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ORIGINAL ARTICLE

Pattern of hMLH1, hMSH2 and hMSH6 expression and clinical characteristics in a sample of Malaysian colorectal carcinoma cases

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Abstract

Malignant transformation from normal colonic mucosa to carcinomas may be accelerated by genetic loss or inactivation of genes of the DNA mismatch repair system. The aim of the study was to determine the local incidence and pattern of immunohistochemical expression of mismatch repair proteins namely: hMLH1, hMSH2 and hMSH6 in a series of colorectal carcinomas (CRCs) and correlate this to their clinical and pathological features. Forty-three out of 298 cases of CRCs (14.4%) showed abnormal staining pattern for mismatch repair proteins with a majority (65.1%) showing single hMLH1 loss. Tumours with mismatch repair defect (MMR-d) were frequently found at the right side of colon (p<0.001), poorly differentiated carcinomas (p<0.001), produced more mucin (p=0.007), exophytic growth (p=0.007) and were bigger (p=0.002) than tumours with no mismatch repair defect. Immunohistochemical stains for mismatch repair proteins could be done in local laboratories on these selected cases before referring for the expensive molecular test.

Keywords: mismatch repair defect, colorectal carcinoma, immunohistochemical stain

INTRODUCTION

Colorectal cancer represents one of the major causes of cancer-related morbidity and mortality in the world. It is common among both males and females, and it is estimated that about 5% of the population worldwide will develop colorectal cancer in their lifetime. Colorectal cancer was the most common cancer in males and second most common cancer in females after breast cancer in Malaysia.[1]

The normal colorectal mucosa undergoes progression from adenoma of varying degrees of dysplasia to invasive carcinoma by a series of gene mutations. These may involve activation of proto-oncogenes and inactivation of tumour suppressor genes that have an influence on cell proliferation and programmed cell death. Malignant transformation may also be accelerated by genetic loss or inactivation of genes of the DNA mismatch repair system.

When there are errors or alterations in DNA metabolism such as DNA replication, recombination or repair, genetic information may be altered. One of the safeguard mechanisms in place to correct this and maintain genomic stability is the DNA mismatch repair (MMR) system. The DNA mismatch repair gene rectifies DNA mismatches generated during DNA replication and blocks DNA recombinations occurring between divergent sequences by recognising complementary base pair errors.[2,3] It also contributes to genomic stability by controlling cell cycle checkpoints and is responsible for controlling programmed cell death in response to damaged DNA products.[4,5] Damaged cells are thus eliminated from progressing further in the cell cycle, preventing tumorigenesis. On the other hand, when there is a defect in the DNA mismatch repair system, damaged DNA escapes repair and are passed down the line of replication. This will result in tumour progression.

The DNA mismatch repair system was first demonstrated in bacteria, *Escherichia coli* about 40 years ago.[6] They consist of proteins encoded by MutH, MutL, MutS and MutU genes. The human homologues of the *E.coli* MutS and MutL have been identified. While the MutS and MutL proteins in the *E.coli* are homodimers, their human counterparts function as heterodimeric

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complexes. MutL interacts with MutS to enhance mismatch recognition. Both possess adenosine triphosphatase (ATPase) activity. The human mismatch repair genes were named after their prokaryotic counterparts, e.g. human mutator L homologue 1 (hMLH1) and human mutator S homologue 2 (hMSH2).

Three human MutS homologues (hMSH2, hMSH3 and hMSH6) have been identified. hMSH2 interacts with either hMSH6 or hMSH3 to form hMutSα and hMutSβ heterodimeric complexes respectively. Both complexes play critical roles in mismatch repair initiation. The human MMR components homologous to MutL in the *E.coli* are hMLH1, hMLH3, hPMS1 and hPMS2. hMLH1 interacts with hPMS2, hPMS1 or hMLH3 to form three heterodimeric complexes: hMutLα, hMutLβ or hMutLγ respectively. hMutLα is needed for mismatch repair and hMutLγ for meiosis but the function of hMutLβ remains unclear.

There are several types of repeated DNA sequences in the human genome including satellites, minisatellites, telomeric families and microsatellites. A microsatellite is a short sequence or runs of one to six dinucleotides that is repeated in a tandem array in a human genome. The length of these microsatellites may vary from individual to individual but each person has microsatellites of a set length. When there is a defect in the gene regulating DNA repair, replication errors result and are reflected by widespread variations in short, repeating sequences of DNA microsatellites. Microsatellite instability (MSI) is a condition manifested by damaged DNA when there are mutations in DNA mismatch repair system. Tumours exhibiting this replication error (RER) phenotype are said to have microsatellite instability (MSI) tumour phenotype.

Molecular testing is the gold standard for assessing the DNA mismatch repair competency. This involves extracting DNA from the tumour and normal tissue and then performing polymerase chain reaction amplification and gel electrophoresis of a few chromosomal loci and comparing the microsatellite sequences to detect the microsatellite instability. However, molecular testing is extremely time consuming, labour intensive and expensive. It is also not widely available in most laboratories in our country. An alternative method of detecting mismatch repair defect is by using immunohistochemical tests for mismatch repair proteins; namely against hMLH1, hMSH2, hMSH6 and hPMS2.

