37) were well differentiated and 29.7% (11/37) and 51.4% (19/37) were moderately and poorly differentiated gastric adenocarcinoma respectively. Twenty seven percent (10/37) of the cases had invaded up to muscularis externa layer and 73% (27/37) up to serosa layer, 75.7% (28/37) cases revealed lymph node involvement and 24.3% (9/37) were lymph node negative.

There were 4 cases (10.81%) with <30%, 16 cases (43.24%) with 30-70% and 17 cases (45.95%) with >70% positively stained cells. Out of 37 cases, 3% (1/37) showed weak staining intensity, 70% (26/37) showed moderate staining intensity and 27% (10/37) showed strong staining intensity. Among the 37 cases of gastric adenocarcinoma, there were 100% positive immunoexpression with MMP-9 of which 45.9% (17/37) showed weakly positive (scoring 1-4) and 54.1% (20/37) showed strongly positive immune-expression (scoring 6-9).

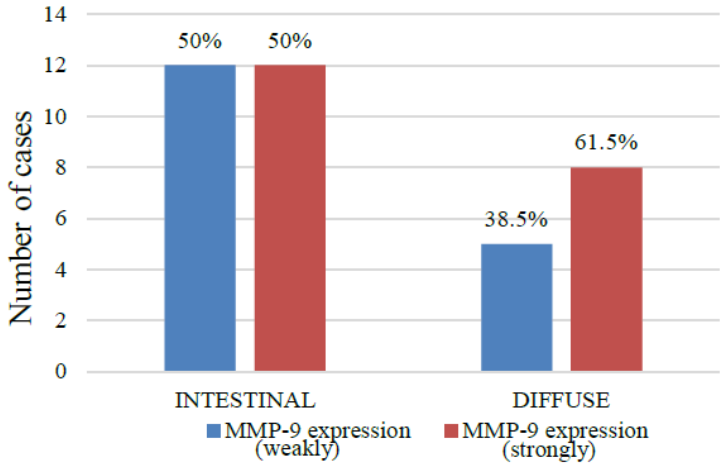

Figure (1) MMP-9 immunoexpression in different histological types of gastric adenocarcinoma(p=0.731)

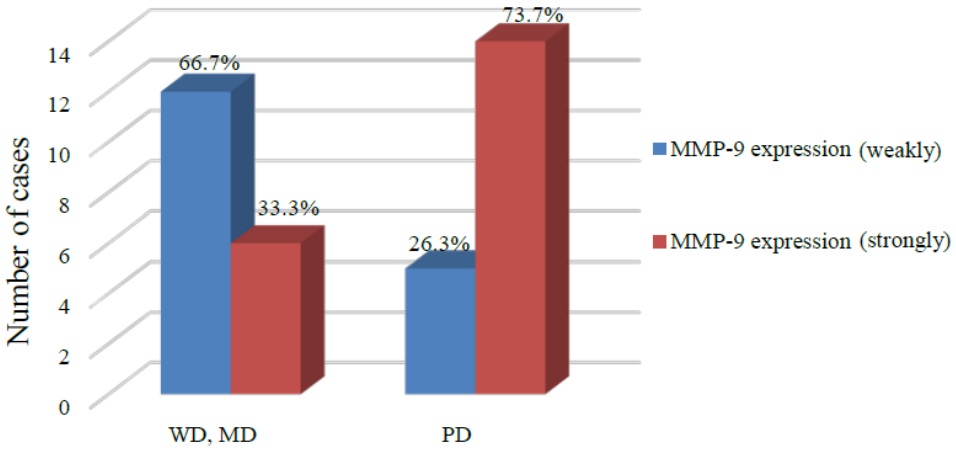

Figure (2) MMP-9 immunoexpression in different histological grading of gastric adenocarcinoma(p=0.022)

Regarding the association between MMP-9 immunoexpression and depth of invasion of gastric adenocarcinoma, weakly positive MMP-9 immunoexpression was seen in 30% (3/10) of cases involving muscularis layer and 51.9% (14/27) of cases involving up to serosa layer. Strongly positive MMP-9 immunoexpression was seen in 70% (7/10) of cases involving muscularis layer and 48.1% (13/27) of cases involving up to serosa layer. However, this result did not reach to statistically significant level between depth of invasion and MMP-9 immunoexpression (p=0.288).

