ORIGINAL ARTICLE

Immunohistochemical Analysis of Mismatch Repair Deficiency in Colorectal Cancer Patients in Kuantan, Pahang

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ABSTRACT

INTRODUCTION: The deficient mismatch repair (dMMR) status of colorectal cancer (CRC) is the hallmark of a defective DNA mismatch repair (MMR) system. Immunohistochemistry (IHC) indirectly detects the affected gene by the loss of its protein product. This study aimed to assess the frequency of different types of dMMR status and associate them with the clinicopathological characteristics of CRC patients diagnosed at Hospital Tengku Ampuan Afzan (HTAA) and the Sultan Ahmad Shah Medical Centre (SASMEC) using an immunohistochemical method. **MATERIAL AND METHODS:** Formalin-fixed paraffin-embedded (FFPE) tissue blocks of 123 CRC cases were retrieved from the Pathology Department of HTAA and SASMEC for patients diagnosed between 1 January 2017 and 31 December 2018. IHC was performed manually using an Envision Flex Polymer detection kit (Dako) along with four primary anti-mouse antibodies (MLH1, PMS2, MSH2, and MSH6) for the MMR proteins to assess dMMR status. **RESULTS:** Out of 123 cases, 21 (17.07%) showed the loss of one or more MMR protein expression and were dMMR. There was no statistically significant association between pMMR (proficient Mismatch repair) and dMMR cases with regards to clinicopathological factors (age, sex, race, site of the tumour, TNM (Tumour Node Metastasis) staging, bowel wall invasion, lymph node metastasis, lymphovascular invasion, histological type, and tumour differentiation). **CONCLUSION:** IHC is the preferred method and most reproducible test for assessing dMMR status in CRC patients in the histopathology diagnostic laboratory.

KEYWORDS: Colorectal cancer, Immunohistochemistry, MMR, MSI

INTRODUCTION

Colorectal cancer (CRC) is the second most frequently diagnosed carcinoma in females and the third in males,[1] and it causes almost 700,000 deaths each year globally.[2] In Malaysia, CRC is now considered among the most incident cancers in men and placed as the second most frequent cancer in women.[3]

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The significance of microsatellite instability (MSI) in CRC has been explored for more than two decades. Microsatellites are simple repeated sequences of DNA, and they are common findings throughout the human genome.[4] Due to their repetitive nature, microsatellites are sensitive to mismatch errors. Thus, the presence of MSI in CRC is a marker of a dMMR.[5,6] The presence of MSI indicates an increased rate of a genetic hyper-mutability condition. Since the discovery of dMMR in familial CRC in the early 1990s, further exposition of the complex mechanisms of dMMR in both the hereditary and sporadic forms of CRC have led to subset characterization of CRC based on distinctive molecular and clinicopathologic features. CRC with a loss of

expression of MMR proteins (dMMR tumours)[7] is present in about 15% of all CRC cases (12% are sporadic, while the remaining 3% are inherited cases of Lynch Syndrome).[8] Screening for MMR status in CRC was initially intended to recognise patients with Lynch Syndrome. However, accumulated evidence has suggested that dMMR is a substantial biomarker for the prognosis and treatment prediction of CRC.[8] Additionally, the immune escape mechanisms recently described in dMMR CRC are a major indicator of the substantial roles of immunotherapy in the treatment of CRC.[8-10]

Based on this new information, testing for MSI or the loss of an MMR protein is currently recommended for most patients with CRC (NCCN, 2014).[8, 11] Of these two methods, MMR protein expression by IHC has the advantage of not requiring additional instruments other than those are already available in a standard histopathology laboratory. Furthermore, the ability to identify the affected gene by detecting the loss of its protein product has a superior advantage over the molecular identification of MSI. The cost of the test is the main hurdle for examining the dMMR system in all cases of CRC and heading the recommendations for CRC patient testing, especially at institutions that are already financially constrained and where the benefit of having a newer therapy for cancer is beyond reach for the majority of patients. An overview of the prevalence of dMMR in CRC cases in a local setting would allow policymakers to plan to offer the service in the near future.

