Immunoexpression of Matrix Metalloproteinase-9 in Colorectal Adenocarcinoma

Saung Hnin Phyu[1], Saw Wut Hmone[2], Myint Myint Nyein[2] & Myat Mon[2]*

[1]National Health Laboratory
[2]Department of Pathology
University of Medicine 1, Yangon

Colorectal carcinoma is one of the most common malignant tumors of gastrointestinal tract and is a contributing factor of cancer mortality in Myanmar. Matrix metalloproteinase-9 is a proteolytic enzyme which breaks down extracellular matrix leading to tumor progression, contributing as a potential prognostic marker nowadays. The study was aimed to determine MMP-9 immunoexpression in colorectal adenocarcinoma and its association with Astler-Coller staging. A cross-sectional descriptive study was done on 42 specimens of colorectal adenocarcinoma. All tissue specimens were studied with haematoxylin and eosin to categorize histological types and grades. Out of 42 cases, 21.4% were well differentiated, 57.2% were moderately differentiated and 21.4% were poorly differentiated. According to Astler-Coller staging, 11.9% were found to be in stage B_1, 40.5% in stage B_2, 7.1% in stage C_1, 33.3% in stage C_2 and 7.1% in stage D. 81% (34/42) of colorectal carcinoma showed positive MMP-9 immunoexpression. Positive MMP-9 immunoexpression was seen in 91% of conventional adenocarcinoma, 40% of mucinous carcinoma and no cases signet ring carcinoma. The findings of the study pointed out that MMP-9 immunoexpression was positively associated with histological types of colorectal adenocarcinoma (p=0.001). MMP-9 immunoexpression was positive in 88.9% of well differentiated adenocarcinoma, 91.7% of moderately differentiated adenocarcinoma and 44.4% of poorly-differentiated adenocarcinoma (p=0.007). Regarding the immunoexpression of MMP-9 in different Astler-Coller staging, positive MMP-9 immunoexpression was seen in 60% of the cases in stage B_1, 76.5% in stage B_2, 66.7% in stage C_1, 92.9% in stage C_2 and 100% in stage D. The findings of the study can be extrapolated to predict prognosis and help in better management of colorectal adenocarcinoma by introducing targeted therapy against MMP-9 in future.

Keywords: MMP-9, Colorectal adenocarcinoma, Immunoexpression

INTRODUCTION

Colorectal carcinoma represents the third most common cancer in the world, with nearly 1.4 million new cases diagnosed in 2012.[1] In Myanmar, the total number of colorectal carcinoma patients admitted to YGH were 407 cases in 2010, 630 cases in 2011, 990 cases in 2012 and 485 cases in 2013 according to annual report of YGH.[2] Colorectal carcinoma is a significant clinical problem and staging of tumor at the time of diagnosis is the most important prognostic indicators of colorectal carcinoma. The prognosis of colorectal cancer is directly correlated with the extent of tumor invasion and metastasis. Tumor cells invasion and metastasis are regarded as a multi-step

*To whom correspondence should be addressed
Tel: +95-9795309442
E-mail: saung11.hninphyu2007@gmail.com
DOI: https://doi.org/1034299/mhsrj.00958

phenomenon, involving the proteolytic degradation of basement membrane and extracellular matrix (ECM).[4]

Matrix metalloproteinases (MMPs) plays an important role in degradation of ECM and basement membrane. There are 28 subtypes of matrix metalloproteinases, from MMP-1 to MMP-28. MMP-9 is called gelatinases B or 92 kDa type IV collagenases.[5] High expression of matrix metalloproteinase-9 (MMP-9) correlates with invasiveness and metastatic potential in many tumors.[6]

Significant expression of MMP-9 was demonstrated in primary colorectal adeno-carcinoma tissue compared with normal mucosa and colorectal adenoma.[6] Tumor cells as well as stromal cells within the primary colorectal tumors are principal MMP-9 producers and may promote invasion and metastasis by cancer cells.[7] MMP-9 also degrades the extracellular matrix resulting in facilitation of tumor progression, including invasion, metastasis and growth.[8]

