Original Article

The Study of Mismatch Repair [MMR] Genes and Clinicopathological Risk Factors in Treatment of Stage-II Colon Cancer: Preliminary Report of 2-Year Follow-up at Chulabhorn Hospital

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Background: Early-stage colon cancer is increasingly detected by colonoscopic screening. The development of adjuvant chemotherapy in patients with stage-II colon cancer remains a challenge. In Thailand, the clinicopathological risk factors [CPR] are currently used as criteria for the selection of adjuvant chemotherapy in each patient. Previous reports showed that mismatch repair gene [MMR] status could be a prognostic factor for the decision on adjuvant chemotherapy.

Objective: To determine the characteristics of MMR status and CPR of Thai colon cancer cases, with treatment follow-up in stage-II colon cancer by MMR and CPR.

Materials and Methods: This was a preliminary report of patients with stage-II colon cancer who received treatment at Chulabhorn Hospital. MMR status was determined by microsatellite instability [MSI] testing and CPR was determined in each patient. Patients with deficient MMR and low CPR received post-surgery surveillance whereas those with proficient MMR and/or high CPR were treated with adjuvant chemotherapy (5-FU/LV). The follow-up of adverse events, serious adverse events, disease-free survival [DFS], and overall survival [OS] was at the third and fifth years.

Results: During July 4, 2014 to December 31, 2016, there were 31 cases of stage-II colon cancer. All of them were at the stage of T3NoMo (IIA). High CPR and low CPR were found in 20 cases (64.52%) and 11 cases (35.48%), respectively. There were 28 cases with MMR testing results. Proficient MMR (MSI-low) was observed in 23 cases (82.14%). There were 3 cases with disease recurrence, all of which were in proficient MMR group and received adjuvant chemotherapy. Serious adverse events were found in 2 cases with infection during febrile neutropenia after chemotherapy but no treatment-related death was observed. DFS and OS could not yet be evaluated.

Conclusion: Incidence of proficient MMR (MSI-low) in Thai patients with stage-II colon cancer was comparable to that of other countries (80 to 90%). Treatment by adjuvant chemotherapy using MMR status and CPR was feasible with low serious adverse events.

Keywords: Colon cancer, Mismatch repair gene, Clinicopathological risk factors, MSI, Proficient MMR

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Colon cancer is a major public health problem in Thailand nowadays. According to the hospital-based cancer registry of the National Cancer Institute, the incidence of colon cancer is ranked the first in both men and women[1]. Currently, colonoscopy is recommended for colorectal cancer screening[2]; however, it has not been implemented as a national policy and most people in Thailand do not receive colon cancer screening.

Currently, adjuvant chemotherapy is suggested for stage-II colon cancer. The data from meta-analysis[3] showed that adjuvant 5-FU is found to be better than observation, with the 5-year disease-free survival [DFS] of 76% vs. 72%; HR 0.7, but no statistical difference was reported on overall survival [OS]. Hence, it is important to have a prognostic, predictive marker for the selection of adjuvant chemotherapy in patients with stage-II colon cancer.

The current National Comprehensive Cancer Network [NCCN] Guideline recommends using clinicopathological factors [CPR] and mismatch repair [MMR] genes to classify high risk patients for further adjuvant chemotherapy[4] (Table 1). Studies showed that the prognosis of patients not only depends on CPR, but also on molecular markers, such as microsatellite instability [MSI] analysis of MMR genes. Patients with high MSI [MSI-H] or MMR deficiency have better survival rates than those with low MSI [MSI-L] or microsatellite stability [MSS][5]. Approximately 10 to 20% of colon cancer cases are found with MSI-H (MMR deficiency)[6].

As most studies have been conducted in the western countries and no data of MSI/MMR status are presently available for guiding treatment of Thai patients with stage-II colon cancer, hence, this prospective study was set out to utilize both CPR and MSI/MMR status for classifying stage-II colon cancer patients for adjuvant chemotherapy.

Table 1. Clinicopathologic factor according to NCCN Guideline[4]

Clinicopathologic factor
1) Poorly differentiated histology [exclusive of those cancers that are MSI-H]
2) Lymphatic/vascular invasion
3) Bowel obstruction
4) <12 lymph nodes examined
5) Perineural invasion
6) Localized perforation
7) Close or positive margin
8) Pathological T4

Materials and Methods

Study Population

The protocol of this research was reviewed and approved by the Ethics Committee for Human research, Chulabhorn Research Institute (EC No. 008/2557). Inclusion criteria were pre-operative clinical stage-II or pathological stage-II, performance status 0 to 2, aged 18 to 65 years, and patients who did not previously receive chemotherapy. Main exclusion criteria were poor performance status, abnormal blood test including Cr >2 mg/dL and/or SGOT and SGPT >3 times and/or total bilirubin >2 mg/dL, pregnant or lactating women, previous surgery but no tissue specimen or bowel surgery over 4 weeks, and rectal cancer. Patients with pre-operative clinical stage-II would receive surgery and if the stage-II pathological result confirmed, they would continue in this trial. All patients' tissues would be reviewed by pathologists and MSI examination.

