COLORECTAL CARCINOMAS IN NATIONAL HOSPITAL ABUJA, NIGERIA

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DECLARATION

I declare that no part or whole of this work which was performed by me under proper supervision has been submitted anywhere either for publication or to any postgraduate medical college for any purpose. The findings and views, expressed are entirely mine, except where duly acknowledged or referenced.

DR. OLAH, FRIDAY GEORGE E.C.

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Signature                                      Date
CERTIFICATION

We hereby, certify that this project titled: THE SURGICAL PATHOLOGY AND MSI STATUSES OF COLORECTAL CARCINOMAS IN NATIONAL HOSPITAL ABUJA, NIGERIA (A RETROSPECTIVE IMMUNOHISTOCHEMICAL STUDY, 2004-2013) was conducted by Olah, Friday George E.C. under our supervision.

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ABSTRACT

Study Background, Aim and Objective: Tumours with microsatellite instability occur more frequently in younger patients, are more frequently located proximal to the splenic flexure and exhibit exophytic growth, poor differentiation, extracellular mucin production, and a Crohn’s-like lymphocytic reaction. Cell lines from tumours with microsatellite instability are less sensitive to alkylating agents. Such tumours have a better survival rate relative to microsatellite-stable (MSS) tumours of similar stage.

The aim of this study is to do a pathological review and to determine the characteristics and frequency of microsatellite instability status in a series of colorectal cancers in Abuja, Nigeria with a view to suggesting possible aetiological factors.

Method: The study is a retrospective histopathological and immunohistochemical study of 175 paraffin tissue blocks with histologically proven colorectal carcinoma and available clinical information spanning a 10 year period.

Results The mean age was 50.8 years (range 21-94 years) with a near-equal gender distribution (M: F ratio-1.08:1). The vast majority of patients (69.8%) were aged between 40 and 69 years with 21.6% patients below age 40 years. Well differentiated carcinomas were the commonest, accounting for 81 or 43.6%. Tumours of poorer grade were relatively more common in the younger age groups. The left sided colonic tumours were more commonly endophytic/annular, the right colonic tumours were more commonly exophytic/fungating (p value = 0.02).

Overall, 140 out of 175 samples were tested for microsatellite instability (MSI) using standard IHC, 45 patients (32.1%) had high frequency MSI (MSI-H) tumours and 75 patients (53.6%) had microsatellite stable (MSS) tumours. A right sided location and poor differentiation correlated with MSI-H tumours (p values of 0.013 and <0.001 respectively). The strongest associations were found between MSI-H status and residual adenomatous tissue, worse tumour grade, more Crohn-like feature and more advanced pathological staging with p values <0.001 each.

Conclusions: High frequency MSI (MSI-H) colorectal carcinomas were relatively more frequent in our study (32.1%). The disease occurs at a much younger age group than in Caucasians and Arabs. Premalignant (adenomatous) lesions are few accounting for the low colon cancer rates in our study. The CIMP pathway is suggested to play a greater role than is currently ascribed to it in the tumorigenesis. The rarity of adenomatous polyps also suggests the possibility of other precursors like inflammation.
DEDICATION

To God Almighty, the author and sustainer of life.

To all who endure colorectal cancer as patients and loved ones.

To all who work for the sufferers through care and research.

To Prof EEU Akang who did ordinary things in extraordinary ways.
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CHAPTER 1.
INTRODUCTION

1.0 BACKGROUND

Over the last few years the tasks of the surgical pathologist have been restructured gradually from those of classifying diseases and making accurate histological diagnosis alone. The pathologist is now much more involved in clinical decision making bordering on prognostication, choice of therapy as well as other findings directly relevant to patient management. Furthermore, early detection and prevention of ailments like cancer especially among at-risk groups have been refined by the input of pathology. Most of these achievements have been made possible by advances in molecular biotechnology and medical genetics. These new disciplines harness modern medicine and basic science to improve our insight into the processes which regulate cell growth and differentiation and the defects that occur in this closely guarded process.\textsuperscript{1,2}

Although molecular diagnostics is rapidly expanding to include all epithelial neoplasms of the gastrointestinal tract and indeed most other cancers, clinical testing has progressed the furthest in colorectal cancer (CRC) and as such, the molecular diagnostics, therapy and prognostics (theranostics) of CRC is the thrust of this work. Molecular tests that are currently in use, including microsatellite instability (MSI) testing to detect inheritable disease, and KRAS and BRAF mutational analysis to predict response to therapy, were discussed.\textsuperscript{1}

Colorectal carcinoma (CRC) was, hitherto thought to be uncommon in the developing countries especially the tropics. However, this disease is gaining in prevalence in our parts of the world sufficiently enough to elicit concern. By the last decade, carcinoma of the colorectum moved from the tenth to the third position.\textsuperscript{3} A reasonable percentage of these cancers are a frequent component of the family cancer syndromes which are developed at a younger age.

Tumours with microsatellite instability occur more frequently in younger patients, are more frequently located proximal to the splenic flexure and exhibit exophytic growth, poor differentiation, extracellular mucin production, and a Crohn’s-like lymphocytic reaction.\textsuperscript{4} Cell lines from tumours with microsatellite instability are less sensitive to alkylating agents.\textsuperscript{5} Such tumours have an improved survival rate relative to microsatellite-stable (MSS) tumours of similar stage.\textsuperscript{6,7} The mechanism underlying tumorigenesis leads to profound divergences in the pathological features, prognosis and response to chemotherapy of both the sporadic and inherited types of colorectal cancer. Pathogenic mutations in a mismatch repair (MMR) gene usually lead to absence of any detectable gene product.
First step is usually to test all tumours for MSI-H phenotype by polymerase chain reaction (PCR) technique. The alternative is to pre-screen tumours with immunostaining for Msh2 and/or Mlh1 protein which is cheaper and technically easier.\textsuperscript{8} Next, germline (specific genetic) testing is performed if the immunohistochemical (IHC) test is positive. With this in mind, a study analysing microsatellite instability was carried out on tissues of colorectal cancer patients received at the National Hospital, Abuja, Nigeria.

For patients with detected high risk of developing synchronous or metachronous cancers, the screening protocol is recommended. Genetic testing of the proband with colorectal cancer will be of benefit to other family members by identifying the family mutation. It will also benefit the proband where Lynch syndrome is identified and appropriate surveillance for associated malignancies can be initiated and maintained.\textsuperscript{8}

Currently, the TNM, Duke and Astler-Coller classifications are the only tools used during the selection process to determine prognosis and therapeutic options in most centres in Nigeria. There is strong evidence that MSI-positive tumours are resistant to 5-fluorouracil and more sensitive to Irinotecan than MSI-negative tumours.\textsuperscript{9} In this regard, recognition of the microsatellite phenotype is important in the management of patients with CRC rather than the blind approach currently being used.
CHAPTER 2.
LITERATURE REVIEW

2.1 EPIDEMIOLOGY OF COLORECTAL CANCER

It is generally believed that colorectal cancer incidence is lower in Africans, Arabs and Indians than in Caucasians.\(^\text{10,11}\) The high colon cancer rates in Caucasians has been ascribed to the tripod of genes, the environment and lifestyle. The adenoma-carcinoma sequence has been suggested to be the common pathway of causal factors. The relative rarity of colon cancer in our parts of the world, coupled with the peculiar biological characteristics of the tumour in these “low colon cancer areas” has led to the suggestion that a novel pathway may account for the mechanism in cancers here.

2.1.0 BURDEN OF THE DISEASE

Colorectal cancer is the fourth most common cancer in men and the third most common cancer in women with an estimated worldwide incidence of 570,000 new cases per annum.\(^\text{12}\) It affects 7% of the population and ranks as the second leading cause of cancer-related mortality.\(^\text{13}\) It has variable geographical distribution. Several studies have reported rapid increases in colorectal cancer incidence rates, particularly in developing countries in many parts of the world, and these increases are thought to reflect changing dietary and physical activity patterns.\(^\text{14,15}\)

Colorectal cancer (CRC) is the third most common cancer and currently second in leading causes of cancer death among both men and women in the United States and African Americans are at the highest risk of developing and dying from colon cancer.\(^\text{16,17}\) About 141,210 new cases and 49,380 deaths were expected in 2011. About 72% of cases arise in the colon and about 28% in the rectum.

Bowel cancer is the second most common cancer in the UK, after breast cancer in women and lung cancer in men. Up to the 1960s it was rare in Japan and Far East countries, but the incidence has increased rapidly, so that age-specific large bowel cancer rates in males are now greater in Japan than in the UK (Bingham et al. 1996)\(^\text{18}\). These changes are attributed to Westernization of the Japanese diet, including increased meat consumption.

In the Philippines, colorectal cancer is one of the first 10 cancers with age-standardized incidence (and mortality) of 18.8 (12.1) per 100,000 among males and 14.8 (9.5) per 100,000 among females.\(^\text{19}\) According to the 2008 Glob can report on the incidence of colorectal
cancers in Nigeria the age standardized incidence rates (and mortality) for males were 7.1(5.8) per 100,000 and 4.4(3.6) per 100,000 for females.\textsuperscript{20}

Over the past decade, the overall incidence of CRC in South Africa has increased markedly. In 1989, CRC was the 10th most common cancer diagnosed in males and females in South Africa but was more recently ranked among the foremost 5 cancers (5th among males and 3rd among females).\textsuperscript{21} The epidemiological profile for CRC in South Africa is similar to what obtains in the western world. The number of CRC patients in South Africa has been on the rise.\textsuperscript{22} Cronje et al studied 1,732 colorectal cancer patients between 1990 and 2003 and found that 83\% of black patients were \textless{}50 years of age compared with only 10\% of younger whites (p\leq0.001).\textsuperscript{23}

Currently, there is no available population statistic on colorectal cancer. However, evidence from hospital studies point to the low colon cancer incidence and a slow rising rate.

Between 1954 and 1967 Williams and Edington counted 166 colorectal cancer patients over an 8 year period. A similar study at that time by Mulligan in Ilesha over a 14 year period reported 27 patients over a period of 14 years, about two patients a year.\textsuperscript{24}

According to the records from Ibadan, between 1971 and 1979 the incidence ranged between 12 and 14 patients per year. Since then, the yearly incidence has risen from an average of 18 patients per year, thirty-five years ago to about 25 patients per year about 18 years ago. The incidence rose to over 50 patients in 2004. It is noteworthy that colorectal cancer moved from the tenth to the fourth position among the commonest malignancies over the period under review.\textsuperscript{25} Data from the cancer registry at the University College Hospital Ibadan show an average of 70 cases per year from 2002 to 2006.\textsuperscript{26}

Ojo \textit{et al} 1991 and Akinola, Arigbabu 1994 reported an average of 10–13 cases per year between 1991 and 1994 from Ife. More recently, Omonisi, Ojo, reviewed 526 cases of gastrointestinal cancers diagnosed histologically in Ile –Ife, from 1999-2008. They reported that gastrointestinal cancer was second only to breast cancers and colorectal cancer was atop accounted for 37.6\% of all GIT tumours.\textsuperscript{27}

Abdulkareem et al studied 420 CRC cases between 1995 and 2007 in Lagos. It was observed that CRC was still the commonest malignant lesion of the GIT. They are commonly located in the recto-sigmoid region. The age and sex prevalence and morphological profiles related well with those from elsewhere.\textsuperscript{28}
Onuigbo in 1975 and Nwafo, Ojukwu in 1980, reported yearly averages of between 3 and 5 from Enugu (Eastern Nigeria). In Benin-City, a study between January 1983 and December 2002 showed colorectal tumours to constitute 2% of the histologically confirmed tumours diagnosed during the period. The rectum was the most common location.29

It is important to note that greater awareness, improved diagnostic services and a better functioning cancer registry across Nigeria may have contributed to the increased numbers recorded.

2.1 AGE AND GENDER DISTRIBUTION

Abdulkareem et al studied 420 CRC cases (237 males and 183 females) between 1995 and 2007. It was observed that CRC was still the commonest malignant lesion of the GIT. It had a slight male predilection (1.3:1), similar to the global ratio.91 Of these, 23% occurred below 40 years. In that study, majority were poor prognostic type with 85% presenting at TNM stages II and III. These features are highly suggestive of a HNPCC profile and familial clustering of cancer syndromes may be discovered with a careful search. Soliman et al postulated that the occurrence of colorectal carcinoma at young age in Egyptians may imply a hitherto unidentified inherited syndromes.30

2.1.2 TOPOGRAPHICAL DISTRIBUTION

Of the 2238 cases in which site was reported, the rectum 1349(60%) was the commonest site. There were about twice as many left-sided tumour (62.3%) than there were right sided tumours (37.7%). The caecum constituted 17%.91

2.2 MOLECULAR MECHANISMS OF COLORECTAL CARCINOGENESIS

Several mechanisms are at play in the transmission of genetic materials from parent cells to their progeny. Other equally vital mechanisms are involved in the regulation of cell cycle, differentiation and aging. Tumours are initiated when these mechanisms falter.

Two broad groups of genes govern tumorigenesis: those that promote growth of tumours (oncogenes) and tumour suppressor genes. Oncogenes can encode growth factors or their receptors, signalling molecules, regulators of the cell cycle, and other factors that regulate cell proliferation and survival. Their oncogenicity can be induced by mutations that lead to overactive gene products, amplifications that alter copy number, alterations or rearrangements that affect promoter function, or modified interactions with regulators of
transcription or epigenetic modification. Tumour suppressors on the other hand restrain growth and proliferation, passage through the cell cycle, motility, invasion, or other functions related to stable differentiation. Genes that encode tumour suppressors are commonly inactivated by deletion, mutations, promoter methylation, or other changes in regulation. Failure of these functions could lead ultimately to development of cancer.

Fearon and Vogelstein proposed a genetic model to explain the stepwise formation of colorectal adenocarcinoma (CRC) from normal colonic tissues.\(^{31}\) This model states that: (1) CRC is the result of permanent changes in genes with important functions in regulating cell proliferation or repair of DNA damages. In other words, CRC is a disease due to accumulated genetic mutations; (2) mutations in more than one gene are required, and (3) the sequence of mutations is important in determining the eventual formation of CRC.

**The adenoma/carcinoma progression sequence**

The large majority of colorectal malignancies develop from an adenomatous polyp (adenoma). These can be defined as well-demarcated masses of epithelial dysplasia, with uncontrolled crypt cell proliferation. The evolution of CRC proceeds on the basis of a relatively uniform and linear sequence of steps, with APC inactivation initiating adenomas and additional genetic changes, notably KRAS mutation, and TP53 inactivation promoting the emergence of increasingly aggressive subclones (Figure 2.1)\(^{32}\).

The original Fearon and Vogelstein model proposed that only tubular and tubulovillous adenomas had the potential to progress to invasive adenocarcinoma. It is now recognized that serrated polyps including sessile serrated adenomas (SSA) and traditional serrated adenomas (TSA) also have the potential for malignant transformation. Premalignant serrated polyps more frequently arise in the proximal colon and are associated with microsatellite instability and DNA hypomethylation at CpG islands, whereas conventional tubular adenomas arise via biallelic inactivation of the APC tumour-suppressor gene and display chromosome instability. Furthermore, other molecular lesions, such as \(BRAF\) V600E mutations, are characteristically found more often in tumours arising via the serrated neoplasia pathway.\(^8\)

The condition familial adenomatous polyposis (FAP), caused by germ-line mutation of APC, was perceived as the hereditary counterpart of the ‘vast majority’ of sporadic CRCs. An additional factor required to explain how the accumulation of multiple genetic changes could occur within the limited lifespan of a cell is genetic instability involving the loss of a mechanism(s) not only critical for the maintenance of genomic fidelity during cell division,
but is also capable of triggering apoptosis in the setting of accumulating genetic damage. Over the ensuing long period this accumulation of genetic alterations eventually overwhelms the control mechanisms built into each cell.\textsuperscript{33}

\textbf{Figure 2.1} Characteristics of the two pathways in colorectal carcinogenesis. APC, adenomatous polyposis coli; BAX, Bcl-2-associated X protein; CIMP, CpG island methylator phenotype; COX, cyclooxygenase; DCC, deleted in colon cancer; IGF-IIIR, insulin-like growth factor II receptor; LOH, loss of heterozygocity; MLH, MutL homologue; MSH, MutS homologue; Smad, mothers against decapentaplegic homologue (Drosophila); TCF, T cell factor; TGF-βR, transforming growth factor β receptor. (modified from Fearon ER, Vogelstein B. A genetic model for colorectal tumorigenesis. Cell 1990;61:759–767).