Furthermore, MMP-9 promotes tumor neovascularization by specifically activating angiogenetic factors at the cancer cell matrix interface.[9] It is also involved in the process of colorectal tissue remodeling and carcinogenesis and metastasis including cell adhesion, migration, tissue invasion, vascular invasion.[6] MMP-9 expression is associated with disease-free survival as well as overall survival of patients with colorectal cancer.[4]

MMP-9 overexpression in primary tumor epithelial cells may represent a highly specific molecular marker for both the prognosis and predictive stratification of patients with colorectal cancer. This study was a preliminary study on MMP-9 expression in colorectal adenocarcinoma. MMP-9 can offer an ideal candidate for targeted anti-metastatic therapy in colorectal carcinoma.[6]

MATERIALS AND METHODS

A cross-sectional descriptive study was done on clinically diagnosed colorectal carcinoma cases attending all surgical units of Yangon General Hospital (YGH). This study was carried out starting from August, 2015 to December, 2016. All resected specimens of histologically proved colorectal adeno-carcinoma tissue sent to Histopathology Department of YGH were included. Scopic biopsies and secondary metastatic carcinoma to colon were excluded. The sample size was calculated by using following formula.[7]

$$N = \frac{4 Z^2 p (1-p)}{W^2}$$

N = Minimum required sample
Z = Standard normal deviate for alpha. Alpha=0.05, z=1.96
P = Proportion of MMP-9 expression=0.69 (Yang *et.al*, 2014)
W = Total width of confidence interval=0.28

Forty-two resected specimens of colorectal adenocarcinoma cases sent to Histopathology Department of YGH were included. Scopic biopsies, secondary metastatic carcinoma were excluded. Biopsied specimens were fixed with 10% buffered formal saline. Macroscopic examination, cutting and careful sampling of the specimen were done.

The site and size of tumor, level of invasion and lymph node involvement were noted. After adequate fixation, tissue processing by automatic tissue processor for histological examination was done. Tissue sections from colectomy specimens were embedded with paraffin wax. After embedding, sectioning were done by microtome and stained with hematoxylin and eosin. H & E stained tissue sections were carefully examined under ordinary light microscope to determine histological type and histological grading.

Staging of colorectal carcinoma was done according to Astler-Coller staging.[8] One representative paraffin block of each case was further processed for IHC staining with MMP-9 monoclonal antibody (Rabbit Anti-human MMP-9 antigen, Clone EP1255Y: Code: AN504-10M BioGenex, Emergo Europe) by using the peroxidase-anti-peroxidase method. Negative control

(normal lung tissue) was used for each batch of IHC staining process.

MMP-9 immnopositivity was observed as the brownish precipitates in the cytoplasm of the tumor cells. MMP-9 immunoexpression was determined by scoring system resulting from the sum of degree of intensity of staining and percentage of the cells stained. Intensity of staining was done according to Chu, *et al*.[4] in which 0 - negative (no staining), 1 - weak (pale yellow), 2- moderate (yellow), and 3 - strong (brown). The percentage of cells stained in 500 tumor cells[12] (0-<15% of the cells stained, 1-16-30% of the cells stained, 2-31-60% of the cells stained, and 3->60% of the cells stained). Scoring=intensity of staining + percentage of cells stained. Score 3-6 was considered as positive immunoexpression and score 0-2 was considered as negative immunoexpression.[13]

Data analysis

Statistical analysis of data was done by using the SPSS software, version 20. The association between MMP-9 immunoexpression and staging of colorectal adenocarcinoma was tested using Chi-square test.

Ethical consideration

This study was carried out according to the Guidelines of Research and Ethical Committee. Written informed consent was taken from the patient after explanation of the procedure prior to the research. Patients had right to refuse to participate in the research as well as right to withdraw from the research at any time without reason. There was no interference of the treatment by refusing to participate in the research. This study was done only on the biopsy specimen of surgical operations (colectomy specimen).

