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Effects of MMR Protein and BRAF V600E Mutation on Survival of Sporadic Young-Onset Colorectal Cancer Cases

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The effect of mismatch repair (MMR) protein and the b-type raf proto-oncogene (BRAF) mutation on survival of young-onset sporadic colorectal cancer (CRC), is debatable. This study aimed to determine the survival of sporadic young-onset CRC cases with MMR protein and BRAF mutation cases in Kelantan. The retrospective cohort study comprised 31 sporadic young-onset CRC cases diagnosed before age 40. Patients' information was extracted from the medical records unit and laboratory information system (LIS). Cases with hereditary CRC, inflammatory bowel disease, concurrent non-colonic cancer and cases under 18 years old were excluded from the study. The Kaplan–Meier survival analysis was used to determine the survival time of CRC cases. The mean age of sporadic young-onset CRC cases was 31.5-year with most were female (61.3%). The majority (41.9%) of cases complained of abdominal discomfort as their primary presenting symptom. Most of cases had a tumour located on the right side of colon (71.0%) and moderately differentiated adenocarcinoma (83.9%). More than half (54.8%) were in stage iv when presented to the hospital. The pattern that is most common was a positive expression in all MMR proteins that made up the group of microsatellites stable (MSS) (64.5%). Microsatellite instability (MSI) was high in nine cases (29.0%) and low in two cases (6.5%). 83.9% of the patients showed positive BRAF v600e expression. The overall median survival time of sporadic young-onset CRC cases was 4.1 years (95% confidence interval (CI): 2.59, 5.62). Histology subtypes and BRAF v600e mutation were found to affect the overall survival of sporadic young-onset CRC cases. Most sporadic young-onset CRC cases were microsatellite stable and displayed positive BRAF V600E. Diagnosis at an early stage would improve survival, in general, among cases with CRC. The most typical symptoms of sporadic young-onset CRC were abdominal discomfort and an advanced cancer stage. Histology subtypes and BRAF V600E mutation affected the overall survival. There is a need for more research involving a wide range of clinical and biological factors.

Keywords: Microsatellite instability; DNA mismatch repair (MMR) genes; B-type RAF proto-oncogene (BRAF) mutation; survival; polymerase chain reaction (PCR); immunohistochemistry

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I. INTRODUCTION

About 1.8 million new cases and 881,000 fatalities from cancer-related causes were caused by colorectal cancer (CRC), making it the second most prevalent cancer-related death, in 2018 (Bray *et al.*, 2018). Malaysia is not an exception to the rising CRC rates that constitute a significant public health problem in the Asia-Pacific region (Nawawi *et al.*, 2021). The rapid socioeconomic development that is causing more people to adopt Western foods and lifestyles might be the cause of these growing rates (Arnold *et al.*, 2017; Center *et al.*, 2009; Ferlay *et al.*, 2015).

Currently, CRC is the second most frequent cancer in Malaysia (Azizah *et al.*, 2019b). According to the Malaysian National Cancer Registry Report 2012-2016, men are more likely to develop CRC (16.9% of all cancers diagnosed) and it is the second most common cancer in women (10.7% of all cancers diagnosed) (Azizah *et al.*, 2019a). The average age of CRC patients, 61.6 years, makes it more common in older age groups (Hassan *et al.*, 2013). However, CRC in the young population is progressively increasing worldwide despite the disease historically affecting the old (Lui *et al.*, 2019; Vatandoust *et al.*, 2016). Approximately 10% of cases involve people who are under 55 years old (Perrott *et al.*, 2020).

The young population are more likely to develop CRC due to familial disorders, however, the majority of cases in this age group are sporadic (Levine & Zbuk, 2019). Red meat intake and sedentary lifestyles are well-known environmental and lifestyle risk factors for CRC in general. However, their specific contributions to young-onset CRC have yet to be established (You *et al.*, 2020). Fast food consumption has increased three- to five-fold among young adults (You *et al.*, 2020). Smoking has been shown to lower the age of CRC onset while type 2 diabetes, obesity, and metabolic syndrome are all becoming more common in younger people (You *et al.*, 2020). Low clinical suspicion in young symptomatic individuals frequently delayed the diagnosis until an advanced stage (You *et al.*, 2020). Compared to their older counterparts, these younger cases typically present with more advanced disease, a delayed diagnosis, and undesirable pathology characteristics (Connell *et al.*, 2017). This can adversely affect the likelihood of their survival (Khan *et al.*, 2016).

