A Review of Colorectal Cancer Research in Malaysia

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SUMMARY

105 articles related to colorectal cancer(CRC) were found in a search through a database dedicated to indexing all original data relevant to medicine published in Malaysia between the years 2000-2013. 56 articles were selected and reviewed on the basis of clinical relevance and future research implications. Research into the genetic basis for colorectal cancer included studies in germline mutations of known syndromes as well as polymorphisms that conferred individuals a higher odds ratio for developing CRC. Several studies also documented the variety of somatic mutations seen in cases of sporadic CRC in Malaysia. Studies into the knowledge and attitudes of Malaysians regarding CRC revealed poor appreciation of the common symptoms, risk factors and available measures for its early detection. This may explain the observed facts that more Malaysians present with late stage CRC than seen in developed countries. The small amount of data recorded concerning the outcome of treatment also suggests overall survival of Malaysian CRC patients for comparable stage of CRC is lower than achieved in developed countries.

KEY WORDS: *Colorectal cancer, Malaysia, Review, genetics, screening, diagnosis, staging, treatment, outcome*

INTRODUCTION

A literature search of articles as detailed in the paper *Bibliography of clinical research in Malaysia: methods and brief results*[1] was undertaken and 105 articles found. Of these, 56 abstracts were considered relevant to basic science and clinical practice by the author [a general surgeon] and full text articles were reviewed. The aim of this review article is to summarise what has already been published on colorectal cancer in Malaysia, to discuss the impact of the research findings to clinical practice, and to identify gaps in colorectal cancer research in Malaysia.

SECTION 1: REVIEW OF LITERATURE

EPIDEMIOLOGY

Colorectal cancer (CRC) is the most common cancer in Peninsular Malaysia among men and the third most common among women, according to the National Cancer Registry Report 2003-2005[2]. There were slightly more affected men than women [1.1:1]. The cumulative lifetime risk of developing CRC was 1:38 in men and 1:50 in women. The Age-Standardised Rate (ASR) was highest among Chinese men (31.5 per 100,000), in whom it is more than twice of that in Indian (15.7 per 100,000) and Malay men (12.3 per 100,000). Chinese women also had an ASR (26.2 per 100,000), which was more than twice that of Indian (12.9 per 100,000) and Malay (9.7 per 100,000) women.

RISK FACTORS

A number of lifestyle (e.g. dietary intake of fibre and red meat) and genetic factors (e.g. hereditary nonpolyposis colorectal cancer) cause an increased risk for colorectal cancer and these are true for Malaysians. However, knowledge of risk factors of colorectal cancer remain low in Malaysia. A survey of 991 subjects from an urban middle class area of Kuala Lumpur between 2006-2008[3], using a standard questionnaire for the Asia Pacific Colorectal Cancer Working Group, found that the majority of Malaysians (57%) could not identify risk factors for the disease. The most commonly recognised risk factor was family history (24%), followed by low fibre diet (16%), age (11%), high fat diet (9%), smoking (9%) and obesity (4.5%).

Diet

A case control study of 59 cases and 59 controls at Hospital Kuala Lumpur (HKL) using quantitative food frequency questionnaires showed that soy bean and soy products (OR=0.38), higher servings of fruits (OR=0.47) and vegetables (OR=0.49) were associated with a reduced risk for colorectal adenomas, while tubers, such as potatoes (OR=4.14) and red meat (OR=2.51) were associated with an increased risk (OR = 4.14)[4].

Metabolic risk factors

Obesity, high fasting blood glucose, hypertension and abnormal blood lipids, which are collectively recognised as features associated with metabolic syndromes, are associated with increased risk to colorectal cancer. A cross-sectional study of 140 colorectal cancer patients diagnosed in 2010 in hospitals in Kuala Lumpur, Putrajaya, Selayang, Alor Star and Penang found that 71% (99/140) had features of metabolic syndromes, and this was more common in men than in women[5]. Consistent with data in other countries, individuals with two or more metabolic syndrome features were at a three-fold increased risk for CRC. Furthermore, a study in Kelantan, shows that patients with type 2 diabetes and hypertension were more likely to present with late stage CRC and with cancers located distal to the transverse colon (89% and 85% in diabetic and hypertensive patients respectively)[6].

Streptococcus gallolyticus

Colonisation by *Streptococcus gallolyticus* (the new name of *S. bovis* biotype I) has been suggested for its association with colorectal cancer, but it remains unclear whether this is causal, or whether colorectal cancer tissue is more easily colonised by the microbe. Ahmed SA *et.al.* reported a higher prevalence of *S. Gallolyticus* in Malaysian colorectal cancer patients compared to healthy volunteers (68% vs 17%), as detected by serology[7]. Other studies reported a higher prevalence of *S. gallolyticus* and its subspecies in faeces of CRC patients compared with matched controls (46% compared to 7%)[8,9], and in tumour tissue of CRC patients compared to normal controls (13%-20% compared to 2%)[9].

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Colonic polyps

Two-thirds of CRC are known to arise from adenomatous polyps and the presence of these polyps are a significant risk factor for the development of CRC. Using a standard colonoscope and methylene blue dye to look for flat adenomas, Rajendra et.al studied 426 consecutive patients who underwent colonoscopy between 1997 and 2000, and reported finding 29 adenomas in 12 patients, 15 of which were polypoid, 14 were flat, and none were depressed lesions[10]. Notably, the flat adenomas were all less than 5 mm which could easily be missed without the methylene blue dye spraying technique at colonoscopy.

In view of the ethnic difference in incidence of CRC in Malaysia, Rajendran *et.al.* also sought to determine whether ethnic differences in the prevalence of adenomas correlated with ethnic differences of CRC. In their series of 311 consecutive patients undergoing colonoscopy, ethnicity was not associated with prevalence of adenomas. However, only 63 adenomas in 36 patients were observed in this cohort and larger studies are required to validate this observation[11].