It was used only for this research. At the end of the research, the leftover tissue samples were discarded according to Universal Safety Precaution. There was no incentive and no extra charges for participation of this research. Confidential of the information collected was strictly maintained. It was researcher's responsibility for scientific misconduct. Result of the study was used only for health care research purpose.

RESULTS

Immunohistochemical staining

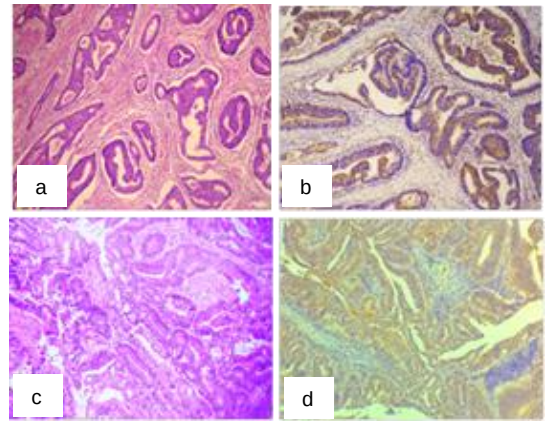

Fig. 1 (a) well differentiated adenocarcinoma (H & E stain x 100), (b) well differentiated adenocarcinoma showing positive MMP-9 immunoexpression (IHC stain x 100), (c) moderately differentiated adenocarcinoma (H & E stain x 100), (d) moderately differentiated adenocarcinoma showing positive MMP-9 immunoexpression (IHC stain x 100)

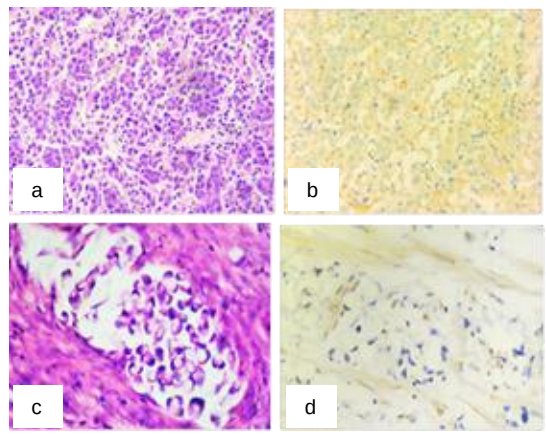

Fig. 2 (a) poorly differentiated adenocarcinoma (H & E stain x 400), (b) poorly differentiated adenocarcinoma showing positive MMP-9 immunoexpression (IHC stain x 400), (c) signet ring carcinoma rectum (H & E stain x 400) (d) signet ring carcinoma rectum showing negative MMP-9 immunoexpression (IHC stain x 400)

According to age group distribution among 42 cases of colorectal adenocarcinoma, the largest numbers of cases were seen in 40-59 years age group. The mean age of this study was 53 years±13.22 years. The youngest age of the study was 19 years and the oldest was 76 years. There was a female preponderance with male: female ratio of 1: 1.8. Conventional adenocarcinoma was found to be predominant histologic pattern. Moderately differentiated was found out to be the commonest histologic grade in this study. In this study, positive MMP-9 immunoexpression was seen in most of conventional adenocarcinoma, however negative in all cases of signet ring carcinoma (Fig. 1, 2 & Table 1).

Table 1. Characteristics of the adenocarcinoma colon cases among study population

Types	Number of patients	Patients (%)
Age		
<20 years	1	2.4
20-39 years	7	16.7
40-59 years	19	45.2
≥60 years	15	35.7
Sex		
Male	15	35.7
Female	27	64.3
Macroscopic types		
Polypoid	14	33.3
Ulcerative	15	35.7
Infiltrative	13	31
Histologic types		
Conventional adenocarcinoma	35	83.3
Mucinous carcinoma	5	11.9
Signet ring carcinoma	2	4.8
Histological grading		
Well differentiated	9	25.4
Moderately differentiated	24	57.2
Poorly differentiated	9	25.4
Depth of invasion		
Muscularis	8	19
Serosa	34	81
Lymph node involvement		
Negative	22	52.4
Positive	20	47.6
Aster-Coller Staging		
Stage B1	5	11.9
Stage B2	17	40.5
Stage C1	3	7.1
Stage C2	14	33.3
Stage D	3	7.1

There was a statistical association between histologic type of colorectal carcinoma and MMP-9 immunoexpression (p=0.001).

