CLINICOPATHOLOGICAL CORRELATION OF ALPHA-METHYLACYL -COENZYME A RACEMASE EXPRESSION IN COLORECTAL NEOPLASIA- A CROSS-SECTIONAL STUDY IN A TERTIARY CARE HOSPITAL

By

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LIST OF ABBREVIATIONS USED

AJCC	:	American Joint Committee on Cancer
AMACR	:	Alpha-Methyl Acyl-Coenzyme A Racemase
APC	:	Adenomatous Polyposis Coli (gene)
CIMP	:	CpG Island Methylator Phenotype
COX-2	:	Cyclooxygenase-2
CRC	:	Colorectal Carcinoma
DAB	:	3, 3'-Diaminobenzidine
DPX	:	Dibutylphthalate Polystyrene Xylene (mounting medium)
FAP	:	Familial Adenomatous Polyposis
GALT	:	Gut-Associated Lymphoid Tissue
H&E	:	Hematoxylin and Eosin
HGD	:	High-Grade Dysplasia
IBD	:	Inflammatory Bowel Disease
IHC	:	Immunohistochemistry
KRAS	:	Kirsten Rat Sarcoma Viral Oncogene
LGD	:	Low-Grade Dysplasia
MMPs	:	Matrix Metalloproteinases
MRD	:	Medical Records Department
MSI	:	Microsatellite Instability
SPSS	:	Statistical Package for the Social Sciences
TNM	:	Tumor, Node, Metastasis (staging system)
TP53	:	Tumor Protein p53
WHO	:	World Health Organization

ABSTRACT

“CLINICOPATHOLOGICAL CORRELATION OF ALPHA-METHYLACYL -COENZYME A RACEMASE EXPRESSION IN COLORECTAL NEOPLASIA- A CROSS-SECTIONAL STUDY IN A TERTIARY CARE HOSPITAL”

BACKGROUND: Colorectal neoplasia incorporates a variety of lesions ranging from benign adenomatous polyps to malignant colorectal carcinomas (CRC), collectively posing a substantial burden on global public health. Recent studies have shown dysregulated expression of Alpha-methyl acyl-coenzyme a racemase (AMACR) in various carcinomas like colorectal carcinoma, hepatocellular carcinoma, renal cell carcinoma, and prostatic carcinoma.

Owing to its tumor heterogeneity, a comprehensive understanding of the clinicopathological correlation of AMACR expression in colorectal neoplasia is essential to unravel its true diagnostic and prognostic potential.

OBJECTIVES: This study aimed to analyse clinicopathological correlation of alpha methyl acyl-coenzyme- a racemase expression with colorectal neoplasia.

METHODS: A total of 51 colorectal neoplasia cases diagnosed during April 2023 to March 2024 were included in this study. Slides stained with H&E, and immunohistochemically for AMACR were evaluated for histopathological examination. Results were subjected to appropriate statistical analysis.

RESULTS: The results of this study show an increased expression of AMACR in colorectal adenomas and carcinomas as compared to normal colonic epithelium. There was significant difference of AMACR expression in moderately and poorly differentiated carcinomas reinforcing AMACR as a marker of tumor differentiation.

AMACR expression was significantly associated with tumor stage but not nodal status.

CONCLUSION: In conclusion, the findings of this study suggest a possible role of AMACR in early neoplastic changes. Further research is needed to establish its precise role in tumor progression and potential clinical applications in colorectal cancer management.

KEYWORDS: Colorectal neoplasia; AMACR; Colorectal polyps; Colorectal carcinoma; prognosis

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INTRODUCTION

Colorectal neoplasia encompasses a spectrum of lesions ranging from benign adenomatous polyps to malignant colorectal carcinomas, collectively posing a substantial burden on global public health. Colorectal carcinoma (CRC) is the 3rd leading cause of morbidity and 2nd most common of mortality globally.[1] Genetic and environmental factors together contribute in the causation of CRC.[2] Epidemiological researches reveal that nutrition, consumption of alcohol, and smoking are the main environmental factors for CRC.[2,3] Increased fatty acid intake and low fiber in the diet are major factors. Consumption of red meat as well as dairy products are the common sources of branching fatty acids.[2-4]

Alpha-methyl acyl-coenzyme a racemase (AMACR) is a mitochondrial and peroxisomal enzyme having a crucial role in branching fatty acid beta-oxidation.[5] Dysregulated expression of AMACR has been implicated in various stages of tumorigenesis, including cellular proliferation, apoptosis evasion, and metastasis formation. Increased expression of AMACR is seen in different carcinomas like prostatic carcinoma, hepatocellular carcinoma, breast carcinoma, and colorectal carcinoma.[5-6] Increased expression in carcinoma as well as pre-malignant lesions implies that it may play an important part in colorectal carcinogenesis.[5,7]

However, the precise role of AMACR in colorectal neoplasia remains incompletely understood, with conflicting findings reported in the literature. Several studies have reported an elevated AMACR expression in colorectal neoplasms in comparison to normal colonic epithelium, indicating potential role in diagnosis and prognosis.

Conversely, other studies have reported a lack of significant association between AMACR expression levels with clinicopathological parameters, highlighting the need for further investigation.

Moreover, the heterogeneity of colorectal neoplasia, encompassing various histological subtypes, molecular alterations, and clinical presentations, adds complexity to the study of AMACR as a biomarker.

Factors like tumor location, tumor size, differentiation status of tumor, and genetic alterations may influence AMACR expression patterns and their clinical significance. Therefore, a comprehensive understanding of the clinicopathological correlation of AMACR expression in colorectal neoplasia is essential to unravel its true diagnostic and prognostic potential.

According to many studies, about 53%-82% of colorectal carcinoma have increased AMACR expression, specifically in well-differentiated and moderately differentiated carcinomas and loss of AMACR expression was reported in poorly differentiated tumors. It suggests expression of AMACR may be associated with histological grade and prognosis of CRC.[4-8] Hence aims to detect AMACR expression and correlation with clinicopathological parameters in CRC.

Hence, the rationale for conducting this cross-sectional study lies in bridging these knowledge gaps and elucidating the intricate relationship between AMACR expression and colorectal neoplasia pathogenesis. By systematically examining AMACR expression levels in a cohort of colorectal neoplasia cases in tertiary care center, this study aimed to evaluate correlation between AMACR expression levels and various clinicopathological parameters.

OBJECTIVES

1. To evaluate AMACR expression in colorectal neoplasm.
2. To correlate AMACR expression with clinicopathological parameters in colorectal neoplasms.

REVIEW OF LITERATURE

❖ Embryological Development of the Colon and Rectum: [9-11]

Embryologically, the development of the colon and rectum involves a complex process originating from the endodermal layer of the embryo, specifically from hindgut part of the primitive gut-tube. Here is a detailedoverview of this process:

1. Primitive Gut Tube Formation

- **Primitive Gut Tube**: It forms during the fourth week of embryonic development when the embryo undergoes folding. This tube is divided in to 3 parts: foregut, midgut, and hindgut.
- **Hindgut**: The hindgut forms the colon and rectum. It extends from the distal 3rd of transverse colon to cloacal membrane.

2. Partitioning of the Cloaca

- **Cloaca**: Initially, hindgut terminates into a cavity called cloaca, which will eventually divide into urogenital sinus and the anorectal canal.
- **Urorectal Septum**: Around 4th to 6th week of embryonic development, the urorectal septum grows caudally separating the cloaca into the urogenital sinus anteriorly and anorectal canal posteriorly.
- **Anal Membrane**: It temporarily closes the anorectal canal, which will later rupture to create the anal opening.

3. Development of the Colon

- **Midgut Rotation**: It undergoes a 270-degree rotation anticlockwise around superior mesenteric artery, which positions future ascending colon, transverse colon, and the initial segment of descending colon.

- **Differentiation**: As midgut differentiates, ascending colon, transverse colon, and descending colon form. Distal portion of hindgut will give rise to the descending colon, sigmoid colon, rectum, and upper portion of anal canal.