The present study aimed to assess the presence of dMMR in all CRC tissue samples of patients diagnosed at HTAA and the SASMEC in Kuantan for two years. The dMMR status was then associated with all available clinicopathological information. The dMMR status was tested using the IHC method.

METHODS

Ethical approvals

The study commenced upon receiving ethical approval from both the IIUM Research Ethics Committee (IREC) of the IIUM (IREC 2019-180) and the Medical Research Ethics Committee (MREC) of the Ministry of Health in Malaysia (NMRR-18-3675-45439).

CRC Cases

All CRC cases diagnosed between 1 January 2017 and 31 December 2018 at two tertiary hospitals in Kuantan (HTAA and SASMEC) were identified through the laboratory information database. Only cases with available tissue blocks from surgical resection of the large bowel were included in this study. There were 123 CRC cases. The FFPE blocks of these cases were retrieved and subjected to dMMR analysis.

Demographics and clinicopathological data

The histopathological reports of all cases were reviewed to obtain the following information: age, gender, race, site of the tumour, pTNM (pathological Tumour Node Metastasis) staging, bowel wall invasion, lymph node metastasis, lymphovascular invasion, histological type, and tumour differentiation.

dMMR status assessment

The dMMR status of the CRC cases was analysed by IHC. Initially, H&E staining was performed to verify the cellularity of the FFPE tissue blocks. The stained slides were then reviewed by an experienced histopathologist. Immunohistochemical staining was performed manually using a polymer detection kit (Envision FLEX, DAKO, Denmark) with the following reagents: wash buffer (20X), antigen retrieval solution (50X), peroxidase-blocking reagent, mouse (linker) for MLH1, HRP (Horseradish peroxidase) and 3.3'-diaminobenzidine tetrahydrochloride chromogen.

The FFPE tissue blocks were sectioned into 3-µm thicknesses for MLH1, MSH2, MSH6, and PMS2 detection. Heat-induced epitope retrieval (HIER) with antigen retrieval solution at a pH of 9.0 was carried out using a pressure cooker with protocols 97˚C for 20 min and at 65˚C for 10 min for MLH1, MSH6, and PMS2, and lastly at 121˚C for 30 sec and then at 95˚C for 10 sec for MSH2. The tissue sections for MLH1, MSH2, and MSH6 expression were incubated with ready-to-use anti-mouse primary antibodies for 20 min each, while the incubation period for PMS2 was 30 min. MMR was considered positive when the tumour's nuclei were stained in the presence of positive internal control. Stromal cells and epithelial cells from the colonic mucosa

were considered as the internal control. Negative expression was considered when a loss of the tumour cell's nuclear staining was observed despite the presence of positive internal control (Figure 1). Cases that showed positive nuclear staining for all four MMR proteins in the cancerous tissue were termed as pMMR. Cases that demonstrated negative nuclear staining for at least one or more of the MMR proteins were termed as dMMR.[12,13] This method of reporting has been recommended also by the College of American Pathologists (CAP).[14]

Statistical analyses for the association between dMMR status and clinicopathological data were performed using the Statistical Package for the Social Sciences (SPSS, version 23.0). A chi-square test was used to assess the association of the categorical variables, and $p<0.05$ was considered statistically significant.

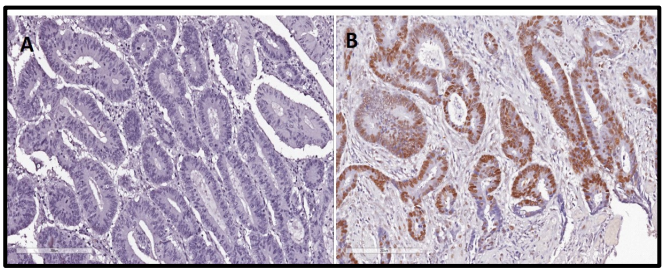

Figure 1: Mismatch repair protein expression (IHC, 200x magnification) A: Negative MMR expression. B: Positive MMR expression.