It is interesting to note that both clinical management and research have proceeded for many years with the assumption that CRC is a homogeneous disease. Again, it has been observed over the last decade that particular morphological subtypes, such as mucinous carcinoma as well as clinical features have been shown to differ according to topography.\textsuperscript{34} It is now known that only a few mutations are common to most colorectal tumours. Accumulating evidence indicates that CRC has substantial genetic heterogeneity, and that CRC actually comprises several different genetic diseases all affecting the same organ.\textsuperscript{35,36} In fact, no two CRCs are alike in every ‘genetic’ way. CRCs are heterogeneous in terms of molecular momentum, regional distribution, pathology of the invasive and precursor lesions and natural history.

\section*{2.3 GENETIC PATHWAYS IN COLORECTAL CANCER}

\subsection*{2.3.1 Genetic and Epigenetic Instability and Chromosomal Alterations}

Traditionally, colorectal cancers are seen as either sporadic or inherited, and can arise through more than one molecular pathway.\textsuperscript{12,37} Two specific types of genetic instability have
been defined in colorectal cancer. One is associated with chromosomal instability (the CIN) and the other with DNA microsatellite instability (MSI), which is due to replication errors.  

Genetic and Epigenetic instability characterize most colorectal tumours. They cause alterations of gene function on a genome-wide scale. The four better understood pathways include: (1) chromosomal instability (CIN), (2) microsatellite instability (MSI), (3) CpG island methylator phenotype (CIMP), and (4) global DNA hypomethylation. The common theme of the first two pathways is genomic instability. The result of genomic instability is the accumulation of mutations that provide a survival advantage to specific clones of cells, which eventually could become carcinogenic. These mutations occur in genes that control cell growth and cell death. There are overlaps between these categories and imprecise use of these terms resulting in difficulty in review of the literature. The different types of genomic and epigenetic instability as well as clinical relevance these mechanisms are next discussed.

2.3.2 Chromosomal Instability Pathway (CIN) or Suppressor Pathway

This is the most common form of genomic instability, found in as many as 70-85% of colorectal cancers. In the chromosome instability, which can be recognised by the presence of aneuploidy, is defined as the presence of numerical chromosome changes or multiple structural aberrations. The CIN pathway is associated with mutation in APC and/or loss of chromosome 5q that includes the APC gene, mutation of the KRAS oncogene, loss of chromosome 18q and deletion of chromosome 17p, which contains the important tumour suppressor gene TP53. The earliest identifiable lesion in this pathway is the dysplastic aberrant crypt focus (ACF), a microscopic mucosal lesion that precedes the development of a polyp. It results in loss of heterozygosity (LOH) and DCC (on chromosome 18). CIN confers poor prognosis in colorectal cancers.

Most CRCs are sporadic and arise through the chromosomal instability (CIN) pathway. A very small percentage of chromosomal instability tumours are inherited and arise secondary to germline mutations in the APC gene (familial adenomatous polyposis; less than 1% of CRCs).

2.3.3 Microsatellite Instability (MSI) or Mutator Pathway

Colorectal tumours generated via the mutator pathway are characterized by microsatellite instability. They account for approximately 15% of colorectal cancers. Loeb proposed the mutator hypothesis, suggesting that mutations in genes that maintain the stability of the genome may induce a “mutator phenotype” that may then drive tumour progression.
Thibodeau and colleagues reported a similar mechanism in tumours of the proximal colon with absence of loss of heterozygosity.\textsuperscript{43} The latter mechanism was subsequently identified in colorectal tumours in HNPCC families.\textsuperscript{44} The mechanisms underlying MSI are relatively well understood and involve inactivation of genes in the DNA Mismatch Repair (MMR) family either by aberrant methylation or by somatic mutation (insertions or deletions).\textsuperscript{20} They are characterised by frame shift mutations and base-pair substitutions that are commonly found in short, tandemly repeated, nucleotide sequences known as microsatellites. When these “spelling errors” are not checked because of defects in the mismatch repair mechanisms (mutated genes), MSI occurs. These microsatellite mutations may lead to genomic instability, which, in turn, may accelerate further accumulation of mutations in other cancer genes during tumorigenesis.\textsuperscript{45,46} Rather than provide a territorial expansion or selective growth advantage like CIN does, the mutator changes propagate gene alterations in proto-oncogenes or in other mutator genes. MSI leads to a dramatic increase in genetic errors and several microsatellites are present in genes implicated in colorectal carcinogenesis, such as \textit{hMSH2}, \textit{hMSH3}, \textit{hMSH6}, \textit{hMLH1}, \textit{hPMS1}, and \textit{hPMS2}, \textit{TGF-BRII}, \textit{BAX}, \textit{CASPASE 5}, \textit{β-CATENIN}, \textit{APC}, \textit{IGF-II}, and \textit{E2F4}.\textsuperscript{47}

MSI is assessed using a panel of 5 nucleotide markers selected for high sensitivity and specificity.\textsuperscript{48} The panel includes two mononucleotide (BAT25 and BAT26) and three dinucleotide microsatellites (D5S346, D2S123, and D17S250). Microsatellite-high (MSI-H) is defined as MSI at $\geq 2$ (40\%) of the five specified sites, MSI-L (low) means MSI at 1 site, and microsatellite stable (MSS) when no instability is demonstrated in a panel selected at a National Cancer Institute consensus conference.\textsuperscript{49}

Colorectal cancer patients with MSI tumours have been shown to have a better prognosis compared to patients with CIN tumours\textsuperscript{50,51,52} and probably respond differently to adjuvant chemotherapy compared to patients with microsatellite stable (MSS) cancer.\textsuperscript{53,54,9} Furthermore, individuals with Lynch syndrome (hereditary non-polyposis colorectal cancer, HNPCC) almost exclusively develop MSI colorectal cancers because they have germline mutations in the MMR genes.

\textbf{2.3.4 The CpG Island Methylator Phenotype (CIMP) or Methylator Pathway}

The CIMP pathway is the second most common pathway to sporadic CRCs accounting for approximately 15\% of sporadic cases.\textsuperscript{55,56,57} Epigenetic instability in colorectal cancer is manifested as both hypermethylation of gene promoters that contain CpG islands (the CpG Island methylator phenotype, CIMP), and global DNA hypomethylation. The CIMP pathway
provides the epigenetic instability necessary for sporadic cancers to methylate the promoter regions of, and thus epigenetically inactivate the expression of key tumour suppressor genes such as MLH1. The strong association between *BRAF* V600E mutations and CIMP colorectal cancer suggests a role for activated *BRAF* in the pathogenesis of the methylator phenotype and a link between sporadic MSI and CIMP. The CIMP panel of genes and/or markers is analogous to the panel of microsatellites used to determine microsatellite status. However, there is no universally agreed battery of CIMP markers or even a gold-standard technique for characterising methylation for the diagnosis of CIMP. CIMP is usually defined as methylation of at least three loci from a selected panel of five gene associated CpG islands. The discovery and classification of CIMP tumours (into CIMP-High or CIMP1 and CIMP-Low or CIMP2) has advanced our understanding of the molecular pathology of colorectal cancer but has not yet impacted clinical care. In addition to aberrant gene methylation, a global decrease in methylation has also been identified in many colorectal cancers and is tightly associated with CIN tumours. Further research is necessary to determine if measurement of global DNA hypomethylation in colorectal cancer has any role in the clinical setting.

CIMP-positive CRCs are characterised by a well-defined cluster of clinicopathological features, including proximal location and a gender and age bias for the development of CIMP in older women. The ultimate phenotype is influenced by the presence or absence of concomitant microsatellite instability, which may arise from gene promoter methylation-induced transcriptional silencing of MLH1. Hence, additional MSI-H status confers better prognosis on CIMP-positive CRCs. In the CIMP pathway, however, sessile serrated adenomas are the chief pathological precursor unlike CRCs developing via the CIN pathway, and also in HNPCC which originate from adenomatous polyps.

Sporadic MSI colorectal cancers most often have loss of MMR activity as the result of silencing of *MLH1* by aberrant methylation. It is also now recognized that sporadic MSI tumours are associated with the serrated neoplasia pathway and frequently carry *BRAF* V600E mutations, while cancers resulting from germline mutations in MMR genes (Lynch syndrome) do not have mutated *BRAF*. Thus, the presence of a *BRAF* mutation in an MSI tumour effectively excludes the possibility that the tumour arose as the consequence of Lynch syndrome.

Jass and colleagues proposed the serrated pathway for colorectal carcinogenesis. In this pathway, the initiating step for colorectal carcinogenesis is not through *APC* mutations, but
caused by epigenetic silencing of genes involved in DNA repair, cell-cycle control, and a control of differentiation. Morphologically, the precursor lesions are serrated and include hyperplastic, mixed hyperplastic/adenomatous polyps, and serrated adenomas. Tumours originating via this pathway may have any level of instability (MSS, MSI-L, and MSI-H).

It may be that genomic instability, whether via CIN or the mutator pathway, occurs after adenoma formation, but prior to frank malignancy.

2.3.5 Landscaper Defect Pathway

In juvenile polyposis and ulcerative colitis, it appears that the defective cells are derived from the stroma and that the epithelial tumorigenesis is the result of an abnormal microenvironment. This pathway has been termed the landscaper defect pathway.

There are still other undescribed pathways that will undoubtedly interact with the pathways described, and may even modify these routes to carcinogenesis.

Once early adenomas are formed, other molecular changes in other tumour-suppressor genes and oncogenes occur. These changes are facilitated by genomic instability which enables the cells to obtain growth advantages, leading to late adenomas (tubular, tubulovillous, and villous), and, in some cases, to carcinoma and metastases.

2.4 MICROSATELLITE INSTABILITY AND MISMATCH REPAIR SYSTEM

Microsatellite DNA sequences are defined as short dinucleotide or mononucleotide repeats. These sequences are usually within noncoding regions. However, some genes contain microsatellites within coding regions e.g., TGF-β receptor II, Insulin like growth factor II receptor, regulators of the cell cycle e.g. E2F4, regulators of apoptosis e.g. BAX and even the MMR genes themselves. These repeats exist in normal cells at frequencies of less than 50. Alterations due to mutations in the pockets of repeat sequences, also called microsatellites, are a feature in some tumours. One type of error called “slippage” can occur during the replication of microsatellite sequences by DNA polymerase. Various repair mechanisms are available to correct these errors (Figure 2.2).

![Figure 2.2. Slippage during DNA replication. (From Chung: Ann Int Med; 2003, vol 138, 2003)](image-url)
The primary function of the MMR system is to eliminate these mismatches and insertion-deletion loops. Mutations in MMR genes result in a failure of the mismatch repair system to repair errors that occur during the replication of DNA in tumour tissue. Such errors are characterized by the accumulation of alterations in the length of simple, repetitive microsatellite (2 to 5 base repeats) sequences that are distributed throughout the genome (MSI). Seven different MMR proteins are known to be related to MSI in CRCs. They are mutS – hMsh2, hMsh3, hMsh6; and mutL – hMlh1, hMlh3, hPms1 and hPms2. Mutations in hMsh2 or hMlh1 usually result in high MSI while mutations in genes such as hMsh6 result in low levels of MSI because the former are the forebears (or ‘carriers’) of the other repair proteins.

![Figure 2.3. Components of DNA mismatch repair system. (From Chung: Ann Int Med; 2003, vol138).](image)

### 2.5 MICROSATELLITE INSTABILITY IN HEREDITARY AND SPORADIC CRC

MSI in hereditary and sporadic colorectal cancer occurs through two different mechanisms. In HNPCC the cause is a germline mutation in a mismatch repair enzyme; alterations of the MutS homologue 2(MSH2) and MutL homologue 1 (MLH1) mismatch repair genes account for more than 90 per cent. Instability in microsatellite sequences in sporadic colorectal cancer exhibiting MSI is often due to loss of expression of a mismatch repair gene (most commonly MLH1) caused by epigenetic silencing (Figure 2.4).

![Figure 2.4. Distribution of CRCs based on genetic and epigenetic instability and chromosomal alterations.](image)
2.5.1 Hereditary non-polyposis colorectal cancer (Lynch Syndrome): germline mutation in mismatch repair genes

In 1966, Dr Henry Lynch and colleagues described an aggregation of colorectal and endometrial cancers inherited in an autosomal dominant manner in two large Midwestern kindred.s,73 The definition of HNPCC is primarily based on family history (the Amsterdam and Bethesda criteria) (see Table 2.1 below). Lynch syndrome, on the other hand, is defined by the documentation of inherited inactivating mutations in the DNA MMR system. Without genetic testing, a diagnosis of Lynch syndrome cannot be made.

<table>
<thead>
<tr>
<th>Name</th>
<th>Criteria</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amsterdam</td>
<td>Amsterdam criteria I: Three or more relatives with colorectal cancer, one of whom is a first-degree relative of the other two, FAP should be excluded</td>
<td>61.0%</td>
<td>67.0%</td>
</tr>
<tr>
<td></td>
<td>Colorectal cancer involving at least two generations</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>One or more colorectal cancer cases diagnosed before the age of 50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amsterdam</td>
<td>Amsterdam criteria II: Three or more relatives with histologically verified LS-associated cancer (colorectal cancer, cancer of the endometrium, small bowel, ureter, or renal pelvis), 1 of whom is a first-degree relative of the other 2; FAP should be excluded</td>
<td>78.0%</td>
<td>61.0%</td>
</tr>
<tr>
<td></td>
<td>Colorectal cancer involving at least two generations</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>One or more cancer cases diagnosed before the age of 50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Revised Bethesda</td>
<td>At least one of the following features</td>
<td>90.9%</td>
<td>77.1%</td>
</tr>
<tr>
<td></td>
<td>1: Colorectal cancer diagnosed in a patient under the age of 50</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2: Presence of synchronous or metachronous colorectal cancer, or other LS-associated tumours**, regardless of age</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3: Colorectal cancer with the MSI-H histology*** under the age of 60</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4: Colorectal cancer in one or more first-degree relatives with an LS-related tumour, with one of the cancers under the age of 50</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>5: Colorectal cancer in two or more first- or second-degree relatives with LS-related tumours, regardless of age</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data on sensitivity and specificity of Amsterdam criteria from Syngal et al74 and Revised Bethesda from Piñol et al75; **Lynch syndrome (LS)-associated tumours include colorectal, endometrial, stomach, ovarian, pancreas, ureter and renal pelvis, biliary tract, brain tumours, sebaceous gland adenomas and keratoacanthomas in Muir-Torre syndrome, and carcinoma of the small bowel; ***Presence of tumour infiltrating lymphocytes, Crohn’s-like lymphocytic reaction, mucinous/signet-ring differentiation, or medullary growth pattern. FAP: Familial adenomatous polyposis; MSI-H: Microsatellite instability-high.
HNPCC is the most common inherited colon cancer predisposition syndrome. It is caused by autosomal dominant inherited mutations in genes that encode proteins responsible for the detection, excision, and repair of errors that occur during DNA replication namely, DNA mismatch repair (MMR) genes. The risk of developing colorectal cancer in patients with Lynch syndrome increases with age. Eighty per cent of the children of HNPCC patients will develop colon cancer as compared to 5% of children of nonsufferers. Lynch syndrome patients who have colorectal cancer also have an estimated 16% risk of a second primary within 10 years. Lynch syndrome includes those with an existing cancer and those who have not yet developed cancer. For consideration here is microsatellite instability (MSI) in which there is mutational and/or epigenetic inactivation of MMR genes, mostly hMLH1 and hMSH2.