Inherited CRC syndromes

There are two most prevalent cancer susceptibility syndromes that result from germline mutation of key genes involved in CRC, namely Hereditary Non Polyposis Colon Cancer (HNPCC) and Familial Adenomatosis Polyposis Coli (FAP). Other rare syndromes are also associated with higher risk for CRC but less clearly defined features, such as Cowden's disease and Peutz-Jegher's syndrome.

Familial Adenomatous Polyposis (FAP)

Familial Adenomatous Polyposis is caused by germline mutations in the APC gene on chromosome 5q and is classically inherited in an autosomal dominant fashion by affected individuals. It is responsible for approximately 1% of all colon cancer. It is characterised by the development of at least a 100 or more adenomatous polyps in the colorectum. A subset of these polyps ultimately acquire additional somatic changes required for the transition to cancer. The mean age for cancer development is 42 years. More than 600 mutations have been reported in the APC gene[12].

Zulqarnain *et al.* reported FAP inheritance in nine individuals in three generations of a Chinese family[13]. Sequence analysis revealed that the affected individuals are heterozygous for a C847T transition that produced a stop codon at amino acid position 283 in place of the usual arginine (Arg283Ter) located in exon 8 of the APC gene.

Lynch Syndrome (Hereditary Non-Polyposis Colorectal Cancer, HNPCC)

The Lynch Syndrome is an old terminology for HNPCC which is responsible for 2-3% of all colon cancer. It is an autosomal dominant inheritance with a penetrance of about 90%. It is caused by mutation in one of five genes that function in DNA mismatch repair (MMR genes -ie. MLH1, MSH2, MSH6, PMS1 and PMS2) which results in development of colonic carcinoma at early age but in the absence of multiple colonic adenoma such as seen with FAP.

Mohd Nizam *et al.* utilised the revised Bethesda Guidelines to identify 34 CRC patients with features of Lynch Syndrome from Kelantan, Kedah and Sabah[14]. The initial immunohistochemistry testing of the tumour samples from these patients found loss of MLH1 and MSH2 protein expressions in three and four patients respectively. Genomic DNA was then extracted from the blood cells of these patients and subjected to polymerase chain reaction (PCR) amplification analysis. Germline mutations were identified in four out of seven patients.

Low penetrance single nucleotide polymorphisms

Besides the known germline mutations that predispose to CRC, it is possible that other inherited mutations or polymorphisms increase an individual's risk for CRC. For example, the genetic variant that predisposes an individual to inflammatory bowel may also constitute a risk factor for CRC. Published studies on a few such polymorphisms have been conducted in Malaysia, but these require validation in large cohorts. Moreover, there have been no studies conducted to determine the significance of variants identified through genome-wide association studies in Caucasian populations in the Malaysian population.

1. MLH1 promoter polymorphism

Besides defective MMR genes, it is thought that the influence of hereditary low penetrance alleles such as the MLH1 promoter polymorphism -93G>A gene may predispose an individual to CRC. The influence of this gene was studied in a case-control study comprising of 104 histopathologically confirmed CRC patients as cases (52 sporadic CRC and 52 suspected Lynch Syndrome patients) and 104 normal healthy individuals from across Malaysia[15]. DNA was extracted from peripheral blood and the polymorphism was genotyped. The genotypes were categorised into homozygous wild type (G/G), heterozygous (G/A) and homozygous variants (A/A). When risk association was investigated for all CRC patients as a single group, the heterozygous (G/A) genotype showed a significantly higher risk for CRC susceptibility with an Odds Ratio (OR) of 2.3. When analysed specifically for the two types of CRC, the heterozygous (G/A) genotype showed significantly higher risk for sporadic CRC susceptibility (OR of 3.7) than for suspected Lynch Syndrome patients (OR: 1.6). The risk was not statistically significant (p=0.253) for suspected Lynch Syndrome patients. Even though homozygous variant (A/A) also showed higher OR value of 2.357 for sporadic CRC risk, the difference was not statistically significant. MLH1 promoter polymorphism -93G>A does appear to modulate susceptibility risk in Malaysian CRC patients, especially those with sporadic disease.

2. p53 polymorphism

In a risk factor prevalence study of blood samples of 202 sporadic CRC patients matched with controls, Abdul Aziz et al reported that the frequency of the P53Arg72Pro Single Nucleotide Polymorphism (SNP) homozygous variant (Pro/Pro) genotype of the p53 genes was significantly higher in cases compared to controls (21% vs 13%), (p=0.013)[16].

3. Interleukin-8-251T>A polymorphism

Chronic inflammation has been linked to increased risk of cancer in patients with inflammatory bowel disease, including Crohn's disease and ulcerative colitis. It is suspected that interleukin (IL)-8, a chemokine mediator of inflammation, may play a role in the pathogenesis of CRC. The mutation IL-8-251T>A may predispose a person to inflammatory bowel disease leading on to CRC. M Aminudin et al. compared DNA from blood samples of 255 CRC patients from Alor Star and Kelantan with age and sex matched controls[17]. They found that individuals with the homozygous variant AA genotype had a 3.6 times higher risk of having CRC compared to those carrying the homozygous wild TT genotype. The variant A allele was calculated to carry a significantly higher risk (OR=1.3) for CRC.

4. Tumour Necrosis Factor-alpha (TNF-α) polymorphism

Tumour Necrosis Factor-alpha is another pro-inflamatory cytokine that was studied in 161 CRC patients and matched

controls by the same investigators in the same hospitals[18]. They found that individuals who were homozygous for the TNF-αG>A allele was 2.6 times more likely to have CRC compared to controls.

MANAGEMENT

Screening

There is currently no population-based screening for colorectal cancer in Malaysia.