The microsatellite instability (MSI) pathway is one of the suggested pathways for the development of CRC. The MSI occurs in 15% of sporadic young-onset CRC (Saizul *et al.*, 2021) and leading to significant heterogeneity in both phenotype and survival. Microsatellite stable (MSS), MSI-low (MSI-L), and MSI-high (MSI-H) are three different types of MSI pathways (Kurzawski *et al.*, 2004). MSS is defined as no mutation in the microsatellite panel. In contrast to MSS, MSH-H happened when the tumour has two or more mutated microsatellite sequences, MSI-L happens when a tumour has just one mutant microsatellite sequence.

A large proportion of all colorectal tumours with MSI is through the acquired methylation of MutL homolog 1 (MLH1) alone (Boland & Goel, 2010; Kurzawski *et al.*, 2004). Tumours with hypermethylation of MLH1 and B-type RAF proto-oncogene (BRAF) mutation almost represent sporadic CRC, which is unrelated to hereditary non-polyposis colorectal cancer (HNPCC) or Lynch syndrome (Boland & Goel, 2010). The characteristic features of sporadic CRC with MSI were no family history with the loss of MLH1 and/or PMS1 Homolog 2, Mismatch Repair System Component (PMS2) expression and the presence of BRAF V600E mutation (Batur *et al.*, 2016). The BRAF V600E mutation is seen in sporadic MSI-H CRC. Typically, sporadic CRC rather than HNPCC exhibit the BRAF V600E mutation (Capper *et al.*, 2013; Thiel *et al.*, 2013). Cases with stage IV CRC, especially those with MMR-proficient tumours, are also at risk due to the BRAF V600E mutation (Toon *et al.*, 2014). Although inconsistent results have been reported, BRAF mutation is also linked to worse outcomes in CRC (Bokemeyer *et al.*, 2012; Ogino *et al.*, 2012; Yokota *et al.*, 2011).

This is the first study reported on the survival of sporadic CRC with MMR protein and BRAF V600E mutation among young adults in Kelantan. Keeping in mind the long-term consequences of CRC in young adults, the researcher conducted a cross-sectional at two hospitals to determine the effect of MMR and BRAF mutations on survival of sporadic young-onset CRC in Kelantan.

II. MATERIALS AND METHOD

A. Study Design and Population

This retrospective cohort study of young-onset CRC aged less than 40 years old diagnosed and treated at Hospital Universiti Sains Malaysia (USM) and Hospital Raja Perempuan Zainab (HRPZ) II from 1 Jan 2006 to 31 Dec 2017. Cases were excluded if they had hereditary CRC, inflammatory bowel disease and concurrent non-colonic cancer.

B. Data Collection

A proforma form was used to collect the data. Cases' clinicopathological characteristics were extracted from the operative notes in medical record unit and laboratory information system. Data on cases' demographics, presenting symptoms, tumour sites (right side and left side), stage, histological subtypes (moderately differentiated adenocarcinoma, mucinous adenocarcinoma, signet ring cell and poorly differentiated adenocarcinoma), MMR protein (MSS, MSI-L and MSI-H) and BRAF V600E (positive, negative and equivocal) were collected. The stage of CRC was based on the 8th edition of the American Joint Committee on Cancer Staging (AJCC-8).

C. Primary Event

All-cause mortality was the main event of interest. For those who were lost to follow-up (LTFU) during the study period or were still alive at the study closure, the observations were censored. The definition of the survival time is the time from the CRC diagnostic date to the death or censoring date. Death status was verified by referring to the electronic medical records in the hospital. As for LTFU cases, the event status was matched with the National Registration Department database. The cases' information, including their full names and National Registration Identity Card numbers were used. If the cases were confirmed dead based on the record, LTFU status would be changed to "died".

D. Statistical Analysis

Data were entered and analysed using IBM SPSS version 27.0 (IBM Corp. Released 2016. IBM SPSS Statistics for Windows, Version 27.0. Armonk, NY: IBM Corp.). The categorical data are presented as frequencies (n) and percentages (%). The mean and standard deviation (SD) were provided for the normal numerical data. The median and interquartile range (IQR) were reported for the non-normally distributed data. Kaplan-Meier method was used to estimate the survival time of CRC diagnosis and to compare the survival distributions of two or more groups. The survival curves of the groups were compared using a log-rank test. In the event of multiple pairwise comparisons, the Bonferroni correction was made by multiplying the p-value by the number of comparisons.