MSI analysis by PCR[7-10]

Formalin-fixed paraffin-embedded [FFPE] tissue samples of both cancer and normal cells (size >1x1 cm) with cancer cells not less than 10% of the whole tissues were obtained. The area and volume of normal and cancer cells was identified by pathologists from H&E slide and a section of FFPE was cut onto the slide and cancer and normal cells were separated by micro-dissection or macro-dissection. DNA was extracted from cancer and normal cells using extraction kits and PCR was performed. If no normal cells available, DNA from the patient's blood was used. Markers of 5 positions were used according to the International guideline for evaluation of MSI in colorectal cancer[7], as shown in Table 2. Fragment analysis with ABI3100 or ABI3730XL Genetic Analyzers was performed at 1st base in Singapore or Macrogen in Republic of Korea. The results were analyzed using Gene Mapper program to compare graph differences between cancer and normal cells with the interpretation as follows:

- MSI-high (H) if abnormal marker ≥2 positions
- MSI-low (L) if abnormal marker = 1 position
- MSS if none of abnormal marker
- In the MSI-L group, if the abnormality was only found to D5S346, D2S123 or D17S250, additional BAT 40 examination was performed. If the BAT 40 was abnormal, then it was called MSI-H[1].

Treatment management

Figure 1 summarizes the flow of treatment for stage-II colon cancer patients in this study. Treatment with adjuvant chemotherapy was considered by CPR and MSI. If patients had proficient MMR [MSI-L], regardless of CPR status, adjuvant chemotherapy would be given as 5-FU [425 mg/m^2 D1-D5] and leucovorin [20 mg/m^2 D1-D5] of all 6 cycles. Patients with deficient MMR (MSI-H) but had high-risk CPR (except poorly differentiated tumors) would also be given adjuvant chemotherapy. Only patients with deficient MMR (MSI-H) and low-risk CPR would receive surveillance.

Statistical methods

Demographic data were analyzed using descriptive statistics. Primary endpoint was to study the treatment outcomes using MSI/MMR and CPR status for adjuvant therapy, with 3-year and 5-year follow-up of DFS and OS. However, this was the preliminary report of 2-year follow-up without DFS and OS. Co-primary endpoint was to determine the prevalence of MSI/MMR and CPR status in Thai colon cancer patients. The side effects of 5-FU/LV therapy were also evaluated.

Results

During July 4, 2014 to December 31, 2016, there were 47 colon cancer patients in the study. Of those, 16 patients were excluded due to post-operative stage-III colon cancer. The remaining of 31 patients with stage-II colon cancer was included, with complete demographic data and adjuvant chemotherapy results. Until December 31, 2016, 28 patients had MMR/MSI and CPR results. The mean age of patients was 54 years, with slightly more males than females. Most of them had colon cancer located on the left side (70.96%), and all of them are stage-IIA.

Of the 28 patients with known MMR/MSI report, there were 23 cases (82.14%) with proficient MMR (MSI-L or MSS). When classified by CPR, there were 20 cases of high-risk CPR out of 31 cases (64.52%) (Table 3). When analyzing 28 patients with known MMR/MSI status, it was noted that those in the proficient MMR (MSI-L or MSS) group had two times higher proportion of high-risk CPR than low-risk CPR. However, patients in the deficient MMR [MSI-H] group had a slightly higher proportion of low-risk CPR than high-risk CPR (Table 4).

Of the 31 patients, there were 3 in the surveillance group, while the other three in the chemotherapy group refused to receive chemotherapy. The remaining of 25 patients received adjuvant chemotherapy, with side effects including oral mucositis, diarrhea, and neutropenia. Meanwhile, the side effects grade >3 were neutropenia (2 cases), febrile neutropenia (2 cases), oral mucositis (1 case), and diarrhea (1 case). No patients died from chemotherapy (Table 5).