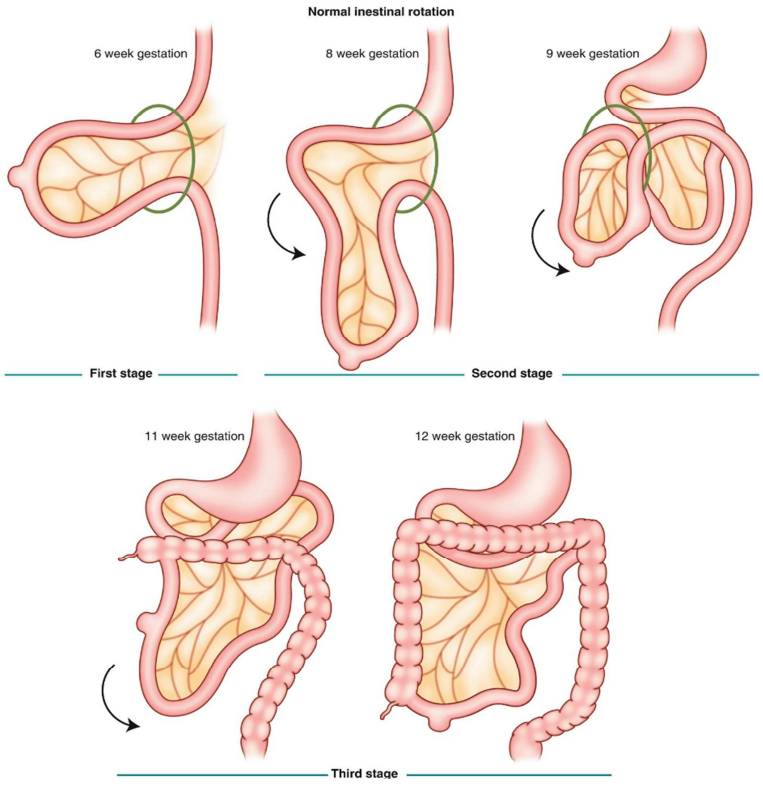

Figure 1: Normal intestinal rotation [10]

(Image taken from Carmichael JC, Mills S. Anatomy and embryology of the colon, rectum, and anus. Springer International Publishing; 2022.)

4. Formation of Rectum and the Anal Canal

- **Rectum**: It forms from hindgut as it differentiates into the terminal part of the digestive tract.
- **Anal Canal**: The upper part of anal canal develops from hindgut, while the lower part from an invagination of the ectoderm called the proctodeum. These two parts meet at the pectinate line.
- **Anal Membrane Rupture**: The anal membrane eventually ruptures around the 8th week, establishing the continuity between the rectum and the external environment.

❖ Anatomy of the Colon and Rectum:[12-17]

- The colon and rectum constitute the large intestine. The colon is divided into following segments: the ascending colon, transverse colon, descending colon, sigmoid colon, and rectum.
- The rectum connects sigmoid colon to anus and serves as a temporary site of storage for feces prior to defecation.
- The colon and rectum are lined by a mucous membrane formed by epithelial cells that absorb water as well as nutrients from digested food.

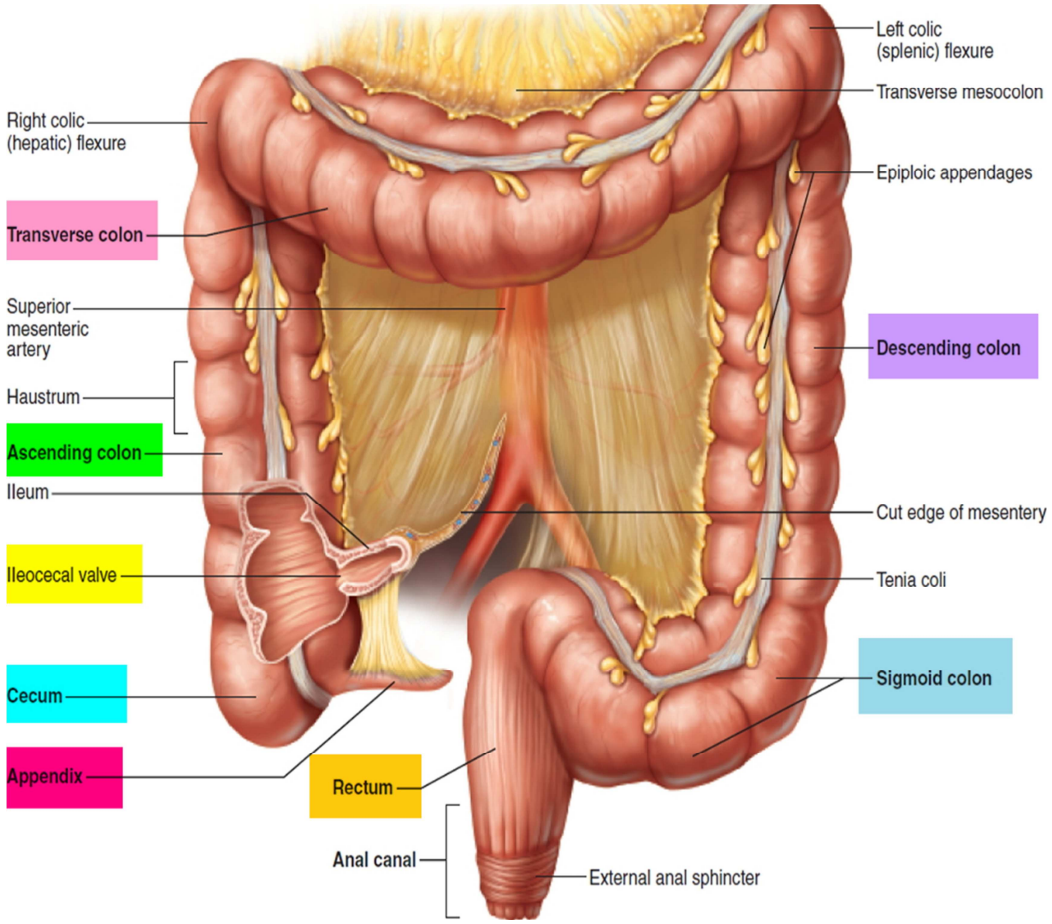

Figure 2: Anatomy of the Colon and Rectum [18]

(Image taken from Standring S, editor. Gray's Anatomy: The Anatomical Basis of Clinical Practice. 42nd ed. Elsevier; 2021)

- Blood Supply

  - ❑ The midgut derivatives, including the proximal two-thirds of the transverse colon, are supplied by the Superior Mesenteric Artery

  - ❑ The hindgut derivatives, including the distal two-thirds of the transverse colon, descending colon, sigmoid colon, and rectum, are supplied by the Inferior Mesenteric Artery

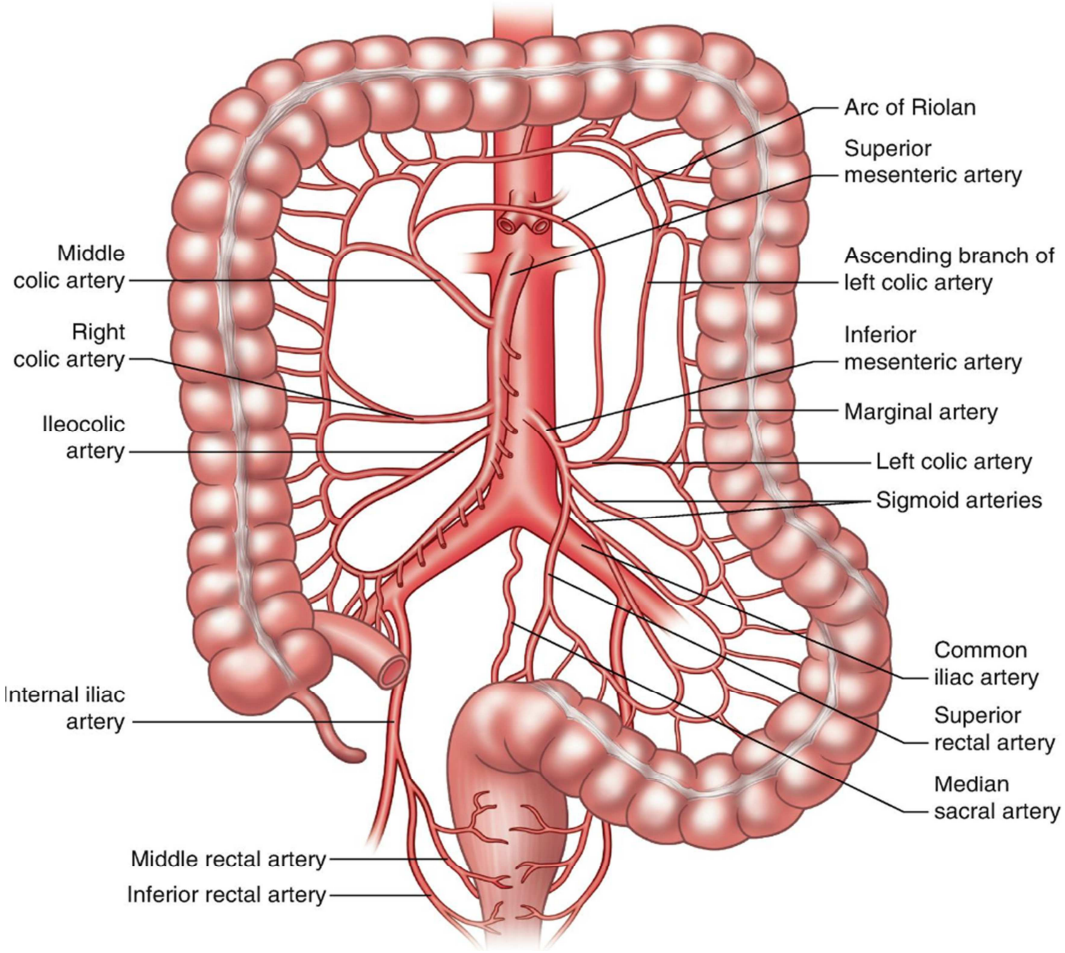

Figure 3: Blood Supply of Colon and Rectum [18]