E. Ethical Issue

The human ethical committee of the School of Medical Sciences, USM Medical Research and Ethics Committee (MREC), Ministry of Health in Malaysia approved the study. All of the data were anonymous; thus, there were no unique identifiers. The data were entered into analysis software in a password-protected computer with limited access. The data were kept strictly confidential.

III. RESULTS

Table 1 summarises the sociodemographic characteristics of 31 sporadic young-onset CRC cases. The age was distributed as follows: five were under 25 years of age, seven were 26-30 years, 11 were 31-35 years and eight were 36-40 years with the overall mean of 31.5 years. There were 12 male and 19 female cases. All cases were Malay. Abdominal pain (41.9%) was the most common complaint, followed by rectal bleeding (6.5%), constipation (6.5%), dark-coloured stool (6.5%), and diarrhoea (3.2%).'

Nine cases (29.0%) had cancer on their left side. Majority of cases were moderately differentiated adenocarcinoma (83.9%), 9.7% had mucinous adenocarcinoma, 3.2% of cases had signet ring cell carcinoma, and 3.2% had poorly differentiated adenocarcinoma. Stage IV, stage III, and stage II were 54.8%, 29.0%, and 16.1%, respectively, in the cases.

Nine cases (29.0%) showed loss of more than one MMR protein expression, two cases (6.5%) showed loss of one MMR protein expression, and twenty cases (64.5%) displayed all four panels of MMR proteins (MSS) (MSI-L). Twenty-six cases (83.9%) of BRAF V600E expression were positive, compared to three cases (9.7%) that were negative and two cases (6.5%) that were ambiguous.

Table 1. Demographics profiles of young CRC cases in Hospital USM (n=31)

Variables		Status, n (%)	
		Dead	Censored
Gender	Male	5 (41.7)	7 (58.3)
	Female	7 (36.8)	12 (63.2)
Symptoms	Abdominal pain	5 (38.5)	8 (61.5)
	Rectal bleeding	0 (0.0)	2 (100.0)
	Dark-coloured stool	1 (100.0)	0 (0.0)
	Constipation	0 (0.0)	1 (100.0)
	Diarrhoea	0 (0.0)	1 (100.0)
Location	Right side	10 (45.5)	12 (54.5)
	Left side	2 (22.2)	7 (77.8)
Histology	Moderate differentiated adenocarcinoma	8 (30.8)	18 (69.2)
	Mucinous adenocarcinoma	2 (66.7)	1 (33.3)
	Signet ring cell carcinoma	0 (0.00	1 (100.0)
	Poorly differentiated adenocarcinoma	0 (0.0)	1 (100.0)
Stage	II	2 (40.0)	3 (60.0)
	III	3 (33.3.)	6 (66.7)
	IV	7 (41.2)	10 (58.8)
Types of MMR protein	MSS	3 (17.6)	14 (82.4)
	MSI-L	1 (50.0)	1 (50.0)
	MSI-H	5 (62.5)	3 (37.5)
BRAF mutation status	Positive	9 (34.6)	17 (65.4)
	Negative	2 (66.7)	1 (33.3)
	Equivocal	1 (50.0)	1 (50.0)

There were 12 deaths (38.7%) and 19 censored cases (61.3%). The overall median survival time was 4.1 years (95% CI: 2.59, 5.62) (Figure 1).

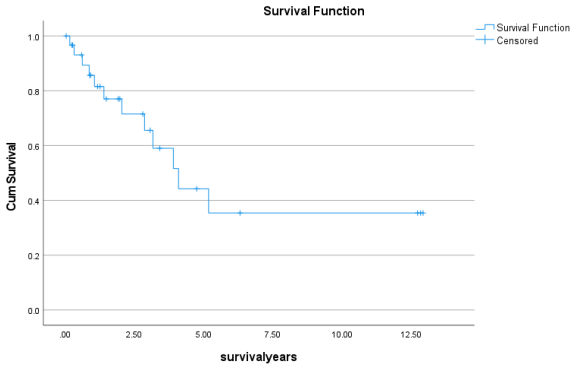

Figure 1. Kaplan Meier estimate for survival time of sporadic young-onset CRC in Hospital USM and HRPZ II (n=31)

Table 2 shows the median survival time of sporadic young-onset CRC.