Microsatellite instability is seen in most hereditary non-polyposis colorectal cancers (HNPCC) and a subset of sporadic colorectal carcinomas. More than 90% of hereditary non-polyposis colorectal carcinomas are associated with germline mutations of one of the mismatch repair genes, most frequently hMLH1or hMSH2. 7-20% of sporadic cancers were shown to have microsatellite instability.[7-10] This group of patients with microsatellite instability tumours share some similar characteristics to patients with HNPCC tumours.

Supporting evidence from various studies[11,12] demonstrated that carriers of mismatch repair mutations had an increased risk of developing colorectal cancer compared to the general population and when these patients developed colorectal carcinomas, their tumours also behaved differently from microsatellite stable tumours. The patients showed better response to adjuvant 5-fluorouracil-based chemotherapy[13] and had better prognosis and survival rates with lower risk of metastasis.[14] Thus, there is benefit in identifying patients with tumours that have microsatellite instability phenotype in order to manage them accordingly with the appropriate therapy including screening for mutations in other family members. It is thus important to study the constellation of features of these tumours with mismatch repair defect in a local setting to identify the characteristics which could predict and identify these tumours. Tumours with associated features could then be selected for testing by immunohistochemistry in a local laboratory and then referred for confirmation with molecular tests for mismatch repair defects.

The main aim of this study was to determine the incidence and pattern of immunohistochemical expression of hMLH1, hMSH2 and hMSH6 in a series of unselected consecutive colorectal carcinomas in a local hospital and identify any associated clinical and pathological features of the tumours.

MATERIALS AND METHODS

This retrospective study included 298 patients with colorectal carcinomas treated in a public hospital in Johor, Malaysia. The clinical presentations of the patients were reviewed from hospital clinical records and interviews with patients. The length of survival was calculated from the date of first presentation to the final follow-up date or date of death.

Tumour sites were grouped as right-sided for

tumours proximal to and including the splenic flexure and left-sided for those located distal to the splenic flexure. The size of the tumour was taken as the largest cross-sectional diameter (in centimetres). The growth appearance of the tumour was described as exophytic if tumour growth protruded beyond the mucosal surface in a polypoidal or fungating fashion and non-exophytic (endophytic) if it invaded deep into the mucosa forming an ulcerating or flat surface.

The histopathological reports and the original microscopy slides were reviewed to determine the histological grade of tumours and the amount of mucin produced. Typing and grading of tumours were performed according to the World Health Organisation (WHO) tumour classification system.[15] The mucin content was defined as the amount of mucin in the tumour and was categorized as less than or equal to 10% and more than 10%.

Two blocks of 10% formalin-fixed, paraffin wax-embedded colorectal carcinoma tissue were selected per case to include a region of normal colonic mucosa in one of the selected blocks. A total of four sections were cut from each block for immunohistochemistry staining for hMLH1, hMSH2 and hMSH6 together with a section for negative control. Positive nuclear staining of more than 10% of tumour cells was considered positive for protein expression: hMLH1, hMSH2 and hMSH6 (Figures 1A & B). Normal colonic mucosal epithelial cells and lymphocytes were reactive to these proteins and served as internal control. Loss of expression was recorded when all malignant cells showed absent nuclear staining or when less than 10% of tumour cells showed positive nuclear staining (Figures 1C & D). Tumours with loss of expression of one or more proteins were considered to be tumours with mismatch repair defects (MMR-d) while tumours with intact expression for all three proteins were considered to be non MMR-d tumours or intact tumours.