(Image taken from Standring S, editor. Gray's Anatomy: The Anatomical Basis of Clinical Practice. 42nd ed. Elsevier; 2021)

Innervation

- **Parasympathetic Innervation** is by the pelvic splanchnic nerves (S2-S4) for the hindgut derivatives.
- **Sympathetic Innervation** is by the lumbar splanchnic nerves (L1-L2).

❖ **Colon and Rectum: Histology**

The colon and rectum have a typical four-layered histological structure, similar to other parts of the gastrointestinal tract:

1. Mucosa

- **Epithelium**:
    - Lined by simple columnar epithelium having many goblet cells for mucus secretion.
    - Crypts of Lieberkühn (intestinal glands) are present, which contain:
        - **Enterocytes (absorptive cells)**: Involved in water and electrolyte absorption.
        - **Goblet cells**: Increase in number towards the rectum to provide lubrication.
        - **Enteroendocrine cells**: Secrete regulatory hormones (e.g., serotonin, somatostatin).
        - **Regenerative stem cells** are localised at base of the crypts for epithelial renewal.[19]
- **Lamina Propria**:
    - Contains loose connective tissue with immune cells (plasma cells, macrophages, lymphocytes).
    - Well-developed GALT (Gut-Associated Lymphoid Tissue) is present.[19]

- **Muscularis Mucosae**:
    - It is a thin layer of smooth muscle, separating the mucosa from the submucosa.[19]

2. Submucosa

- Made of dense irregular connective tissue.
- Contains blood vessels, lymphatics, and Meissner's plexus (submucosal nerve plexus for secretion regulation).[19,20]

3. Muscularis Externa

- Composed of smooth muscle arranged in two layers:
    - **Inner circular layer**: Controls luminal diameter.
    - **Outer longitudinal layer**: Arranged into three thickened bands called taeniae coli (except in the rectum, where it forms a continuous layer).
- Contains Auerbach's (myenteric) plexus, which regulates motility.[21,23]

4. Serosa/Adventitia

- Serosa (in intraperitoneal parts): A thin layer of mesothelium and connective tissue.
- Adventitia (in extraperitoneal parts like the rectum): Composed of fibrous connective tissue, anchoring it to surrounding structures.[23,24]

Differences Between the Colon and Rectum (Table 1)

Feature	Colon	Rectum
Goblet cells	Abundant	Most abundant
Taeniae coli	Present	Absent (continuous muscle layer)
Lymphoid tissue	Present (GALT)	More prominent
Serosa/Adventitia	Serosa in free parts	Adventitia in lower part

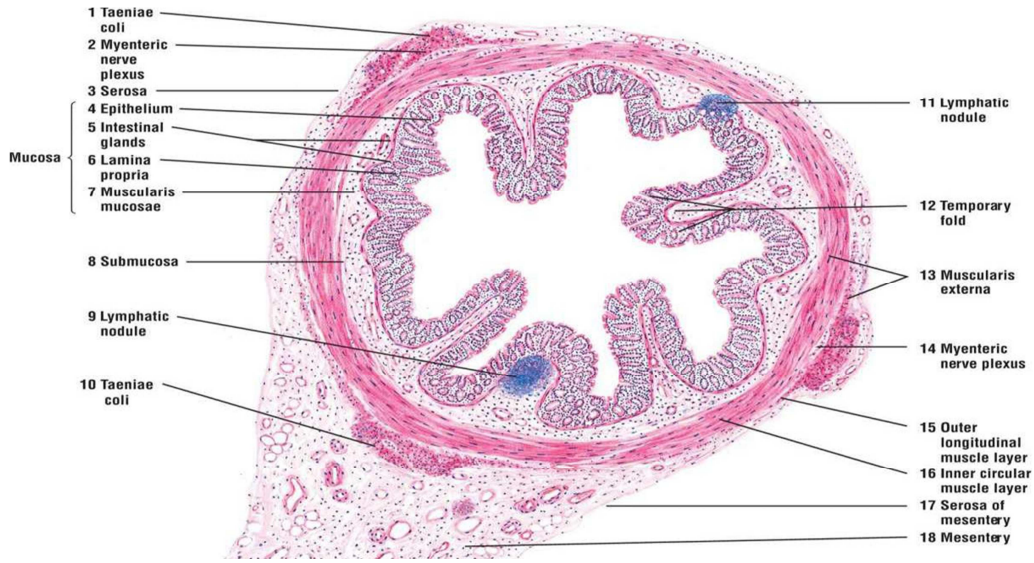

Figure 4: Large intestine: Transverse section of the colon with mesentery (H and E stain, Low magnification view).[24]

(Image taken from Eroschenko VP., DiFiore's atlas of histology with functional correlations / Victor P. Eroschenko. 13th edition, 2017)

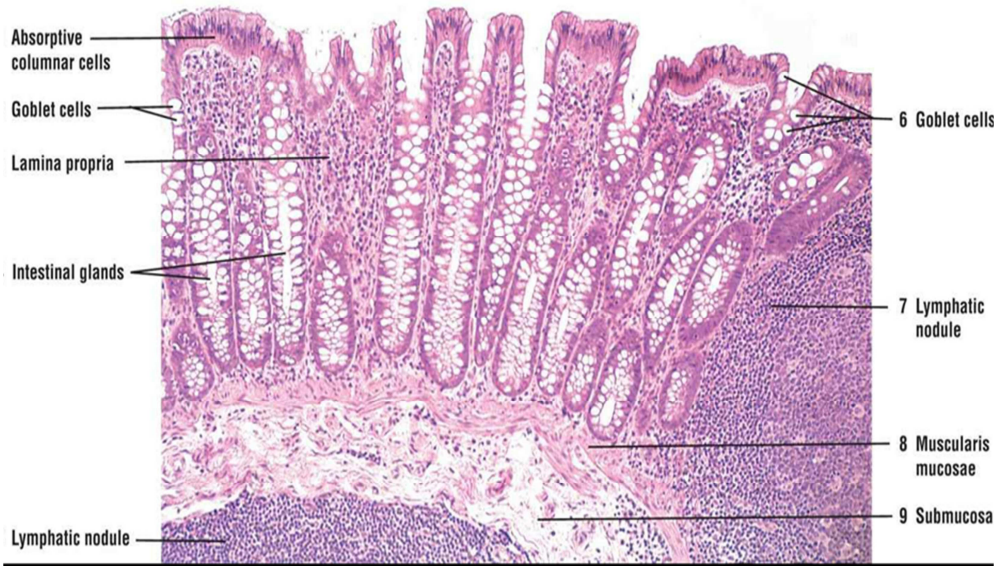

Figure 5: Large intestine: Wall of the colon. (H and E stain,40x).[24]

(Image taken from Eroschenko VP., DiFiore's atlas of histology with functional correlations / Victor P. Eroschenko. 13th edition, 2017)

- Physiology of the Colon:

- Its primary function is absorption of water and electrolytes from the undigested food, and to form feces (solid waste) for elimination.[25]

- Peristalsis, rhythmic contractions of the colon's muscular wall, propels fecal matter toward the rectum.