Patients with Lynch syndrome are also at an increased risk for a wide variety of extra-colonic malignancies, most notably endometrial cancer. Among women, endometrial cancer is the second most common cancer associated with Lynch syndrome, with an estimated lifetime risk of 40 to 60%. Sebaceous neoplasms of the skin are seen in the Lynch syndrome variant, Muir-Torre syndrome, and Turcot syndrome is associated with brain tumours, including medulloblastomas, glioblastomas and astrocytomas. The spectrum of Lynch syndrome-associated malignancies also includes cancers of the stomach, small intestine, pancreas, biliary tract, and urothelial carcinoma of the renal pelvis and ureter. Of the 95 analyzed African American samples, Brim et al reported that 29 (30.5%) were MSI-H, at a mean age of 65.7 years. Four MSI-H patients had a strong family history of colon cancer, and met the Amsterdam criteria for HNPCC.

2.5.2 Sporadic colorectal cancer: microsatellite instability through epigenetic silencing

Deficient mismatch repair occurs in 12 to 15 per cent of all sporadic colorectal cancers (Figure 2.4). It is caused by biallelic or hemiallelic methylation of cytosine residues of the cytosine and guanine (CpG)-rich promoter sequences of MLH1. Simply stated, the epigenetic change affects gene function (without genetic changes) by aberrant methylation of DNA that prevents the gene(or gene-region) from being transcribed, thus ‘silencing’ the gene and causing deficiency in protein expression. Epigenetic silencing is now recognized as a third pathway in Knudson’s model of tumour suppressor gene inactivation in cancer. As an example, loss of the tumour suppressor gene phosphatase and tensin homologue (PTEN) located at 10q23 occurs through promoter hypermethylation in colorectal cancer with high-frequency MSI (MSI-H). MSI in sporadic colorectal cancer usually arises because of
epigenetic silencing of the DNA mismatch repair gene $MLH1$. CIMP appears to bear a defining event in about half of all sporadic colorectal cancers. CIMP-positive colorectal cancers are characterized by distinct pathological, clinical and molecular genetic features. CRCs that are CIMP+/MSI+ are thought to arise from sessile serrated adenomas admixed with dysplasia, and MSI is thought to be a late event in the multistep progression to carcinogenesis in this molecular subtype. In contrast, MSI is an initiating event and driving force for the progression of the lesion in the CIMP-/MSI+ subtype (hereditary nonpolyposis colon cancer). CIMP+/MSI- tumours arise from either sessile serrated adenomas or traditional serrated adenomas.

### Table 2.2: Characteristics of different CRC pathways

<table>
<thead>
<tr>
<th>PREDOMINANT PATHWAY</th>
<th>MSI/ CIMP STATUS</th>
<th>SITE OF CANCER</th>
<th>PROGNOSIS</th>
<th>CIN</th>
<th>GERMLINE MMR</th>
<th>SOMATIC BRAF (V600E)</th>
<th>MUTATION</th>
<th>MLH1</th>
<th>MSH2</th>
<th>MSH6</th>
<th>MGMT</th>
<th>MLL AT11</th>
<th>DMADJATE PRECURSOR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mutator</td>
<td>MSI-H/ CIMP-</td>
<td>Proximal</td>
<td>Better</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Advanced adenoma</td>
</tr>
<tr>
<td>Methylator/Mutator</td>
<td>MSI-H/ CIMP+</td>
<td>Proximal</td>
<td>Better</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Serrated polyp</td>
</tr>
<tr>
<td>Methylator</td>
<td>Non-MSI-H/ CIMP+</td>
<td>Proximal</td>
<td>Worse</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Serrated polyp</td>
</tr>
<tr>
<td>&quot;Alternate&quot; methylator</td>
<td>MSI-L/ CIMP+</td>
<td>Distal</td>
<td>Unclear</td>
<td>No</td>
<td>No</td>
<td>No(K-ras)</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Serrated polyp</td>
</tr>
<tr>
<td>Suppressor</td>
<td>Non-MSI-H/ CIMP-</td>
<td>Distal</td>
<td>Standard</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Advanced adenoma</td>
</tr>
</tbody>
</table>

### 2.5.3 AETIOLOGICAL FACTORS

A. RED MEAT, DIET, BACTERIAL METABOLISM, INFLAMMATION, AND HYPERPROLIFERATION.

A.1. The role of ‘Red’ meat

Cross AJ, et al. in a large prospective study looked at the association of meat consumption (especially red meat) and colorectal cancer risk. ‘Red’ meat is usually taken to include beef, lamb and pork, including that in meat products. They concluded that there was indeed an association. The meat consumption in resource poor settings is much lower and corresponds to low colon cancer risk. The potential aetiological factors underlying these associations include haem iron, nitrate/nitrite and heterocyclic amines (HCAs). High-meat-eating fast-acetylator phenotypes are at increased risk of acquiring colonic adenomatous polyps and cancers. Evidently, the Japanese population contains a higher
proportion of fast acetylators, which may account for the striking rise in colon cancer associated with Westernization and, for example, meat consumption.\textsuperscript{97}

In a UK Department of Health (1998) report, the evidence was classified as ‘moderately consistent’ of a positive association between red and processed meat consumption and colon cancer. Two of three cohort studies showed a dose response, with relative risks in the order of 2 associated with ten to twelve portions of red meat per week.\textsuperscript{98} The report also found that there was moderately consistent evidence that poultry (white meat) and fish consumption are not associated with risk of colon cancer. A major difference between red and white meat is in their content of iron (Fe), which is poorly absorbed from the small intestine. This difference in effect may be due to the fact that Fe and Molybdenum (Mo) are integral components of nitrate reductase and are essential for enzyme activity.\textsuperscript{99} Faecal nitrate reductase is a key step in determining the levels of production of nitrosating agents such as nitrite from nitrate and \textit{N}-nitrosation.\textsuperscript{134}

Meat also alters nitrogen metabolism and enhances the production of endogenous promoters and possible carcinogens such as \textit{N}-nitroso compounds (NOC) within the colon. Patients with ureterosigmoidostomies who have very high lumen NH\textsubscript{3} concentrations have a greatly increased risk of developing tumours distal to the site of ureteric implantation.\textsuperscript{100}

In rodents, heterocyclic amines are carcinogenic in a wide variety of organs, mainly liver, but including skin, lung, colon and mammary gland. One, 2-amino-1-methyl-6-phenylimidazo(4,5-b)pyridine, has attracted particular attention because it tends to be the most abundant, and colon tumours that are produced from it in rats have a high frequency of microsatellite instability, which is similar to that seen in human inherited and sporadic colorectal cancers. Furthermore, G–C base pair mutations are produced in cell lines, which are similar to those produced in \textit{APC} gene mutations of colon tumours in rodents. Even though the dose response ratio is not exactly known, it has been proven that the carcinogenic form, the exocyclic amine of heterocyclic amines is hydroxylated by cytochrome P450 enzymes, mainly CYP1A2. Covalent binding to deoxyguanosine in DNA then occurs. Much higher amounts of these adducts are formed in human colon than in rats.\textsuperscript{101}

A.2. The Role of Microbiota in Colorectal carcinogenesis.

It has been proposed by O’Keefe in 2008 that the differences in the factors that regulate mucosal proliferation and therefore cancer risk between individuals may be a result of the differences in the colonic environment, which represents the microbial interface between the
external environment (namely the diet) and the colonic mucosa. High dietary intake of red meat by African Americans increases microbiota populations of sulfur-reducing bacteria (SRBs), which produce cytotoxic and genotoxic hydrogen sulfide as a terminal product. These products cause chronic mucosal inflammation and hyper-proliferation, thus increasing cancer risk. Native Africans on the other hand, are protected by high populations of methanogenic bacteria which thrive in high resistant carbohydrate and vitamins, meat-free conditions and produce the non-toxic terminal product, methane.

Chen et al utilized pyrosequencing based analysis of 16S rRNA genes to determine the overall structure of microbiota in patients with colorectal cancer and healthy controls. The tumour microbiota exhibited lower diversity compared to normal controls. The structures of the intestinal lumen microbiota and mucosa-adherent microbiota were different in CRC patients compared to matched microbiota in healthy individuals. *Lactobacillales* was enriched in cancerous tissue, whereas *Faecalibacterium* was reduced. In the mucosa-adherent microbiota, *Bifidobacterium*, *Faecalibacterium*, and *Blautia* were reduced in CRC patients, whereas *Fusobacterium*, *Porphyromonas*, *Peptostreptococcus*, and *Mogibacterium* were enriched. In the lumen, predominant phylotypes related to metabolic disorders or metabolic exchange with the host, *Erysipelotrichaceae*, *Prevotellaceae*, and *Coriobacteriaceae* were increased in cancer patients. Thus, the intestinal microbiota is associated with CRC risk and intestinal lumen microflora potentially influence CRC risk via co-metabolism or metabolic exchange with the host. For instance, a number of these facultative and anaerobic colonic bacteria are able to catalyse the formation of N-nitroso compounds at an optimum pH of 7.5. However, mucosa-associated microbiota potentially affects CRC risk primarily through direct interaction with the host. The microbiota mediates the effect diet has on colon cancer risk by their generation of butyrate, folate, and biotin, molecules known to play a key role in the regulation of epithelial proliferation.

**A.3. Inflammatory Bowel Disease (IBD) and Colorectal Cancer (CRC)**

The association between inflammatory bowel disease (IBD) and colorectal cancer (CRC) has been recognized since 1925 and still accounts for 10%-15% of deaths in IBD. IBD-associated CRC (IBD-CRC) affects patients at a younger age than sporadic CRC. The prognosis for sporadic CRC and IBD-CRC is similar, with a 5-year survival of approximately 50%. It has been proposed that T helper (Th)2 immune response characterizing ulcerative colitis determines an elevated risk of developing colitis associated colorectal cancer (CAC). In Crohn's disease, while a Th17-mediated immune response could cause inflammation and
enhance CAC risk, the shift towards a Th1-mediated colitis could lower the incidence of CAC.\textsuperscript{106} The increased risk of colorectal cancer in association with IBD is thought to be due to genetic and acquired factors. Inflammation promotes tumorigenesis both extrinsically (driven by chronic inflammatory conditions) and intrinsically (driven by inflammation and inflammatory cells recruited to and contained within tumours).\textsuperscript{107,108} Multiple pathways are likely to play a role, including production of reactive oxygen species and cytokine and chemokine expression by immune cells, which increase the risk of mutagenesis, and interactions between cancer stem cells and the local tumour microenvironment, including immune cells and myofibroblasts.\textsuperscript{109} Inflammation also affects DNA methylation patterns and histone modification.

Confirmed risk factors for IBD-CRC are duration, severity and extent of colitis, the presence of co-existent primary sclerosing cholangitis (PSC) and a family history of CRC. Evidence-based guidelines advise surveillance colonoscopy for patients with colitis 8 to 10 years after diagnosis, with the interval for further surveillance guided by risk factors (extent of disease, family history of CRC, post-inflammatory polyps, concomitant PSC, personal history of colonic dysplasia and colonic strictures).\textsuperscript{110}

**A.4. High-fat and high-protein, low complex carbohydrate diet**

Studies have shown that people who have diets high in fresh fruit and vegetables are at reduced risk of colorectal cancer due to the high vitamin and micronutrient content of such food items.\textsuperscript{136} The food types of people in low colon cancer risk areas like Nigeria consists of carbohydrate accompanied with vegetables. The human colonic bacteria ferment starch and nonstarch polysaccharides to short-chain fatty acids, mainly acetate, propionate, and butyrate.\textsuperscript{111} Also cassava contains Tamarin which yields hydrocyanide which is toxic to cancer cells.\textsuperscript{112}

It has been suggested that the higher risk of colon cancer in Americans may be partly explained by their high-fat and high-protein, low complex carbohydrate diet, which produces colonic residues that induce microbes to produce potentially carcinogenic secondary bile acids and less antineoplastic short chain fatty acids.\textsuperscript{113} Studies have also shown higher populations of secondary bile salt producing bacteria in African Americans which are stimulated by high animal fat diets to produce carcinogenic secondary bile salts. In contrast, a high resistant starch diet stimulates mucosal-protective \textit{Lactobacillus} species, found to be more common in Africans.
Thus, the risk of developing cancer of the colon is determined by the interaction between diet and resident microbiota, which influences the level of chronic inflammation and epithelial proliferation - and therefore cancer risk - in the colonic mucosa.

A.5. Faecal transit time

Faecal transit time is inversely related to faecal weight. Refined carbohydrates, food low in fibre with consequent prolonged faecal transit time leads to constipation and prolonged contact with toxic metabolites, which together with the use of cathartics is a risk factor for colorectal cancer.

Conversely, lactose intolerance which is observed commonly in native Africans has a protective effect on the colonic mucosa.\textsuperscript{114} This is partly due to the increased transit time of undigested food and the butyrates produced from subsequent fermentation of the lipid component of milk by colorectal bacteria.

A.6. Spices and Phytonutrients

Colorectal cancers has a low incidence in Africans, Indians and Arabians who tend to eat very spicy and hot meals.\textsuperscript{115} It is possible that some of these food items may contain phytochemicals which are mucosa-protective or toxic to cancer cells.

B. OBESITY AND PHYSICAL INACTIVITY

Activity levels both pre- and post-diagnosis are associated with outcomes in colorectal cancer.\textsuperscript{116} In low income regions like Nigeria, relative lack of material resources, means that people get to work hard physically. This, coupled with lower caloric intake is protective against cancer development.

Obesity is a risk factor for colorectal cancer based on its molecular and metabolic effects on insulin and IGF-1, leptin, adipokines, and sex hormones. The association between obesity and rectal cancer is weaker than with colon cancer. There is a weaker association between obesity and colon cancer in women than in men.\textsuperscript{117} People who are physically active are at lower risk of developing colorectal cancer. Researchers analysed data from women in the U.S. Nurses’ Health Study and men in the Health Professionals Study to determine if there was a link between weight, exercise and the risk for CTNNB1-positive or CTNNB1-negative colorectal cancer. CTNNB1 is a molecule implicated in cancer and obesity. A higher body-mass index (BMI) (25 kg/m\textsuperscript{2} or greater) and low activity (less than 9 MET-h/week) were 82% more likely to have CTNNB1-negative colorectal cancer. BMI and physical activity had no effect on the risk for CTNNB1-positive colorectal cancer. The mechanistic basis of the
association between estimated body weight (EBW) and carcinogenesis remains incompletely understood. Postulated mechanisms include increased insulin and insulin-like growth factor signalling and chronic inflammation (both linked to the metabolic syndrome), as well as signalling via adipokines, such as leptin. In addition, data suggest a reduction in risk of several cancers in the first 10 years after bariatric surgery. EBW also impacts negatively on gastrointestinal cancer outcomes.118

C. COLONIC ADENOMATOUS POLYPS

Only four cases of FAP have been reported in Nigeria (from Ibadan) in the last 35 years119,120 and two cases of hereditary non-polyposis colon cancer have been reported within the last 15 years. This relative absence of pre-malignant conditions like adenomatous polyps in the West African has been reported by several authors.121,122 non-FAP adenomatous polyps were reported in 51 cases (2%) of the tumours reviewed by Rotimi and Abdulkareem.140 Paucity of a detectable adenoma-carcinoma sequence in Africans may mean a different aetiopathogenesis of colorectal cancer. Colon cancers associated with adenomatous polyps occur later in life and mostly among caucasians. The observation of the tumour at a much younger age in blacks supported by rarity of polyps suggests a yet to be identified pathway.