Methodology

Faecal occult blood test is one method that could be used in screening for CRC, but the guaiac-based faecal occult blood tests (gFOBT) is hampered by the need to impose dietary restrictions prior to testing, whereas the faecal immunochemical tests (FIT) does not require it. The sensitivity for detecting any neoplasia in a study of 103 subjects screened at an endoscopy unit, comparing the two tests where dietary restriction was not imposed, was 53% for FIT and 40% for gFOBT. The specificity for excluding any neoplasia was 91.7% and 74% respectively. Of the 69 with normal colonoscopic findings, 4.3% were positive for FIT and 23% for gFOBT[19].

A seven-gene biomarker panel analysing gene expression of biomarkers (ANXA3, CLEC4D, TNFAIP6, LMNB1, PRRG4, VNN1 and IL2RB) that are differentially expressed in CRC patients as compared with controls was tested in blood samples from 210 individuals undergoing colonoscopy at Lam Wah Ee Hospital in Penang between 2007 and 2009[20]. The test had been previously validated in a North American population. Ninety nine were patients with CRC, 111 were controls. Logistic regression analysis of seven-gene panel found it had a 77% specificity, 61% sensitivity and 70% accuracy rate, comparable to the data obtained in the North American study making it not a proposed stand-alone test or screening tool.

Awareness of symptoms and risk factors

Despite the increasing incidence of colorectal cancer, awareness of the symptoms of CRC, its risk factors and availability of screening for early diagnosis remains low in the general Malaysian population. Indeed, all of the >1,000 patients diagnosed with CRC in Universiti Malaya Medical Centre (UMMC) and Kuching between 2000-2006 were symptomatic at presentation and none were diagnosed from a screening test[21].

Knowledge of symptoms and risk factors of colorectal cancer has been reported to be disturbingly low (Table I). The survey of 991 subjects in urban Kuala Lumpur found that 42% were unable to identify symptoms of CRC without being prompted or given a list of options and 57% could not identify any risk factor for CRC.3 On the positive side, 24% could identify family history as a risk factor. Other risk factors identified were low fibre diet (16%), age (11%), high fat diet (9%) smoking (9%) and obesity (4.5%). Surprisingly, ignorance was highest among the Chinese (53%). A survey of 2,379 participants from households across small towns in Perak found that the most frequently recalled symptoms were abdominal pain (15%, 346/2,379), followed by "bleeding from the back passage" (6.6%, 158/2,379). All other symptoms were identified by less than 5% of the subjects. When prompted with a list of symptoms, only 30% of the population were able to accurately identify CRC symptoms. Chinese had poorer recognition of CRC symptoms compared to Malays, despite having the highest incidence of CRC[22]. Symptom recognition appears to be higher in a cross-sectional study of 1,905 average risk individuals identified from 44 primary care clinics in West Malaysia from August 2009 to April 2010, with 35% to 74% accurately identifying each CRC symptom, albeit from a given list[23].

Awareness and uptake of screening

Given the low awareness of risk factors and signs and symptoms of colorectal cancer, it is perhaps not surprising that the majority of Malaysians were not aware of screening methods for CRC and uptake of screening was low. In the study of 991 participants in Kuala Lumpur3 the majority (65%) were not aware of any available screening tests for CRC, 33% were aware of colonoscopy and 14% were aware of the faecal occult blood test (FOBT). Two other cross-sectional studies also report low awareness of colorectal cancer screening methods. The first study of 300 students from the Management and Science University found the majority of the participants had no knowledge of colonoscopy (61%) or FOBT screening (62%)[24].

A second cross-sectional study involving 1,905 average risk individuals (those aged 50 years and older who were not known to have personal history of CRC or diseases with increased risk for CRC) from 44 primary care clinics throughout West Malaysia from August 2009 to April 2010 found that only 7% of respondents were aware of screening. Only 13 (0.7%) of respondents had undergone any form of CRC screening in the preceeding five years. The main reason for undergoing screening was advice from health care providers (84.6%)[25]. The main factors for not participating were embarrassment (35.2%) and feeling uncomfortable (30.0%). There were 11.2% of respondents who had never received advice to do screening. In the KL study of 991 subjects, only 15 (1.5%) had previously undergone a screening procedure (13 colonoscopy, two FOBT) and even after being provided with information on risk for CRC, only 39% were agreeable to undergo screening[3]. Malays and Indians were twice more likely compared to the Chinese to be agreeable for screening. Taken together, despite being at the highest risk, ignorance was highest among Chinese (53%) and Chinese were twice less likely to be agreeable to undergo screening.

Regional Comparison

In terms of regional comparison, Malaysia fairs poorly. In a large study (7,915 subjects) across 14 countries in the Asia-Pacific region* in 2007, Malaysia ranked second highest in terms of ignorance after India. Half of the 501 Malaysians surveyed were unaware of any symptoms of CRC, 58% were unaware of any risk factors for CRC, and 80% did not know of any test for colorectal cancer[26]. Malaysians gave the lowest score for the perceived severity of CRC and correspondingly, Malaysians saw the least need for screening. Despite many of the other countries having a lower per capita income than Malaysia, Malaysians were the least likely to have participated in CRC screening, with only 1.2% (3% among those >50 years old) of Malaysians reported previous screening compared to 49% in the Philippines, 38% in Australia and an average participation of 18% across the 14 countries surveyed. Only 38% of Malaysians expressed an intention to undergo screening, compared to 62% in Singapore and 95% in Thailand, both of which are our immediate neighbours. Overall, 20% of the subjects had received physician's recommendations to undergo CRC screening, but this rate was only 1% among Malaysians.