Among the cases, 46.4% (13/28) of lymph node positive tumour showed weakly positive staining and the remaining 53.6% (15/28) showed strongly positive staining. Out of 9 cases of lymph node negative tumour, 44.4% (4/9) were weakly positive and 55.6% (5/9) were strongly positive staining (p=1.0).

DISCUSSION

Gastric adenocarcinoma is a curable cancer if it is detected in early stages. However, the prognosis is still poor because of extensive local tumour invasion and metastasis at the time of diagnosis. Gastric cancer mortality is mainly determined by tumor invasion and metastasis which involve degradation of basement membrane and intercellular matrix and molecules participating in these steps like matrix metalloproteinases are regarded as potential prognostic factors [4]. Matrix metalloproteinase-9 cleave extracellular matrix, invade the surrounding tissue and promote tumor neovascularization by specifically activating angiogenetic factors at the cancer cell matrix interface [10]. So, it is involved in process of tumor biology ranging from initiation to angiogenesis, dessimination, immunological surveillance and metastatic growth [7]. In this study, 37 cases of gastric adenocarcinoma were studied. Histological type and grade were determined by H & E stain and immunoexpression of MMP-9 was detected by PAP method of immune-histochemistry. The association between immunoexpression of MMP-9 and different histological types and grades of carcinoma stomach were found out.

Age distribution of gastric adenocarcinoma

The findings of the current study was also consistent with Ko-Ko-Tun's study in which the highest percentage belonged to the age group 50-70 years [12]. In a study done by Soe-Sandar, the age of most of the gastric cancer patients was found to be 58 years. The mean age was 57.63 years [13].

Rakhshani et al found out that out of 101 gastric carcinomas, the mean age was 60.13±11 years (ranged from 32 to 82 years), and the majority (53.5%) of the patients were involved in 61-82 years of age [14]. Wang et al also pointed out that there were 33 cases under 60 years and 36 cases above 60 years in a study of 69 gastric cancer patients in China [15]. So, the results from the present study were consistent with data from previous studies and showed that the risk of carcinoma of stomach is increased with increasing age.

Gender distribution in gastric adeno-carcinoma

The findings of this study were consistent with Soe-Sandar's study in which the ratio of male to female patients was 1.8:1 showing that male were more affected than females [12]. Most of the study in other countries showed that there was a slight male preponderance with male to female ratio of 2:1 in Kumar et al [16] and 2.3:1 in Shan et al [17]. The findings of the present study were consistent with other international reports. So, the findings of the current study support the results of local and international studies.

Histological types of gastric adeno-carcinoma

According to histological types, the findings were similar to the study done by Soe-Sandar in which majority of patients (56.3%) were diagnosed with intestinal type of gastric adenocarcinoma [13]. There were controversial results regarding the distribution of histological types among the studies worldwide. Shan et al found that the commonest histological type was the intestinal type (44.4%) followed by diffuse type (38.6%) and mixed type (17%) [17]. But, Roberts [18] and Zhu et al [19] showed that diffuse type was the commonest with 53% and 57.3% respectively in their studies.

It has been shown that, the intestinal type seemed to be predominated in high-risk areas and associated with intestinal dysplasia. Diffuse type seems to be less related to environmental influences. As Myanmar is considered as high risk region for the development of gastric carcinoma, the intestinal type may predominate.

Histological grades of gastric adeno-carcinoma

Regarding to the histologic grades, the findings of this study were consistent with Soe-Sandar's study in which the majority of cases (64.6%) were poorly differentiated and the remaining 6.2% and 29.2% were well differentiated and moderately differentiated respectively [13]. Shan et al also found out that there were 73.1% of poorly differentiated adenocarcinoma, 25.2% of moderately differentiated and only 1.7% of well differentiated adenocarcinoma [17]. So, the findings of the present study had supported the findings of both local and international studies in which poorly differentiated adenocarcinoma was the commonest histological grade of gastric adenocarcinoma.