Most writers agree that familial adenomatous polyposis (FAP) carries 100% risk of developing cancer of the colon by age 40 years. In FAP there are multiple adenomatous polyps throughout the GIT due to mutation in the APC gene on chromosome 5q21. It can be classical (>500 polyps-cancer risk 100% by age 40 years) or attenuated (about 30 polyps-cancer risk 50%). Other features include desmoid tumours, epidermoid cysts, adrenal adenoma etc. Somatic mutations in the APC gene occur in sporadic colorectal cancers, whereas in FAP, a germ line mutation is inherited and a somatic mutation acquired.

Hereditary non-polyposis colon cancer syndrome (HNPCC) or Lynch syndrome is autosomal dominant disorder described by Henry Lynch. It is characterised by 50-80% increased risk of colon cancer and 40% risks of endometrial and ovarian cancer. Other lesions include sebaceous adenomas and epithelioma, keratoacanthomas, café-au-lait spots and brain tumours. HNPCC is associated with defective DNA mismatch repair due to inherited mutation in one of the mismatch repair genes leading to microsatellite instability with loss of expression of mismatch repair proteins like MLH1, MLSH2, MSH6, and PMS2. HNPCC is accounts for about 6% of all colon cancers. Use of aspirin is said to reduce the risk.
Other precancerous mucosal conditions include juvenile polyposis syndromes, Peutz-Jegher syndrome, MUTYH-associated polyposis (MAP), serrated polyposis, Cowden syndrome, Turcot and Gardener syndromes.

Increasing age, history of cancer especially in women who have had cancer of the ovary, uterus, or breast and smoking of tobacco are other factors which may contribute to colorectal carcinogenesis include.

**Other factors** which have an inverse causal relationship to colorectal cancers are vegetables and other high fibre diets as mentioned earlier. Hormone replacement therapy and use of calcium intake have been suggested by some studies. One of the better studied habits is regular use of aspirin after diagnosis of colorectal cancer. It was associated with longer survival among patients with mutated-PIK3CA colorectal cancer, but not among patients with wild-type PIK3CA cancer. This makes PIK3CA a likely predictive biomarker for preventive therapy with aspirin.\(^{123}\)

### 2.6 MOLECULAR TESTING FOR MSI IN COLORECTAL CANCER

Screening for HNPCC using the recommended Bethesda MSI markers is performed in trials for detection of HNPCC.\(^{124}\) Molecular testing is usually required for accurate assessment of specific gene mutations or genomic instability that provides prognostic and predictive information beyond clinicopathological features. Genetic testing for germline MMR mutations is complicated, time-consuming, and expensive. Consequently, it is generally recommended that patients at increased risk for Lynch syndrome undergo pre-screening with microsatellite instability (MSI) analysis and immunohistochemistry (IHC). Use of this step-wise approach detects virtually all cases of Lynch syndrome in a cost-effective manner. Individuals with germline mutations in one of the MMR genes are defined as having Lynch syndrome.

Testing for MSI using the polymerase chain reaction (PCR) is straightforward. For MSI analysis, DNA is usually extracted from paraffin-embedded tumour tissue and normal tissue or peripheral blood. Reliable demonstration of MSI requires that at least 30% of the tumour specimen is composed of tumour cells. Polymerase chain reaction is used to amplify the region containing the microsatellite, and the products are then separated on the basis of size. Mutations that alter microsatellite length (by deletion or insertion) are visualized as band shifts on electrophoresis. A microsatellite is considered unstable if the distribution of the fragments from the tumour sample differs from that of the normal tissue.
2.6.1. Immunohistochemistry for Microsatellite Instability

IHC analysis for the DNA MMR proteins MLH1, MSH2, MSH6, and PMS2 is now available on a clinical basis. The vast majority of colorectal tumours that demonstrate an MSI-H phenotype are found to show loss of expression of one or more of the DNA MMR proteins. Loss of expression of one of the four MMR proteins nearly always means that the CRC tumour will show MSI and is detected by the absence of nuclear staining in the tumour cells with the presence of nuclear staining in lymphocytes and normal colon epithelial cells. Since these proteins are present in complexes, loss of expression of one MMR protein is often associated with the loss of the partner MMR protein. Loss of expression of MLH1 is almost always accompanied by loss of PMS2 expression, and loss of expression of MSH2 is almost always accompanied by loss of MSH6 expression. In contrast, loss of MSH6 expression and loss of PMS2 expression can sometimes be seen without accompanying loss of MSH2 or MLH1 expression.

2.6.2. Germline mutation analysis

Demonstration of the loss of particular MMR proteins in a given family direct which genes should be examined by clinical germline mutation analysis. Since DNA MMR IHC results can strongly suggest the presence of a germline DNA MMR mutation, many institutions require the patient be provided with genetic counselling by an appropriate health care provider prior to initiating IHC studies.

Methods of detecting MSI continue to develop, and correlation with genetic defects explored with cDNA microarray analysis is expected to improve understanding in the future.

Whatever the number of microsatellite markers used in a panel, instability in 40 per cent of markers or more (two of five markers in the Bethesda panel) is defined as MSI-H, whereas instability in 20–40 per cent of markers is defined as low-frequency MSI (MSI-L). Tumours with no proven instability (20 per cent or less) are termed microsatellite stable (MSS).

Immunohistochemistry may miss rare MSI cases that are caused by germline mutations by other genes and does not discriminate germline mutation from epigenetic alteration when loss of MLH1 protein expression is detected.

Figure 2.4: Testing strategies for Lynch Syndrome (HNPCC)
Tumours that display MSI and loss of MLH1 protein expression by IHC are subjected to testing for BRAF V600E mutation status and MLH1 promoter hypermethylation to help distinguish sporadic MSI tumours (~35% BRAF-mutant and 99% MLH1-methylated) from Lynch syndrome MSI tumours (BRAF-WT, infrequent MLH1-methylation).127,128

<table>
<thead>
<tr>
<th>Biomarkers</th>
<th>Frequency</th>
<th>Sporadic</th>
<th>Lynch syndrome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microsatellite instability (MSi)</td>
<td>15%</td>
<td>&gt;95%</td>
<td></td>
</tr>
<tr>
<td>BRAF V600E Mutations</td>
<td>35% of sporadic MSI</td>
<td>&lt;1%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5% of MSS</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>10% overall</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mismatch Repair Protein Loss by IHC</td>
<td>10-15%, mostly MLH1</td>
<td>~90%</td>
<td></td>
</tr>
<tr>
<td>MLH1 Promoter Hypermethylation</td>
<td>~99% of sporadic MSI</td>
<td>&lt;1%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>&lt;1% of MSS</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>15% overall</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Source: Synthesis of foregoing reviews

Table 2.3. Biomarkers used in the diagnosis of Lynch Syndrome (HNPCC)*

The order of genes to be tested is influenced by the IHC results and that approximately 80% of identified germline mutations in the MMR genes of Lynch syndrome families are detected in MLH1 and MSH2. The identification of germline MMR gene mutations significantly impacts the care of colorectal cancer patients and their families, and a step-wise approach allows the efficient and cost-effective identification of Lynch syndrome cases.

**Determination of tumour microsatellite status**

Determination of tumour microsatellite status by polymerase chain reaction has been suggested in some studies, as the best method to recognize MSI-positive CRC.29 Although MMR gene sequencing of all patients is the most sensitive strategy, it is highly inefficient and cost-ineffective and not recommended. Rather, a screening strategy of MSI or IHC testing (with or without optional BRAF testing) is recommended and retains a relatively high sensitivity.129,130,131

In other studies, MSI status by PCR and IHC screening tests for MMR mutations were shown to have similar sensitivity and specificity. MSI screening has a sensitivity of about 89% for MLH1 and MSH2 and 77% for MSH6, and a specificity of about 90% for all. It is likely that, using high quality MSI testing methods, these parameters can be improved. IHC screening has sensitivity for MLH1, MSH2, and MSH6 of about 83% and a specificity of about 90% for all. Therefore, the IHC method testing for MSH2, MLH1, PMS2 and MSH6 were employed in this study. In addition, it is cheaper, requires less technical handling and is readily available in this environment.
2.7 IMPLICATIONS OF MSI STATUS IN COLORECTAL TUMOURS

1. MSI-H is observed more frequently in women and in colorectal cancers that occur proximal to the splenic flexure.

2. These tumours also exhibit poor differentiation, a mucinous cell type and frequently peritumoral lymphocytic infiltration (‘Crohn’s like inflammation’). Studies suggest interplay between TGF-β and peritumoral lymphocytes.

3. Colorectal cancer exhibiting MSI is associated with larger primary tumours (T3 tumours), but with a more favourable stage (less lymph node involvement and reduced occurrence of metastasis). The pronounced genetic instability of cells with MSI may increase susceptibility to apoptosis because of an accumulation of mutations in genes that are required for cell growth.

4. Cancers demonstrating MSI have a higher incidence of synchronous and metachronous tumours. Most of the synchronous cancers were associated with MLH1 hypermethylation and as such are more likely to be sporadic.

![Figure 2.5. Pathological distinctions between tumours exhibiting microsatellite instability (MSI) and chromosomal instability (CIN). Percentages indicate the anatomical distribution of colorectal cancers (TNM refers to the tumour node metastasis staging system) *Source: Synthesis of foregoing reviews](image)

2.8 HISTOLOGICAL STAGING SYSTEM (TNM)

The extent of spread of the cancer is estimated for prognostication and therefore, determination of management modality. It is also used for research purposes. The systems for staging colorectal cancers largely depend on the level of local invasion, the degree of lymph node involvement and whether there is distant metastases or not.

Despite the promising advances in the molecular pathology of colorectal cancer, it is important to emphasize that clinicopathological staging of tumour tissue is still the
cornerstone of prognostication and treatment selection. The WHO/AJCC tumour-node-metastasis (TNM) classification system 7th Edition is recommended, although the original Dukes staging system is still used by some clinicians.\textsuperscript{135} See Table 2.4. TNM and Modified Dukes classifications for colorectal cancers and survival rates attached as Appendix 1.

The intra-tumour stroma ratio is also an additional prognostic factor. This parameter could be a valuable and low cost addition to the TNM status and next to current high-risk parameters such as microsatellite instability status used in routine pathology reporting. Adding the stroma parameter to the ASCO criteria reduced the rate of 'undertreated' patients from 5.9\% to 4.3\%, the 'overtreated' increased with 6.8\% but the correctly classified increased with an additional 14\%.\textsuperscript{136}

### 2.9 HISTOLOGICAL TYPES/ GRADING SYSTEMS

Colonic epithelial malignancies are graded depending on the percentage of the tumour which has well-formed glands compared to those without, or infiltrating individual tumour cells.\textsuperscript{137} Signet-ring cell carcinoma, small cell, and mucinous carcinomas (where signet ring or small cell form, or mucin production is equal to or greater than 50\% of the total surface area in the slides respectively) are classified as poorly differentiated. The World Health Organization grading system monograph on colonic adenocarcinoma is presented next.

<table>
<thead>
<tr>
<th>Grade</th>
<th>Differentiation</th>
<th>AJCC</th>
<th>% glands descriptive grade</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Well differentiated</td>
<td>Low grade</td>
<td>&gt;75</td>
</tr>
<tr>
<td>2</td>
<td>Moderately differentiated</td>
<td>Low grade</td>
<td>50–75</td>
</tr>
<tr>
<td>3</td>
<td>Poorly differentiated</td>
<td>High grade</td>
<td>&lt;50</td>
</tr>
<tr>
<td>4</td>
<td>Undifferentiated</td>
<td>High grade</td>
<td>&lt;5</td>
</tr>
</tbody>
</table>

In a study of CRC in Western Nigeria, Abdulkareem et al showed that about half of the colonic adenocarcinomas are well differentiated. Those classified as moderately or poorly-differentiated account for some 30\%. In that study mucinous carcinomas are a distant third and 6 times more frequent than signet ring types.\textsuperscript{90}
CHAPTER 3.
STUDY RATIONAL, AIMS AND OBJECTIVES

3.0 RATIONALE FOR STUDY

Colorectal carcinomas are heterogeneous in terms of molecular momentum, regional distribution, pathology of the invasive and precursor lesions and natural history. While these have been studied extensively in the developed countries where CRC incidence is high, not much is available in literature about the molecular characterisation, the precursor lesions or the natural history of the disease in our region of the world where the tumour occurs in relatively lower rates. Also, not enough has been said about the molecular explanation of the aetiology or why CRCs are relatively infrequent in these parts of the world.

In this study will the histological profile of colorectal cancer in National Hospital Abuja will be reviewed with the background of what is presently known. It will determine the tumour MSI status immunohistochemically by demonstrating loss of expression of MMR proteins. More precise characterization of colorectal carcinogenesis and a better appreciation of the interplay between genetic predisposition, environmental exposure and luminal events, will generate new opportunities to improve surveillance, chemoprevention and therapeutic strategies.

The outcome of the search for familial predispositions, environmental factors and lifestyle interactions that are responsible for our observations will impact strategies for prevention as well as advance knowledge of the pathways involved in the molecular pathogenesis of colorectal carcinoma especially in low incidence regions such as ours.

3.1 AIM AND OBJECTIVES

Aim

The aim of this study is to do a pathological review and to determine microsatellite instability status with a view to suggesting possible aetiological pathways in a series of colorectal cancers in National Hospital, Abuja.
Specific Objectives

1. To describe the pathological characteristics of CRCs in National Hospital Abuja, detailing the topography, gross characteristics, microscopic features and TNM staging, where possible.

2. To determine the tumour MSI status immunohistochemically by demonstrating loss of expression of MMR proteins (Msh2, Mlh1, Pms2 and Msh6).

3. To correlate the MSI status of the colorectal cancers with the pathological features observed.

4. To determine if the pathological features, MSI statuses and the biodata of the patients bear any aetiopathological relationship to the low-incidence CRC.
CHAPTER 4.
MATERIALS AND METHODS

4.0 ETHICAL CLEARANCE

Ethical clearance was obtained in writing from the Ethics Committee of the National Hospital Abuja. However, consent is implied by the very act of submitting tissue samples for tests. In addition, informed written consent was not obtained from each participating patient or the next of kin since genetic testing was not done in this study.

4.1 METHODS

4.1.0 STUDY SITE AND POPULATION

This work was conducted on all the colorectal cancer cases at the Histopathology Department of National Hospital, Abuja between June 1, 2004 and December 31, 2013 which met the exclusion criteria. The study included all gross tissues and paraffin blocks which were diagnosed with colorectal carcinoma in the archives of the laboratory.

4.1.2 STUDY DESIGN AND DATA ANALYSIS

The study was a retrospective cohort study of all colorectal cancers submitted for tests at the National Hospital Abuja.

Data analysis was stratified as (i) Histopathological data and (ii) MSI data. Statistical comparisons between histopathological features and MSI status were completed using the two-sided Fisher’s exact test. Patients were analysed on the basis of age, gender (male v. female) and tumour site. All statistical analyses were compiled by Excel and SPSS 21. Two-sided P < 0.05 was considered statistically significant.

4.1.3 SAMPLE SIZE DETERMINATION

All cases of colorectal cancer which were received within the period under study were tested. The percentage prevalence for colorectal cancer in this population group ranges from 5.8 to 7 percent.\textsuperscript{86, 92,105}

The minimum sample size was calculated using the formula for proportions in descriptive studies: 
\[N = \frac{Z_{\alpha}^2pq}{d^2}\]
Where \( N = \) desired sample size, \( Z_{\alpha^2} = \) standard normal deviation set at 1.96 (95% confidence interval), \( p = \) prevalence as a proportion using 7% (0.07), \( q = 1 - p(0.93) \) and \( d = \) error margin of 5% in proportion (0.05).

Therefore \( N = (1.96)^2 \times 0.07 \times 0.93 / (0.05)^2 = 100.035264 \)

Sample size, \( N = 100 \).

Minimum sample size is 105, considering an attrition rate of 5%.

### 4.1.4 SAMPLING METHOD/CRITERIA

The data was collated from all biopsy and resection specimens and accompanying request form as contained in the electronic records.