Table 2. The correlation of matrix metalloproteinase-9 (MMP-9) expression and pathological features in colorectal cancer patients

	MMP-9 immunoexpression		p value
	Positive n(%)	Negative n(%)	
Histologic types			
Conventional adenocarcinoma	32(91.4)	3(8.6)	0.001*
Mucinous carcinoma	2(40.0)	3(60)	
Signet ring carcinoma	0(0)	2(100)	
Histologic grading			
Well differentiated	8(88.9)	1(11.5)	0.007*
Moderately differentiated	22(91.7)	2(8.2)	
Poorly differentiated	4(44.4)	5(55.6)	
Depth of invasion			
Muscularis	5(62.5)	3(37.5)	0.325
Serosa	29(85.3)	5(14.7)	
Lymph node involvement			
Positive	18(90)	2(10)	0.243
Negative	16(72.7)	6(27.3)	
Astler-Coller staging			
Stage B1	3(60)	2(40)	0.3244
Stage B2	13(76.5)	4(23.5)	
Stage C1	2(66.7)	1(33.3)	
Stage C2	13(92.9)	1(7.1)	
Stage D	3(100)	0(0)	

*p< 0.05 (Statistically significant)

There was a statistical association between histologic grading and MMP-9 immunoexpression (p=0.007). MMP-9 immunoexpression was significantly increased in well and moderately differentiated adenocarcinoma whereas poorly differentiated adenocarcinoma showed relative reduction of MMP-9 immunoexpression than other 2 types (Table 2).

DISCUSSIONS

Colorectal carcinoma is one of the most common malignant tumors of gastrointestinal tract in the world. Despite earlier diagnosis and progression in radical surgery, radiotherapy, and neoadjuvant chemotherapy, many colorectal cancers remain incurable. The mortality of colorectal cancer has been increasing due to early metastasis. The prognosis of colorectal cancer is directly correlated with the extent of tumor invasion and metastasis.[4]

In this study, 42 cases of colorectal carcinoma were studied. Histological types, grading and

Astler-Coller Staging were determined by H & E stain. Immunoexpression of MMP-9 was detected by peroxidase-antiperoxidase method of immunohistochemistry and the association between MMP-9 and Astler-Coller staging were evaluated.

Age and gender distribution among colorectal carcinoma

In Thein Myint's study,[14] age distribution ranged from 33-79 years.The peak incidence was also found between 40-59 years old. The mean age was 56.7 years.[14] Although age is the most important risk factor in colorectal cancer,[15] the peak incidence of colorectal cancer in this study is between 40-59 years old. This may be due to the fact that incidence of colorectal cancer might be increasing in younger population in this country due to the genetic, dietary pattern such as consuming high fat diet, red meat, alcohol and lack of physical activity. In this study, male to female ratio was 1: 1.8. Khin Thida Aung and Win Shwe Zin accounted that male to female ratio of colorectal cancer was 1: 1.1 and 1: 1.5, respectively.[16, 17] By comparing the above finding, female preponderance and the male to female ratio in the present study was consistent with the findings of other studies.