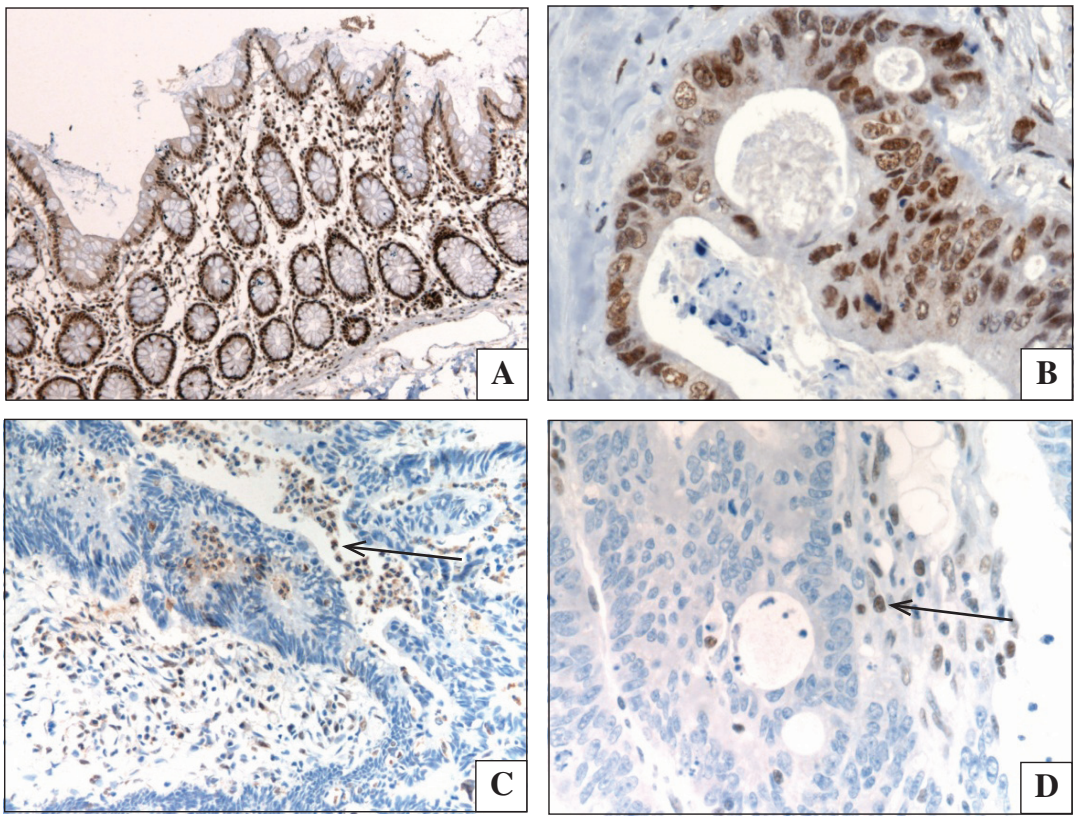

FiG. 1: (A) Normal colonic mucosa epithelium reactive to hMSH2. IHC stain with hMSH2, original magnification x 100. (B) Colorectal carcinoma showing positive reaction to hMLH1. IHC with hMLH1, original magnification x 400. (C) This MMR-d tumour was negative for hMSH2. The lymphocytes (arrow) were reactive to hMSH2 and acted as internal control. IHC with hMSH2, original magnification x 200. (D) Higher magnification showing only lymphocytes reactive to hMLH1 in this MMR-d tumour. IHC with hMLH1, original magnification x 400.

Statistical analysis was performed using the Predictive Analytic Software (PASW), Statistical version 18.0 software program, formerly known as Statistical Package for the Social Sciences (SPSS). The variables were compared using Pearson's Chi-square test, student's t-test or the Mann-Whitney U test, according to the data type. Statistical significance was defined as a *p*-value of less than 0.05. Kaplan and Meier survival curves were plotted. Comparisons of survival rates and their statistical significance were tested using the log rank test where $p<0.05$ was considered significant.

RESULTS

Demography

The age at presentation of the patients in the study ranged between 25.0 and 91.0 years of age and the mean age at presentation was 61.0 years with a standard deviation of 13.0 years. There were more males than females with colorectal carcinomas; with a ratio of 1.26:1. Chinese patients predominated (46.6%), followed by Malay patients (45.0%), Indian patients (6.3%) and patients of other races (2.0%, Figure 2). Chinese were more likely to have colorectal carcinomas than Malays or other races (p=0.001, OR 2.984, 95% CI 1.568 to 5.679).

Of a total of 298 cases of colorectal carcinomas, 255 cases (85.6%) demonstrated normal nuclear expression for all mismatch repair proteins namely hMLH1, hMSH2 and hMSH6, while 43 cases (14.4%) showed abnormal staining patterns for at least one of the three mismatch repair proteins. Among mismatch repair defect tumours, 28 showed complete loss of hMLH1, 7 cases with loss of both hMSH2 and hMSH6, 6 cases of loss of hMSH6 and 2 cases of loss of hMSH2. None of the cases lost all three MMR proteins (Table 1).

More than half of the 43 cases of mismatch repair defect tumours were Malay patients (55.8%) followed by Chinese patients (41.9%) and Indian patients (2.3%). Although Chinese was the predominant race with colorectal carcinomas, it was found that Malay patients had the highest proportion of mismatch repair defect tumours. 17.9% of Malay patients with colorectal carcinomas had mismatch repair defect tumours as compared to 12.9% of Chinese patients and 5.3% of Indian patients.

There was no statistical difference for patients with mismatch repair defect tumours when compared with patients with intact tumours with regards to the gender (p=0.497). However, analysis of female patients revealed