- Bacterial fermentation of the undigested carbohydrates and fiber in colon produces short-chain fatty acids, which provide energy for the colonic mucosal cells and help maintain gut health.[26,27]

- Epidemiology of Colorectal Neoplasia

Colorectal neoplasia, which includes both benign adenomas and malignant CRC, remains a crucial global health concern. It is the 3rd most frequently diagnosed cancer worldwide and 2nd leading cause of cancer-related mortality. As per WHO estimates, colorectal carcinoma accounted for approximately 1,931,590 new cases (10% of all cancer diagnoses) and 935,173 deaths (9.4% of all cancer-related fatalities) globally.[28] Incidence rates vary geographically, with higher prevalence in developed countries, although emerging trends show rising rates in developing regions due to lifestyle changes and aging populations.[29]

- **Risk Factors for Colorectal Neoplasia:**

In addition to well-established risk factors, emerging research highlights potential links between colorectal neoplasia and factors such as microbiota composition, chronic inflammation, and environmental exposures. An in depth understanding of these influences may give valuable information into disease mechanisms and novel preventive approaches.[30]

Colorectal neoplasia encompasses a range of lesions, from benign adenomatous polyps to malignant colorectal carcinomas, and is affected by genetic, lifestyle, and environmental. Identifying and understanding these risk factors can provide details for early detection and prevention. Below are some key factors associated with colorectal neoplasia:

- **Age:** Advancing age is a major risk factor, with majority of cases diagnosed in individuals over 50 years old. Screening is generally recommended starting at 50, or earlier for those with other risk factors.[31]

- **Family History:** A history of CRC or adenomatous polyps in family can augment susceptibility. Individuals with a 1st degree relative diagnosed with CRC are at increased risk and may require earlier or more frequent screenings.[32-34]

- **Personal Medical History:** Previous history of CRC, adenomatous polyps, or chronic inflammatory bowel diseases including ulcerative colitis and Crohn's disease increase the risk. Routine surveillance is typically advised for such individuals.[35,36]

- **Genetic Syndromes:** Certain inherited conditions like Lynch syndrome (hereditary nonpolyposis colorectal cancer) and familial adenomatous polyposis (FAP), substantially increase the risk of colorectal neoplasia.[34]

- **Diet and Lifestyle Factors:** Consuming excess amounts of red and processed meats, and diet with reduced amounts of fibre, fruits, and vegetables, is associated with a risk of CRC. Other contributing factors are obesity, lack of physical activity, alcohol intake, and smoking.[32]

- **Inflammatory Bowel Disease (IBD):** Chronic inflammatory diseases such as ulcerative colitis significantly raises the likelihood of colorectal neoplasia. Individuals with IBD often require regular surveillance colonoscopies to detect early precancerous changes.[35]

- **Diabetes and Metabolic Syndrome:** Conditions like diabetes, obesity, hypertension, and dyslipidaemia are linked with an elevated risk of colorectal neoplasia. Proper management of these conditions may help lower the risk.[37]

- **Race and Ethnicity:** Research indicates that African American population

have higher occurrence of CRC and are often diagnosed at a younger age than other racial and ethnic groups. These disparities may be influenced by genetic, environmental, and healthcare access factors.[38]

- **Radiation Therapy:** Individuals who have undergone abdominal or pelvic radiation therapy, especially childhood cancer survivors, are at increased risk for colorectal neoplasia later in life. Close monitoring and early detection strategies may be necessary for this population.[39]

- **Environmental Exposures:** Occupational and environmental exposure to harmful substances such as industrial chemicals, heavy metals, and radiation may contribute to the risk of developing colorectal neoplasia. Regulatory measures aim to mitigate these risks.[40]

Overall, colorectal neoplasia causation is multifactorial and depends on genetic predisposition, environmental factors, and living habits. Increasing awareness, adhering to screening recommendations, and adopting healthier lifestyle habits play a crucial role in prevention and early detection.[41]

Clinical Manifestations:

- **Symptoms:** Colorectal neoplasia may present with a variety of symptoms, including changes in bowel habits (e.g., constipation, diarrhea), rectal bleeding, abdominal pain or discomfort, unintended weight loss, fatigue, and symptoms of bowel obstruction (e.g., bloating, nausea, vomiting).[42]

- **Asymptomatic Presentation:** Early-stage colorectal neoplasia may be asymptomatic, particularly in the absence of obstructive lesions. As a result, screening programs aimed at detecting neoplastic changes in asymptomatic

individuals have become an essential component of colorectal cancer prevention and early detection efforts.[43,44]

Pathogenesis:

- Genetic and Epigenetic Alterations: Colorectal neoplasia arises due to the accumulation of genetic and epigenetic modifications that disrupt normal cellular processes and contribute to tumor development. These changes may involve mutations in critical oncogenes and tumor suppressor genes, chromosomal instability, DNA methylation alterations, and variations in microRNA expression.

- Mutation in adenomatous polyposis coli (APC) Gene: Earliest genetic changes in colorectal neoplasia involves mutations in APC gene. The loss of function leads to abnormal Wnt signalling, uncontrolled cellular proliferation, and forming adenomatous polyps.

- Mutations in KRAS and TP53: Mutations in KRAS oncogene and TP53 tumor suppressor gene are frequently found in colorectal cancer and are linked to cancer progression and metastasis. KRAS mutations result in persistent activation of MAPK signalling pathway, promoting cellular growth and survival, whereas mutations in TP53 impair cell cycle control and apoptosis.

- Microsatellite Instability (MSI): MSI occurs due to defects in DNA mismatch repair, causing accumulation of mutations in microsatellite sequences in genome. This phenomenon is particularly common in colorectal cancers associated with Lynch syndrome and is linked to an improved prognosis and response to immunotherapy.

- CpG Island Methylator Phenotype (CIMP): CIMP is characterised by extensive hypermethylation of CpG islands within the promoter sequence of the tumor suppressor genes, resulting in their transcriptional inactivation. Colorectal cancers exhibiting CIMP are associated with distinct clinical features, such as a higher prevalence in the proximal colon, a greater occurrence in females, and an increased likelihood of BRAF mutations.[43,44]

❑ Histopathological Features:

➢ **Adenoma-Carcinoma Sequence**: Adenomatous polyps progress to invasive colorectal cancer by a well-defined sequence of histopathological changes, including increasing dysplasia, loss of normal glandular architecture, and invasive growth into the submucosa and beyond.[45]

➢ **Histological Subtypes:** Colorectal adenocarcinomas can be classified into various histological subtypes based on their architectural and cytological features. Common subtypes include mucinous adenocarcinoma, tubular adenocarcinoma, medullary carcinoma and signet ring cell carcinoma with each having distinct histological characteristics and clinical implications.[46,47]

❑ WHO Classification of Colorectal Neoplasia [48]

Colorectal epithelial tumors are classified in different categories by the WHO (2019) (Annexure III)

- Histological Grading Systems for Colorectal neoplasia:

a. Adenoma Grading: Adenomas, which are precursor lesions to colorectal cancer, are often graded based on their degree of dysplasia, which reflects the abnormality of the cells within the lesion. The grading typically includes:

i. Low-Grade Dysplasia (LGD): Cells show mild atypia and retain some resemblance to normal colonic epithelium.

ii. High-Grade Dysplasia (HGD): Cells showing loss of normal architecture and increased nuclear atypia. HGD adenomas are considered to have a higher risk of progressing to invasive carcinoma.

b. Carcinoma Grading:

Adenocarcinomas are typically classified into three grades based on the extent of tubule (acinar) formation and cellular arrangement. The grade is determined by the percentage of tumors showing well-formed glands.

1) Grade 1 or well-differentiated adenocarcinoma shows glands in > 95% of the tumor.
2) Grade 2 or moderately differentiated adenocarcinoma shows 50% to 95% of glands in the tumor.
3) Grade 3 or poorly differentiated adenocarcinoma shows 5% to 49% of glands in the tumor.
4) Grade 4 or undifferentiated carcinoma shows less than 5% of glands in the tumor.

Signet ring carcinoma and mucinous adenocarcinoma are considered grade 3 carcinomas.[49]

- Staging System:

AJCC TNM Staging for Colorectal Cancer (Annexure IV)[50]

The AJCC staging system provides clinicians with a standardized method for describing the extent of colorectal cancer, which helps guide treatment decisions, predict prognosis, and facilitate communication among healthcare providers. It is regularly updated to incorporate new knowledge and advances in cancer research.[51-54]

- Expressive Enzymes in Colorectal Neoplasia: [55-59]

- **AMACR:** Overexpression of AMACR, observed in metabolism of branching fatty acid, is observed in colorectal adenomas, carcinomas, and pre-malignant lesions, suggesting its involvement in early tumorigenesis. Elevated AMACR levels correlate with tumor grade, stage, and prognosis, indicating its potential as a biomarker for disease detection and risk stratification.

- **Cyclooxygenase 2 (COX-2):** It is involved in prostaglandin synthesis and is associated with inflammation, cell proliferation, and angiogenesis in colorectal cancer. Its aberrant expression contributes to tumor growth and progression.

