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Original Research Article

KRAS and P53 Gene Mutation Patterns Linked to Colorectal Cancer in Sudan

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Abstract

Background*:** Molecular pathogenesis of CRC represents a major target for understanding and controlling of the disease in the Sudan. Therefore, the present study aimed to explore the clinicopathological pattern of CRC in the Sudan with specific emphases in the role KRAS and P53 genes mutations. Methodology: This study was carried out in El-Obeid, North Kordofan state, Sudan, using CRC samples from patients who presented to the Al-Obeid teaching hospital between 2017 and 2022 and had a biopsy for histopathological diagnosis. This series featured everyone who has CRC. ***Results*:** The most prevalent CRC subtype is adenocarcinoma, which is followed by mucinous adenocarcinoma and metastatic adenocarcinoma, which constitute 74%, 20%, and 6%, respectively. KRAS mutation was found in 40% patients 48% males and 33.3% females). P53 mutation was found in 46% individuals 43.5% men and 48% females. Positive correlations between KRAS and P53 were found in 26% cases, while negative correlations were found in 40% cases. ***Conclusion: Many CRC patients in Sudan present at advanced stages of the illness and at a younger age, necessitating early screening measures. CRC patients have been discovered to have KRAS, P53, or both mutations. As a result, precise preventative and therapeutic methods are considered essential.

Keywords: Colorectal cancer, KRAS, P53, Sudan, Adenocarcinoma.

INTRODUCTION

Colorectal cancer (CRC) is the third highest cause of cancer death globally, affecting both men and women (25% less in women). It is the third most prevalent cancer in men, behind lung and prostate cancer, and the second most common cancer in women, following breast cancer [1, 2].

CRC affects males more than females in Sudan, with the majority of victims being young adults [3]. The prevalence rates of CRC vary geographically due to changes in dietary and environmental factors, which account for more than 90% of CRCs, and inherited risk factors account for roughly 5-10% of CRCs [2]. About 70% of the CRC occurs sporadic, up to 25% in patients with family history, and about 10% are hereditary.

Adenomas are benign lesions that develop into premalignant neoplastic lesions when certain genes, such as APC, KRAS, SMAD4, and TP53, are altered, resulting in an adenoma-carcinoma genetic model of colorectal carcinogenesis [4].

Most sporadic CRCs have accumulated mutations, with around 85% having mutations in APC, TP53, KRAS, TTN, PIK3CA, FBXW7, and SMAD4. Approximately 15% are caused by hypermethylation of the DNA mismatch repair (MMR) gene [5]. KRAS is an oncogene, and its mutations occur in around one-third of CRC patients but not in those without CRC. It occurs most frequently in Codon 12 and least frequently in Codon 13 [6]. 30% to 50% of patients have KRAS positivity [7].

TP53 is a tumor suppressor gene and most mutations occur in exon 4-8 [6]. TP53 occurs in (50% to 60%) of CRC patients [8, 9]. However, there is a complete lack of evidence from Sudan on the significance of hereditary risks of genes such as KRAS and P53 in the etiology of Sudan's rising CRC burden. As a result, the current study sought to examine the role

of the KRAS and P53 genes in a group of Sudanese patients with CRC.

MATERIALS AND METHODS

Fifty tissue blocks from histopathology laboratories in El-Obeid, North Kordofan state, were used in this investigation. Tissue blocks embedded in wax and preserved with formalin were obtained from CRC patients undergoing surgical excision. Every tissue had been wax-embedded and formalin-fixed. The sample consisted of all CRC patients revealed to histopathology laboratories in El-Obeid between 2017 and 2022. For the purpose of diagnosis, conventional histology was used to make the main diagnosis of CRC. In each case, a consultant histopathologist confirmed the diagnosis of CRC. Following that, immunohistochemistry methods were used to detect two MMR proteins (KRAS and P53).