* Australia, Brunei, China, Philippines, Hong Kong, India, Indonesia, Japan, Korea, Malaysia, Pakistan, Singapore, Taiwan and Thailand

Diagnosis

In view of the anticipated need for colonoscopies to screen for CRC, adequate facilities and appropriate guidelines need to be in place. Chan and Goh have examined the usage of colonoscopies at the UMMC[27]. Of 380 patients referred for colonoscopy, 58% were classified as appropriate according to the American Society of Gastrointestinal Endoscopy guidelines. The most common appropriate indications were unexplained rectal bleeding (21%) followed by CRC surveillance (12%). The most common inappropriate indication was inappropriately timed colonic cancer surveillance (8.4%). Chronic constipation in 36 cases (9.5%) was the most common 'unlisted' indication. A positive colonoscopic finding was detected in 35% of examinations and CRC was found in 36 patients (9.5%). Appropriateness of indication was not a predictive factor for positive findings of CRC and there was no difference in the proportion of cases with positive findings or CRC in the three 'appropriateness categories'.

Tan *et al.* reported a prospective study of 485 consecutive patients who underwent colonoscopy during a 22-month period to determine the predictive factors for detecting CRC[28]. Analysis revealed that independent predictors were the presence of rectal bleeding (OR 4.3) and iron deficiency anaemia (OR 4.0). In those aged 50 and older, male gender (4.5) and abdominal pain (3.1) were also significant positive predictors for cancer.

The rate of detection of CRC was reported to be 6% (22/375) in one series[29] and 7% (228/3404) in another30. The first series from Universiti Kebangsaan Malaysia (UKM) found that 73% (16/22) of cancers were located within the recto-sigmoid area. The diagnostic yield for CRC was highest when the indication was rectal bleeding (13%, 11/88) and altered bowel habit (9%, 5/56)[29]. There was a total of 53 (14%) cases of adenomas detected with 79% (42/53) located within the recto-sigmoid area in the UKM series[40], while polyps were noted in 14% (470) of the patients in the UMMC series. Polyps detected concomitantly with cancer were noted in 55 patients (2%) Tumours were mainly left sided (80%, 198/248)] with the majority located in the recto-sigmoid region[30]. Adenomas were found most frequently at colonoscopies for cancer surveillance (24%, 14/59) and rectal bleeding (19%, 17/88)[29]. Four patients were diagnosed to have FAP and 8% (19) had synchronous lesions[30].

Delays in diagnosis

A five year retrospective audit from 1999-2004 involving 137 CRC patients was undertaken in UMMC[31]. The median time to diagnosis was nine days after the first UMMC Surgical Unit consultation with a mean of 19 days. Eleven percent had to wait more than four weeks for diagnosis. The median time from confirmation of diagnosis to surgery was 11 days with a mean of 19 days. Sixty two percent of patients underwent surgery within two weeks of diagnosis and more than 88% by four weeks. However, 10% of them had delayed surgery which was done beyond four weeks from diagnosis. Long colonoscopy waiting time was the main cause for delay in diagnosis while delay in staging CT scans were the main reason for treatment delays.

Patient delay in seeking consultation was examined in a cross-sectional study of patients presenting at the UKM endoscopy unit, between 2008 and 2009[32]. Among the 80 patients, aged 40 and older who presented with rectal bleeding, 60% had delayed consulting medical practitioners by more than two weeks. Fifty three percent (42/80) were not worried or little worried about the symptom, and those who delayed consultation were ten times more likely to not worry or worry less. Sixty four percent correctly identified rectal bleeding as a symptom of CRC but were not aware of the best screening method to detect colorectal cancer.

PRESENTATION AND TREATMENT

Patient characteristics

There have been four hospital-based series of CRC patients that recorded the sites of CRC published the last ten years, from Kuala Lumpur[30], Kota Bharu[33], Kuantan[34] and Penang[35]. Table II summarises the distribution of CRC seen throughout the colon and rectum. Left sided cancers predominate. About two-thirds of all CRC occur from the sigmoid colon to the anus. Malays, not surprisingly, accounted for 77% (88/115) of the patients in Kota Bharu and 59% (70/119) in Kuantan, where they account for a larger proportion of the population. The mean age of diagnosis was 64.4 years and the male to female ratio was 1.15 in the Kuala Lumpur series[30]. The mean age of CRC patients in Kota Bharu was 55.7 years. Eighty two percent of patients were older than 50 years old in Kuantan. In addition to the four, the National Cancer Patient Registry-Colorectal Cancer (NCPR-CC) which was set up in 2007 with pilot centres in Alor Star, Kuala Lumpur, Selayang, Serdang, Kota Bharu, Hospital Universiti Sains Malaysia (HUSM) Kubang Kerian, Johor Bahru, Kuching and Kota Kinabalu hospitals gives a similar picture of the location of CRC in Malaysian patients covering a more widespread sample[36]. Out of the 622 patients enrolled, 60% were males and 40% were females. Forty two percent were Malay, 38% Chinese, 6% Indians and other races accounted for the rest. The mean age was 61 years. The age profile and ethnic distribution in all the above series are reflective of the age standardised rates of CRC noted in Malaysia more accurately documented in the results of the National Cancer Registry[2].

A family history of CRC was noted in 11%[30] and 7%[36] of patients in two different studies. The NCPR study noted that 94% presented with symptoms, only 1% (4/622) was detected through screening[36].

Mohd Radzniwan et al. found that on average, 107 of their CRC patients had symptoms for 13 weeks before consultation[37].

Staging

Information on staging of CRC in Malaysia could be gleaned from a few sources, but exclusion of patients or incomplete data confound the findings. Eighty three percent (409/622) of patients in the NCCR report were not appropriately staged or had missing data for staging[36]. Azmi et al. noted that 41% of their patients were found to have Stage B2 disease and 45% had Stage C2 disease. Malays presented with later stage of cancer compared to Chinese. Fifty four percent of Malays had Stage C2 while 58% of Chinese had Stage B2. Fifty percent of the patients younger than 50 years old were diagnosed with stage C2[34].