- **Matrix metalloproteinases (MMPs):** MMPs facilitate tumor invasion and metastasis by remodeling the extracellular matrix. Dysregulated expression of MMPs in colorectal neoplasia promotes tumor aggressiveness and metastatic potential.

- **Enzymes involved in drug metabolism:** Differences in the expression and function of enzymes responsible for drug metabolism and detoxification, such as cytochrome P-450 enzymes and glutathione S-transferases, influence

the efficacy and toxicity of chemotherapy agents used in colorectal cancer treatment. Inter-individual differences in enzyme expression levels can affect drug metabolism, bioactivation, and elimination, impacting treatment response and adverse effects.

Prognostic Factors:

- **Tumor Stage:** The stage of a tumor is the most crucial factor in prognosis of CRC, and is based on depth of tumor invasion, lymph node involvement, and distant metastases. Tumors at an advanced stage are linked to poorer prognosis and necessitate more intensive treatment strategies.[60-62]

- **Histological Grade**: The histological grade, which indicates the level of tumor differentiation, plays a key role in determining prognosis. Tumors with poor differentiation have a greater likelihood of recurrence and metastasis when compared to well-differentiated tumors.[63-65]

- **Molecular Biomarkers:** Prognostic insights in colorectal cancer can be obtained through molecular biomarkers, including KRAS and BRAF mutation statuses, microsatellite instability, and the CpG island methylator phenotype. These biomarkers not only influence prognosis but also aid in selecting appropriate treatment approaches and predicting therapy responses.[66-68]

- **Tumor Budding:** Tumor budding is characterized by the presence of small isolated clusters of tumor cells at invasive margin of CRCs. It is linked to aggressive tumor progression, lymph node involvement, and an unfavorable prognosis, making it a key pathological indicator in colorectal cancer.[69]

❑ Role of AMACR in Colorectal Neoplasia: [70-73]

Previous studies have implicated AMACR in the pathogenesis of colorectal neoplasia. AMACR, involved in fatty acid metabolism, is dysregulated in colorectal adenomas and carcinomas.

- **Prognostic Implications:** Studies suggest that high AMACR expression levels in colorectal neoplasia correlate with adverse clinical outcomes such as disease recurrence, metastasis formation, and decreased overall survival. However, conflicting evidence exists regarding its prognostic value.[70]
- **Molecular Mechanisms of Dysregulation:** Various factors like genetic alterations, epigenetic modifications, and dysregulated signaling pathways contribute to AMACR dysregulation in colorectal neoplasia, highlighting the need to investigate its molecular mechanisms further.[70]
- **Expression in Precancerous Lesions:** Studying AMACR expression dynamics in precancerous lesions of the colorectum offers valuable insights into early neoplastic transformation, helping in early detection and intervention strategies.[70,71]
- **Metastasis and Disease Progression:** Elevated AMACR expression levels have been correlated with tumor aggressiveness, Lymph node and Distant organ metastases, and advanced tumor stage, indicating its prognostic significance in predicting metastatic potential in colorectal neoplasia.[72]
- **Interplay with Tumor Microenvironment:** AMACR expression in tumor cells may interact with the tumor microenvironment, influencing tumor behavior and treatment response, thus offering potential therapeutic targets for combination therapies.[73]

- Challenges:

Despite advancements in screening and treatment modalities, challenges persist in the management of colorectal neoplasia.

- **Early Detection:** One of the primary challenges in managing colorectal neoplasia is early detection. Many cases of colorectal cancer develop slowly over several years, often without causing noticeable symptoms in the early stages. As a result, delayed diagnosis limits treatment options and worsens prognosis, necessitating effective screening and need for early detection.[74]

- **Screening Compliance**: Despite the availability of various screening modalities for colorectal cancer, such as fecal occult blood tests, colonoscopy, sigmoidoscopy, and stool DNA tests, screening compliance remains suboptimal in many populations. Barriers to screening include lack of awareness, concerns about discomfort or embarrassment, financial constraints, and logistical challenges. Overcoming these barriers and improving screening uptake among eligible individuals are critical for early detection and prevention of colorectal cancer.[75,76]

- **Health Disparities**: Colorectal cancer disproportionately affects underserved populations due to disparities in screening, diagnosis, and care. Targeted interventions and equitable healthcare access are crucial to improving outcomes.[77-79]

- **Risk Factor Modification:** Obesity, inactivity, smoking, and poor diet are modifiable risk factors for colorectal neoplasia.[80-82]

However, promoting behavior change and encouraging individuals to adopt healthier lifestyles can be challenging. Overcoming barriers related to patient motivation, cultural norms, socioeconomic factors, and access to resources requires multifaceted approaches, including public health campaigns, education, policy changes, and community-based interventions.

- **Genetic and Molecular Heterogeneity:** Colorectal neoplasia encompasses a spectrum of lesions with diverse genetic and molecular characteristics, ranging from benign adenomatous polyps to invasive carcinomas. Understanding the complex molecular pathways involved in colorectal tumorigenesis and identifying biomarkers for early detection, prognosis, and targeted therapy present significant challenges. Advances in genomic research, molecular profiling technologies, and personalized medicine hold promise for addressing these challenges and improving patient outcomes through precision oncology approaches.[78]

- **Surveillance and Follow-up:** Patients having history of colorectal neoplasia, require regular surveillance colonoscopies to detect recurrent or new lesions and to monitor for disease progression. However, ensuring adherence to surveillance recommendations and optimizing the timing and frequency of follow-up examinations can be challenging. Barriers to surveillance include patient factors (e.g., anxiety, fear of the procedure), healthcare system factors (e.g., availability of resources, scheduling issues), and provider-related factors (e.g., communication, patient counseling). Efforts to enhance surveillance protocols, patient education, and provider-patient communication are essential to optimize long-term outcomes for colorectalneoplasia patients.[81]

The study addresses the need for standardized methodologies for AMACR assessment, clarification of its biological role in colorectal carcinogenesis, and validation of its clinical utility. This research seeks to clarify AMACR's role in colorectal neoplasia and pave the way for future clinical applications.

MATERIALS AND METHODS

A total of 51 specimens of colorectal neoplasms cases from patients diagnosed clinically and histopathologically at KLE's Dr. Prabhakar Kore Hospital and Medical Research Centre, Belagavi during April 2023 to March 2024 were included in this study.

Study design: One-year cross sectional study

Study period: April 2023 to March 2024

Study population: Patients clinically and histologically diagnosed with colorectal neoplasms at KLE's Dr. Prabhakar Kore Hospital and Medical Research Centre, Belagavi during the study period.

Inclusion criteria: Histologically proven cases of colorectal neoplasms, biopsies and resection specimens

Exclusion criteria: All poorly preserved specimens

Sample size: Fifty-one cases in total which fulfilled the inclusion criteria were taken in the study (universal sampling).

Ethical clearance: The present study was approved by Jawaharlal Nehru medical college's Institutional Ethics committee on Human Subjects research (Reference number: MDC/JNMCIEC/137).

Data collection: The colorectal neoplasm cases that were operated in KLE's Dr. Prabhakar Kore Hospital and Medical Research Centre, Belagavi were taken for this study. These specimens were grossed according to the standard procedure, and bits were given. Slides were stained with H&E, and were screened. Suitable blocks

were selected for AMACR IHC staining with clone 13H4 (Vitro) antibody. Information regarding clinical data were obtained from Medical Records Department (MRD) records.

Histopathology Evaluation: All slides were evaluated and histological grading was done.

IHC Staining Procedure: Thin tissue sections of 3-4 microns were cut with microtome and then placed on coated slides.

Steps for IHC staining for AMACR is as follows:

1. The slides were baked at 60 degree centigrade for 1 hour prior to start deparaffinisation.
2. Then deparaffinised in fresh xylene for two changes 10minutes each.
3. Rinsed in absolute alcohol for two changes 10minutes each.
4. Then were rinsed in water for-5minutes, followed by distilled water for-1minute.
5. They were subjected to antigen retrieval using pressure cooker method using TRIS EDTA buffer solution.
6. After that they were allowed to cool at room temperature for 15minutes.
7. Rinsed with wash buffer 2times with gap of 30seconds each.
8. Placed in 0.3% Hydrogen peroxide-8 to 10 minutes to inhibit endogenous peroxidase activity.
9. Then washed with wash buffer 3times with gap of 30seconds each.
10. Primary antibody incubated for 45-60 minutes at room temperature in a closed room
11. Then they were washed with wash buffer 3times with gap of 30seconds each

12. HRP polymer was applied and incubated for 25-30 minutes at room temperature in a closed chamber
13. Washed with wash buffer 3times with gap of 30seconds each
14. DAB substrate was applied to sections for 10minutes
15. Washed in water-2minute, followed by wash with distilled water-1minute
16. Counter stained with Haematoxylin-3minutes
17. Washed in running water for 2 minutes.
18. Blotted and cleared in xylene & mounted with DPX.