Immunohistochemical testing was carried out using two monoclonal antibodies KRAS: Mono clonal mouse anti-human CK5,6, clone D5/16B4. P53: Mono clonal mouse anti-human EGFR, clone, as follows:

Two formalin-fixed, paraffin-embedded tumor slices (3m) were cut and mounted on salinized slides (Dako). Following xylene deparaffinization, slides were rehydrated in a graded series of alcohol before being placed in running water. Then, using a PT connection, antigen retrieval for KARS and P53 was performed. Endogenous peroxidase activity was stopped for 10 minutes with 3% hydrogen peroxidase and methanol, followed by 20 minutes at room temperature in a moisture chamber with 100-200 l of primary antibodies, followed by a washing in Phosphate buffer saline. The KRAS and P53 primary antibodies were ready for use (Dako, Carpintera). After 3 minutes of washing with PBS, antibody binding was detected by incubating for 20 minutes with dextran-labeled polymer (Dako-EnVision TM Flex kit). Finally, the sections were washed three times in PBS before being stained with 3, 3 diaminobenzidine tetra hydrochloride (DAB) (Dako) for up to 5 minutes to produce the distinctive brown stain for the observation of the antibody/enzyme combination. Hematoxylin was then used to stain the slides. Positive and negative control slides are also generated for each staining session. The positive control slides included the antigen under research, while the negative control slides were made from the same tissue block but treated with PBS rather than the primary antibody. Each slide was assessed by an investigator, and the results were validated and rated by a consultant histopathologist.

Ethical Consent

Beside obtaining ethical acceptance from relevant authorized bodies. The Human Research Ethics Committee (HREC) at Prof Medical Research Consultancy Center has approved the research protocol. HREC 0001/MRCC.12/23).

Statistical Analysis

After preparing the data in a standard master sheet, the variables were entered into SPSS software for analysis. Percentages, frequencies, cross-tabulations were obtained.

RESULTS

In this study there were 23 (46%) males and 27 (54%) females among the 50 patients, who ranged in age from 20 to 75 years, with a mean age of 48. As indicated in Table 1, Fig 1, the majority of patients were aged 41-60 years, followed by 61-75 years and ≤ 40 years, with 23/50 (46%), 11(22%), and 8(16%), respectively.

Rectal lesions were the most common, followed by bowel and colon lesions, accounting for 21/50 (42%), 12 (24%), and 10%, respectively. Rectal is the most prevalent site for men, followed by colon and bowel, accounting for 10/23 (43.5%), 5(21.7%), and 4(17.4%), respectively. The most prevalent tumor site in females was rectal, followed by intestine and colon, representing 11/27(%), 8%, and 5%, respectively, as shown in Table 1, Fig 1.

Table 1: Distribution of the study subjects by sex, age and lesion site

Variable	Males	Females	Total
Age			
≤40 years	4	4	8
41 - 60	12	11	23
61 - 80	3	8	11
mislaid	4	4	8
Total	23	27	50
Lesion site			
Colon	5	5	10
Bowel	4	8	12
Rectal	10	11	21
Sigmoid	0	3	3
Colorectal	4	0	4
Total	23	27	50