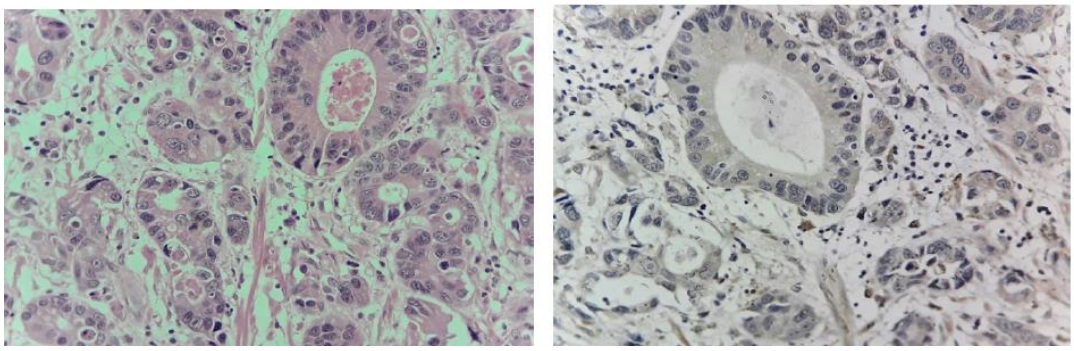

Figure (3) Well differentiated adenocarcinoma (H &E and IHC stain x400X)
MMP-9 immunoexpression score 4 (Case No.25)

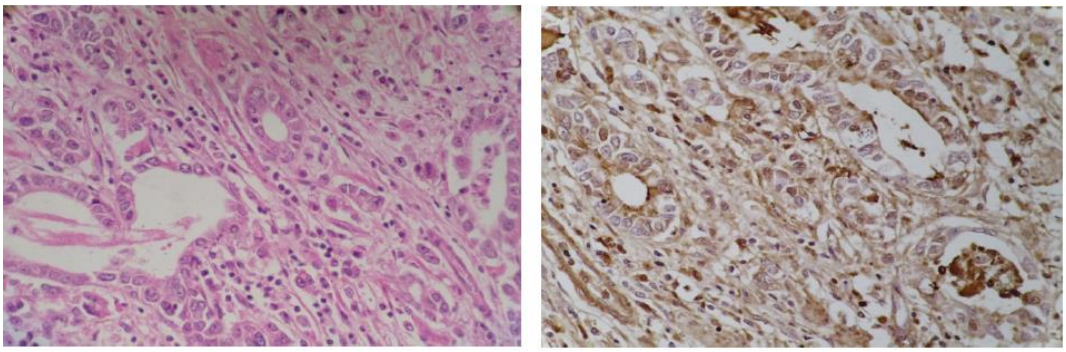

Figure (4) Moderated differentiated adenocarcinoma (H &E and IHC stain x400X)
MMP-9 immunoexpression score 9 (Case No.27)

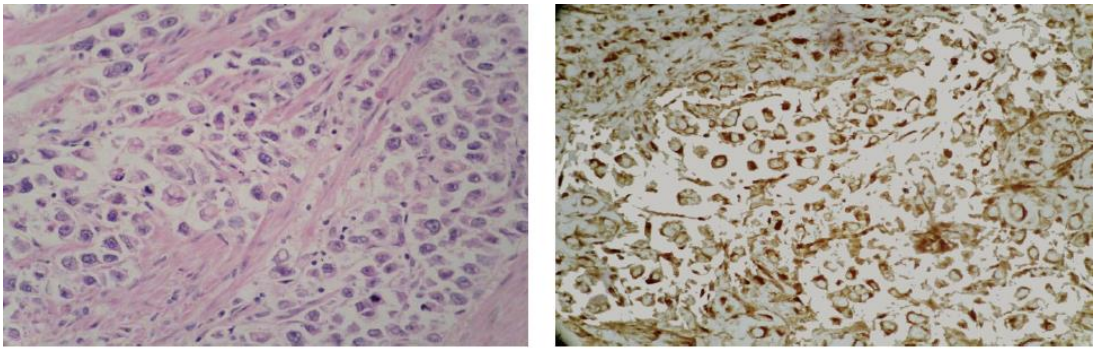

Figure (5) Poorly differentiated adenocarcinoma (H &E and IHC stain x400X)
MMP-9 immunoexpression score 9 (Case No.21)