### 4.2 DATA COLLECTION

#### 4.2.1 Histopathological Review of Tumours

The histopathological review was based on the protocol of provided by the College of American Pathologist (relevant sections attached as Appendix 2). For all tumours, all haematoxylin-eosin–stained slides were reviewed and the original histological details independently confirmed by the two supervising pathologists, without knowledge of patient demographics and tumour sites. The specimen type (classified as biopsy or resection), were noted. The tumour locations were classified into three sites: right colon (caecum, ascending colon and transverse colon); left colon (descending colon and sigmoid colon); and rectum. Tumours were graded as low grade (well differentiated and moderately differentiated) or high grade (poorly differentiated) based to accepted morphological features. According to the extent of mucin production, mucinous cancers were those containing more than 50% of extracellular mucin. Mucinous and signet-ring tumours were considered to be of high grade.

The tumour growth patterns were described as expanding or infiltrating. The presence of Crohn-like inflammatory infiltrates were defined as intense lymphoid reaction with numerous lymphoid aggregates with frequent germinal centres and intraepithelial lymphocytes (classified as conspicuous when more than 30 are present per 10 high-power fields). Other prognostic features (tumour budding, elastic lamina invasion, lymphocytic infiltration, lymphovascular permeation, perineural invasion, tumour margin. The tumours were classified using the UICC TNM classification of malignant tumours, 7th edition TNM staging system. Presence of residual adenomatous tissue around the cancer and of distant colonic polyps
(hyperplastic, adenomatous, or serrated) were noted. Samples taken from distant (noncolonic sites) biopsied or resected for histological examination were noted too.

4.2.2 Immunohistochemistry

All samples which met the selection criteria were then subjected to IHC analysis. Samples were taken to include relatively normal tissue for comparison of tumour and normal cells, and a minimum proportion of tumour to help ascertain the quality of the test results.

Table 3.1. Details of antibodies used for the immunohistochemistry study

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Antibody clone</th>
<th>Dilution</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>hMLH1</td>
<td>G168-728</td>
<td>1:40</td>
<td>BD PharMingen, San Diego, California</td>
</tr>
<tr>
<td>hMSH2</td>
<td>FE11</td>
<td>1:30</td>
<td>EMD Millipore, Temecula, California</td>
</tr>
<tr>
<td>MSH6</td>
<td>44/MSH6</td>
<td>1:200</td>
<td>BD PharMingen, San Diego, California</td>
</tr>
<tr>
<td>PMS2</td>
<td>A16-4</td>
<td>1:50</td>
<td>BD PharMingen, San Diego, California</td>
</tr>
</tbody>
</table>

Immunohistochemical staining MMR proteins were scored as either no or positive staining. Loss of expression were recorded when nuclear staining for each of the proteins hMLH1 (hMLH1, hPMS2), hMSH2 (hMSH2, hMSH6), and hMSH6 (hMSH6), is absent from all malignant cells but preserved in normal epithelial and stromal cells. Definite nuclear staining of adjacent non-tumor cells (e.g. lymphocytes, fibroblasts and endothelial cells) in the tissue array served as an internal positive control. Tissue specimens were analyzed blinded to all other analyses and clinical information by two independent observers. Cases with discrepant scores were re-evaluated jointly on a second occasion and agreement finally reached (no loss of expression). The supervising pathologists were unaware of patient outcomes when reassessing the histological features or determining the MSI phenotype by IHC analysis.

Table 3.2. Usual Immunohistochemical Patterns in MSI-H CRC

<table>
<thead>
<tr>
<th></th>
<th>IHC MLH1</th>
<th>IHC PMS2</th>
<th>IHC MSH2</th>
<th>IHC MSH6</th>
</tr>
</thead>
<tbody>
<tr>
<td>MLH1 mutation</td>
<td>Lost</td>
<td>Lost</td>
<td>Preserved</td>
<td>Preserved</td>
</tr>
<tr>
<td>MSH2 mutation</td>
<td>Preserved</td>
<td>Preserved</td>
<td>Lost</td>
<td>Lost</td>
</tr>
<tr>
<td>MSH6 mutation</td>
<td>Preserved</td>
<td>Preserved</td>
<td>Preserved</td>
<td>Lost</td>
</tr>
<tr>
<td>PMS2 mutation</td>
<td>Preserved</td>
<td>Lost</td>
<td>Preserved</td>
<td>Preserved</td>
</tr>
</tbody>
</table>

All specimens were examined and photographed on a Leica DC 750 microscope.
4.3 EXCLUSION CRITERIA FOR THIS STUDY

1. Slides are considered unsuitable for analysis when there is complete absence of staining in both mucosa and tissue lymphocytes and when there is insufficient tissue for full immunohistochemical analysis.

2. Cases of anal cancer were excluded from this study.
CHAPTER 5.
RESULTS

A. HISTOPATHOLOGICAL DATA ANALYSIS

5.1 GENERAL FINDINGS
A total of 223 colorectal carcinoma (CRC) cases were received at the Department of Histopathology, National Hospital Abuja from June, 2004 and December, 2013. (See Figure 5.1 (Page 4)). This represented 1.13% of the 19,817 cases received in the period and 6.16% of malignancies seen in the 10 year period. Colorectal carcinoma (CRC) was the most common malignant gastrointestinal tumour accounting for 67% of all GIT malignancies and representing 96% of all malignant tumours of the colorectal and anal regions. A total of 175 (78.48%) CRC cases met the inclusion criteria. Biopsies were 96 (54.86%) while 79 (45.14%) were resection specimens.

5.2 AGE DISTRIBUTION
Figure 5.2 (Page 47) demonstrates the age spread in which the youngest patient was 21 years and the oldest, 94 years (mean age - 50.8 years, SD-14.52, Range-63 years). The disease peaked in the 40-49yrs age group (mode 48 years). Thirty five patients (21.6%) had the tumour below 40 years. Fourteen patients (8.6%) were 70 years or more. The vast majority of patients (69.8%) were aged between 40 and 69 years. Those within the age range of 90-99 years were the least (0.67%).

5.3 GENDER DISTRIBUTION
The study included 91 males and 84 females (M: F ratio-1.08:1(Figure4.2). The mean age of the male patients was 50.38 ± 13.42 years, ranging from 25 to 85 years while that of the females was 51.27 ± 15.72 years, ranging from 21 to 94 years.

5.4 LOCATION OF TUMOUR
There were about twice as many left-sided (distal colonic and rectal) tumour (62.3%) as there were right sided (caecal to splenic flexure) tumours (37.7%). Of the left sided tumours, two-thirds originated in the rectum. See Table 4.5A on Page 52 and Table 5.1 on Page 56.

5.5 CLINICAL FEATURES ASSOCIATED WITH TUMOUR LOCATION
Although left sided tumours were twice as many as right, the latter clustered about the middle age groups (30 to 69) with a p value of 0.01. Left sided tumours were more likely to present with rectal bleeding while right sided lesions were more likely to give abdominal pain (p value of 0.08 each). Other features were not shown to relate to location of tumour.
5.6 MACROSCOPIC FEATURES

There were 96 biopsy samples making up 55% and 79 resection specimens (45%). The gross tumour sizes ranged from 1 to 25 cm, most of which were left sided. They were fungating or had intact mucosa with endophytic/annular (constricting) lesions. Lymph node yields were from 0 to 7.

5.7 GRADES OF THE CARCINOMAS

The grades of the carcinomas are shown in Figure 5.4 (Page 49). The commonest was the well differentiated group, which accounted for 81 or 43.6%. The moderately differentiated category constituted 50 (28.6%). The least common grade was the poorly differentiated tumour with 44 (25.1%). Pearson’s Chi-Square ($\chi^2$) test showed that there was no statistically significant association between the gender and grade of tumour even though there was a slightly higher proportion for females with moderately differentiated tumour.

Tumours of poorer grade were relatively more common in the age groups between 40 and 69 years. While most of the well differentiated tumours were in this range, it is striking that most of the 20-29 age group had moderately to poorly differentiated tumours.

The more poorly differentiated tumours were comparatively more likely to be in the right colon (Figure 5.5, Page 50).

5.8 GROWTH PATTERN OF THE CARCINOMAS

Figure 5.6 (Page 51) shows the growth patterns to be equally split between endophytic/annular and exophytic/fungating patterns. While the rectal and colonic tumours were more commonly annular (constricting), the right colonic tumours were more commonly exophytic pattern ($\chi^2=8.17$, df=2, p=0.02).

5.9 HISTOLOGICAL TYPES OF THE CARCINOMAS

Table 5.2 (Page 57) shows the histological types of colorectal carcinoma based on the WHO classification. The predominant type in all age groups was adenocarcinomas with 64% of cases (112 of 175) and is proportionately distributed across age groups. Tumour types with worse categorization were seen only in younger individuals; mucinous and signet ring carcinomas were not observed beyond 60 years of age. The signet ring and small cell carcinomas were rare. There were no significant associations between histological type and the age group ($\chi^2=34.03$, df 56, p 0.99) or gender ($\chi^2=4.12$, df 8, p 0.85).

5.10 T-STAGE OF THE CARCINOMAS

Table 5.3 (Page 58) shows the depth of primary tumour invasion (T) for resection and biopsy
specimens. Seventy nine cases were resection while 96 were biopsy specimens. Fifty-four (68.1%) of the resection specimens were T3 or T4 while 29.1 and 2.5% were T2 and T1 respectively. Tumours with high stage were more likely to occur on the right ($\chi^2 = 30.729$, df 8, p 0.00). There were strong associations between the T stage and the gender ($\chi^2=30.729$, df 8, p<0.001) and age groups ($\chi^2=30.729$, df 8, p<0.001).

5.11 TNM STAGING OF COLORECTAL CARCINOMAS

Pathological (TNM) staging was done for all 79 resection samples using the UICC TNM classification of malignant tumours, 7th edition TNM staging system. The distribution is shown in Figure 5.7 (Page 52). Eighteen, 21, 12 and 28 presented at TNM stages I, II, III and IV respectively. Twenty eight resection specimens had incomplete data for staging. Tumour stage was strongly associated with the location of the tumour as more of the right sided tumours were either stage IV or I ($\chi^2=46.88$, df 12, p 0.00). However there was no statistically significant relationship between the stage of the disease and the patient’s age ($\chi^2=26.69$, df 42, p 0.97) or gender ($\chi^2=3.54$, df 6, p 0.74). See Table 5.4 (Page 59)

5.12 OTHER PATHOLOGICAL FEATURES

Table 5.4 (Page 59) is a frequency distribution of pathological features which are of special prognostic significance in colorectal studies. All subjects had more than one of the features. About half of the cases had involved tumou margins as well as tumour budding. The most frequent features were lymphocytic infiltration and Crohn-like features. Adenomatous tissue accompanied the carcinoma in 53(30.3%) of the patients.

B. MICROSATTELITE INSTABILITY DATA ANALYSIS

5.14 IMMUNOHISTOCHEMISTRY STAINING FOR MSI STATUS

Mismatch repair proteins (MMR) testing was performed using IHC in 140 patients of the 175 patients. Loss of protein expression was observed for MLH1 (26), MSH2 (34), PMS2 (57) and MSH6 (41). These are shown in Table 5.6 (Page 61).

As described early for Table 3.2 of Chapter 4, when MLH1 is deficient, both MLH1 and PMS2 are immunohistochemically absent because the PMS2 protein is rapidly degraded in the absence of MLH1. Similarly, in MSH2-deficiency, both MSH2 and MSH6 protein expression are absent. In contrast, in the case of either PMS2 or MSH6-deficiency, only the gene of interest is not expressed. One case each had stand-alone MSH2 loss and MLH1 loss.
Table 5.7 (Page 62) demonstrates MSI status in 140 patients. Overall, 45 patients (32.1%) had high frequency MSI (MSI-H) tumours, and 75 patients (53.6%) had microsatellite stable (MSS) tumours. The remaining 20 (14.3%) had low frequency (MSI-L) tumours.

Cytoplasmic staining was seen in about 10% of the paraffin blocks examined. However, only cases with simultaneous strong nuclear staining were accepted as positive.

5.15 CLINICOPATHOLOGIC CHARACTERISTICS ASSOCIATED WITH MSI STATUS

There were no recognizable patterns between MSI status and age, gender or histological type of the tumour (p values of 0.904, 0.999 and 0.302 respectively).

A right sided location and poor differentiation were observed in 51.0% and 66.7% of MSI-H tumours, respectively, versus 49.0% and 33.3% of MSS tumours, respectively (p values of 0.013 and 0.00, respectively). Exophytic growth pattern was more common for MSI-H tumours. The annular tumours were more likely to be MSS (p value .023). The strongest associations were found between MSI status and residual adenomatous tissue, worse tumour grade, Crohn-like feature and pathological staging with p values<0.001. Although MSI-H colorectal cancers were diagnosed at a significantly greater depth of tumour invasion, these tumours had a significantly lower overall pathological stage than cancers with microsatellite stability. (See Table 5.8, Page 63).
Figure 5.1: ANNUAL RATES OF COLORECTAL CARCINOMA
HISTOPATHOLOGY LABORATORY OF NHA

\[ y = 0.4061x + 15.267 \]

FREQUENCY

YEARS

04 05 06 07 08 09 10 11 12 13
0 5 10 15 20 25 30
Figure 5.2: AGE AND GENDER DISTRIBUTION OF CRC PATIENTS IN HISTOPATHOLOGY LABORATORY OF NHA
FIGURE 5.3: CROSSTABULATION OF GENDER AND TUMOUR GRADE OF CRC

<table>
<thead>
<tr>
<th>Tumour Grade</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Well differentiated</td>
<td>45</td>
<td>35</td>
</tr>
<tr>
<td>Moderately differentiated</td>
<td>25</td>
<td>20</td>
</tr>
<tr>
<td>Poorly differentiated</td>
<td>30</td>
<td>15</td>
</tr>
</tbody>
</table>
FIGURE 5.4

TUMOUR GRADE * AGE GROUP

TUMOUR GRADE
- Well differentiated
- Moderately differentiated
- Poorly differentiated

Count

Age Group


49
FIGURE 5.5: LOCATION VERSUS TUMOUR GRADE OF CRC

- LEFT COLON
- RECTUM
- RIGHT COLON

Well differentiated
Moderately differentiated
Poorly differentiated
FIGURE 5.6: GROWTH PATTERN AND LOCATION OF COLORECTAL CARCINOMA, NHA
FIGURE 5.7: TNM PATHOLOGICAL STAGES OF COLORECTAL CARCINOMA
FIGURE 5.8A: MSI STATUS * TNM STAGE

FIGURE 5.8B: CROHNLIKEFEATURE * MSI.HvsMSS

FIGURE 5.8C: MSI*TUMOUR GRADE
FIGURE 5.8D: MSI*ADENOMATOUS TISSUE

![Bar chart showing the comparison between MSS and MSI.H for adenomatous tissue not seen and present.]

FIGURE 5.8E: MSI*TUMOUR LOCATION

![Bar chart showing the comparison between MSS and MSI.H for tumor location on the left and right.]

FIGURE 5.8F: MSI*TUMOUR GROWTH PATTERN

![Bar chart showing the comparison between MSS and MSI.H for tumor growth pattern—exophytic and endophytic.]