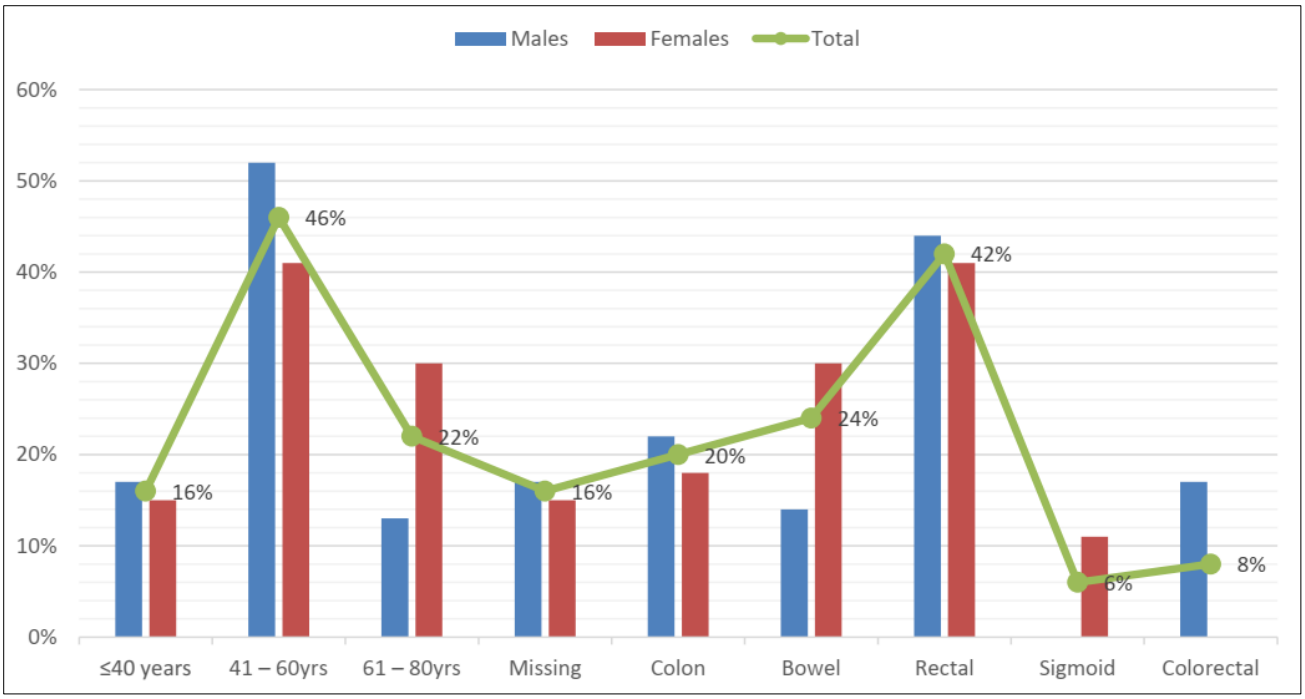

Figure 1: Description of patients by sex, age and tumor site

The distribution of research subjects by histology, grade, and stage among males and females is summarized in Table 2 and Fig 2. The most prevalent CRC subtype is adenocarcinoma, which is followed by mucinous adenocarcinoma and metastatic adenocarcinoma, which constitute 37/50 (74%), 10 (20%), and 3(6%), respectively. In males, adenocarcinoma accounted for 18/23 (78.3%), with mucinous adenocarcinoma contributing to 4/23 (17.4%). In females, adenocarcinoma accounted for 19/27 (70.4%), with mucinous adenocarcinoma representing 6/27 (22.2%).

In regard to cancer grade, the majority of patients had Well Differentiated carcinoma, followed by Moderately Differentiated and Poorly Differentiated carcinoma, accounting for 19/33 (57.6%), 9/33 (27.3%), and 3/33 (9%), respectively. 1/16 (6.3%) young men and 3/17 (17.6%) females were found to be poorly differentiated. A female was the lone example with poorly differentiated cancer. Dukes C 13/22 (59%) was the most common cancer stage.

Table 2: Distribution of the study subjects by histopathology, grade and stage

Variable	Males	Females	Total
CRC type			
Adenocarcinoma	18	19	37
Adenocarcinoma with mucinous	4	6	10
Metastatic Adenocarcinoma	1	2	3
Total	23	27	50
Cancer Grade			
Well Differentiated	10	9	19
Moderately Differentiated	5	4	9
Poorly Differentiated	1	3	4
Undifferentiated	0	1	1
Missing	7	10	17
Total	23	27	50
Cancer stage			
Dukes B	3	6	9
Dukes C	6	7	13
mislaid	14	14	28
Total	23	27	50