Treatment

At UMMC, 84% (147/176) of the patients underwent surgery, 28% (50/176) received either adjuvant or palliative chemotherapy[30]. Ghazali *et al.* at HUSM excluded patients who had more than 30% incomplete information in their medical records and noted 27% (31/115) had surgery alone while 69% (79/115) had surgery with chemotherapy and/or radiotherapy. Another 4% (5/115) had only chemotherapy and/or radiotherapy[33]. The NCCR records noted that 492 of the 622 patients with CRC underwent surgery, 16 of whom had two

surgeries. Eighty two (16%) received only palliative surgery. Two hundred forty one patients (39%) underwent chemotherapy; 175/241(73%) had adjuvant chemotherapy, i.e. postoperatively; and 36/241 (15%) had neoadjuvant chemotherapy. Seventy eight patients received radiotherapy, most with chemotherapy. Only 12 had radiotherapy as palliative monotherapy.

Neoadjuvant Chemoradiation

Lee *et al.* have retrospectively analysed all newly diagnosed patients with rectal adenocarcinoma who underwent long course preoperative RT at the Department of Radiotherapy and Oncology, HKL between 2004 and 2010[39]. Sixty seven out of 507 CRC patients who underwent long course preoperative RT were eligible for this study. The median tumour location was 6 cm from the anal verge. Most patients (95%) had suspicion of mesorectum involvement while 28.4% of patients had enlarged pelvic nodes on staging CT scan. The median age of this group at diagnosis was 60 years old with a range of 26-78 years. All patients underwent preoperative chemo-irradiation except for five who had preoperative RT alone. The radiation dose prescribed was 45Gy in 25 fractions given daily over five weeks. The chemotherapy regime given concurrently with RT for all patients consists of intravenous bolus 5-Flourouracil (5FU)300-325mg/m^2 and folinic acid 20mg/m^2 administered daily for five days on weeks 1 and 5 of pelvic RT. Only 38(57%) patients underwent definitive surgery. Post-operatively, patients received another four cycles of adjuvant chemotherapy. Five patients were deemed to be inoperable radiologically and three patients were found to have unresectable disease intraoperatively. The remaining 21 (31%) patients defaulted surgery.

Complications

The NCPR[36] records show 30 (6%) patients had to return to the operation theatre because of surgical complications, the commonest cause being an anastomotic leak (n=15). Medical complications occurred in 19% (94/508) of surgical operations. Sixty one of these complications were not specified. Chest infection and cardiac events occurred only in 16 and ten cases respectively. Medical complications were more likely in patients who had emergency surgery (26%) compared to those who had elective surgery (16%). In contrast, surgical complications were not related to whether the surgery was elective or emergency. Inpatient mortality was 6% (36/431).

Teoh *et al.* evaluated various risk factors associated with anastomotic leakage after anterior resection surgery for rectal cancer in 64 patients whom were operated from 2001 until 2003 in HUKM[40]. Ten (16%) patients who had demonstrated anastomotic leakage were further analysed. There was significantly more anastomotic leakage in patients with very distal tumour less than 4 cm from anal verge (42% - 3/17) when compared to very proximal tumour of more than 15 cm from anal verge (4.3% -1/23). There was a higher percentage of anastomosis leakage in patients with diabetes, low albumin level, higher staging, poorly differentiated tumours and who had neoadjuvant radiotherapy but the difference was not statistically significant because of the small sample size.

Pathology

Histological information was available from 466 patients in the NCPR report. Ninety six percent (446/466) had adenocarcinomas and of these 81% (301/446) were moderately differentiated. Fifty three percent (118/224) of specimens with lymph nodes showed tumour involvement of the nodes. From 296 resected specimens, 12(4%) had proximal or distal margins involved[36].

Biomarkers

The mutational events that occur in sporadic CRC can serve as biomarkers to differentiate and prognosticate the disease of patient groups. In addition they may indicate what future therapy may benefit subsets of patients. However, many of these biomarkers have yet to possess any clinical relevance today, but are windows to the future. The presence of KRAS gene mutation for example, has recently been found to indicate a poor response to anti-EGFR monoclonal antibody therapy[41].

Gene mutations that occur early in CRC tumourigenesis include the APC gene and KRAS proto-oncogene. The DCC gene and P53 gene mutations occur later, although the exact order may vary. Different types of mutation can occur in each of these genes. The array of somatic genetic mutations that promote CRC include point mutations, small insertion/deletion events, translocations, copy number changes, and loss of heterozygosity (LOH), which eventually attenuate gene expression.

Variety

Sporadic gene mutations are generally known to occur in a particular locus, but these defective gene mutations can occur at many different loci. The different types of mutations that occur in the genes associated with colorectal cancer has been described for the APC, KRAS, MSH2 and MLH1 genes in tissue samples of 76 Malaysian colorectal cancer patients[42]. Seventeen types of missense mutations were found in 38 of these 76 patients. Nine different mutations were identified in the APC gene, five different mutations were detected in the KRAS gene, and two types of mutations were identified in the MSH2 gene. Only one mutation was identified in MLH1. Out of these 17 mutations, eight types of mutations (47%) were predicted to be pathogenic. Seven patients were identified with multiple mutations (3: MSH2 and KRAS, 1: KRAS and APC, 1: MLH1 and APC, 2: APC and APC). Another study examined mutations in the APC and beta-catenin (CTNNB1) genes (genes in the Wnt signalling pathway) as well as MMR genes[43]. They found 15/73 (21%) cases with mutations in the APC gene. Fourteen were exonic mutations, of which 12 were found within the mutation cluster region concurring with studies by Miyoshi *et al.*[44] and Polaski[45]. They found only one CTNNB1 mutation and 23% (16/70) of the cases also had some form of MMR defect. They looked for racial differences in the prevalence of these mutations but found none.