54
FIGURE 5.8G: MSI*HISTOLOGICAL_TYPE

FIGURE 5.8H: MSI*AGE GROUP

FIGURE 5.8I: MSI*GENDER
<table>
<thead>
<tr>
<th></th>
<th>Frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>LEFT</strong></td>
<td></td>
</tr>
<tr>
<td>LEFT COLON</td>
<td>45(25.7)</td>
</tr>
<tr>
<td>RECTUM</td>
<td>64(36.6)</td>
</tr>
<tr>
<td><strong>RIGHT</strong></td>
<td>66(37.7)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>175(100)</td>
</tr>
<tr>
<td>Age Group</td>
<td>ADENOCARCINOMA</td>
</tr>
<tr>
<td>-----------</td>
<td>----------------</td>
</tr>
<tr>
<td>20-29</td>
<td>6</td>
</tr>
<tr>
<td>30-39</td>
<td>10</td>
</tr>
<tr>
<td>40-49</td>
<td>29</td>
</tr>
<tr>
<td>50-59</td>
<td>25</td>
</tr>
<tr>
<td>60-69</td>
<td>24</td>
</tr>
<tr>
<td>70-79</td>
<td>7</td>
</tr>
<tr>
<td>80-89</td>
<td>4</td>
</tr>
<tr>
<td>90-99</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>106</td>
</tr>
</tbody>
</table>
Table 5.3: CROSSTABULATION OF SPECIMEN TYPE AGAINST T STAGE OF CRC

<table>
<thead>
<tr>
<th>T STAGE</th>
<th>BIOPSY</th>
<th>RESECTION</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>12 (12.5)</td>
<td>2 (2.5)</td>
<td>14 (8)</td>
</tr>
<tr>
<td>T2</td>
<td>35 (36.5)</td>
<td>23 (29.1)</td>
<td>58 (33.1)</td>
</tr>
<tr>
<td>T3</td>
<td>6 (6.3)</td>
<td>28 (35.4)</td>
<td>34 (19.4)</td>
</tr>
<tr>
<td>T4</td>
<td>15 (15.6)</td>
<td>26 (32.9)</td>
<td>41 (23.4)</td>
</tr>
<tr>
<td>TX</td>
<td>28 (29.2)</td>
<td>0 (0.0)</td>
<td>28 (16.0)</td>
</tr>
<tr>
<td>TOTAL</td>
<td>96 (100)</td>
<td>79 (100)</td>
<td>175 (100)</td>
</tr>
<tr>
<td>METASTATIC SITE</td>
<td>Frequency</td>
<td>%</td>
<td></td>
</tr>
<tr>
<td>-----------------------</td>
<td>-----------</td>
<td>-------</td>
<td></td>
</tr>
<tr>
<td>PERITONEUM</td>
<td>21</td>
<td>31.8</td>
<td></td>
</tr>
<tr>
<td>OMENTUM</td>
<td>14</td>
<td>21.2</td>
<td></td>
</tr>
<tr>
<td>MESENTERY</td>
<td>7</td>
<td>10.6</td>
<td></td>
</tr>
<tr>
<td>LIVER</td>
<td>6</td>
<td>9.1</td>
<td></td>
</tr>
<tr>
<td>UMBILICUS</td>
<td>5</td>
<td>7.6</td>
<td></td>
</tr>
<tr>
<td>OVARY</td>
<td>4</td>
<td>6.1</td>
<td></td>
</tr>
<tr>
<td>LUNG</td>
<td>2</td>
<td>3.0</td>
<td></td>
</tr>
<tr>
<td>SKIN</td>
<td>2</td>
<td>3.0</td>
<td></td>
</tr>
<tr>
<td>APPENDIX</td>
<td>1</td>
<td>1.5</td>
<td></td>
</tr>
<tr>
<td>BRAIN</td>
<td>1</td>
<td>1.5</td>
<td></td>
</tr>
<tr>
<td>CHEST WALL</td>
<td>1</td>
<td>1.5</td>
<td></td>
</tr>
<tr>
<td>DUODENUM</td>
<td>1</td>
<td>1.5</td>
<td></td>
</tr>
<tr>
<td><strong>Total (METASTATIC SITE)</strong></td>
<td><strong>65</strong></td>
<td><strong>100.0</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Total (grand)</strong></td>
<td><strong>175</strong></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
## TABLE 5.5: FREQUENCIES OF CARDINAL PATHOLOGICAL FEATURES OF COLORECTAL CARCINOMAS

<table>
<thead>
<tr>
<th>Feature</th>
<th>Present</th>
<th>Absent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Margin Freedom</td>
<td>89 (50.9)</td>
<td>86 (49.1)</td>
</tr>
<tr>
<td>Tumour Budding</td>
<td>88 (50.3)</td>
<td>87 (49.7)</td>
</tr>
<tr>
<td>Elastic Lamina Invasion</td>
<td>133 (76)</td>
<td>42 (24)</td>
</tr>
<tr>
<td>Lymphocytic Infiltration</td>
<td>118 (67.4)</td>
<td>57 (32.6)</td>
</tr>
<tr>
<td>Lympho-vascular Permeation</td>
<td>68 (38.7)</td>
<td>107 (67.4)</td>
</tr>
<tr>
<td>Perineural Invasion</td>
<td>41 (23.4)</td>
<td>134 (76.6)</td>
</tr>
<tr>
<td>Mucin Production</td>
<td>38 (21.7)</td>
<td>137 (78.3)</td>
</tr>
<tr>
<td>Crohn-like Feature</td>
<td>147 (84)</td>
<td>28 (16)</td>
</tr>
<tr>
<td>Residual Adenomatous Tissue</td>
<td>53 (30.3)</td>
<td>122 (69.7)</td>
</tr>
<tr>
<td>Total</td>
<td>175 (100)</td>
<td></td>
</tr>
</tbody>
</table>
### TABLE 5.6: OUTCOME OF MMR PROTEIN TESTS OF CRC BY IHC

<table>
<thead>
<tr>
<th>TEST</th>
<th>MLH1</th>
<th>MSH2</th>
<th>PMS2</th>
<th>MSH6</th>
</tr>
</thead>
<tbody>
<tr>
<td>NEGATIVE</td>
<td>26</td>
<td>34</td>
<td>57</td>
<td>41</td>
</tr>
<tr>
<td>POSITIVE</td>
<td>114</td>
<td>106</td>
<td>83</td>
<td>99</td>
</tr>
<tr>
<td>Total Tested</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not tested*</td>
<td></td>
<td></td>
<td></td>
<td>35</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td></td>
<td>175</td>
</tr>
</tbody>
</table>

*Samples were not tested due to damage or insufficient tissue left on the paraffin block.
Table 5.7: PATTERN OF MSI STATUS IN COLORECTAL CARCINOMAS

<table>
<thead>
<tr>
<th>NUMBER OF MMR PROTEIN EXPRESSION LOST</th>
<th>Frequency</th>
<th>Valid %</th>
<th>Cumulative %</th>
</tr>
</thead>
<tbody>
<tr>
<td>MSI-H</td>
<td>4</td>
<td>19</td>
<td>13.6</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>10</td>
<td>7.1</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>16</td>
<td>11.4</td>
</tr>
<tr>
<td>MSI-L</td>
<td>1</td>
<td>20</td>
<td>14.3</td>
</tr>
<tr>
<td>MSS</td>
<td>0</td>
<td>75</td>
<td>53.6</td>
</tr>
<tr>
<td>Total Tested</td>
<td></td>
<td>140</td>
<td>100.0</td>
</tr>
<tr>
<td>Not tested</td>
<td></td>
<td>35</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>175</td>
<td></td>
</tr>
<tr>
<td>Category</td>
<td>MSS (n)</td>
<td>MSLH (n)</td>
<td>$\chi^2$ Value</td>
</tr>
<tr>
<td>---------------------------</td>
<td>---------</td>
<td>----------</td>
<td>----------------</td>
</tr>
<tr>
<td><strong>PATHOLOGICAL STAGING</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biopsies</td>
<td>53(81.5)</td>
<td>12(18.5)</td>
<td>26.585</td>
</tr>
<tr>
<td>1</td>
<td>5(38.5)</td>
<td>8(61.5)</td>
<td></td>
</tr>
<tr>
<td>2A</td>
<td>2(20.0)</td>
<td>8(80.0)</td>
<td></td>
</tr>
<tr>
<td>2B</td>
<td>2(33.3)</td>
<td>4(66.7)</td>
<td></td>
</tr>
<tr>
<td>3A</td>
<td>2(0.0)</td>
<td>3(60.0)</td>
<td></td>
</tr>
<tr>
<td>3B</td>
<td>0(0.0)</td>
<td>1(100.0)</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>10(52.6)</td>
<td>9(47.4)</td>
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</tr>
<tr>
<td><strong>CROHNLIKE FEATURE</strong></td>
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<td></td>
</tr>
<tr>
<td>None</td>
<td>14(87.5)</td>
<td>2(12.5)</td>
<td>18.628</td>
</tr>
<tr>
<td>Mild</td>
<td>39(73.6)</td>
<td>14(26.4)</td>
<td></td>
</tr>
<tr>
<td>Moderate</td>
<td>18(48.6)</td>
<td>19(51.4)</td>
<td></td>
</tr>
<tr>
<td>Severe</td>
<td>3(23.1)</td>
<td>10(76.9)</td>
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</tr>
<tr>
<td><strong>TUMOUR GRADE</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Well differentiated</td>
<td>41(78.8)</td>
<td>11(21.2)</td>
<td>16.758</td>
</tr>
<tr>
<td>Moderately differentiated</td>
<td>23(62.2)</td>
<td>14(37.8)</td>
<td></td>
</tr>
<tr>
<td>Poorly differentiated</td>
<td>10(33.3)</td>
<td>20(66.7)</td>
<td></td>
</tr>
<tr>
<td><strong>RESIDUAL ADENOMATOUS TISSUE</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not seen</td>
<td>58(70.7)</td>
<td>24(29.3)</td>
<td>8.193</td>
</tr>
<tr>
<td>Present</td>
<td>16(43.2)</td>
<td>23(56.8)</td>
<td></td>
</tr>
<tr>
<td><strong>TOPOGRAPHY</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left</td>
<td>49(72.1)</td>
<td>19(27.9)</td>
<td>6.578</td>
</tr>
<tr>
<td>Right</td>
<td>25(49.0)</td>
<td>26(51.0)</td>
<td></td>
</tr>
<tr>
<td><strong>GROWTH PATTERN</strong></td>
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<td></td>
</tr>
<tr>
<td>Exophytic/Fungating</td>
<td>47(71.2)</td>
<td>19(28.8)</td>
<td>5.135</td>
</tr>
<tr>
<td>Endophytic/Annular</td>
<td>27(50.9)</td>
<td>26(49.1)</td>
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</tr>
<tr>
<td><strong>HISTOLOGICAL TYPE</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>52(66.7)</td>
<td>26(33.3)</td>
<td>9.355</td>
</tr>
<tr>
<td>Mucinous</td>
<td>2(25.0)</td>
<td>6(75.0)</td>
<td></td>
</tr>
<tr>
<td>Signet ring</td>
<td>1(25.0)</td>
<td>3(75.0)</td>
<td></td>
</tr>
<tr>
<td>Squamous cell</td>
<td>1(100.0)</td>
<td>0(0.0)</td>
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</tr>
<tr>
<td>Adenosquamous</td>
<td>6(60.0)</td>
<td>4(40.0)</td>
<td></td>
</tr>
<tr>
<td>Medullary</td>
<td>7(70.0)</td>
<td>3(30.0)</td>
<td></td>
</tr>
<tr>
<td>Small cell</td>
<td>1(50.0)</td>
<td>1(50.0)</td>
<td></td>
</tr>
<tr>
<td>Undifferentiated</td>
<td>3(60.0)</td>
<td>2(40.0)</td>
<td></td>
</tr>
<tr>
<td><strong>AGE GROUP</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>20-29</td>
<td>8(66.7)</td>
<td>4(33.3)</td>
<td>3.279</td>
</tr>
<tr>
<td>30-39</td>
<td>9(75.0)</td>
<td>3(25.0)</td>
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<td>40-49</td>
<td>16(61.5)</td>
<td>10(38.5)</td>
<td></td>
</tr>
<tr>
<td>50-59</td>
<td>14(56.0)</td>
<td>11(44.0)</td>
<td></td>
</tr>
<tr>
<td>60-69</td>
<td>14(56.0)</td>
<td>11(44.0)</td>
<td></td>
</tr>
<tr>
<td>70-79</td>
<td>5(62.5)</td>
<td>3(37.5)</td>
<td></td>
</tr>
<tr>
<td>80-89</td>
<td>2(66.7)</td>
<td>1(33.3)</td>
<td></td>
</tr>
<tr>
<td>90-99</td>
<td>0(0.0)</td>
<td>1(100.0)</td>
<td></td>
</tr>
<tr>
<td><strong>GENDER</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>41(62.1)</td>
<td>25(37.9)</td>
<td>0</td>
</tr>
<tr>
<td>Female</td>
<td>33(62.3)</td>
<td>20(37.7)</td>
<td></td>
</tr>
</tbody>
</table>
PLATE 5.5A: POORLY DIFFERENTIATED COLONIC CARCINOMA METASTAZING TO THE THIGH OF A 26 YEAR OLD FEMALE. H&E x200. (S752_11)

PLATE 5.5B: WELL DIFFERENTIATED ADENOCARCINOMA FROM A 47 YEAR OLD MAN. H&E x400. INSET SAME TUMOUR x40. (S462_07)

PLATE 5.5C: MODERATELY DIFFERENTIATED ADENOCARCINOMA OF A RESECTED RECTOSIGMOID SPECIMEN IN A 48 YEAR OLD FEMALE H&E x200. (S1976_13)

PLATE 5.5D: SIGNET RING CARCINOMA OF THE RECTUM IN A 71 YEAR OLD MAN. H&E x400. INSET H&E x40. (S442_08)

PLATE 5.5E: MUCINOUS CARCINOMA OF THE DESCENDING COLON IN A 21 YEAR OLD WOMAN PRESENTING AT STAGE IV. H&E x40. (S1562_10)
PLATE 5.5F: MSH6 NEGATIVE CONTROL. A MUCINOUS CARCINOMA x400. INSET SAME TUMOUR. x400. (S288_06)

PLATE 5.5G: MSH6 POSITIVE CONTROL. TYPICAL NUCLEAR STAINING FOR ALL THE MMR PROTEINS. CRYPT CELLS, LYMPHOCYTES AND FIBROBLASTS ARE POSITIVE INTERNAL CONTROLS. x100

PLATE 5.5H: MSH2 NEGATIVE TUMOUR. x400. (S1657_04)

PLATE 5.5I: MSH2 POSITIVE. x40. INSET x400. (S685_06)

PLATE 5.5J: PMS2 NEGATIVE x100. INSET IS A x40. (S2018_05)

PLATE 5.5K: PMS2 POSITIVE SIGNET RING CARCINOMA. x400. (S1643_07)
PLATE 5.5L: MSH1 NEGATIVE PAPILLARY ADENOCARCINOMA. X40. (S542_04)

PLATE 5.5M: MLH1 POSITIVE TUMOUR X400. (S357_06). Inset is AN MLH1 POSITIVE SIGNET RING CARCINOMA. X40. (S358_06)

PLATE 5.5N: MSH6NEGATIVE POORLY DIFFERENTIATED TUMOUR. x100. INSETX40. (S643_04)

PLATE 5.5O: MSH6 POSITIVE POORLY DIFFERENTIATED TUMOUR METASATION TO LYMPH NODE. x100. INSETX40. (S637_05)
CHAPTER 6
DISCUSSION

6.0 GENERAL CONSIDERATION

Malignancies arise from an accumulation of mutations. Consequently, cancers that emerge from different mutational pathways differ clinically. Mutation and/or DNA MMR gene silencing precede the accumulation of mutations within genes and general genome instability in colorectal cancer. This is the case for the subgroup of colorectal cancers that are characterized by high-frequency microsatellite instability. This study of 175 colorectal carcinoma samples offer understanding of the biology of CRC and identifies a potential therapeutic target.

6.1 PATHOLOGICAL CHARACTERISTICS OF CRCS

Individuals with CRC in this cohort are frequently within the 3rd to 10th decade of life with ages ranging from 21-94 years with mean of 50.8 years. This is in agreement with the findings of Cronje et al who found that 83% of black patients were ≤50 years of age compared with only 10% of younger whites (p≤0.001). Carethers et al reported a mean age of 67.8 for Caucasians and 61.9 for African Americans.