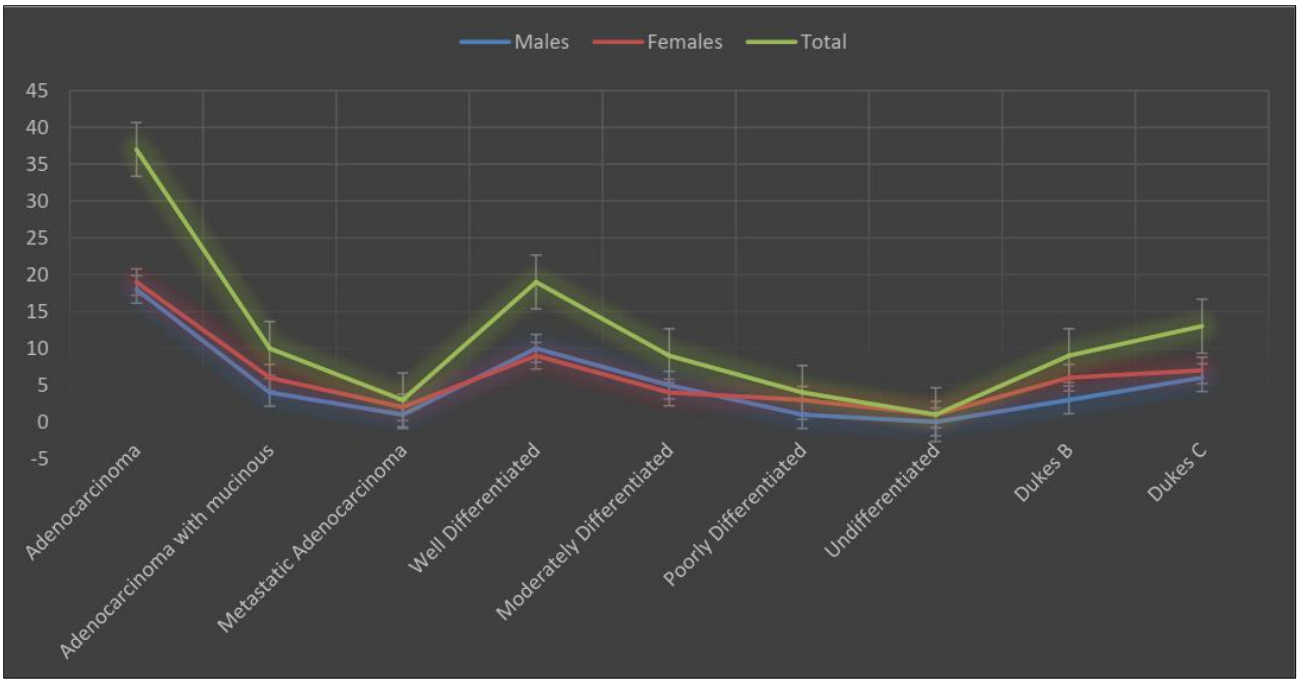

Figure 2: The study subjects by histopathology, grade and stage

Table 3 summarizes the distribution of patient ages by histology and grade. About 8/50 (16%) of the CRC were found in people under the age of 40, while 23/50 (46%) were found in those between the ages of 41 and 60. There is no apparent relationship between age and characteristics such as grade and stage.

Table 3: Distribution of patients' ages by histopathology and grade

Variable	≤40 years	41-60	61-75	AN	Total
Histopathology					
Adenocarcinoma	5	17	8	7	37
Adenocarcinoma with mucinous	2	6	2	0	10
Metastatic Adenocarcinoma	1	0	1	1	3
Total	8	23	11	8	50
Grade					
Well Differentiated	2	10	2	5	19
Moderately Differentiated	3	5	1	0	9
Poorly Differentiated	0	2	2	0	4
Undifferentiated	0	0	1	0	1
Unavailable	3	6	5	3	17
Total	8	23	11	8	50
Stage					
Dukes B	1	5	2	1	9
Dukes C	3	6	4	0	13
Unavailable	4	12	5	7	28
Total	8	23	11	8	50

KRAS mutation was found in 20/50 (40%) patients (11/23 (48%) males and 9/27 (33.3%) females), as shown in Table 4, Fig 3. The age groups 41-60, 61-80, and 40 years saw the most KRAS positive cases, accounting for 11/20(55%), 3/20(15%), and 2/20(10%), respectively. Out of the 20 positive cases, 16/20 (80%) had adenocarcinoma, 3/20 (15%) had mucinous adenocarcinoma, and 1/20 (5% had metastatic adenocarcinoma.