RESULTS

Demographic and clinicopathological data

The mean age of patients in the study was 61.58 (range: 31–86) years. Of the 123 patients, 73 (59.35%) and 50 (40.65%) were male and female, respectively. Nineteen (15.45%) patients were ≤50 years old, while 104 (84.55%) patients were >50 years old. Most patients were Malays (90 patients, 73.17%), followed by Chinese (30 patients, 24.39%), Indians (2 patients, 1.63%), and other races (1 patient, 0.81%). The tumours' characteristics are shown in Table I.

Mismatch repair status

Out of 123 CRC cases, 102(82.93%) demonstrated positive nuclear staining for MMR proteins and were pMMR, while 21(17.07%) showed a loss of the expression of at least one or more of the MMR proteins and were dMMR. The expression profiles of patients with dMMR are illustrated in Table II. There were no significant associations between MMR status and the demographic and clinicopathological characteristics of the patients (Table III).

Table I: Clinical characteristics of the study sample (n=123)

Demographic variables	**n (%)**
Age	
≤ 50	19 (15.45)
> 50	104 (84.55)
Sex	
Female	50 (40.65)
Male	73 (59.35)
Race	
Malay	90 (73.17)
Chinese	30 (24.39)
Indian	2 (1.63)
Indonesian	1 (0.81)
Clinicopathological data variables	**n (%)**
Tumour site	
Right-sided	28 (22.76)
Left-sided	95 (77.24)
TNM staging	
Stage I	17 (13.82)
Stage II	36 (29.27)
Stage III	65 (52.85)
Stage IV	5 (4.06)
Bowel wall invasion	
pT1	4 (3.25)
pT2	26 (21.14)
pT3	76 (61.79)
pT4	17 (13.82)
Lymph node metastasis	
pN0	56 (45.53)
pN1	44 (35.77)
pN2	22 (17.89)
pN3	1 (0.81)
Lymphovascular invasion	28 (22.76)
Histological differentiation	
Well differentiated	20 (16.26)
Moderately differentiated	100 (81.3)
Poorly differentiated	3 (2.44)
Histological type	
Mucinous	20 (16.26)
Non-mucinous	103 (83.74)

Table II: Details of the immunohistochemistry profiles of the dMMR colorectal cases (n=21)

Types of MMR protein loss	n (%)
hMLH1	1(4.76%)
hMLH1 and hPMS2	6(28.57%)
hMLH1, hMSH2 and hPMS2	2(9.52%)
hMLH1, hMSH2, hMSH6and hPMS2	1(4.76%)
hMSH2	4(19.1%)
hMSH2 and hMSH6	1(4.76%)
hMSH2 and hPMS2	2(9.52%)
hMSH2, hMSH6 and hPMS2	1(4.76%)
hPMS2	3(14.29%)

Table III: The association between the clinicopathological characteristics and the MMR protein expression profile (n=123)

Variables	dMMR (n=21) n(%)	pMMR (n=102) n(%)	*p*-value
Age			
≤ 50	4(19.05)	15(14.71)	0.616
> 50	17(80.95)	87(85.29)	
****Male vs Female***	9 (42.86)	41(40.2)	0.821
Race			
Malay	15(71.43)	75(73.53)	0.626
Chinese	5(23.81)	25(24.51)	
Indian	1(4.76)	1(0.98)	
Other	0(0)	1(0.98)	
Site of tumour			
Right-sided	6(28.57)	22(21.57)	0.486
Left-sided	15(71.43)	80(78.43)	
pTNM staging			
I	2(9.52)	15(14.71)	0.564
II	8(38.1)	28(27.45)	
III	11(52.38)	54(52.94)	
IV	0 (0)	5(4.9)	
Bowel wall invasion			
pT1	0(0)	4(3.92)	0.711
pT2	4(19.05)	22(21.57)	
pT3	13(61.9)	63(61.77)	
pT4	4(19.05)	13(12.74)	
Lymph node metastasis	10(47.62)	46(45.1)	0.833
Lymphovascular invasion	4 (19.05)	24(23.53)	0.656
Histological differentiation	2 (9.5)	18(17.7)	0.515
Well	18(85.7)	82(80.4)	
Moderate	1(4.8)	2(2.0)	
Poor			
****Mucinous vs non-mucinous***	5(23.81)	15(14.71)	0.303