Antibody in the study:

Antibody: AMACR
Localisation: Cytoplasmic
Clonality: Monoclonal Rabbit Anti Human AMACR (Clone 13H4)
Dilution: Ready to use
Manufacturer: Vitro

Negative control (without adding primary antibody) was done in all batches.

Evaluation of immunoreactivity[84]:

- A semi quantitative system based on intensity of reaction product and percentage of cells showing cytoplasmic positivity were used.
- Staining intensity was scored as
    - Negative (0),
    - Weak (1+),
    - Moderate (2+),
    - Strong (3+).

- The extent of staining was represented as percentages of positive staining areas in relation to the whole carcinoma area.
- It was scored as
    - 0-5% (Score 0),
    - 6-20% (Score 1),
    - 21-40% (Score 2),
    - 41-60% (Score 3),
    - 61–80% (Score 4),
    - 81-100% (Score 5)
- The product of intensity and extent of staining scores were used as final staining scores (0-15).
- For the purpose of statistical analysis, tumors having a final staining score of ≥1 were considered to be positive.
- The final scores of AMACR expression were graded as
    - Negative (Score 0),
    - Poor (Score 1-5),
    - Moderate (Score 6-10),
    - Strong (Score 11-15)

Statistical analysis was done using SPSS software 26.0. To study association between analysed AMACR expression and clinicopathological parameters, chi square was used.

RESULTS

In the current study, 51 cases of histologically proven colorectal neoplasms were taken which included 16 resection specimens and 35 biopsies that were received in the Department of Pathology, KLE's Dr. Prabhakar Kore Hospital and Medical Research Centre, Belagavi during the study period.

The mean age of cases in the study was 49.45 ± 7.18 years.

Table 2: Gender based Distribution of study participants. (n=51)

Gender	Frequency	Percentage
Female	20	39.2
Male	31	60.8
Total	**51**	**100.0**

Out of the 51 study participants, a major proportion was males (60.8%) as compared to females (39.2%).

Graph 1: Gender wise Distribution of study participants. (n=51)

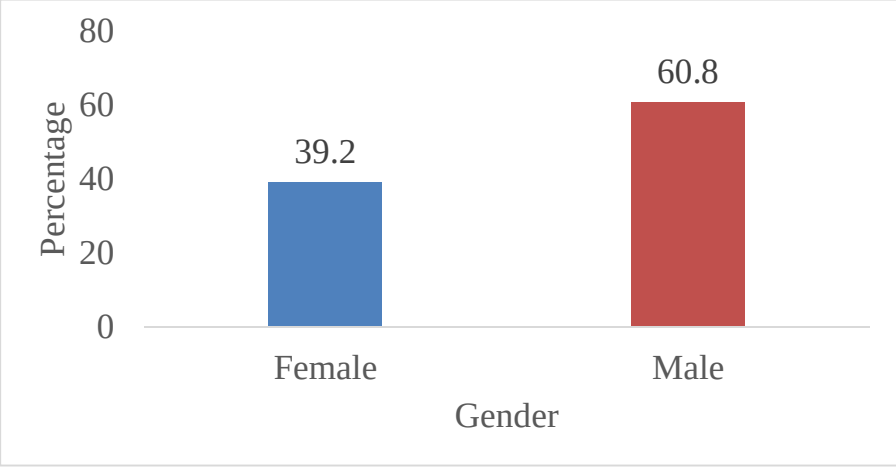

Table 3: Distribution of Tumor Site in study participants. (n=51)

Site	Frequency	Percentage (%)
Rectum (including biopsies & resections)	26	51
Sigmoid (including biopsies & resections)	10	19.6
Ascending Colon (including biopsies & hemicolectomy)	4	7.9
Caecum	3	5.9
Descending Colon (including biopsies)	3	5.9
Hepatic Flexure	3	5.9
Splenic Flexure	1	1.9
Transverse Colon (including biopsies)	1	1.9
Total	**51**	**100**

The most commonly affected site among study participants was the rectum (51%), followed by the sigmoid colon (19.6%). The ascending colon accounted for 7.9% of cases, while the caecum, descending colon, and hepatic flexure each contributed 5.9%. The splenic flexure and transverse colon were the least affected, with 1.9% of cases each. This distribution highlights the predominance of colorectal neoplasms in the rectum and sigmoid colon.

Graph 2: Distribution of Tumor Site in study participants. (n=51)

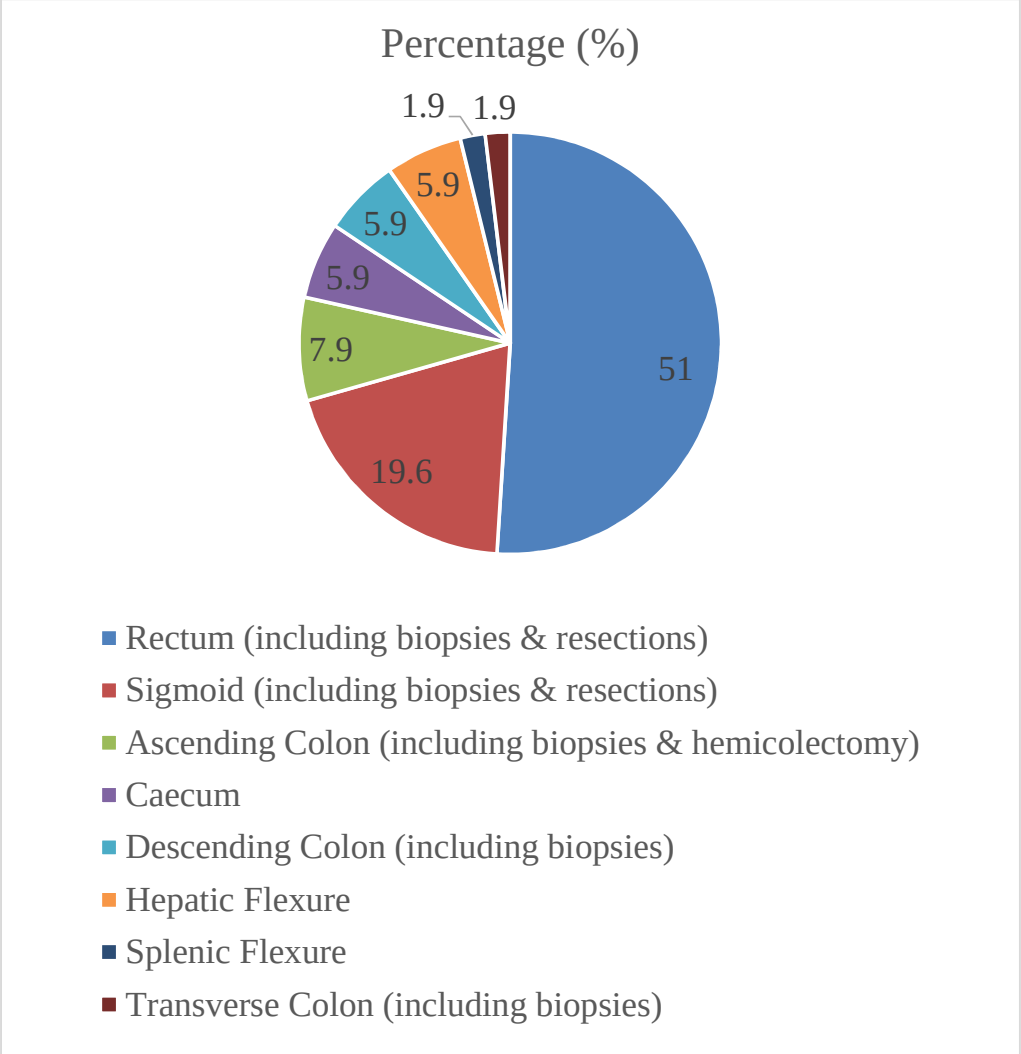

Table 4: Distribution of Histopathological Diagnosis of study participants. (n=51)

Histological Diagnosis	Frequency	Percentage (%)
Adenocarcinoma	37	72.5
Mucinous Carcinoma	2	3.9
Signet Ring cell carcinoma	1	2
Adenoma	11	21.6
Total	**51**	**100**

The distribution of the histological diagnosis of the 51 study participants showed that adenocarcinoma was the most common diagnosis, observed in 72.5% of cases. Adenoma accounted for 21.6%, while mucinous carcinoma (3.9%) and signet ring cell carcinoma (2%) were less frequent. This distribution underlines the predominance of adenocarcinoma as the primary histological diagnosis in colorectal neoplasms.