Table 4: Distribution of KRAS by sex, age, cancer type

Variable = KRAS	Positive	Negative	Total
Sex			
Males	11	12	23
Females	9	18	27
Total	20	30	50
Age			
≤40 years	2	6	8
41 - 60	11	12	23
61 - 80	3	8	11
Unavailable	4	4	8
Total	20	30	50
Histopathology			
Adenocarcinoma	16	21	37
Adenocarcinoma with mucinous	3	7	10
Metastatic Adenocarcinoma	1	2	3
Total	20	30	50

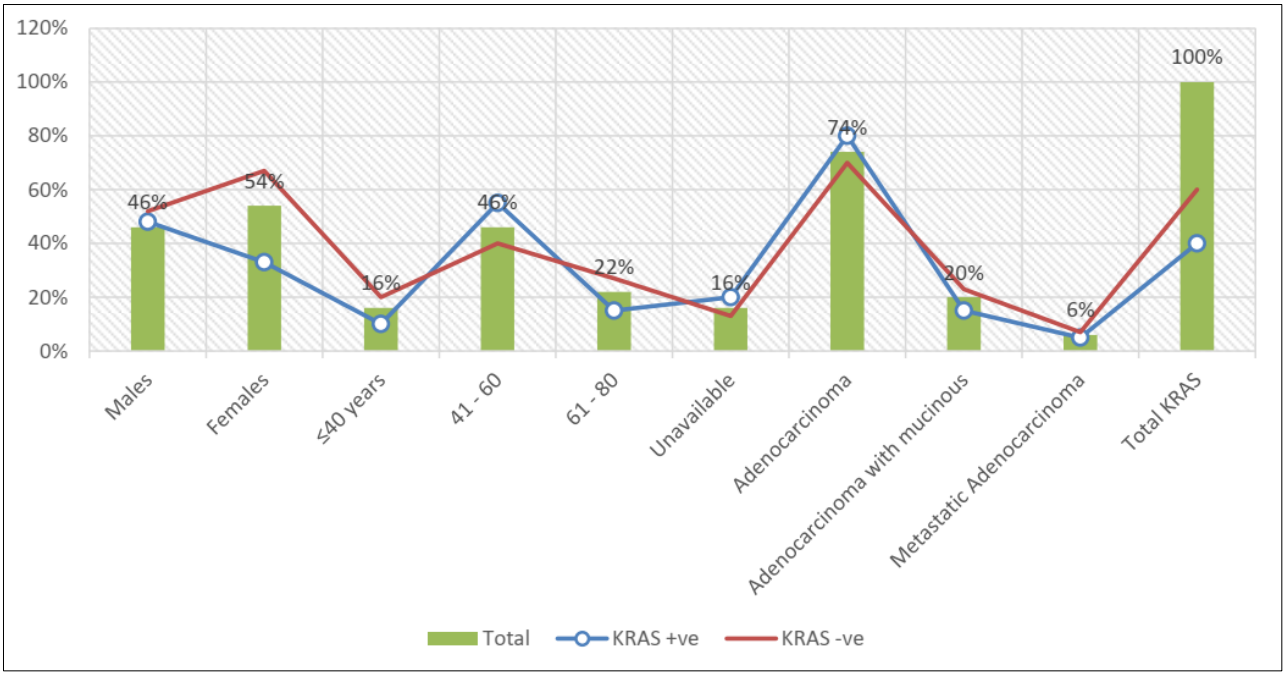

Figure 3: KRAS by sex, age, cancer type

P53 mutation was found in 23/50 (46%) individuals (10/23 (43.5%) men and 13/27 (48%) females), as shown in Table 5. The age groups 41-60, 61-80, and 40 years saw the most P53 positive cases, accounting for 10/23 (43.5%), 6/23 (26%), and 3/23 (13%), respectively. Out of the 23 positive patients, 18/23 (78.3%) had adenocarcinoma, 4/23 (17.4%) had mucinous adenocarcinoma, and 1/23 (4.3%) had metastatic adenocarcinoma.