*Data for *male, ** Mucinous type. p-value is significant at < 0.05. Chi-square test.*

DISCUSSION

The study included 123 CRC cases from two tertiary public hospitals (HTAA and SASMEC) in Kuantan, Pahang. The cases were diagnosed with CRC in 2017 and 2018. Based on data from the Malaysian Statistics Department in 2010, the three main races in Malaysia were Malays (54.7%), Chinese (24.6%), and Indians (7.3%).[15] However, according to the Malaysian National Cancer Registry, CRC is more prevalent among the Chinese (38.2%) than in Malays (19.4%) and Indians (19%).[16] In the current study, most CRC cases were diagnosed in Malays [n=90 (73.2%)], while Chinese patients constituted all but a few of the remaining cases [n=30 (24.4%)]. The predominant Malay CRC subjects were well correlated with the geographical location of the study samples, where Pahang is a densely Malay-populated community. The male-to-female ratio of CRC cases in our study was 1.5:1, which is similar to the national statistic of 1.3:1.[16]

Molecular analysis for MSI and IHC for MMR proteins (MLH1, PMS2, MSH2, and MSH6) are the two main methods for determining a patient's MSI or dMMR status.[17] In this study, an IHC method was used to assess mismatch repair status. This method indirectly specifies the possible gene responsible for the mismatch repair deficiency[13, 18] through an assessment of the corresponding protein products.[19] As compared to MSI molecular analysis, IHC is an inexpensive and reproducible method[7] with a highly significant sensitivity (96.7%) and specificity (100%).[20, 21]

Out of the 123 CRC cases studied, dMMR was identified in 17.1% (n=21) [pMMR (n=102 or 82.9%)]. The incidence was almost identical to a recently concluded study (17.1% vs. 16.4%) predominantly done in Malaysian Chinese subjects.[11] The incidence rate was also comparable to various published data with a reported dMMR incidence ranging from 10–18 %.[11, 12, 22-26] There was no statistically significant association on the incidence of dMMR status between the different races. The incidence rates of dMMR cases in Malays, Chinese, and Indians were 71.4% (n=15), 23.8% (n=5), and 4.8% (n=1), respectively, which follows the study's ethnic distributions. At the same time, the dMMR cases among Malays and Chinese were 16.7% (15 out of 90) and 16.7% (5 out of 30), respectively. These same types of non-ethnic biases of dMMR status were also reported in other studies done on similar ethnic groups.[22, 27] This finding is in contrast to a significant tendency for

dMMR CRC among African-Americans compared to Caucasian-Americans.[28]

In our cohort, the most frequently seen dMMR was the concomitant loss of MLH1 and PMS2 in 6 out of 21 cases. This is not a rare finding; many studies have reported similar results.[24,25] In addition, various combinations of protein losses were discovered in this study ranging from a single protein deletion to the absence of all four proteins (1 case). A literature search revealed only a few reports of patients lacking all four proteins.[27]

This study found no significant association between dMMR status and various clinicopathological characteristics. Although there were reports of a relationship between dMMR and CRC in the right colon,[12, 20, 22, 29] this trend was not observed in the current study. Similarly, the study also did not find any association between dMMR status and lymphovascular invasion, as previously reported.[29] Many studies are also in agreement with this finding. The inconsistent association between studies may indicate that the association of dMMR with the site of tumour and lymphovascular invasion are incidental findings.

In this study, most dMMR tumours were the non-mucinous type [76.2% (16 out of 21], which was similar to the findings of a study done by Cheat et al[23] on the multiracial Malaysian population. An insignificant association between the dMMR status and the grade of CRC tumour[27, 29] and the dMMR status and lymph node metastasis[29] has also been discovered and explained by others.

CONCLUSION

This study found a comparable percentage of CRC cases with dMMR status (17%) as prior studies reported elsewhere. Due to the value of MMR status in the prognostication and management of CRC and the discovery of its relative prevalence here, this study, therefore, proposes that MMR status should be routinely identified in all CRC cases. IHC is known to be sensitive, specific, and reproducible, so this method could be the preferred choice of clinicians in many locations.

CONFLICT OF INTEREST

There was no conflict of interest among the authors concerning this study.

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