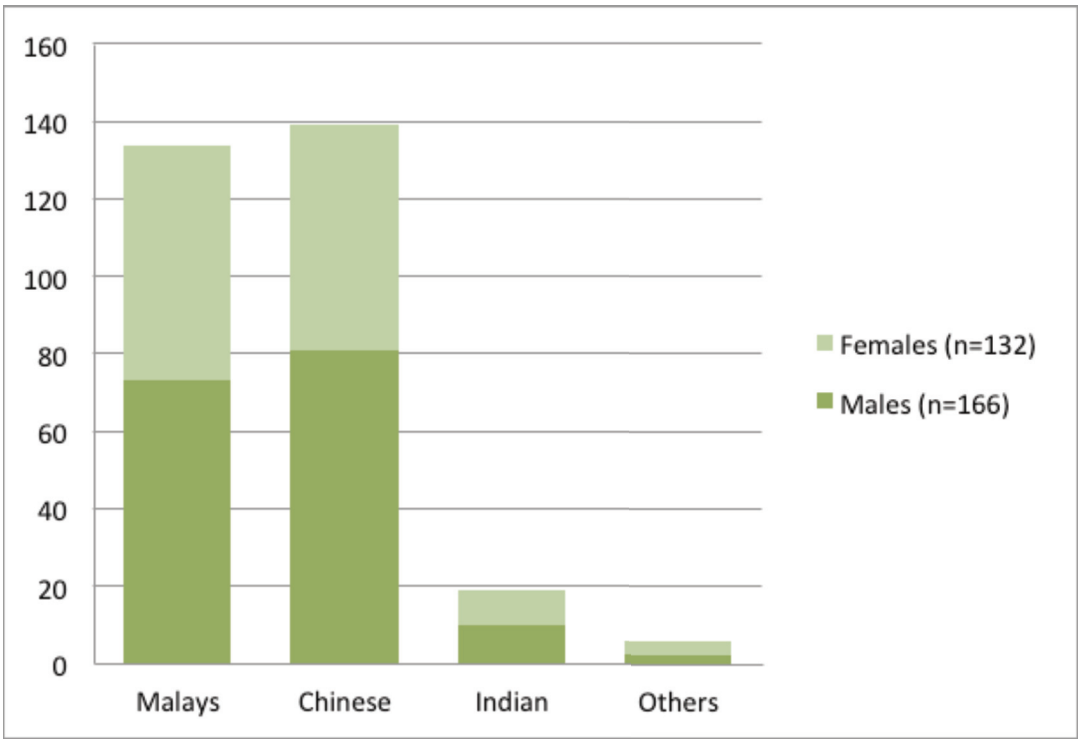

FIG. 2: Patients with colorectal cancer by race and gender.

TABLE 1: hMLH1, hMSH2 and hMSH6 protein expression in mismatch repair defect colorectal cancers (n=43).

Immunohistochemistry results	No. of tumours
hMLH1negative/ hMSH2 positive / hMSH6 positive	28 (65.1%)
hMLH1 positive/ hMSH2 negative / hMSH6 positive	2 (4.70%)
hMLH1 positive/ hMSH2 positive / hMSH6 negative	6 (14.0%)
hMLH1 positive / hMSH2 negative / hMSH6 negative	7 (16.3%)
Total	43 (100%)

a significantly higher proportion of mismatch repair defect tumours (19.7%) among Malay females when compared to non-Malay females (7.00%, p=0.031, Table 2). This suggested that mismatch repair defect tumours were more likely to occur in Malay females than non-Malay females (OR 3.24, 95% CI 1.07-9.80). However, this difference was not observed in the analysis of male patients (p=0.808). Patients with mismatch repair defect tumours presented at a younger age [mean age and standard error (SE) = 58.9 $\pm$ 2.2 years] than patients with non MMR-d tumours (mean age and standard error = 61.4 $\pm$ 0.8 years, p=0.292).

Tumour characteristics

In this study, 207 cases (69.5%) were located at the left side of the colon. Out of the 43 cases of mismatch repair defect tumours, 26 were right-sided tumours (60.5%), in contrast to only 17 cases located on the left side of the colon (39.5%). Right-sided tumours had an odds ratio of 4.47 (95% CI 2.28-8.76) for being mismatch repair deficient compared to left-sided tumours (p<0.001).

The majority of colorectal carcinomas in this study were endophytic tumours with deep ulceration. There were 199 cases (66.8%) with endophytic growth pattern while the remaining 99 cases (33.2%) were polypoidal or exophytic in growth. Although approximately 2/3 of the tumours were endophytic lesions and only 1/3 were exophytic lesions, a significant percentage (22.2%) of exophytic tumours had loss of mismatch repair protein as compared to only 10.6% of endophytic tumours (p=0.007, OR 2.42, 95% CI 1.26-4.67).

Tumour size ranged from 1.0 cm to 17.0 cm. The mean size of all tumours was 4.8 cm with a standard deviation of 2.1 cm. The majority of tumours (199 cases, 66.8%) were smaller than 5.0 cm in diameter. The mean sizes of MMR-d tumours and non MMR-d tumours were 5.7 $\pm$ 2.4 cm and 4.7 $\pm$ 2.0 cm respectively (p=0.009). More than half of MMR-d tumours were larger than 5 cm (53.5%) as compared to only 29.8% of intact tumours. Tumours with mismatch repair protein defects were significantly larger compared to non MMR-d tumours (p=0.002, OR 2.71, 95% CI 1.41-5.22).

TABLE 2: Distribution of patients by race and gender: analysis of MMR-d tumours among Malay vs. non-Malay female and male patients respectively.

Gender	Race	MMR status: All patients (n=298)	MMR-d group (n=43)	Non-MMR-d group (n=255)	*P* value	Odds Ratio (95% CI)
		All females (n=132)	MMR-d group (n=17)	Non-MMR-d group (n=115)		
Female	Malay	61	12	49	0.031	3.24
	Non-Malay	71	5	66		(1.07-9.80)
		All males (n=166)	MMR-d group (n=26)	Non-MMR-d group (n=140)		
Male	Malay	73	12	61	0.808	1.11
	Non-Malay	93	14	79		(0.48-2.57)

The majority of the colorectal carcinomas (246/298, 82.6%) were classified as well to moderately differentiated carcinomas while only 52 cases (17.4%) were classified as poorly differentiated carcinomas. Only 13.3% with non MMR-d tumours had poorly differentiated carcinomas. This was in stark contrast to the group of patients with mismatch repair defect tumours where 41.9% of mismatch repair defect tumours were poorly differentiated colorectal carcinomas. There was a statistical significant positive correlation between mismatch repair defects and poorly differentiated tumours as compared to intact tumours (p<0.001, OR 4.68, 95% CI 2.31–9.47).

Most colorectal carcinomas (228 cases or 76.5%) were associated with minimal mucin, that is, less than 10% mucin. Most tumours (202 out of 255 cases, 79.2%) with intact MMR protein staining had less than or equal to 10% mucin. In contrast, 39.5% (17 out of 43 cases) of mismatch repair defect tumours produced more than 10% mucin (p=0.007).