The disease occurs at a relatively younger age peaking in the 40-49 yrs age group (mode 48 years) in this study. This may not be unconnected with the relatively high MSI-H rate, aggressive tumour biology, frequent alarm signals among other reasons. Similarly Rotimi & Abdulkareem presented an age incidence of 46 years (peak=41-50 age group) and 674(32%) of all the cases were below 40 years. However, presentation was early in this study when juxtaposed against the corresponding mean age of over 70 years in Caucasians.

The male: female ratio of 1.08:1 recorded in the present study concurs with the finding of Abdulkareem et al who studied 420 CRC cases (237 males and 183 females) between 1995 and 2007 and reported a slight male predilection (1.3:1). There were about twice as many left-sided tumour (62.3%) than there were right sided tumours (37.7%). Of the left sided tumours, two-thirds originated in the rectum. Our observed gender ratio is comparable to other studies in this country and worldwide.

In our study, majority of the tumours were either moderately or poorly differentiated, mostly adenocarcinomas with growth pattern almost split equally between annular and fungating margins. Mucinous carcinomas are the third most common and 6 times more frequent than
signet ring types similar to the study from Lagos. Other studies reported a similar profile, the poor prognostic type with 85% presenting at TNM stages II and III. In our study 68.1% of the resection specimens were T3 or T4. In particular, morphological subtypes, such as mucinous carcinoma featured prominently on the right. These features are highly suggestive of a HNPCC profile and familial clustering of cancer syndromes may be discovered with a careful search. Preliminary clinical data and the strong relationship between the MSI-H status and right sidedness, mucinous and Crohn-like features, relatively younger age and poorer tumour stage all further buttress this suggestion.

About 38% of the tumours studied occurred on the right side with residual adenomatous tissues occurring in a third of them. Premalignant serrated polyps more frequently arise in the proximal colon and are associated with microsatellite instability and DNA hypomethylation at CpG islands, whereas conventional tubular adenomas arise via biallelic inactivation of the APC tumour-suppressor gene and display chromosome instability. Furthermore, other molecular lesions, such as BRAF V600E mutations, are characteristically found more often in tumours arising via the serrated neoplasia pathway. This study reported twice as many tumours on the left as there are on the right sided. In the American survey of 2011, about 72% of cases arise in the colon and about 28% in the rectum.

The lymph node yield was relatively poor, ranging from mostly 0 to a single case of 7 nodes per patient. Again, other features like circumferential margins (for left sided tumours) or apical nodes for right sided tumours were not mentioned either because of suboptimal surgery or grossing technique. The minimum recommended by the College of American Pathologists is 12 nodes. The implication is that either surgery is not adequate, pathology reporting protocol was not strictly followed or both.

The reasons why patients in our region of the world present at advanced stages may be linked to reluctance to seek health checks on noticing specific early symptoms of colorectal carcinoma that may include dyspepsia. This may also partially explain why there are more rectal tumours which more frequently present with bleeding and rectal mass as was the case in this study. The 53 year study of Nigerian CRC cases by Rotimi & Abdulkareem reported that all patients with clinical data had one or more alarm features just like this study.

Precursor adenomatous lesions and consequently colon cancer rates are high in the Western world. Few premalignant lesions were identified in this study. This may partially account for the corresponding low rate of colorectal carcinomas in this study. Two cases of adenomatous polyps preceding a diagnosis of CRC were reported. However, over 30% of the tumours
examined had adenomatous tissue. This relative absence of pre-malignant conditions like adenomatous polyps in the West African has been reported by several authors.\textsuperscript{117} Only four cases of FAP have been reported in Nigeria (from Ibadan) in the last 35 years\textsuperscript{111} and two cases of hereditary non-polyposis colon cancer have been reported within the last 15 years. The study on African-Americans in South Africa has demonstrated higher incidence of microsatellite instability (MSI-H) tumours.\textsuperscript{141} In a case report on hereditary non-polyposis colon cancer (HNPCC) in Nigeria, Adebamowo et al studied five colorectal cancer patients for MSI and found that 2 out of 5 patients had MSI-H tumours.\textsuperscript{142}

In another dimension, paucity of reported premalignant adenomas may be pointing to other causal factors and pathways. For instance hamartomatous polyps such as juvenile polyposis syndrome and Peutz-Jeghers syndrome, and chronic inflammatory diseases can also lead to invasive colorectal neoplasms. The natural history of colorectal carcinomas associated with chronic colitis differs from that of ordinary adenomas both morphologically and with respect to the type and sequence of genetic alterations.

### 6.2 TUMOUR MSI STATUS

In accord with other studies MSI-H tumours rate is highest in Africans followed by Asians and least in Caucasians. Forty five patients (32.1\%) had high frequency MSI (MSI-H) tumours, and 75 patients (53.6\%) had microsatellite stable (MSS) tumours. The remaining 20 (14.3\%) had low frequency (MSI-L) tumours. In a study from Ibadan, Duduyemi et al reported MSI in 5 of 26 (19.2\%) patients, 3 of whom were less than 40 years.\textsuperscript{143} In a Danish study by Jensen et al absence of mismatch repair protein expression was stated in 52 (17.0\%) tumours.\textsuperscript{144} Of the 95 analyzed African American samples, Brim et al reported that 29 (30.5\%) were MSI-H, at a mean age of 65.7 years. Four MSI-H patients had a strong family history of colon cancer, and met the Amsterdam criteria for HNPCC.\textsuperscript{145} In that study, fourteen (26\%) of the Iranian samples were MSI-H tumours with a mean age of 59.8 years. Of the 53 patients who were examined, 12 (22.6\%) were with MSI-H with poor prognostic features.\textsuperscript{146} In a large series reported by Aaltonen et al, a 12 percent incidence was found.\textsuperscript{147} There was a similar result (17\%) among patients whose family histories fulfilled the Amsterdam criteria for hereditary nonpolyposis colorectal cancer.

Interestingly, in addition to the majority of HNPCC-related colorectal cancer that accounts for 2 to 4\% of all colorectal cancer, 10 to 15\% of sporadic colorectal cancers also display the MSI-H phenotype\textsuperscript{17,22} In a multiracial study by Carethers et al, the prevalence of MSI in the cancers differed between African Americans and Caucasians. There were 39/276 (14\%)
Caucasians with MSI cancers, compared to 15/227 (7%) African Americans with MSI cancers.\textsuperscript{152} The differences noted are likely to reflect the fact that our population was relatively young (all received a diagnosis at 50 years of age or younger) and thus may have included a greater proportion of patients with hereditary nonpolyposis colorectal cancer which can only be confirmed with determination of events at genetic level.

Thus, the majority of unselected MSI-H colorectal cancers are sporadic in nature and do not occur in the context of HNPCC. Similar to HNPCC, sporadic MSI-H colorectal cancer arises due to deficiencies in DNA mismatch repair proofreading function. Again, in most of the sporadic MSI-H colorectal cancers, the MLH1 gene has been silenced (translation and transcription have been blocked) by hypermethylation of the promoter region of the MLH1 gene.\textsuperscript{148} The presence of a $BRAF$ mutation in an MSI tumour effectively excludes the possibility that the tumour arose as the consequence of Lynch syndrome. $BRAF$ mutation status testing is outside the scope of this study.

The existence of MSI-L is controversial. However, the DNA repair gene O-6-methylguanine DNA methyltransferase (MGMT) is epigenetically silenced more frequently in MSI-L tumours than in both MSI-H and MSS cancers\textsuperscript{149} which suggests a different method of DNA error repair. MSI-L status has been related to poor prognosis in patients with stage C cancers\textsuperscript{150} Although techniques for improved detection of these subtle differences are developing, such as hypermethylation of MGMT.\textsuperscript{151} The true clinicopathological relevance of MSI-L status remains to be established.

This study did not show association between age or gender and the location, or MSI status of the tumour. Similar findings were reported by Carethers et al.\textsuperscript{152}

\subsection*{6.3 OBSERVABLE PATHOLOGICAL PATTERNS AND MSI STATUS}

In the analysis of outcome, MMR-deficient, compared to MMR-proficient tumours were significantly associated with right sided location and poorer differentiation. Tumours with fungating growth pattern were more commonly MSI-H. Although MSI-H colorectal cancers were diagnosed at a significantly greater depth of tumour invasion (pT), these tumours had a significantly lower overall pathological stage than cancers with microsatellite stability. In addition to the relative absence of familial adenomatous polyps, these are all features of CIMP tumours having implications for prognosis and possible outcome of disease.\textsuperscript{35} Again CIMP has been established as a unique epigenetic phenotype in colorectal cancer, and CIMP-high colorectal tumours have a distinct clinical, pathological, and molecular profile, such as associations with proximal tumour location, female sex, poor differentiation, MSI, and high
BRAF and low TP53 mutation rates. However, our findings seem to concur with studies from other parts of the world. For instance, Jensen et al reported that patients having MMR deficient compared to MMR proficient tumours had significantly lower risk of recurrence (HR = 0.4; 95% CI: 0.3–0.8 P = 0.003) and death (HR = 0.5; 95% CI: 0.3–0.9; P = 0.02), when controlling for the influence of other independent predictors of recurrence; disease stage (P = 0.001), perineural invasion (P = 0.05), and ileus (P = 0.0002). \(^{145}\)

The fact that MSI-H was strongly associated with a relatively lower disease stage, even after we controlled for size of tumour, is fascinating. It suggests that MSI status might be an independent prognostic factor. Secondly, it is able to predict lower pathological stage. It is still not clear how this is possible but it may be related to the kind of mutations or the genetic targets involved in colorectal cancers that are deficient in DNA-mismatch repair. \(^{16}\), \(^{17}\) For example, colorectal cancers which are MSI-H have fewer mutations of the adenomatous polyposis coli (APC) and p53 genes and more frequent mutations of the β-catenin (CTNNB1) and transforming growth factor b receptor type II (TGF-BRII) genes than colorectal cancers with microsatellite stability. \(^{45}\) Distinct clinical and pathological features, such as the intense lymphocytic infiltrates observed in tumours with high-frequency microsatellite instability, may result from the unique genetic alterations posited earlier and contribute to the less aggressive nature of these cancers. \(^{153}\) The strongest associations in this study were found between MSI-H status and residual adenomatous tissue, worse tumour grade, more Crohn-like feature but comparatively lower pathological staging with p values ≈ 0.

From the forgoing, it may be posited that the joint occurrence of high MSH-H rates and the paucity of adenomatous polyps may account for the relatively low colon cancer rates in this study and possibly in other low colon cancer zones.

It may also be that the loss of DNA repair mechanisms makes DNA vulnerable to the effects of DNA-damaging chemotherapeutic agents, such as fluorouracil. In vitro, MSI-H cell lines are less responsive than MSS cell lines to various chemotherapeutic agents. Furthermore, the targeting of DNA cells that are deficient in mismatch repair may offer an opportunity for specific interventions that do not affect normal tissues which have retain mismatch-repair function. The latest example of this is the encouraging response of MSI-H colorectal cancer to PD1 inhibitors, the use of which was approved by the FDA in the USA earlier in 2015. \(^{154}\) Such targeted therapies will be of immense benefit to our index cohort with such highest MSH-H rate of 32%.
CHAPTER 7

CONCLUSION

The tumour occurred more in the middle age group, mostly between 40 and 69 years and have no significant gender bias. More commonly they are left sided with rectal tumours being the most frequent presentation. Poorly differentiated tumours occurred more in the younger age groups and more commonly on the right side. While the rectal and colonic tumours were more commonly annular, the right colonic tumours were more commonly exophytic. The predominant histological type in all age groups was adenocarcinoma. Types with worse prognostic features were better correlated with younger age group. Tumours with worse tumour depth were more likely to occur on the right.

We detected high frequency MSI (MSI-H) tumours in 32.1\% (45/140) of the 175 cases which is high in comparison to the rates in areas with high colon cancer rates. A relative high MSH-H rate versus fewer adenomatous polyps may account for the relatively low colon cancer rates in this study and possibly in other low colon cancer zones.

The disease appears to occur at a much younger age group than in Caucasians and Arabs. High-frequency microsatellite instability was found to be associated with tumours of lower pathological stage, worse tumour biology, proximal location and more immunological response. Even though most of these features are in keeping with the CpG island methylator phenotype (CIMP), transcriptional or translational modifications such as somatic MLH1 mutation need to be shown.
CHAPTER 8

FURTHER STUDIES

Even though there is a hint of possible specific pathway for some of the colorectal cancers, the study did not clearly identify a special pathway for African colorectal cancers based on these observed tumour behaviours. In the CIMP pathway, sessile serrated adenomas are the chief pathological precursor unlike CRCs developing via the CIN pathway, and also in HNPCC which originate from adenomatous polyps.

A larger sample size with adequate documentation of biodata and patients’ management modalities/survival is needed in order to fully characterize the clinicopathological profile of colorectal cancer patients. In this regard, a multicentre study to compare the colorectal cancers in this study with similar tumours from elsewhere in order to investigate why individuals present at a younger age, and with a worse disease profile will be conducted.

As a sequel to this study, a wider genetic profiling using next generation sequencing need to be performed to confirm if these alterations in protein expression are transcriptional or translational. A closer look at the CIMP pathway which correlates with most serrated adenomas, MSI-H status and MLH1 inactivation in particular merits a special study. Classification of polyps and adenomatous tissues, as well as inflammatory bowel lesions, preferably done prospectively will help unravel the nature of this narrative as it pertains to our immediate milieu.

For patients who are found to have MSI-H tumours, future studies may include complete clinical data on management and treatment outcomes. This will enable us include their survival/response to immunological therapies like PD1 and EGFR inhibitors versus conventional drugs (FOLFOX, FOLFIRI).155
CHAPTER 9

STUDY LIMITATIONS

Firm conclusions about colon cancer rates are limited by the scope of this study being laboratory based. More so, the hospital in which this study was conducted is an apex hospital with the inclination for selecting only patients who can afford treatment at that level. Again, even though the number of cases used in this study is above the calculated sample size, the cases with incomplete data, missing and inadequate blocks had to be excluded and so reducing the statistical power of the study.

The lymph node yield was low, circumferential margins and apical nodes were not mentioned. For this reason, large proportion of the cases reviewed did not meet the requirements for reporting as stipulated in the CAP protocol. This limited the pathological characterization of the disease or objective assessment of the quality of surgery. Future studies will employ strict adherence to a widely accepted synoptic scheme for characterizing colorectal tumours.

Preoperative chemo-radiation of rectal cancer may have complicated IHC interpretation and/or decreased tumour mass in patients who had received some treatment. It also made MSI testing difficult. Ideally, evaluation of pretreatment biopsies would have been the preferred option.

From the way colorectal cancers are screened for and reported presently, it is difficult to determine if de novo adenocarcinomas without a benign histopathological precursor lesion ever occur in the large bowel. More so, when it is known that adenocarcinoma can overgrow the precursor lesion.