Yam *et al.* used a commercially available single-nucleotide polymorphism genotyping array to detect both copy number abnormalities and copy-neutral loss of heterozygosity (LOH) in sporadic colorectal carcinomas[46]. Matched tumour and normal tissues of 13 colorectal carcinomas were analysed using a 250K single nucleotide polymorphism array. Copy number gain (92.3%) was most common, followed by copy number loss (53.8%) and copy-neutral LOH (46.2%). Frequent copy number gains and losses were observed on chromosomes 7p, 8, 13q, 17p, 18q, and 20q, and copy-neutral LOH was observed on chromosomes 2, 6, 12, 13q, 14q, 17, 20p, 19q, and 22q. Even though genomic alterations are associated with colorectal cancer development and progression, the results showed that DNA copy number abnormalities and copy-neutral LOH did not reflect disease progression in at least 50% tumours. Copy-neutral LOH was observed in both early and advanced tumours, which favours the involvement of these genomic alterations in the early stages of tumour development.

Prevalence

As the genetic basis of cancers continue to be unravelled in the 21st century, it remains to be seen if the various mutations

responsible for colorectal carcinoma are similar throughout the world, or if different mutations play different roles in different localities. There have been several studies describing the prevalence of the genes responsible for CRC in Malaysia.

Zulhabri found a 20% (14/70) prevalence of KRAS mutations in his series from Universiti Kebangsaan Malaysia, Kuala Lumpur. This gene mutation was significantly more common in larger tumours (>35cm) but were not significantly different when compared according to different races, sex, stage, and microscopic differentiation. There was a tendency for left sided colon tumours to be KRAS mutated[47].

Another study of 49 CRC samples by Yip WK et al., reported a frequency of 25% (11/44) for KRAS mutation (codons 12, 13, and 61), 2.3% (1/43) for BRAF mutation (V600E), and 77% (33/43) for phosphoinositol-3-kinase, catalytic, alpha (PIK3CA) amplification mutations[48]. No mutations for the Phosphatase and tensin homolog (PTEN) mutation was detected, a finding which was confirmed in another study (0/27)[49].

Loss of the normal P53 tumour suppressor gene, is also associated with CRC. However, the loss of a gene product rather than an emergence of a rogue molecule is unlikely to be a potential target for chemotherapy. Nonetheless, its occurrence in CRC is quite common in Malaysia. One study at UMMC reported a 68% (79/116) rate of P53 overexpression[50]. No significant association of P53 overexpression with stage (Dukes' stage) and grade of tumours was found, nor was there any significant relationship between P53 positivity with overall recurrence-free disease interval and survival. A notable finding was a significantly lower rate in P53 overexpression in the tumours among Indian patients (39%, 5/13) when compared to non-Indian patients.

Gene expression

Genetic mutations can either result in the loss or overexpression for some protein products. However gene expression is also mediated by alterations other than changes in the primary base pair sequence of DNA, i.e. epigenetics.

Using immunohistochemical staining, Yip WK *et al.* demonstrated a 55% (24/44) loss of the PTEN protein in their study even though no mutations of the gene were found[48].

Balraj *et al.* found that mutations that produce amplification of PIK3CA produced no significant difference in PI3K p110 alpha expression between CRCs and the adjacent normal colonic mucosa.49 However, a male:female difference was found. It was noted that 100% of male cases vs 56% of female cases harboured amplified PIK3CA($p = 0.002$). PI3K p110 alpha expression was significantly higher in poorly/moderately differentiated carcinoma compared with well-differentiated carcinoma. K-ras mutation, PIK3CA amplification, PTEN loss, and PI3K p110 alpha expression did not correlate with Akt phosphorylation or Ki-67 expression. K-ras mutation, PIK3CA amplification, and PTEN loss were not mutually exclusive. This report on CRC in Malaysia shows comparable frequency of K-ras mutation and PTEN loss, lower BRAF mutation rate, higher PIK3CA amplification frequency, and rare PTEN mutation, in Malaysia compared with other published reports.48 These results have implication for designing targeted therapy drugs. Khor et al. had shown that PI3K/Akt overexpression, found in 21/47 (45%) of their patients by immunohistochemistry, was associated with increased expression of two downstream proteins. They were, glycogen synthase kinase-3β (GSK-3β) in the pathway that promotes cell proliferation, and BCL-2 antagonist of cell death (BAD) in the pathway that blocks cell death[51]. Except for age, there was no correlation between the immunohistochemical scores of the various biomolecules with sex, race and stage and grade of tumour.

Loss of any of the MMR genes (MLH1, MSH2, PMS1, PMS2, GTBP/MSH6) leads to incapacity to recognise and repair errors that occur during DNA replication, resulting in microsatellite instability[52]. The loss of DNA MMR activity accelerates the rate of accumulation of mutations in other genes involved in apoptosis and growth control that predispose to a more rapid adenoma-to-carcinoma transition. Proteins associated with the MMR genes can be detected by immunohistochemistry. A total of 150 colorectal carcinomas from 148 patients from Penang, not distinguishing sporadic and hereditary types, were subjected to immunohistochemistry study[52]. Three patients had synchronous tumours. Twenty eight cancers (18.6%) from 26 subjects (17.6%) had no immunohistochemical expression of any MMR gene proteins, indicating protein inactivation from an MMR gene defect. This comprised three cases with absent MLH1 only, three with absent MSH2 only, two with absent MSH6 only, three with absent PMS2 only, 14 with absent MLH1 and PMS2, two with absent MSH2 and MSH6 and one with absent MLH1, MSH6 and PMS2. There was significant association between abnormal MMR gene protein expression and proximal colon cancers, mucinous, signet ring and poorly differentiated morphology. However, this study did not examine the germline mutations of these genes.

Three synchronous adenocarcinomas has been reported in one patient with histopathological loss of expression of MLH1 and MSH, believed to be a sporadic case[53].

CD133 is a cell surface marker for the AC133 antigen which is the human homologue of murine Prominin-1 found in hematopoietic and neural stem cells and considered a marker of cancer. Studying 56 formalin-fixed, paraffin-embedded tissue blocks of CRC at the UMMC, Chew et al. demonstrated that CD133 expression was present in significantly higher frequency in 49 (88%) colorectal adenocarcinoma tissue compared with 15 (26.8%) adjacent benign colorectal epithelium[54].