Graph 3: Distribution of Histopathogical Diagnosis of study participants. (n=51)

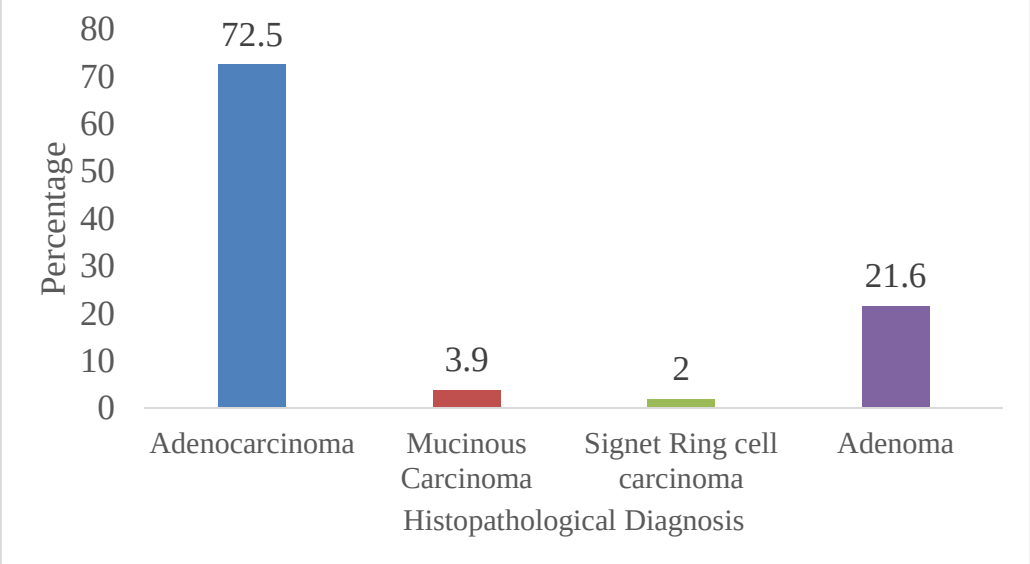

Table 5: Histopathological Grades in Colorectal Carcinoma Patients (n=40)

Diagnosis	Frequency	Percentage
Moderately differentiated carcinoma	31	77.5
Poorly differentiated carcinoma	9	22.5
Total	**40**	**100.0**

In this study, a total of 40 patients with colorectal carcinoma were classified based on histopathological grading. The majority of cases (77.5%) were categorized as moderately differentiated carcinoma.

On the other hand, poorly differentiated carcinoma was observed in 22.5% of patients.

The findings highlight that moderately differentiated carcinoma is the predominant histopathological grade in colorectal neoplasms.

Graph 4: Histopathological Grades in Colorectal Carcinoma Patients (n=40)

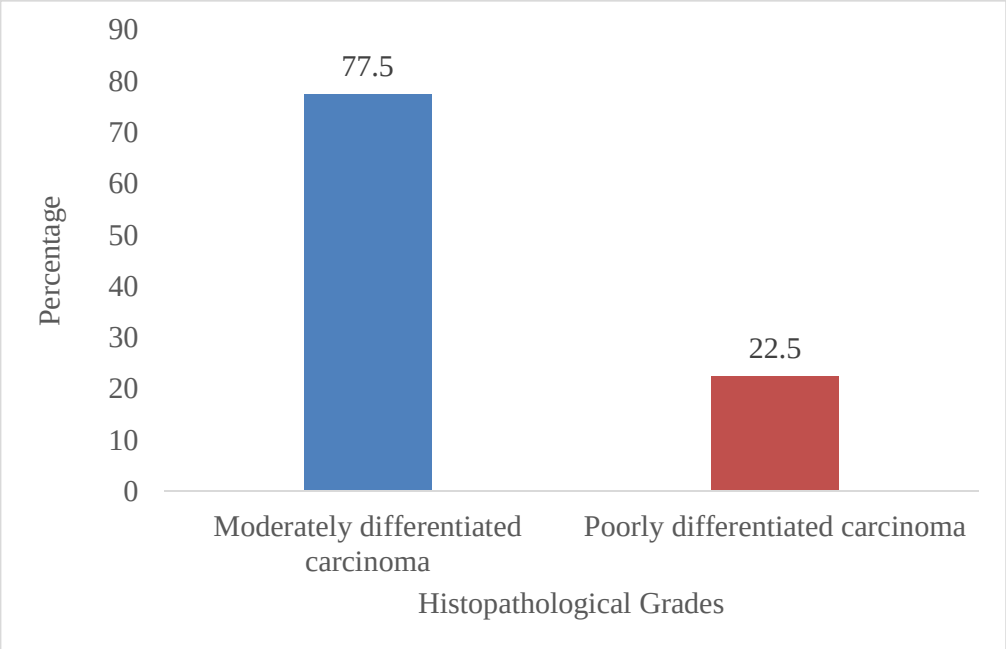

Table 6: Distribution of Tumor Stage in study participants. (n=16)

Tumor Stage	Frequency	Percentage (%)
T1	1	6.25
T2	2	12.5
T3	10	62.5
T4	3	18.75

Among the 16 cases of resection with tumor staging data, the majority were classified as T3 (62.5%). T4 stage tumors representing accounted for 18.75% of cases. T2 stage tumors were observed in 12.5%, while T1 stage tumors, limited were the least common at 6.25%.

Graph 5: Distribution of Tumor Stage in study participants. (n=16)

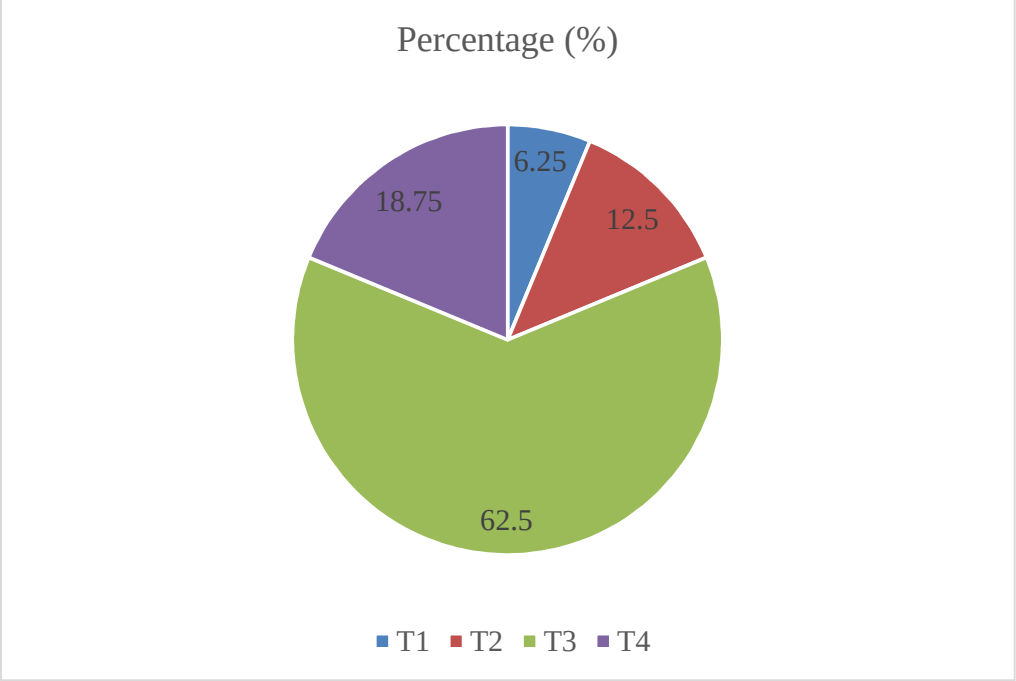

Table 7: Distribution of Nodal Status in study participants. (n=16)

Nodal Status	Frequency	Percentage (%)
N0	9	56.25
N1	4	25
N2	3	18.75

The nodal status could be retrieved for 16 cases, out of which the majority (56.25%) had N0 status. N1 status was observed in 25% of participants, while N2 status was present in 18.75% of cases.