Survival

Forty patients were lost to follow-up. The remaining 258 patients were followed up between 1.0 to 54.0 months, with a mean follow up period of 18.1 months. At the end of the study period, 146 patients were still alive, while a total of 112 patients had died. The overall mean survival was 31.2 ± 1.6 months. The mean survival of patients with mismatch repair defect tumours was 31.4 ± 4.6 months compared with mean survival of patients with intact tumours at 31.0 ± 1.6 months (Figure 3). This was not statistically significant (p=0.615).

DISCUSSION

Demography

The Malaysian population was predominantly made up of Malays (54.3%) followed by Chinese (25.1%), Indians (7.5%) and other race.[16] The Malaysian National Cancer Registry, 2006 reported that 52.4% of patients with colorectal cancer nationwide were made up of Chinese,

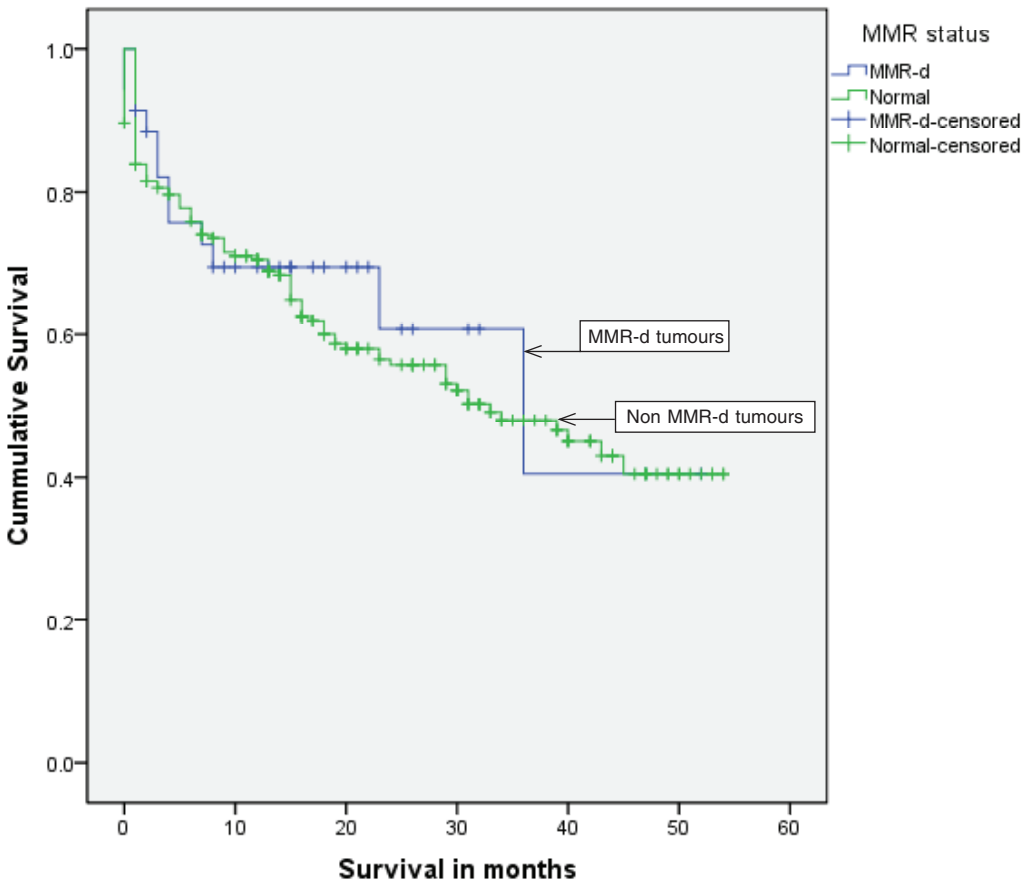

FIG. 3: Survival curves for patients categorised according to mismatch repair status.

followed by Malays (42.7%) and Indians (5%).[1] Chinese patients had a higher risk of developing colorectal carcinomas than Malays or Indians in Malaysia. This study also found a predominance of Chinese patients with colorectal carcinomas: 46.6% of the patients studied were Chinese compared to 45.0% Malays and 6.3% Indians (p=0.001, 95% CI 1.568 to 5.679).

In the census population for the year 2006 there were 24.8 million people in Peninsular Malaysia, with a male to female ratio of 1.02:1.[16] In this study there were more males than females with colorectal carcinomas with a male to female ratio of 1.26:1. In the group of Malay patients, the male to female ratio was 1.20:1 and this male predominance was even more obvious among non-Malays with a ratio of 1.31:1.

Immunohistochemistry vs. Molecular testing

Immunohistochemistry (IHC) was used to evaluate the microsatellite instability status of the tumours rather than using molecular testing that consists of polymerase chain reaction (PCR) and gel electrophoresis to examine the DNA sequences; as the molecular testing was an expensive and time consuming test and not readily available. Previous studies[17-22] had found the use of IHC to be a good alternative and highly specific to assess the status of mismatch repair gene in these tumours. Lindor *et al*[17] tested over 1000 colorectal cancers for DNA mismatch repair deficiency with both methods namely PCR and IHC detection for hMLH1 and hMSH2 and showed that IHC was 92.3% sensitive and 100% specific for screening DNA mismatch repair defects. IHC was also able to suggest which gene was defective, which was not possible with PCR testing. Debniak *et al*[23] estimated that immunohistochemistry cost less than a quarter of the price of MSI testing done with the PCR and gel electrophoresis method. IHC for mismatch repair gene could be introduced in most local laboratories and included as a routine test for selected cases of colorectal carcinomas. This could be followed with confirmatory genetic testing and further screening of family members for possible carriers. Patients with defective mismatch repair genes had an increased risk for multiplicity of tumours (metachronous or synchronous cancers) and required closer and longer term follow-up.[20, 24] They also responded differently to various chemotherapeutic agents including 5-fluorouracil and irinotecan.[25-28] Hence, this information on the MMR-d status will definitely help clinicians in their management of these patients.