The prolonged time interval usually required for precursor lesions to become invasive carcinomas provides us with the opportunities to interfere with the process and reduce mortality. However, inadequate follow-up is a serious pitfall to any study or intervention.
APPENDIX 1: Table 2.4. TNM and Modified Dukes classifications for colorectal cancers and survival rates

<table>
<thead>
<tr>
<th>Stage</th>
<th>T</th>
<th>N</th>
<th>M</th>
<th>Modified Astler-Coller classification</th>
<th>Modified Dukes’ classification</th>
<th>Overall 5 year survival rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>T1,</td>
<td>No</td>
<td>Mo</td>
<td>A</td>
<td>A</td>
<td>80-95</td>
</tr>
<tr>
<td></td>
<td>T2</td>
<td></td>
<td></td>
<td>B1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>II A</td>
<td>T3</td>
<td>No</td>
<td>Mo</td>
<td>B2</td>
<td>B</td>
<td>72-75</td>
</tr>
<tr>
<td>II B</td>
<td>T4</td>
<td>No</td>
<td>Mo</td>
<td>B3</td>
<td></td>
<td>65-66</td>
</tr>
<tr>
<td>II A</td>
<td>T1,2</td>
<td>N1</td>
<td>Mo</td>
<td>C1</td>
<td>C</td>
<td>55-60</td>
</tr>
<tr>
<td>II B</td>
<td>T3,4</td>
<td>N1</td>
<td>Mo</td>
<td>C2/C3</td>
<td></td>
<td>35-42</td>
</tr>
<tr>
<td>II C</td>
<td>Any T</td>
<td>N2</td>
<td>Mo</td>
<td>C1/C2/C3</td>
<td></td>
<td>25-27</td>
</tr>
<tr>
<td>IV</td>
<td>Any T</td>
<td>Any N</td>
<td>M1</td>
<td>D</td>
<td></td>
<td>0-7</td>
</tr>
</tbody>
</table>

Key to Tumour-Node-Metastasis (TNM) Staging System

**T- Primary Tumour**

| Tx       | Primary tumour cannot be assessed |
| To       | No evidence of primary tumour     |
| Tis      | Carcinoma in-situ: intraepithelial or invasion of lamina propria |
| T1       | Tumour invasion of the submucosa  |
| T2       | Tumour invasion of the muscularis propria |
| T3       | Tumour invasion through the muscularis propria into the subserosa or into non-peritonealised pericolic/perirectal tissues |
| T4       | Direct tumour invasion of other organs or structures and/or perforation of visceral peritoneum |

**N- Regional Lymph Nodes**

| Nx       | Regional lymph nodes cannot be assessed |
| No       | No regional lymph nodes metastasis    |
| N1       | 1 to 3 regional lymph nodes metastasis |
| N2       | 4 or more regional lymph nodes metastasis |

**M- Distant Metastasis**

| Mx       | Distant metastasis cannot be assessed |
| Mo       | No distant metastasis                |
| M1       | Distant metastasis                  |
APPENDIX 2A:
Surgical Pathology Cancer Case Summary

Protocol web posting date: October 2013

COLON AND RECTUM: Excisional Biopsy (Polypectomy)

Select a single response unless otherwise indicated.

Tumor Site (Note A)
___ Cecum  
___ Right (ascending) colon  
___ Hepatic flexure  
___ Transverse colon  
___ Splenic flexure  
___ Left (descending) colon  
___ Sigmoid colon  
___ Rectum  
___ Other (specify): ________________________  
___ Not specified

+ Specimen Integrity
+ ___ Intact  
+ ___ Fragmented

+ Polyp Size
+ Greatest dimension: ___ cm  
+ Additional dimensions: ___ x ___ cm  
+ ___ Cannot be determined (see Comment)

+ Polyp Configuration
+ ___ Pedunculated with stalk  
  + Stalk length: ___ cm  
+ ___ Sessile

Size of Invasive Carcinoma
Greatest dimension: ___ cm  
+ Additional dimensions: ___ x ___ cm  
___ Cannot be determined (see Comment)

Histologic Type (Note B)
___ Adenocarcinoma  
___ Mucinous adenocarcinoma  
___ Signet-ring cell carcinoma  
___ High-grade neuroendocrine carcinoma  
  ___ Large cell neuroendocrine carcinoma  
  ___ Small cell neuroendocrine carcinoma  
___ Squamous cell carcinoma  
___ Adenosquamous carcinoma  
___ Medullary carcinoma  
___ Undifferentiated carcinoma  
___ Other (specify): ________________________  
___ Carcinoma, type cannot be determined
**Histologic Grade (Note C)**
- Not applicable
- Cannot be determined
- Low-grade (well-differentiated to moderately differentiated)
- High-grade (poorly differentiated to undifferentiated)

**Microscopic Tumor Extension (Note D)**
- Cannot be determined

Invasion (deepest):
- Lamina propria
- Muscularis mucosae
- Submucosa
- Muscularis propria

**Margins (select all that apply)**

**Deep Margin (Stalk Margin)**
- Cannot be assessed
- Uninvolved by invasive carcinoma
  - Distance of invasive carcinoma from margin: ___ mm or ___ cm
- Involved by invasive carcinoma

**Mucosal Margin (required only if applicable)**
- Cannot be assessed
- Uninvolved by invasive carcinoma
- Involved by invasive carcinoma
- Involved by adenoma

**Lymph-Vascular Invasion (Notes D and E)**
- Not identified
- Present
- Indeterminate

**Additional Pathologic Findings (select all that apply)**
- None identified
- Inflammatory bowel disease
  - Active
  - Quiescent
- Other (specify): ________________

**Ancillary Studies**
*Note: For reporting molecular testing and immunohistochemistry for mismatch repair proteins, and for other cancer biomarker testing results, the CAP Colorectal Biomarker...*
Template should be used. Pending biomarker studies should be listed in the Comments section of this report.

+ Comment(s)

APPENDIX 2B:
Surgical Pathology Cancer Case Summary

Protocol web posting date: October 2013

COLON AND RECTUM: Resection, Including Transanal Disk Excision of Rectal Neoplasms

Select a single response unless otherwise indicated.

Specimen (select all that apply) (Note A)
___ Terminal ileum
___ Cecum
___ Appendix
___ Ascending colon
___ Transverse colon
___ Descending colon
___ Sigmoid colon
___ Rectum
___ Anus
___ Other (specify): ______________________________ 
___ Not specified

Procedure
___ Right hemicolectomy
___ Transverse colectomy
___ Left hemicolectomy
___ Sigmoidectomy
___ Rectal/rectosigmoid colon (low anterior resection)
___ Total abdominal colectomy
___ Abdominoperineal resection
___ Transanal disk excision (local excision)
___ Other (specify): ______________________________ 
___ Not specified

+ Specimen Length (if applicable)
+ Specify: ___ cm

Tumor Site (select all that apply) (Note A)
___ Cecum
___ Right (ascending) colon
___ Hepatic flexure
___ Transverse colon
___ Splenic flexure
___ Left (descending) colon
___ Sigmoid colon
___ Rectosigmoid
___ Rectum

78
__ Ileocecal valve
__ Colon, not otherwise specified
__ Cannot be determined (see Comment)

+ **Tumor Location**
  + __ Tumor is located above peritoneal reflection
  + __ Tumor is located below the peritoneal reflection
  + __ Not specified

**Tumor Size**
Greatest dimension: __ cm
+ Additional dimensions: __ x __ cm
__ Cannot be determined (see Comment)

**Macroscopic Tumor Perforation (Note G)**
__ Present
__ Not identified
__ Cannot be determined

+ **Macroscopic Intactness of Mesorectum (Note H)**
  + __ Not applicable
  + __ Complete
  + __ Near complete
  + __ Incomplete
  + __ Cannot be determined

**Histologic Type (Note B)**
__ Adenocarcinoma
__ Mucinous adenocarcinoma
__ Signet-ring cell carcinoma
__ High-grade neuroendocrine carcinoma
  __ Large cell neuroendocrine carcinoma
  __ Small cell neuroendocrine carcinoma
__ Squamous cell carcinoma
__ Adenosquamous carcinoma
__ Medullary carcinoma
__ Undifferentiated carcinoma
__ Other (specify): ____________________________
__ Carcinoma, type cannot be determined

**Histologic Grade (Note C)**
__ Not applicable
__ Cannot be assessed
__ Low-grade (well-differentiated to moderately differentiated)
__ High-grade (poorly differentiated to undifferentiated)
__ Other (specify): ____________________________

+ **Histologic Features Suggestive of Microsatellite Instability (Note I)**
+ Intratumoral Lymphocytic Response (tumor-infiltrating lymphocytes)
+ ___ None
+ ___ Mild to moderate (0-2 per high-power [X400] field)
+ ___ Marked (3 or more per high-power field)

+ Peritumor Lymphocytic Response (Crohn-like response)
+ ___ None
+ ___ Mild to moderate
+ ___ Marked

+ Tumor Subtype and Differentiation (select all that apply)
+ ___ Mucinous tumor component (specify percentage: ___)
+ ___ Medullary tumor component
+ ___ High histologic grade (poorly differentiated)

**Microscopic Tumor Extension**
___ Cannot be assessed
___ No evidence of primary tumor
___ No invasion of lamina propria
___ Intramucosal carcinoma, invasion of lamina propria/muscularis mucosae
___ Tumor invades submucosa
___ Tumor invades muscularis propria
___ Tumor invades through the muscularis propria into the subserosal adipose tissue or the nonperitonealized pericolic or perirectal soft tissues but does not extend to the serosal surface
___ Tumor penetrates to the surface of the visceral peritoneum (serosa)
___ Tumor is adherent to other organs or structures (specify: _________________)
___ Tumor directly invades adjacent structures (specify: _________________)
___ Tumor penetrates to the surface of the visceral peritoneum (serosa) and directly invades adjacent structures (specify: _________________)

**Margins (select all that apply) (Note J)**
If all margins uninvolved by invasive carcinoma:
   Distance of invasive carcinoma from closest margin: ___ mm or ___ cm
   Specify margin: __________________________

**Proximal Margin**
___ Cannot be assessed
___ Uninvolved by invasive carcinoma
   ___ No adenoma or intraepithelial neoplasia / dysplasia identified
   ___ Adenoma (low-grade intraepithelial neoplasia / dysplasia) present
   ___ High-grade intraepithelial neoplasia / dysplasia or intramucosal carcinoma present
      (specify): ________________________________
___ Involved by invasive carcinoma

**Distal Margin**
___ Cannot be assessed
___ Uninvolved by invasive carcinoma
   ___ No adenoma or intraepithelial neoplasia / dysplasia identified
   ___ Adenoma (low grade intraepithelial neoplasia / dysplasia) present
High-grade intraepithelial neoplasia / dysplasia or intramucosal carcinoma present (specify): __________________________

Involved by invasive carcinoma

Circumferential (Radial) or Mesenteric Margin

Not applicable
Cannot be assessed
Uninvolved by invasive carcinoma
Involved by invasive carcinoma (tumor present 0-1 mm from margin)

Deep Margin (endoscopic mucosal resections) (required only if applicable)
Cannot be assessed
Uninvolved by invasive carcinoma
Involved by invasive carcinoma

Mucosal Margin (noncircumferential transanal disk excision) (required only if applicable)
Cannot be assessed
Uninvolved by invasive carcinoma
Involved by invasive carcinoma
Distance of invasive carcinoma from closest mucosal margin: ___ mm or ___ cm
Specify location (eg, o’clock position), if possible: ________________
Involved by invasive carcinoma
Specify location (eg, o’clock position), if possible: ________________
Uninvolved by adenoma
Involved by adenoma

Other Margin(s) (required only if applicable)

Specify margin(s): __________________________
Cannot be assessed
Uninvolved by invasive carcinoma
Involved by invasive carcinoma

Treatment Effect (applicable to carcinomas treated with neoadjuvant therapy) (Note K)
No prior treatment
Present
No residual tumor (complete response, grade 0)
Moderate response (grade 1, minimal residual cancer)
Minimal response (grade 2)
No definite response identified (grade 3, poor response)
Not known

Lymph-Vascular Invasion (Note E)
Not identified
Present
Indeterminate

Perineural Invasion (Note E)
Not identified
Present
Indeterminate
Tumor Deposits (discontinuous extramural extension) (Note L)
___ Not identified
___ Present (specify number of deposits: ____)
___ Indeterminate

+ Type of Polyp in Which Invasive Carcinoma Arose (Note F)
+ ___ None identified
+ ___ Tubular adenoma
+ ___ Villous adenoma
+ ___ Tubulovillous adenoma
+ ___ Traditional serrated adenoma
+ ___ Sessile serrated adenoma
+ ___ Hamartomatous polyp
+ ___ Indeterminate

Pathologic Staging (pTNM) (Note M)
TNM Descriptors (required only if applicable) (select all that apply)
___ m (multiple primary tumors)
___ r (recurrent)
___ y (posttreatment)

Primary Tumor (pT)
___ pTX: Cannot be assessed
___ pT0: No evidence of primary tumor
___ pTis: Carcinoma in situ, intraepithelial (no invasion of lamina propria)
___ pTis: Carcinoma in situ, invasion of lamina propria/muscularis mucosae
___ pT1: Tumor invades submucosa
___ pT2: Tumor invades muscularis propria
___ pT3: Tumor invades through the muscularis propria into pericolic or perirectal tissues
___ pT4a: Tumor penetrates the visceral peritoneum
___ pT4b: Tumor directly invades or is adherent to other organs or structures

Regional Lymph Nodes (pN)
___ pNX: Cannot be assessed
___ pN0: No regional lymph node metastasis
___ pN1a: Metastasis in 1 regional lymph node
___ pN1b: Metastasis in 2 to 3 regional lymph nodes
___ pN1c: Tumor deposit(s) in the subserosa, or non-peritonealized pericolic or perirectal tissues without regional lymph node metastasis
___ pN2a: Metastasis in 4 to 6 regional lymph nodes
___ pN2b: Metastasis in 7 or more regional lymph nodes
___ No nodes submitted or found

Number of Lymph Nodes Examined
Specify: ____
___ Number cannot be determined (explain): ______________________

Number of Lymph Nodes Involved
Specify: ____
___ Number cannot be determined (explain): ______________________
Distant Metastasis (pM)
___ Not applicable
___ pM1: Distant metastasis
       + Specify site(s): ______________________________
___ pM1a: Metastasis to single organ or site (eg, liver, lung, ovary, nonregional lymph node)
___ pM1b: Metastasis to more than 1 organ/site or to the peritoneum

+ Additional Pathologic Findings (select all that apply)
+ ___ None identified
+ ___ Adenoma(s)
+ ___ Chronic ulcerative proctocolitis
+ ___ Crohn disease
+ ___ Dysplasia arising in inflammatory bowel disease
+ ___ Other polyps (type[s]): __________________________
+ ___ Other (specify): ____________________________

+ Ancillary Studies
   Note: For reporting molecular testing and immunohistochemistry for mismatch repair proteins, and for other cancer biomarker testing results, the CAP Colorectal Biomarker Template should be used. Pending biomarker studies should be listed in the Comments section of this report.

+ Comment(s)
Emory University Hospital/ Winship Cancer Centre Core Laboratories Atlanta

Immunohistochemistry Protocol

1. From the tissue specimens 1.5 mm cylinders were punched out and collected in tissue microarray (TMA) paraffin blocks each containing about 80 samples each.

2. TMA blocks are kept overnight in the oven to improve adherence of the tissue cores to the well. Tissue from normal colonic mucosa, ovaries were included in the tissue arrays serving as reading frame and controls.

3. Sections of formalin-fix, paraffin-embedded tissue were tested for the presence of primary antibodies using Bond Polymer Refine Detection kit (DAB chromogen), (Leica Microsystems, Bannockburn, ILL). The detection system avoids the use of streptavidin and biotin, and therefore eliminates nonspecific staining as a result of endogenous biotin. All steps were performed on the Leica Bond Maxx III automated system.

4. Specimens were deparaffinised and antigen retrieved on the instrument.

5. All slides were incubated with the primary (monoclonal) antibodies for 15 minutes, with post primary polymer for 8 minutes, blocked with 3% hydrogen peroxide for 5 minutes, 3,3-diaminobenzidine (DAB, brown chromogen) for 10, minutes and haematoxylin was used as counterstain for 5 minutes. These incubations were performed at room temperature; between incubations, sections were washed with Tris-buffered saline (Bond wash solution).

6. Coverslipping was done using the Tissue Tek SCA (Sakura Finetek USA, Inc. Torrance, CA) coverslipper.

7. Positive controls of known positive tissue (colonic mucosa and endometrial, and lymphoid tissue) and negative control (section of ovarian tissue) with primary antibody replaced with Tris buffer were run with the study slides.

The Bond Polymer Refine Detection is a biotine-free, polymeric horseradish peroxidase (HRP)-linker antibody conjugate system for the detection of tissue-bound mouse and rabbit IgG and some mouse IgM primary antibodies.
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