Table 5: Distribution of P53 by sex, age, cancer type

Variable = P53	Positive	Negative	Total
Sex			
Males	10	13	23
Females	13	14	27
Total	23	27	50
Age			
≤40 years	3	5	8
41 - 60	10	13	23
61 - 80	6	5	11
NA	4	4	8

Variable = P53	Positive	Negative	Total
Total	23	27	50
Histopathology			
Adenocarcinoma	18	19	37
Adenocarcinoma with mucinous	4	6	10
Metastatic Adenocarcinoma	1	2	3
Total	23	27	50

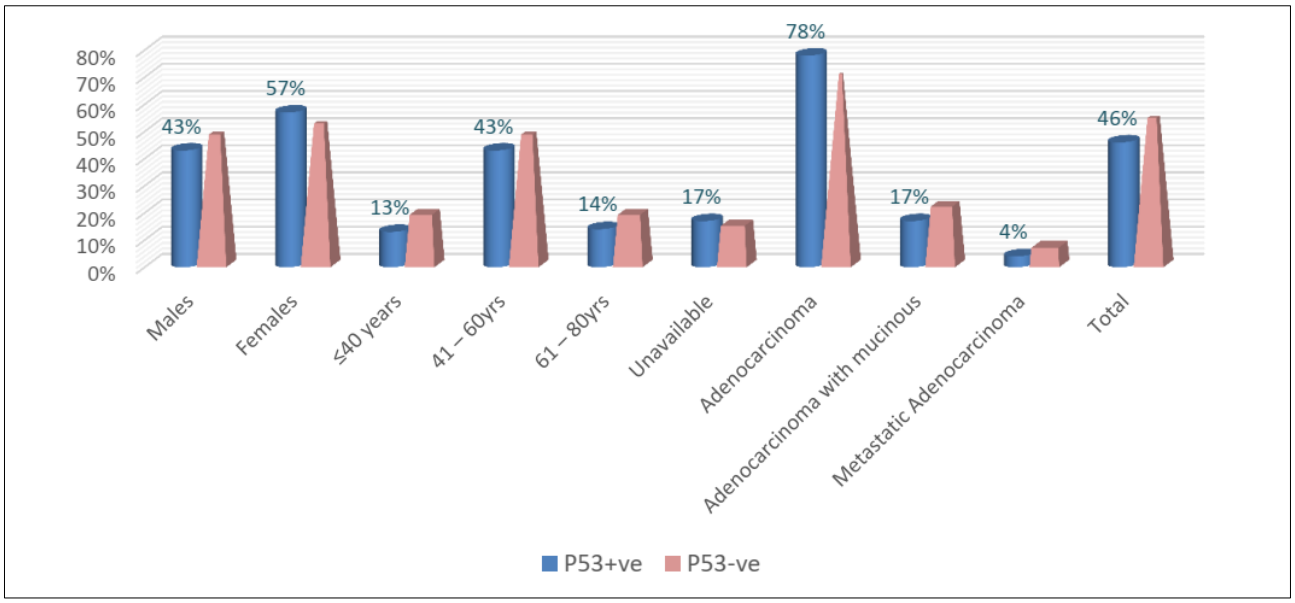

Figure 4: P53 by sex, age, cancer type

Positive correlations between KRAS and P53 were found in 13/50 (26%) cases, while negative correlations were found in 20/50 (40%) cases. As shown in Table 6, 7/20 (35%) of the 20 positive cases by KRAS were found negative by P53, resulting in 10/23 (43.5%) of the 23 positive cases by P53 being found negative by KRAS.

Table 6: Distribution of KRAS by P53

Variable	P53		
KRAS	**Positive**	**Negative**	**Total**
Positive	13	7	20
Negative	10	20	30
Total	23	27	50

DISCUSSION

Colorectal cancer is a major health issue in Sudan, and there is a lack of published data. As a result, the current study attempts to reveal some data about the pattern of colorectal cancer in Sudan, as well as the CRC links with KRAS and P53 mutations.