The Wnt proteins are regulators of signalling pathways that attenuate p53-mediated apoptosis and progression of the phases of the cell cycle. Wnt-1 (26/47) and its downstream effectors WISP-1 (15/47), cyclin D1 (5/47) and survivin (28/47) were found to be overexpressed in 47 samples of CRC tissue from Kuala Lumpur between 1999-2000[55]. They were overexpressed in relation to 40 samples of adjacent normal tissue, 26vs7 for Wnt-1, 15vs5 for WISP-1, 5vs13 for surviving and 28vs0 for cyclin D1. WISP-1 in CRC tissue was positively correlated with patients older than 60 years and with well-differentiated tumours. Cyclin-D1 expression was associated with poorly differentiated tumour.

Notes on experimental chemotherapy

Gene therapy targeting cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) could prospectively modulate treatment of colorectal cancer, if tumour tissue expressed the right profile. A study on 101 archival, formalin-fixed, paraffin-embedded tissue samples of colorectal cancers that were surgically resected found COX-2 production was detected more in tumour tissue compared to adjacent normal tissue (60vs34). More tumours expressed iNOS (82/101, 81.2%) than COX-2. No iNOS expression was detected in adjacent normal tissue[56]. Poorly differentiated tumours had significantly lower total beta-catenin ($p = 0.009$) and COX-2 scores ($p = 0.031$). No significant relationships were established between pathological stage and beta-catenin, COX-2 and iNOS scores.

These findings suggest COX-2 and iNOS inhibitors may be potentially useful as chemotherapeutic agents in the management of colorectal cancer.

Malaysian investigators have reported that alpha-Mangostin enhances betulinic acid cytotoxicity and inhibits cisplatin cytotoxicity on HCT 116 colorectal carcinoma cells[57]. Experimental studies have also shown one fraction of Kenaf seed oil (Hibiscus cannabinus) appears to have cytotoxic effects on an HT29 colorectal cancer cell line[58].

It has also been reported that ciglitazone treatment suppressed colon cancer cell growth via induction of apoptosis[59].

OUTCOME

In the HUSM series over 10 years from 1996–2005, which excluded patients with more than 30% incomplete information in their medical records, the five-year survival rate noted was 68% for Duke's B patients and 12% for Duke's C patients[33]. Comorbidities were not important prognostic factors. Tumour site was not a predictor of survival. The pre-operative CEA level was only significantly related to survival prognosis in univariate analysis but not an independent factor in multivariate analysis (i.e. taking staging into account).

In a five-year follow-up study, Mohd Radzniwan *et al.* were able to review their experience with 107 CRC patients. All patients were traced by telephone interview and their outcome determined. More than half had defaulted follow-up and this happened most frequently (62%) during the first two years following treatment. Adjuvant chemotherapy and/or radiotherapy was offered to Stage C patients and those with insufficient margin clearance for rectal carcinomas. Local recurrence occurred at a rate of 9.7% for early and 19.6% for late cancers respectively. Metastases were seen in 26% of patients who had adjuvant therapy compared to 6% of those who did not. The overall survival at five-year follow-up was 40%[37].

Kong CK *et al.* compared patients presenting with CRC in UMMC, Kuala Lumpur and Sarawak General Hospital (SGH), Kuching over seven years from 2000-2006[21]. They were interested in the differences that may be seen at presentation and in survival, noting that per capita Gross Domestic Product (GDP) and monthly household income in Kuala Lumpur are double of that in Sarawak. They found no significant difference in terms of age, gender, ethnic group, socio-economic class, duration of symptoms or stage at presentation between the two centres, although patients in Kuching tended to have a longer duration of symptoms and more advanced disease at presentation. There were 565 new cases of CRC at UMMC and 642 patients in SGH. Within centres, however, lower socio-economic class was a significant factor for late and more advanced stage at diagnosis at both centres. As a result they also had poorer three-and five-year survival rates. Five-year survival rates by stage were: Stage I (79%) Stage II (65%) Stage III (44%) and Stage IV (9.3%) at UMMC and Stage I (75%) Stage II (53%), Stage III (36%) and Stage IV (5.2%) in SGH. Survival was lower for patients in Kuching compared to Kuala Lumpur, even after matching for socio-economic class. Reasons cited for this were no colorectal-trained surgeons at SGH and relatively more junior surgeons at SGH compared to UMMC. Besides, CRC patients in Sarawak had limited options for adjuvant treatment and as Sarawak is a larger state, its patients may have had more difficulty accessing health services.

Neoadjuvant Chemoradiation

Lee *et al.* studying neoadjuvant chemoradiation observed a three-year overall survival rate of 57.3% for 67 patients. All patients with pathological positive Circumferential Resection Margin status died within four years. With a median follow-up of 38.8 months, there were 25 patients who were alive without recurrence. Three patients were alive with recurrence, six alive with unknown status and 33 patients had died. The main result of this study was the three-year local recurrence rate of 33% which was much higher compared to the current accepted rate of below 10%. The high rate of local recurrence is worrying and is mainly due to patient defaulting post-preoperative chemoirradiation or delayed definitive surgery.

QUALITY OF LIFE

Sharifa Ezat *et al.* surveyed the quality of life in a sample of 160 CRC patients from three public hospitals using the EORTC QLO C-30 questionaire[60]. Ninety one percent of respondents had stage III and IV CRC (mean age of 58 years. The median global health status (GHS) score was 83. Scoring in this system ranges from 0-100, with a higher score representing a higher quality of life. Male respondents had better cognitive and social function compared to females. Functional status deteriorated measurably with stage of disease. The more advanced stage of disease, the higher the symptom scores (fatigue, pain, nausea/vomiting, constipation, diarrhoea, insomnia, dyspnoea, loss of appetite). Women had worse scores for pain, fatigue and dyspnoea. Diarrhoea was significantly worse in younger patients. Overall, the findings of this study were comparable with studies done in developed countries.