Table 2. International guideline for evaluation of MSI in colorectal cancer[7]

Reference panel			
Marker	Repeating unit		Location
BAT25	Mononucleotide		4q12/c-kit
BAT26	Mononucleotide		2p16.3/hMSH2
D5S346	Dinucleotide		5q21/APC
D2S123	Dinucleotide		2p16/hMSH2
D17S250	Dinucleotide		17q11.2/BRCA1
Criteria for interpretation			
No. of markers	5 loci	> 5 loci	Interpretation
	≥2	≥30-40%	MSI-H (high)
	1	< 30-40%	MSI-L (low)
	0	0	MSS (microsatel lite stable)

Discussion

Currently, treatment of 5-FU chemotherapy in patients with stage-II colon cancer in Thailand is often

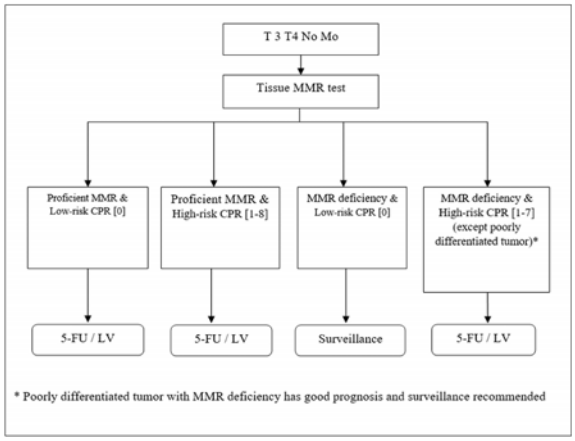

Figure 1. Adjuvant chemotherapy by clinicopathological risk factors [CPR] and MMR status.

based on clinicopathological risk. According to NCCN guideline[4], MSI status is also recommended for treatment consideration. MSI is a major hallmark of MMR with an increase or absence of two or more single or multiple nucleotide units, such as A or CA. Studies showed that patients with MSI-H had better survival rates than those with MSI-L or MSS[5], but do not respond to 5FU[11], whereas other findings revealed that MSI status is not related to the effect of chemotherapy[5]. In Thailand, a retrospective study in 140 patients to identify the association of MMR gene and prognosis in colon cancer patients receiving 5-FU chemotherapy was reported[12]. It was found that the abnormality of MMR could not determine prognosis in those with colon and rectal cancer receiving 5-FU chemotherapy. Unlike other studies, Kanjanapradit et al included patients in stage-II, -III and -IV, not just stage-II and -III. In addition, MMR deficiency was noted in 25.7% of their cases, which was slightly high compared to other countries and our study of 17.86%. In this study, the use of MMR status with CPR increased the number of cases who needed post-surgical chemotherapy. Nonetheless, whether treatment outcomes would be satisfactory or not should be pending upon the results of DFS and OS at 3- and 5-year follow-up. When considering the side effects of adjuvant chemotherapy in this study, the most common included oral mucositis, diarrhea, and neutropenia. There were 2 cases of febrile neutropenia and completely cured after treatment. No patients died from chemotherapy.

Table 3. Characteristics of colon cancer patients in this study (n = 31)

Patient characteristics	Number	Percent
Age (years)		
(Mean, SD)	(54.03, 10.84)	
Gender		
Male	16	51.61
Female	15	48.39
CA colon site		
Ascending	4	12.90
Cecum	1	3.23
Descending	4	12.90
Left splenic flexure	0	0.00
Right hepatic flexure	1	3.23
Sigmoid	18	58.06
Transverse colon	3	9.68
Pathological staging		
T3NoMo (stage IIa)	31	100.00
T4NoMo (stage IIb)	0	0.00
Result of tissue for MMR*		
Proficient MMR (MSS)	23	82.14
MMR deficiency (MSI-H)	5	17.86
Clinical pathological risk factors [CPR]		
High risk	20	64.52
Low risk	11	35.48

*28 cases

Table 4. Management classified by clinicopathological factors [CPR] and MMR status

Flow of management			N	%
Proficient MMR	Low-CPR	5-FU/LV	8	28.57
Proficient MMR	High-CPR	5-FU/LV	15	53.57
MMR deficiency	Low-CPR	Surveillance	3	10.72
MMR deficiency	High-CPR	5-FU/LV	2	7.14
Total			28	100.00

Conclusion

Incidence of proficient MMR (MSI-L) in Thai patients with stage-II colon cancer was 82.14%, which was similar to 80 to 90% reported from other countries.

Table 5. Reported number of adverse events

Adverse	Grade 1 to 2	Grade ≥3	Total
GI side effect			
Oral mucositis	11	1	12
Nausea/vomiting	2	-	2
Hiccup	1	-	1
Diarrhea	7	1	8
Abdominal pain	1	-	1
Transminitis	2	-	2
Hematologic SE			
Neutropenia	5	2	7
Febrile neutropenia	-	2	2
Dermatologic SE			
Rash	1	-	1
Skin hyperpigmentation	2	-	2
Others			
Fever	1	-	1
Dizziness	1	-	1
Fatigue	1	-	1
Hyponatremia	1	-	1
Hypokalemia	1	-	1
Insomnia	1	-	1

The use of MMR status with CPR increased the number of patients receiving post-surgical chemotherapy. Treatment by adjuvant chemotherapy with MMR status and CPR is feasible with low serious adverse events.