Mismatch repair defect tumours

There were 43 cases (14.4% of colorectal carcinomas) with loss of one or more mismatch repair proteins. These results were comparable with those published previously in other countries,[7-10, 29-30] that ranged from 7 to 20%.

Mismatch repair defects in colon cancers were commonly reported to be hMLH1, hMSH2 or hMSH6 defects. A large majority of MMR-d tumours in many published reports[10, 17, 21, 22, 31] were due to loss of either hMLH1 or hMSH2 proteins. hMSH2 defects usually resulted from germline mutations[32] while hMLH1 could be due to germline or somatic hypermethylation of its promoter.[33,34] Herman *et al*[35] reported that the predominant loss in their study on sporadic colorectal carcinomas was hMLH1 as a result of promoter methylation while other researchers reported that hereditary non-polyposis colorectal cancers could arise from either hMLH1, hMSH2 germline mutations[36,37] or hMLH1 promoter region methylation.[38-40] Wu *et al*[41] reported that a small minority of HNPCC cases could also be caused by defects in hPMS1 or hMSH6 that resulted from germline mutations.

Our study showed the predominant mismatch repair protein loss was hMLH1. Twenty eight out of 43 cases or two thirds of the MMR-d tumours were deficient for hMLH1, and 15 cases (or one third of the cases) showed either loss of hMSH2 or hMSH6 or both. There was no case with loss of all three proteins namely hMLH1, hMSH2 and hMSH6. Similarly, previous studies[8, 42, 43] reported the rate of loss of hMLH1 in colorectal cancer to be between 8.0% and 18.6% while the rate of hMSH2 loss was between 1.0% and 2.1%.

MMR proteins interacted in the form of heterodimers; commonly hMSH2 and hMSH6; and hMLH1 and hPMS2.[44] Thus mutations of hMLH1 may entail concurrent loss of protein of hPMS2 and similarly hMSH2 with loss of hMSH6. This occurred through degeneration of the corresponding heterodimerizing protein partner.[41, 45] In their study of 214 colorectal carcinomas examined for MMR protein expression (hMSH1, hMSH2, hMSH6 and PMS2) Rigau *et al*[46] found that there were only two combinations possible when there were loss of two proteins, namely hMLH1/hPMS2 and hMSH2/hMSH6. Other studies found that besides hPMS2, hMLH1 could also form heterodimers with hMLH3 or hPMS1. In their study of sporadic MSI tumours and HNPCC, Young *et al*[45] found that all tumours lacking hMLH1 showed absence of hPMS2 as well. The

close relationship between hMLH1 and hPMS2 had also been reported by Leung *et al* and Ma *et al.*[47,48] This suggested that hPMS2 may be degraded in the absence of its binding partner, hMLH1.

On the other hand, hMSH2 commonly formed heterodimers with both hMSH6 and hMSH3 as well. Young *et al*[45] reported that many tumours lacking hMSH2 were also not staining up for hMSH6. In our study, 7 out of 9 cases with loss of hMSH2 were defective for hMSH6 but none of the cases with loss of hMLH1 were found to be defective for hMSH2 or hMSH6. The results of our study supported the proposal that hMSH2 can form heterodimers with hMSH6 thus forming loss of two proteins namely hMSH2/hMSH6 but hMLH1 lacked any such relationship with hMSH2 or hMSH6.

Although the diagnosis of colorectal cancer at a younger age was frequent in HNPCC, most studies found that sporadic colorectal carcinomas with mismatch repair defect genes were seen in all ages with no significant correlation between patients with mismatch repair defect tumours and patients with intact tumours with regards to their mean presenting age. In this study, patients with MMR-d tumours presented at a slightly younger age than the patients with non MMR-d colorectal carcinomas but was not significantly different (p=0.292). The absence of mismatch repair protein in these patients was a sporadic event and thus the events leading to tumorigenesis would take time than if it were an inherited genetic defect like in HNPCC. However, there were a few studies,[49,50] which found that patients with MMR-d tumours presented at an earlier age when compared to the patients with microsatellite stable tumours. Messerini *et al*[49] studied sporadic mucinous and non-mucinous colorectal carcinomas, and found that the mean age of patients with microsatellite instability was younger at 56.6 years when compared to their microsatellite stable cases with the mean age of 65.0 years. This was even more striking for the studies conducted by Molaei *et al.*[50] They found the mean age of presentation for patients with mismatch repair defect was very much younger at 42.8 years as compared to 53.0 years for those with no mismatch repair protein defect. They reported that patients had an odds ratio of 5.950 (95% CI 2.687-13.175) of presenting at an age younger than 50 years among tumours with mismatch repair defect (p<0.001).

Although Chinese patients were the predominant racial group with colorectal carcinomas, we found that Malay patients had the highest proportion of colorectal carcinomas with mismatch repair defects. This however, was not significantly different. Similarly, most published reports[27, 51] suggested no differences between ethnic groups for patients with MMR-d tumours and microsatellite stable tumours. However, one report[52] found that African-American patients had a significantly higher frequency of MSI-positive tumours than Caucasian patients.