Macroscopic types of colorectal adenocarcinoma

The finding of this study supported the study of Omran and Thabet in which 46.63% were ulcerative pattern, 38.18% were polypoid pattern and 10 cases 18.18% were infiltrative pattern in 55 cases of colorectal adenocarcinoma.[12]

Histological grades of colorectal carcinoma

These findings were similar to those in western countries in which well differentiated adenocarcinoma was 20%, moderately differrentiated adenocarcinoma 60% and poorly differentiated adenocarcinoma 20%.[18]

Astler-Coller staging in colorectal carcinoma

In the study done by Swe Zin Myint, there were no cases in stage A, 9.6% in stage B1, 26.9% in stage B2, 3.8% in stage C1 and 34.6% in stage C2 and 25% in stage D.[19] Regarding the figures in western countries when screening programs were effective and investigative procedures were more advanced, 5.4% of colorectal carcinoma were diagnosed as Astler-Coller stage A, 19% as stage B1, 32.4% as stage B2, 4.5% as stage C1, 30.6% as stage C2 and 8.1% as stage D in total 111 colorectal carcinoma.[20] It can be concluded from all the above findings that most of the colorectal cancer are detected at the minimum of stage B2 in our country unlike western countries. Therefore, early screening in high risk patients can help to diagnose disease at the early stage and also the treatment and it may be beneficial for reducing mortality.

MMP-9 immunoexpression in colorectal carcinoma

According to the study done by Yang, *et al*. positive MMP-9 immunoexpression was observed in 69.1% of colorectal carcinoma.[13] The study done by Omran and Thabet found out MMP-9 immunopositivity in 78.2% of colorectal carcinoma.[12]

MMP-9 immunoexpression in different histological types of colorectal carcinoma

Regarding the association of MMP-9 immunopositivity with histologic types, 91.4% of conventional adenocarcinoma, 40% of mucinous carcinoma and no cases of signet ring carcinoma showed positive MMP-9 immunoexpression (p=0.001). There was a statistical association between histologic types of colorectal adenocarcinoma and MMP-9 immunoexpression.

In the current study, MMP-9 immunoexpression was not increased in mucinous carcinoma and signet ring carcinoma even though these two types are associated with poor prognosis, high grade and aggressiveness.[20] MMP-9 immunostaining was seen in the mucin and stroma indicating that it might be due to the extracellular leakage of MMP-9 into the mucin and stroma. Since MMP-9 has gelatinase activity, the extracellular matrix was degraded

resulting in facilitation of tumor progression and invasion.[13]

MMP-9 immunoexpression in different histologic grading of colorectal carcinoma

In this study, MMP-9 immunoexpression was significantly increased in well and moderately differentiated adenocarcinoma whereas poorly differentiated adenocarcinoma showed relative reduction of MMP-9 immunoexpression than other 2 types. This may be due to the presence of mucinous and signet ring carcinoma classified as poorly differentiated in this category in which MMP-9 immunoexpression was reduced. [8]

The present study was consistent with the study by Yang, *et al.*[13] in that 71.87% of well differentiated adenocarcinoma, 73.68% of moderately differentiated carcinoma and 58.82% of poorly and undifferentiated adenocarcinoma. However, there was no significant differences between MMP-9 immunoexpression and histological grade of tumor differentiation (p=0.565).[13] More cases of mucinous and signet ring cells types should be further studied comfirm the association between these two types and MMP-9 immunoexpression.

MMP-9 immunoexpression in depth of invasion and lymph node involvement of colorectal carcinoma

The study by Chu, *et al.* stated that MMP-9 was highly associated with depth of invasion (p=<0.001) and positive expression was seen in 79.5% of node positive tumor and 46.78% of node negative tumor.[4] In the present study, even there was no significant association of MMP-9 and depth of invasion and lymph node involvement, increased MMP-9 expression was seen in more deeply invaded case and positive nodal involvement cases. It indicated that MMP-9 is an important role in extracellular matrix degradation leading to stromal invasion and lymphatic spread.[10] More available IHC panels for lymphovascular invasion such as D2-40 and CD34 in conjunction with MMP-9 would be much more beneficial in assessing prognostic factors of colorectal cancer.[21]