SECTION 2: RELEVANCE OF FINDINGS FOR CLINICAL PRACTICE

The range of publications from Malaysia looking into the genetic causes and biomarkers of CRC shows investigators are keeping their finger on the pulse of scientific research into the biology of CRC, which may one day give rise to a breakthrough in the treatment of this disease. It is difficult to judge how productive such research will be and whether greater investment will yield more benefit. It is not easy to translate any of this research into clinical practice but as often in basic science research, unexpected finding may surprise us.

More predictable however, is research and audit into clinical practice. These may be less exciting but has revealed perhaps the findings of greatest clinical relevance to CRC management. Audit of patient delay and hospital delay in scheduling diagnostic tests for detecting and assessing CRC patients need to be on-going processes. The capacity for colorectal surgery and delays from time of diagnosis to treatment should also be investigated.

In addition, the revelation that Malaysians are placed in jeopardy by their poor knowledge and attitude towards CRC calls out for action to translate the findings into clinical practice[26]. Health education measures to raise the awareness of our population regarding the severity of the disease as well as its symptoms and risk factors should be mapped out and implemented. Awareness of screening has to be developed alongside provision for colonoscopy. Incentives for the populace to undergo screening colonoscopy, such as tax relieve or through SOCSO benefits or EPF funds, might be considered.

In view of the poor awareness of symptoms and risk factors of CRC among Malaysians, and in addition, the low perception of its severity, it is of great interest to know if Malaysians present with a later stage of the disease compared with other countries. Table IV shows results of CRC disease stage in a few other countries to compare with the data in Table III. If the un-

Table I: Knowledge and Attitude Regarding Colorectal Cancer Screening among Malaysians

	Unaware of Symptoms	Unaware of Risk Factors	Unaware of Screening	Undergone Screening	Agreeable for Screening
Hilmi 2006-8[3] (n=991)	42%	57%	65%	1.5%	39%
Koo 2007[26] (n=501)	50%	58%	80%	1.2%	38%
Harmy 2009-10[32] (n=1905)				0.7%	
Al-Naggar 2013[24] (n=300)			61%		

Table II: Location of Colorectal Cancer in Malaysia Patients

	N	Rectum	Rectosigmoid	Sigmoid	Descending	Transverse	Ascending	Caecum
Goh KL[30] (1999-2003)	248	36%		32%	11%	6%	7%	6%
Ghazali AK[25] (1996-2005)	115	36%	23%	42%				
Azmi[34] (2001-2005)	119	55%		26%			19%	
Kaur G[35] (2001-2005)	148	46%		20%	8%	6%	9%	10%
NCPR[36] (2007-2008)	622	33%	16%	18%	4% +splenic 4%	3% +hepatic 6%	5%	6%

Table III: Location of Colorectal Cancer in Malaysia Patients

	N	Stage A (%)	Stage B (%)	Stage C (%)	Stage D (%)	Unstaged
M Radzniwan[37] (1997-2000)	107	3	36	40	21	Includes only patients with compete five-year follow up
Goh KL[30] (1999-2003)	154	5	42	15	39	Includes only those who had surgery
Ghazali AK[33] (1996-2005)	115	0	44	33	24	Patients with 30% of records incomplete excluded
Penang CR[38] (2004-2008)	1642	12	31	28	29	721 (excluded)

Table IV: Stage of Colorectal Cancer at Presentation in Other Countries

	n	Carcinoma in situ	Stage A (%)	Stage B (%)	Stage C (%)	Stage D (%)	Unknown
Singapore[61] (2003-2007)	7303	1%	10%	25%	32%	19%	14%
United States[62] (1996-1998)	12,099		17% (14-23)	28% (24-36)	38% (29-46)	10% (7-18)	7% (3-10)
Europe[63] (1996-1998)	3,337		17% (11-28)	30% (25-37)	21% (24-30)	21% (11-33)	10% (4-24)
Xin Jiang, China[64] (2000-2007)	1,210		11%	30%	45%	14%	

Table V: Five-year Survival of Colorectal Cancer Patients

	Stage I	Stage II	Stage III	Stage IV
Ghazali AK[33] (1996-2005)		68%	12%	
UMMC (Kong[21]) 2000-2006	79%	65%	44%	9.3%
SGH (Kong[21]) 2000-2006	75%	53%	36%	5.2%
United Kingdom[65] (1996-2002)	93%	77%	48%	6.6%
United States[62] (1991-2000)	93%	79%	64%	8%

staged group in the Malaysian series was included in the total, the percentage in all the other stages falls so low, comparisons cannot made. However, it should be suspected that un-staged patients are more likely to have late stage disease. Even so, when comparing data with the Singapore Cancer Registry over a similar period and a collection of United States and European studies a decade earlier, Malaysian records show a much lower percentage with Stage A disease and a high proportion with late (Stage C and D) disease. The true percentage of Malaysian patients presenting with late disease still needs to be determined. The overall picture, however, indicates that more Malaysian patients are presenting with later stage disease than in developed countries, even if we cannot quantify by exactly how much.

The poor survival rate for all stages of CRC reported in Malaysia in Table V compared with other international studies is also of great concern. There is a difference or up to more than 10% in survival in patients presenting in early colorectal cancer compared to the best centres. Issues such as delay in treatment, optimum use of neo-adjuvant and adjuvant therapy, as well as safe and effective surgery in Malaysia need to be studied and audited.

SECTION 3: FUTURE RESEARCH DIRECTION

As disease prevention is always more effective than curing cancer, studies into the prevalence of known risk factors for CRC and how they might be reduced in the Malaysian population is an area to be explored. Reducing obesity and promoting a healthier diet of less carcinogenic food and greater intake of fibre in the diet to reduce constipation need not only to be studied but also implemented. To document the way Malaysians present and audit outcomes of treatment, the National Cancer Patient Registry-Colorectal Cancer needs to be supported and expanded.

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