Table 2. Median survival time of sporadic young-onset CRC in Hospital USM and HRPZ II (n=31)

Variables	Median survival time (years) (95% CI)
Gender	
Male	-
Female	3.91 (2.63, 5.20)
Location	
Right side	4.10 (2.21, 5.99)
Left side	3.91 (0.00, 8.23)
Histology	
Moderate differentiated adenocarcinoma	5.19 (2.59, 7.78)
Mucinous adenocarcinoma	2.88 (0.00, 7.19)
Signet ring cell carcinoma	-
Poorly differentiated adenocarcinoma	-
Stage	
II	3.18 (0.00, 6.62)
III	5.19 (0.36, 10.01)
IV	3.91 (2.61, 5.22)
MMR protein	
MSS	5.19 (3.24, 7.13)
MSI-L	-
MSI-H	3.18 (0.92, 5.43)
BRAF V600E	
Positive	5.12 (2.40, 7.97)
Negative	4.10 (2.14, 6.06)
Equivocal	-

The log-rank test used for comparing the survival showed a significant difference in histology and expression of BRAF V600E (Table 3).

Those with moderately differentiated adenocarcinoma had a longer median survival time than those with mucinous adenocarcinoma, 5.19 years ((% % CI: 2.59, 7.78). There was a significant difference between those with moderately differentiated adenocarcinoma and poorly differentiated adenocarcinoma (p<0.001) after Bonferroni correction (α=0.0125).

Those sporadic young-onset CRC cases with positive BRAF had a longer median survival time compared to those with negative BRAF, 5.12 years (2.40, 7.97). There was a significant difference in survival between those who had

BRAF positive with equivocal (p=0.007) after Bonferroni correction (α=0.017).

Table 3. Comparison of median survival time of sporadic young-onset CRC in Hospital USM and HRPZ II by using log-rank statistics (n=31)

Variables	Log-rank statistics (df)	*p*-value
Histology		
Moderate differentiated adenocarcinoma & Mucinous adenocarcinoma	0.463	0.496
Moderate differentiated adenocarcinoma & Signet ring cell carcinoma	0.135	0.713
Moderate differentiated adenocarcinoma & Poorly differentiated	23.000	<0.001
Mucinous adenocarcinoma & Signet ring cell carcinoma	0.011	0.918
Mucinous adenocarcinoma & Poorly differentiated	0.424	0.515
Signet ring cell carcinoma & Poorly differentiated	1.000	0.317
BRAF V600E		
Positive & Negative	0.013	0.909
Positive & Equivocal	7.205	0.007
Negative & Equivocal	3.000	0.083

IV. DISCUSSION

There were only 31 cases of sporadic young-onset CRC cases in Hospital USM and HRPZ II. Mostly, this disease occurred among the older population. However, due to changes in lifestyle and demographic profiles, this disease occurred among the younger population. In the literature, young-onset CRC defined differently. Most articles specify young as being under 40 (Chan *et al.*, 2010; O'Connell *et al.*, 2004; Zbuk *et al.*, 2009). Therefore, in order to define young-onset in the current study, 40 years of age was utilised as the cut-off point.

This study examined the effect of MMR protein and BRAF mutations on survival of sporadic young-onset sporadic CRC. Survival time varied between studies. The overall median survival time was 4.1 years (95% CI: 2.59, 5.62). Using the same definition of young-onset CRC, the previous study reported a lower median survival time of 2.4 years (Tawadros *et al.*, 2015). The reason may be the previous study involved a multicentre study using surveillance, epidemiology and results (SEER) cancer registry. Other studies conducted in Japan reported 1.3 years of median survival time. This study only involved stage IV CRC with histologic diagnoses of adenocarcinoma, which might be the reason of lower median survival time (Shida *et al.*, 2018).

Previous studies conducted in Malaysia and Turkey reported the overall median survival time was 4.9 years and 3.7 years (Wong *et al.*, 2021). However, both previous studies defined young cases as those aged below 50 years old. Chang *et al.* (2012) involved a single centre (n=55) and reported that five-year survival was 53% (Chang *et al.*, 2012). Chou *et al.* (2011) reported five-year survival was 44.1% (n=69) (Chou *et al.*, 2011). Dai et al. reported three-year overall survival rates of 23.7% and five-year overall survival rates of 17.3% (Shida *et al.*, 2018). All three studies presented late stages of diagnosis. Other studies reported several five-year survival rates, ranging between 12% and 66.4% (Al-Barrak *et al.*, 2011; Kneuertz *et al.*, 2015; Kocian *et al.*, 2017; Zhao *et al.*, 2017).