On the other hand, the incidence rates of MSI-tumours had been noted to differ from country to country or region to region. Microsatellite instability had been reported to occur in 7 to 20% of colon tumours.[7-10] Studies done in Mediterranean region found a lower rate of 7 to 8% [29,30] as compared to studies in Iran where the rate of MSI-tumours was as high as 14%.[50] It had been proposed that dietary, toxic or other environmental factors could be causes of epigenetic disruption of hMLH1 (such as promoting hypermethylation of the gene) in a particular population. Red meat ingestion, higher frequency of using different cooking practices that increased intake of heterocyclic amines like frying, barbequing or boiling,[53] as well as high consumption of high-grade alcoholic beverages[54] were possible risk factors as these were frequently practised in countries with high MSI incidence rates. Perhaps the differences in incidence rates between different populations may be due to their different dietary habits and to a lesser extent the inherent genetic properties.

Although there was no significant difference of MMR-d tumours for gender or race, it was interesting to note that Malay females instead of non-Malay females in our study were significantly associated with MMR-d tumours (p=0.031). The reason for this race selection occurring only in females of this ethnic group (Malay) was not apparent in this study. Perhaps a more detailed analysis and comparison of the cultural and social habits of Malay females with other racial groups in the country could be undertaken in another future study to explore this finding.

On the other hand, we did not find any statistical difference for gender predilection for tumours with mismatch repair defect (p=0.497). However, many other studies[8, 9, 31, 33, 42] found a strong association of tumours with mismatch repair gene defect for females. This has been directly attributed to the effects of oestrogens. It was uncertain why or how oestrogen levels and oestrogen receptors (ER)

were associated with MSI tumours. The roles of endogenous (reproductive status), exogenous (hormone replacement therapy) and metabolic (obesity-associated) oestrogens in preventing ER methylation and thus MMR genes were unclear. There had been several hypotheses about this. Slattery *et al*[55] hypothesised that at least one major mismatch repair gene may be oestrogen responsive and thus loss of oestrogen could result in loss of DNA mismatch repair capacity. Whatever the possible explanations may be, the data observed in these studies[55-59] supported the finding that hormones played an important aetiological role in colon cancer via the MSI related pathway and that oestrogens prevented MSI tumours whether endogenous, exogenous or obesity associated. The excess of microsatellite instability colon cancers in women were explained by the excess of these tumours at an older age when there was a reduction or withdrawal of oestrogens at the time when these women became post-menopausal. This was supported by the fact that there were fewer MSI phenotype tumours in pre-menopausal young women than young men in their studies.

Anatomical location

Most of the colorectal carcinomas were found in the left side of the colon. On the other hand we showed a significant predilection of MMR-d colorectal carcinomas to the right side of the colon ($p<0.001$). Right sided tumours had an odds ratio of 4.47 with a 95% confidence interval of 2.28 to 8.76 of being mismatch repair deficient compared to left-sided tumours. Our results were similar and comparable to many other published reports.[8, 10, 14, 50, 60-62] In addition, Chapusot *et al*[10] also found that right sided location was a clinically useful positive predictor of mismatch repair status expression. It had a positive predictive value of 33%, second only to tumour with poor differentiation. The negative predictive value was 97%.

Growth appearance and size of tumours

Most of the colorectal carcinomas (70.4%) in this study were of the endophytic type. However, a significant percentage (22.2%) of the cases of exophytic tumours had mismatch repair defect. This study showed that tumours with mismatch repair defect were significantly associated with an exophytic growth appearance ($p=0.007$). The odds ratio was 2.42 with a 95% confidence interval of 1.26 to 4.67. Feeley *et al*[61] and Messerini *et al*[49] studied the growth appearance of tumours found in MMR-d colon cancers. They noted that there was a significant correlation between mismatch repair deficient tumours and exophytic growth. Our study including several others[10, 61, 62] showed that MMR-d tumours were significantly associated with larger tumours. A larger and exophytic tumour was more likely to have mismatch repair defect.

Histological features

Patients with MMR-d colorectal carcinomas were more likely to have poorly differentiated tumours than those with intact tumours with an odds ratio of 4.68 (95% CI 2.31 to 9.47). This was similarly reported by many studies[8, 10, 14, 60, 62] when they compared mismatch repair defect tumours with microsatellite stable tumours. Chapusot *et al*[10] not only found that tumours with microsatellite instability were significantly associated with poorer differentiation but poor differentiation was the most accurate predictor of lack of MMR expression with a positive predictive value of 50% and negative predictive value of 89%. By multivariate analysis, they also demonstrated that poor differentiation was a significant independent factor associated with loss of expression of hMLH1 and hMSH2 proteins.

MMR-d tumours produced more mucin than intact tumours ($p=0.007$). The odds ratio was 2.49 (95% CI 1.26 to 4.93). Many reports[31, 49, 62-65] also claimed that mucinous histology was the hallmark of MMR-defective carcinomas. Chapusot *et al*[10] demonstrated in a univariate analysis that MMR-defective tumours were shown to be significantly associated with a distinct pattern of extracellular mucin production ($p=0.0001$) but was not significant when adjusted for other factors on multivariate analysis. Messerini *et al*[49] studied mucinous sporadic tumours with non-mucinous sporadic tumours as controls and found that mucinous carcinomas showed microsatellite instability more frequently than the controls. In addition they noted this was more marked when the tumours had lost two or more microsatellite alterations. Mismatch repair defects or replication errors may directly influence mucus production both in sporadic and familial cases (HNPCC). Altered mismatch repair genes may be involved in mucin synthesis or degradation resulting in increased amount of mucin in these tumours compared to stable tumours.