According to the current data, CRC is more common in younger populations and is more prevalent in females. The number of cases of early-onset colorectal cancer, which is defined as CRC diagnosed before the age of fifty, is rising, but its diverse etiology, which sets it apart from CRC in general, is still unknown [10]. Our data reflect previous reports that CRC affects Sudanese individuals at a younger age (43.84% were under 50 years old), with a peak frequency in the fifth and sixth decades. All physicians must be made more aware of the possibility of colorectal cancer in young people [11].

Although the vast majority of publications in this context suggest that CRC is more common in men, several researches have linked CRC to particular risk factors in each geographical region. CRC, on the other hand, is mostly avoidable by adjustments in modifiable risk factors, as well as the diagnosis and removal of precancerous lesions. With rising prevalence in developing nations especially among young adults, there is an urgent need to better understand and act on results in order to prevent future cases and fatalities from the disease [12].

Adenocarcinoma was the most common CRC subtype in the current investigation, followed by adenocarcinoma with mucinous, with the most common

presentation as well-differentiated carcinomas. The majority of CRCs are carcinomas, with more than 90% being adenocarcinomas and other uncommon types (adenosquamous, spindle, squamous, and undifferentiated). It is possible to further differentiate CRC adenocarcinoma into medullary, micropapillary, serrated, mucinous, and signet-ring cell types. Adenocarcinomas are classified as well (>95%), moderately (>50%) and poorly (<49%) differentiated based on the proportion of gland formation. They are further subdivided into two tiers: low-grade (well-moderate) and high-grade (poor), with the latter having prognostic importance. More than half of the stain cells are classified as mucinous or signet ring cells when they have that specific trait [13].

The majority of the cases in this series had Dukes C followed by B stages. Stage A cancers were restricted to the rectal wall, which was not found in this investigation. Stage B is for individuals who progress beyond the wall but do not have lymph node (LN) metastases, and Stage C is for those who have positive LN metastases [14].

The prevalence of KRAS mutation in the current study was 40% (48% males and 33.3% females). KRAS mutations are common in cancers. It is mutated in over 30% of all cancer cases and nearly 50% of metastatic colorectal cancer (CRC), the third greatest cause of cancer-related deaths globally. Recent advances in CRC biology and genetics have revealed the importance of KRAS mutations in CRC progression. The KRAS gene encodes a small GTPase (Guanosine TriPhosphatases) that is engaged in signaling pathways that involve critical proteins involved in amplifying growth factor and receptor signals. KRAS mutations at codons 12 and 13 are common, and these mutations have oncogenic characteristics. In CRC, abnormal KRAS protein activation dramatically promotes signals linked with different cancer-related activities such as cell proliferation, migration, and neoangiogenesis [15].

P53 mutations were discovered in 46% of the participants in this study, 43.5% of whom were men and 48% of whom were women. It has previously been observed that the frequency of p53 mutations in CRC ranges between 40 and 50% [16]. P53 is a tumor suppressor gene that causes cell-cycle arrest, DNA repair, senescence, or apoptosis in response to cellular stressors [17]. The TP53 mutation is a critical stage in the progression of adenoma to adenocarcinoma. The functional effects of TP53 mutations in tumor formation have been thoroughly studied. The TP53 mutation was linked to a poor prognosis and chemoresistance in CRC. A p53 mutant's gain of function (GOF) enhances cell proliferation, migration, and invasion via various mechanisms. Potential treatment options include restoring wild type p53 function, depleting p53 mutants, or intervening by targeting oncogenic downstream [18].

IN CONCLUSION

In Sudan, many cases of CRC present at rather advanced stages of the disease, necessitating early screening initiatives. KRAS, P53, or both mutations have been found in CRC patients. As a result, precise preventative and treatment protocols are deemed critical.

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Authors Contribution

BE: Conceptual, consultation, funding, and approval of the final version
SG: Conceptual, data analysis, funding, and approval of the final version
AAA: Conceptual, manuscript drafting, and approval of the final version
HGA: Conceptual, manuscript drafting, and approval of the final version

Funding: Self-funded.

Data Availability: The data presented in this study are available on request to the corresponding author.

Disclosure of Interest: No interest to declare

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