On review of cancer staging, the current study reported sporadic CRC cases were more likely to present with stages III and IV. The lack of suspicion in these individuals may lead to a delay in diagnosis, resulting in more advanced disease at presentation and poorer outcomes (Christodoulides *et al.*, 2020; Stapley *et al.*, 2017). Currently, there are no nationwide, population-based screening programmes for CRC in Malaysia (Fuzi *et al.*, 2015; Veettil *et al.*, 2017). Hence, public awareness of CRC and the participation rate for opportunistic screening in Malaysia remained low (Koo *et al.*, 2012). Strategies to increase awareness of the symptoms of CRC among the general population along with the implementation of screening programmes in Malaysia are necessary for early detection of CRC, which could lead to improved survival.

In cases with stage III disease, Zhao *et al.* (2017) reported significantly lower five-year survival in the younger group (Zhao *et al.*, 2017). A large population-based study of stage IV reported a five-year stage-specific survival of 18.1% in younger cases (aged 20 to 40 years old] based on data from the SEER database from year 1991 to 1999 (Shida *et al.*, 2018).

In addition to the major findings, the current study adds to the growing body of literature confirming histology subtypes and BRAF V600E mutation affect the overall survival of sporadic CRC among the young population. Many studies

have reported that cases with young-onset CRC with poorer histological features had shorter survival (Chalya *et al.*, 2013; Chou *et al.*, 2011; Ganapathi *et al.*, 2011; Goldvaser *et al.*, 2016). Consistent with the studies, the current findings showed a shorter survival time for mucinous adenocarcinoma compared to moderately differentiated adenocarcinoma. The findings reported a median overall survival of 2.88 years for mucinous adenocarcinoma compared to 5.19 years for moderately differentiated adenocarcinoma. There was a significant difference between those with moderately differentiated adenocarcinoma and poorly differentiated adenocarcinoma after Bonferroni correction. The possible explanations are that more cases with young-onset CRC received combination therapy, hence compensating for worse tumour biology, or those younger cases had fewer comorbidities and better baseline life expectancy independent of the cancer diagnosis (Shemesh-Bar *et al.*, 2010).

The choice of treatment method had a significant impact on overall survival for young cohorts, where combination therapy (systemic and surgical) was superior to monotherapy for improving better survival outcomes. According to the results of the current study, cases with young-onset CRC tend to receive combination therapy more frequently. Due to the small sample size, this finding did not attain statistical significance.

The current finding reported a longer survival time of cases with positive BRAF V600E compared to negative BRAF V600E (5.12 years versus 4.10 years). Inconsistent with other published series, cases with BRAF V600E mutation showed worse overall survival compared with negative BRAF with overall survival of 1.44 years versus 2.77 years (Tan *et al.*, 2022). Another study also found that individuals with tumours exhibiting the BRAF V600E mutation were significantly more likely to die from their disease than individuals without this mutation (Phipps *et al.*, 2012). However, both previous studies defined young CRC cases of age less than 50 years old.

To the best the knowledge, this study is the first study in Kelantan to determine the overall survival of CRC among young population sporadic CRC patients. There are several limitations in the study. Results drawn from the study may not reflect the entire Malaysian population since the study only involved two centres in Kelantan. Working with other institutions in other states will allow the researcher to increase the sample size for the young-onset CRC. In order to better understand the genetic component of young-onset CRC, molecular and genetic testing for these cases should be investigated. A national study would be helpful to investigate clinical trends and evaluate the advantages of CRC early screening.

V. CONCLUSION

In conclusion, diagnosis at an early stage would improve survival, in general, among cases with CRC. The most typical symptoms of sporadic young-onset CRC were abdominal discomfort and an advanced cancer stage. Hence, clinicians need to be suspicious when young adults present with abdominal pain even in the absence of predisposing factors. Histology subtypes and BRAF V600E mutation affected the overall survival. There is a need for more research involving a wide range of clinical and biological factors.

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