MMP-9 immunoexpression in different histological types of gastric adenocarcinoma

Regarding to the association of MMP-9 immunopositivity with histologic types, out of 24 cases of intestinal type gastric adenocarcinoma, 50% (12/24) showed weakly positive and the remaining 50% (12/24) showed strongly positive MMP-9 immunoexpression. In the diffuse type gastric adenocarcinoma, 38.5% (5/13) showed weakly positive and 61.5% (8/13) showed strongly positive MMP-9 immunoexpression. The result did not reach to the statistically significant level to show the association between MMP-9 immunoexpression and different histological types of gastric adenocarcinoma (p=0.731). In a study done by Lee et al.,90.5% (38/42) of intestinal type gastric adenocarcinoma and 47.8%(22/46) of diffuse type gastric adenocarcinoma revealed MMP-9 immunopositivity showing significant association between MMP-9 immunoexpression and different histological types of gastric adenocarcinoma($p<0.001$) [8].

MMP-9 immunoexpression in different histological grading of gastric adenocarcinoma

Regarding to the association of MMP-9 immunopositivity with different histologic grades, out of 18 cases of well and moderately differentiated adenocarcinoma, 66.7% (12/18) showed weakly positive immunoexpression and 33.3% (6/18) were strongly positive immunoexpression. Out of 19 cases of poorly differentiated adenocarcinoma, 26.3% (5/19) showed weakly positive immunoexpression and 73.7% (14/19) showed strongly positive immunoexpression (p=0.022). There was a positive association between MMP-9 immunoexpression and different histological grades of gastric adenocarcinoma. MMP-9 immunopositivity was found to be increased when the tumor became poorly differentiated. The study done by Lee et al showed that 77.8% of well differentiated, 80.6% of moderately differentiated and 58.3% of poorly differentiated adenocarcinoma showed MMP-9 immunopositivity. However, there was no statistically significant association between MMP-9 immunoexpression with different histological grade (p=0.406) [8].

MMP-9 immunoexpression with depth of invasion of gastric adenocarcinoma

In the present study, the results did not reach to statistically significant level between depth of invasion and MMP-9 immunoexpression (p=0.288). In the study done by Hwang et al., there was a statistically significant association between MMP-9 immunoexpression and depth of invasion with MMP-9 immunopositivity in 74.5% of tumors with invasion up to submucosa layer, 91.4% of tumors with invasion up to muscularis propia layer, 95.7% of tumors with invasion up to serosa layer and 93.3 % of tumors with invasion to adjacent structures [20].

MMP-9 immunoexpression in lymph node involvement of gastric adenocarcinoma

In the present study, there was no statistical association between lymph node involvement and MMP-9 immunoexpression (p=1.0). Hwang et al found out that there was a signigicant positive association between MMP-9 immunoexpression and lymph node involvement (p=0.038) with MMP-9 immunoexpression in 82.6% of lymph node negative tumor and 95.1% of lymph node positive tumor [20]. In the study done by Lee et al, 62.5% of lymph node negative tumor and 71.4% of lymph node positive tumor showed positive MMP-9 immunoexpression. However, there is no significant correlation between MMP-9 immunoexpression and lymph node involvement (p=0.085) [8].

So, the findings of the present study cannot support international studies and this may be due to the different geographical background and limitations.

CONCLUSIONS

The findings of the study showed that intestinal type was more common than diffuse type and poorly differentiated adenocarcinoma was the most common histological grade. Most of the cases showed tumor penetration up to serosa layer and positive lymph node involvement. MMP-9 immunoexpression was detected in 100% (37/37) of gastric adenocarcinoma of which 45.9% showed weakly positive and 54.1% showed strongly positive immunoexpression.

The finding of this study showed that there was statistically significant association between MMP-9 immunoexpression and different histological grades of gastric adenocarcinoma (p=0.022) with strongly positive MMP-9 immunoexpression in 73.7% of poorly differentiated adenocarcinoma and 33.3% of well and moderately differentiated adenocarcinoma.

Regarding the association of MMP-9 immunoexpression with different histological types, depth of tumor invasion and lymph node involvement, it seemed to have positive association between MMP-9 immuno-expression and increasing stage of tumour but did not reach to statistically significant level. Further studies are recommended to conduct with larger sample size to get more accurate data for MMP 9 immunoexpression. In addition, molecular epidemiology and gene mutation studies of MMP 9 gene should be studied. Other prognostic factors should also be studied to be able to predict the outcome of this disease more accurately. More available IHC panels and reagents would be much more beneficial in assessing other prognostic factors of tumors in the near future.

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