Survival

Many studies had looked at patients with

TABLE 3: Association of various clinical and pathological features with mismatch repair status of tumours

Parameters	Category	All patients	MMR status		P value	Odds ratio (95% CI)
			MMR-d	Non MMR-d		
Gender	Male	166	26	140	0.497	1.26
	Female	132	17	115		(0.65-2.43)
Age	≤ 50 years	53	22	42	0.148	1.74
	>50 years	245	32	213		(0.82-3.73)
Anatomical Location	Left side	207	17	190	<0.001	4.47
	Right side	91	26	65		(2.28-8.76)
Tumour growth	Exophytic	99	22	77	0.007	2.42
	Endophytic	199	21	178		(1.26-4.67)
Size of tumour	Small (<5cm)	199	20	179	0.002	2.71
	Large (≥5cm)	99	23	76		(1.41-5.22)
Histological grade	Mod-well diff	246	25	221	<0.001	4.68
	poorly diff	52	18	34		(2.31-9.47)
Mucin production	Minimal(≤10%)	228	26	202	0.007	2.49
	Marked (>10%)	70	17	53		(1.26-4.93)
Survival in months		31.2	31.4	31.0	0.615	

colorectal carcinomas to determine the possible prognostic factors for survival. The results had been conflicting and this was because of the different pathogenetic mechanisms of tumorigenesis involved in sporadic and familial types of colorectal carcinomas.

Although the mean period of survival of patients with MMR-d tumours was slightly better than patients with intact tumours, there was no statistical difference found in our study (p=0.615). This differed from other published reports[9, 14, 24, 62, 63, 66, 67] that found patients with mismatch repair defect tumours demonstrated better disease specific survival than patients with microsatellite stable tumours. Gafa *et al*[62] found that patients with MSI tumours had a significant survival advantage when only patients with tumours localised to the right side were included in their analysis. The prognostic significance became more evident in the subgroup of those with poorly differentiated carcinomas. They reported the 5-year survival rate of patients with MSI poorly differentiated tumours was 79.2% compared to patients with microsatellite stable (MSS) poorly differentiated tumours of 36.7% (p<0.05). Lim *et al*[14] reported that the overall 5-year survival for patients with MSI was more than 90% but those with microsatellite stable tumours was less than 60% (p<0.05). The improved prognosis and longer survival were seen together with a lower number of distant metastases in these tumours. This may be due to the up-regulated immune response as demarcated by prominent peri-tumoural and Crohn-like lymphocytic responses or presence of tumour-infiltrating lymphocytes. Alternatively, it may be due to the high mutation rate of defective DNA mismatch repair genes that did not allow time for emergence of the genes contributing to tumour metastases. All these could have prevented emergence of metastatic deposits and restricted growth of the tumour with a final better outcome and prolonged survival.

Management

Definitive surgery was the main modality of treatment in most patients with or without adjuvant chemotherapy or radiotherapy. The results of reported studies had been inconsistent on the response of patients with mismatch repair deficient tumours to chemotherapy. One published study[68] showed no difference in response between patients with MSI tumours and patients with MSS tumours when they were treated with 5-fluorouracil (5-FU) in the presence of metastasis. On the other hand, another larger

series[69] found that MSI status and administration of chemotherapy were independent favourable prognostic parameters and suggested that this was due to increased chemo-sensitivity of MSI tumours. Hemminki *et al*[13] found that patients with MSI colorectal cancers who were treated with the same adjuvant 5-FU based chemotherapy which was the standard treatment for Stage C colon cancers performed significantly better when compared with patients with microsatellite stable tumours. They suggested that patients with MSI tumours were potentially curable despite regional lymph node metastases. It also supported the hypothesis that MSI tumours were more sensitive to 5-FU.

Studies by Ruschoff[70] and Yamamoto[71] demonstrated a reduction of proportion of hMLH1 or hMSH2 deficient cell lines exhibiting microsatellite instability when treated with non-steroidal anti-inflammatory drugs (NSAIDs). This had lead to new drug trials to change the phenotypic manifestation of this mismatch repair deficiency and hence hopefully alter the course of cancers. Steinbach *et al*[72] found that there was an actual reduction in the number of adenomas in patients with colorectal cancers when treated with NSAIDs. This may reduce the recurrences of tumours in these patients with colorectal carcinomas.

As the number of cases studied in our study was small and the types of chemotherapeutic agents used were varied it was difficult to obtain a statistical evaluation of patients for each type of treatment received.

Conclusion

Our study showed 14.4% of colorectal cases had loss of mismatch repair defect. There was no gender or racial predilection but Malay females were significantly associated with MMR-d tumours. These patients with MMR-d colorectal carcinomas had distinct clinical and -pathological features (Table 3). The tumours were more likely to be right-sided, larger with exophytic growth and produced more mucin. They were also more likely to be poorly differentiated. Immunohistochemical staining for MMR-d could be done on these cases selected by their associated characteristics; as a preliminary test in most laboratories locally before confirming with the more sophisticated and expensive molecular test for DNA microsatellite instability. This information on the MMR-d status will definitely help clinicians in their management of the patients.

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