MMP-9 immunoexpression in Astler-Coller staging of colorectal carcinoma

Other studies associated MMP-9 immunoexpression with Duke staging of colorectal carcinoma. Herszenyi, *et al.*[6] found out that MMP-9 immunoexpression were slightly higher in advanced Duke stage than that in Duke stage A however the differences were not statistically significant with respect to Duke classification.[6] Yang, *et al.*[13] stated that there was a significant association of MMP-9 immunoexpression with staging of colorectal carcinoma with respect to Duke classification (p=0.029).[13] In this study, MMP-9 immunoexpression was increased with stages according to Astler-Coller staging however there was no significant statistical association between MMP-9 immunoexpression and Astler-Coller staging (p=0.324). MMP-9 was highly expressed in stage B2, stage C2 and stage D supporting the fact that MMP-9 enhance tumor cell progression, lymphovascular invasion and spread of malignancy.[10] Further studies are recommended to conduct with equal distribution of all stages of Astler-Coller staging and larger sample size to get more accurate data for MMP-9 immunoexpression.

Conclusion

The finding of this study showed that moderately differentiated adenocarcinoma was the commonest histological grading. Most of the colorectal carcinoma was diagnosed at Astler-Coller stage B2. In this study, MMP-9 immunoexpression was detected in 81% of colorectal adenocarcinoma. MMP-9 immunoexpression was significantly associated with histological types of colorectal adenocarcinoma (p=0.001) in which 91.4% of conventional adenocarcinoma, 40% of mucinous carcinoma and 0% of signet ring carcinoma showed positive MMP-9 immunoexpression.

There is also significant association between histological grading of colorectal adenocarcinoma and MMP-9 immunoexpression (p=0.007). Positive MMP-9 immunoexpression was seen in 88.9% of well

differentiated adenocarcinoma, 91.7% of moderately differentiated adenocarcinoma and 4.4% of poorly differentiated adenocarcinoma.

Regarding the immunoexpression of MMP-9 in different Astler-Coller staging, positive MMP-9 immunoexpression was seen in 60% of the cases in stage B1, 76.5% in stage B2, 66.7% in stage C1, 92.9% in stage C2 and 100% in stage D. Although MMP-9 immunoexpression was seemed to be increased in higher Astler-Coller staging but it was not reached to statistically significant level (p=0.324). Other parameters such as depth of invasion and lymph node involvement also showed increased MMP-9 immunoexpression with increasing stage, but not significant statistically.

Recommendation

Further studies are recommended to conduct with equal distribution of all stages of Astler-Coller staging and larger sample size by using 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 for lymphovascular invasion such as D2-40 and CD34 would be much more beneficial together with MMP-9 in assessing prognostic factors of tumors in future.[21]

Competing interests

The authors declare that they have no competing interests.

ACKNOWLEDGEMENT

We would like to express our sincere gratitude to Professor Dr. Zaw Wai Soe, Rector, UM (1), Yangon for allowing us to perform this research. We would like to thank Professor Dr. Thein Myint, Professor Dr. Thein Lwin and Professor Dr. Myo Myat Thu, surgical units, Yangon General Hospital for their kind permission in collection of clinical data from the patients. We greatfully acknowledge Professor Dr. Daw Cho Cho Nyunt, Department of Pathology, Yangon General Hospital and Dr. Su Lei Htay, Consultant Pathologist, Yangon General Hospital for their kind permission for specimen collection and suggestions, Associate Professor Dr. Hsu Htar Lwin, Department of Preventive and Social Medicine for giving the advices in statistical calculation. We also would like to thank laboratory staffs of Department of Pathlogy, YGH and Pathology Research Laboratory, University of Medicine 1, Yangon for their support.