What is already known on this topic?

In Thailand, a retrospective study in 140 patients to identify the association of mismatch repair gene and prognosis in colon cancer patients receiving 5-FU chemotherapy found that the abnormality of MMR could not determine prognosis in those with colon and rectal cancer receiving 5-FU chemotherapy. Unlike our study, the present study included patients in stage-II, -III, and -IV, not just stage-II and -III[12]. MMR deficiency was noted in 25.7% of their patients, which was slightly high compared to other countries and our study of 17.86%.

What this study adds?

This prospective study is the first study in Thailand that uses both CPR and MSI/MMR status for adjuvant chemotherapy in patients with post-surgical stage-II colon cancer. No study in Thailand had previously shown data of CPR. Our study showed that those in the proficient MMR (MSI-L) group had two times higher proportion of high CPR than low CPR. However, patients in the MMR deficiency (MSI-H) group had slightly higher proportion of low CPR than high CPR. The use of MMR status with CPR increased the number of patients receiving post-surgical chemotherapy. Nonetheless, whether treatment outcomes would be satisfactory or not will be pending upon the results of DFS and OS at 3-and 5-year follow-up.

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Potential conflicts of interest

The authors declare no conflict of interest.

References

1. National Cancer Institute, Department of Medical Services, Ministry of Public Health. Hospital-based cancer registry [Internet]. 2011 [cited 2017 Jul 12]. Available from: http://www.nci.go.th/th/File_download/Nci%20Cancer%20Registry/Hospitalbase2011.pdf.
2. Rex DK, Johnson DA, Anderson JC, Schoenfeld PS, Burke CA, Inadomi JM. American College of Gastroenterology guidelines for colorectal cancer screening 2009 [corrected]. Am J Gastroenterol 2009;104:739-50.
3. Gill S, Loprinzi CL, Sargent DJ, Thome SD, Alberts SR, Haller DG, et al. Pooled analysis of fluorouracil-based adjuvant therapy for stage II and III colon cancer: who benefits and by how much? J Clin Oncol 2004;22:1797-806.
4. National Comprehensive Cancer Network. NCCN Guidelines® & Clinical Resources [Internet]. 2017 [cited 2017 Oct 1]. Available from: http://www.nccn.org/professionals/physician_gls/f_guidelines.asp.
5. Kim GP, Colangelo LH, Wieand HS, Paik S, Kirsch IR, Wolmark N, et al. Prognostic and predictive roles of high-degree microsatellite instability in colon cancer: a National Cancer Institute-National Surgical Adjuvant Breast and Bowel Project Collaborative Study. J Clin Oncol 2007;25:767-72.
6. Roth AD, Delorenzi M, Tejpar S, Yan P, Klingbiel D, Fiocca R, et al. Integrated analysis of molecular and clinical prognostic factors in stage II/III colon cancer. J Natl Cancer Inst 2012;104:1635-46.
7. Boland CR, Thibodeau SN, Hamilton SR, Sidransky D, Eshleman JR, Burt RW, et al. A National Cancer Institute Workshop on Microsatellite Instability for cancer detection and familial predisposition: development of international criteria for the determination of microsatellite instability in colorectal cancer. Cancer Res 1998;58:5248-57.
8. Dietmaier W, Wallinger S, Bocker T, Kullmann F, Fishel R, Ruschoff J. Diagnostic microsatellite instability: definition and correlation with mismatch repair protein expression. Cancer Res 1997;57:4749-56.
9. Bocker T, Diermann J, Friedl W, Gebert J, Holinski-Feder E, Karner-Hanusch J, et al. Microsatellite instability analysis: a multicenter study for reliability and quality control. Cancer Res 1997;57:4739-43.
10. Drobinskaya I, Gabbert HE, Moeslein G, Mueller W. A new method for optimizing multiplex DNA microsatellite analysis in low quality archival specimens. Anticancer Res 2005;25:3251-8.
11. Ribic CM, Sargent DJ, Moore MJ, Thibodeau SN,

French AJ, Goldberg RM, et al. Tumor microsatellite-instability status as a predictor of benefit from fluorouracil-based adjuvant chemotherapy for colon cancer. N Engl J Med 2003;349:247-57.

12. Kanjanapradit K, Kanngurn S, Boonpipattanapong T, Sangkhathat S. Prognostic significance of the mismatch repair gene in colorectal cancer patients treated with 5-fluorouracil chemotherapy. Songklanagarind Med J 2011;29:27-37.