REFERENCES

1. Cancer research UK. Worldwide cancer statistics 2012. London: England. Available from: <http:// www.cancer researchuk.org/ health-professional/ cancer-statics/ worldwide cancer/ incidence> (Accessed on 14th May 2015)
2. Yangon General Hospital. *Annual Statistical Report of Oncology Department. YGH.* 2012, 2013, 2014.
3. Zuzga DS, Gibbons AV, Lubbe WJ, Onervoneva I & Mario G. Overexpression of matrix metalloproteinase 9 in tumor epithelial cells correlates with colorectal cancer metastasis. *Clinical and Translational Science* 2008; 1(2): 136-141.
4. Chu D, Zhao Z, Zhou Y, Li Y, Li J, Zheng J, Zhao Q & Wang W. Matrix metallo-proteinase 9 (MMP-9) is associated with relapse and prognosis of patients with colorectal cancer. *Annals of Surgical Oncology* 2012; 19(1): 318-325.doi: 10.1245/ s10434-011-1686-3. Epub 2011 Apr 1.
5. Lohi J, Wilson CL, Roby JD & Parks WC. Epilysin, a novel human matrix metallo-proteinase (MMP-28) expressed in testis and keratinocytes and in response to injury. *The Journal of Biological Chemistry* 2001; 276 (13): 10134-10144.
6. Herszenyi L, Sipos F & Galamb O. Matrix metalloproteinase-9 expression in the normal mucosa-adenoma-dysplasia-adenocarcinoma sequence of the colon. *Pathology & Oncology Research* 2008; 14(1): 31-37.
7. Hulley SB. Estimating Sample Size and Power. In: *Designing Clinical Research*. Chapter 6. 3rd ed. Lippincott Williams & Wilkins, 2001; p. 91.

8. Hamilton SR & Aaltonen LA (Eds.). Tumors of colon and rectum. In: *World Health Organization Classification of Tumors. Pathology and Genetics of Tumours of the Digestive System*; IARC Press: Lyon: 2000; 110-111.
9. Roeb E, Dietrich CG, Winograd R, Arndt M, Breuer B, Fass J, *et al*. Activity and cellular origin of gelatinases in patients with colon and rectal carcinoma differential activity of matrix metalloproteinase-9. *Cancer* 2001; 92(1): 2680-2691.
10. Farina AR & Mackay AR. Gelatinase B/MMP-9 in tumor pathogenesis & progression. *Cancer* 2014; 6(1): 240-296.
11. Yu Q & Stamenkovic I. Cell surface-localized matrix metalloproteinase-9 proteolytically activates TGF-beta and promotes tumor invasion and angiogenesis. *Genes and Development* 2000; 14(2): 163-176.
12. Omran OM & Thabet M. Gelatinase A and B expression in human colorectal cancer in Upper Egypt: A clinicopathological study. *Ultrastructural Pathology* 2012; 36(2): 108-116.
13. Yang B, Tang F, Zhang B, Zho Y, Feng J & Rao Z. Matrixmetalloproteinase-9 overexpression is closely related to poor prognosis in patients with colon cancer. *World Journal of Surgical Oncology* 2014; 12(24): 1-6.
14. Thein Myint. Total mesorectal excision (TME) in patients with carcinoma rectum. [Dr MedSc *thesis*], University of Medicine (1): Yangon; 2013.
15. Baxter NN, Virnig DJ, Rothenberger DA, Morris AM, Jessurun J & Virnig BA. Lymph node evaluation in colorectal cancer patients: A population-based study. *Journal of the National Cancer Institute* 2005; 97(3): 219-225.
16. Khin Thida Aung. The significance of apoptosis in colorectal carcinoma. [PhD *thesis*], University of Medicine (1): Yangon; 2005.
17. Win Shwe Zin. Clinicopathological study of colorectal carcinoma in YGH and NYGH (1999-2000). [MMedSc *thesis*], University of Medicine (1): Yangon; 2001.
18. Spence RAJ, Watt PCH & Sloan JM. *Pathology for Surgeon.* 2nd ed. Butterworth-Heineman; 1994; 111-119.
19. Swe Zin Myint. Significant of CEA, CA 19-9, βhCG serum tumor markers in colorectal carcinoma. [PhD *thesis*], University of Medicine (1): Yangon; 2012.
20. Weichert W, Knosel T, Bellach J, Dietel M & Kristiansen G. ALCAM/CD166 is overexpressed in colorectal carcinoma and correlates with shortened patient survival. *Journal of Clinical Pathology* 2004; 57(11): 1160-1164.
21. Muralidharan V, Nguyen L, Banting J & Christophi C. The prognostic significance of lymphatics in colorectal liver metastasis. *HPB Surgery* 2014: ID 954604: 9 pages.