Graph 6: Distribution of Nodal Status in study participants. (n=16)

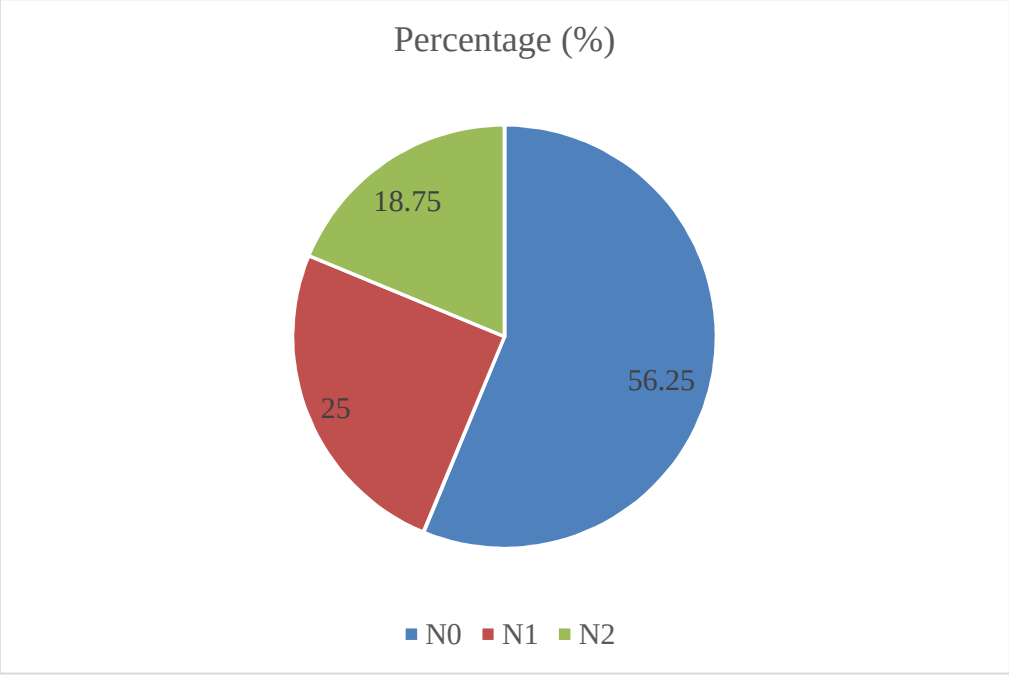

Table 8: AMACR Expression in Colorectal neoplasm Patients.

AMACR Expression	Frequency	Percentage (%)
Strong	2	3.9
Moderate	15	29.4
Poor	22	43.1
Negative	12	23.5
Total	**51**	**100**

In this study of 51 colorectal neoplasm patients, AMACR (Alpha-Methyl acyl-CoA Racemase) expression was analysed and classified.

Poor expression was observed in 43.1% of the cases, moderate expression was seen in 29.4% of cases and negative expression was found in 23.5% of patients.

Strong expression was the least common, seen in only 3.9% of cases.

Graph 7: AMACR Expression in Colorectal neoplasm Patients

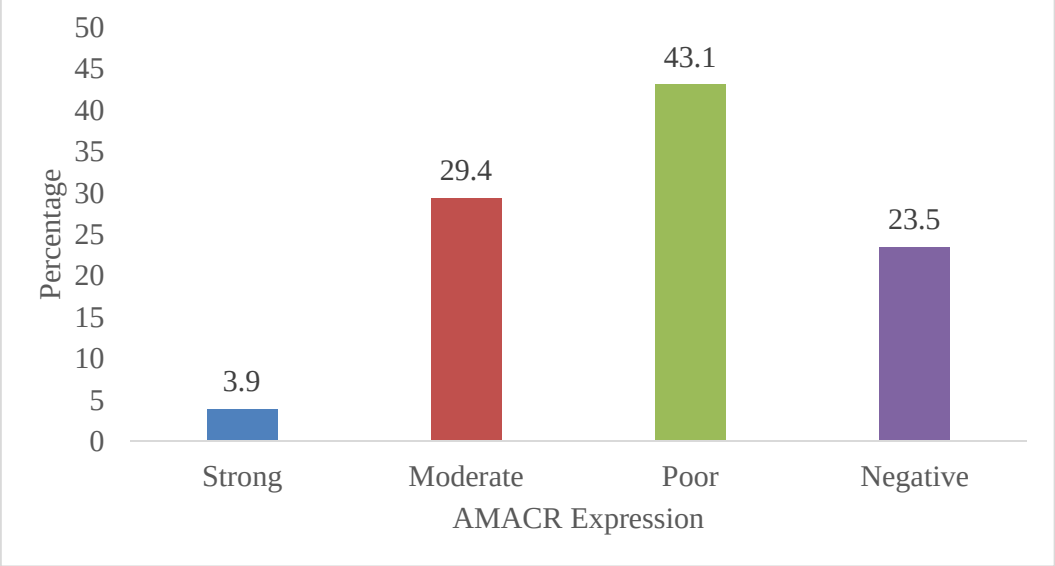

Table 9: Association of AMACR Expression with Histopathological Diagnosis in Colorectal Neoplasms (n=51)

	ADENOMA	CARCINOMA	Chi Square (p value)
Strong	**0**	**2**	**9.58 (0.022) ***
Moderate	**7**	**8**	
Poor	**4**	**18**	
Negative	**0**	**12**	
	11	**40**	

Statistical analysis of AMACR expression showed a significant difference between adenoma and carcinoma cases ($p<0.05$).

Table 10: Association of AMACR Expression with Histopathological Grade in Colorectal Carcinoma (n=40)

AMACR Expression	Histopathological Grade		Chi Square value (p-value)
	Moderately differentiated Carcinoma	Poorly differentiated carcinoma	
Strong	2	0	**8.45 (0.04)**
Moderate	8	0	
Poor	15	3	
Negative	6	6	

Significant association was found between Histopathological grade and AMACR expression in colorectal carcinoma as analysed using the Chi-square test ($\chi^2 = 8.45$, $p = 0.04$).

- Strong AMACR expression was observed only in moderately differentiated carcinoma (n = 2, 3.9%), with no cases in poorly differentiated carcinoma.
- Moderate AMACR expression was seen exclusively in moderately differentiated carcinoma (n = 8, 15.7%).
- Poor AMACR expression was the most frequent, identified in 15 cases (29.4%) of moderately differentiated carcinoma and 3 cases (5.9%) of poorly differentiated carcinoma.
- Negative AMACR expression was detected in 6 cases (11.8%) of moderately differentiated carcinoma and 6 cases (11.8%) of poorly differentiated carcinoma.

Graph 8: AMACR Expression with Histological Grade in Colorectal Carcinoma

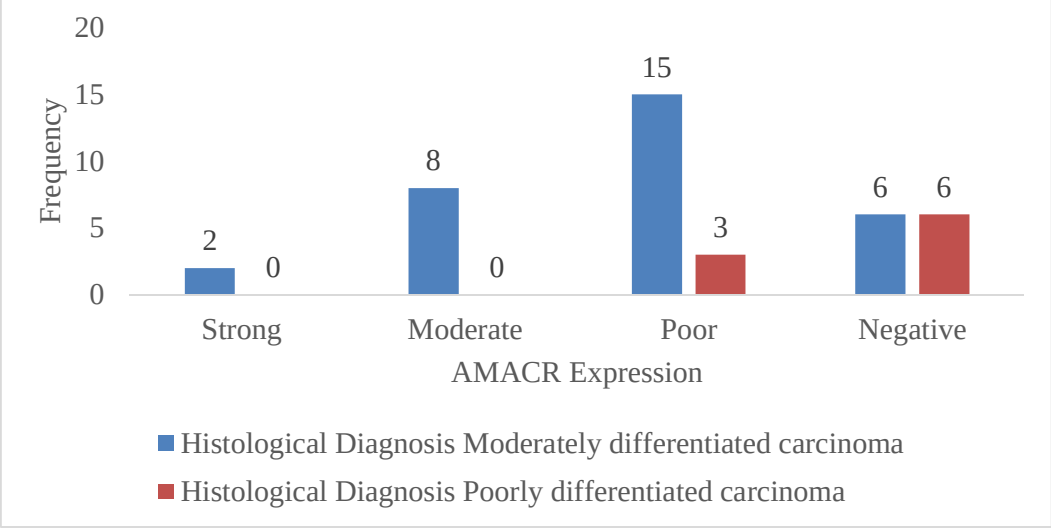

Table 11: Association of AMACR Expression with Tumor Stage in Colorectal Carcinoma

AMACR Expression	Tumor stage				Chi Square value (p-value)
	T1	T2	T3	T4	
Strong	0	1	0	0	**17 (0.0487) ***
Moderate	0	1	2	0	
Poor	0	0	7	1	
Negative	1	0	1	2	

Significant association was found between tumor stage and AMACR expression in colorectal carcinoma cases as analysed using the Chi-square test (χ^2=17, p=0.0487).

Graph 9: AMACR Expression with Tumor Stage in Colorectal Carcinoma

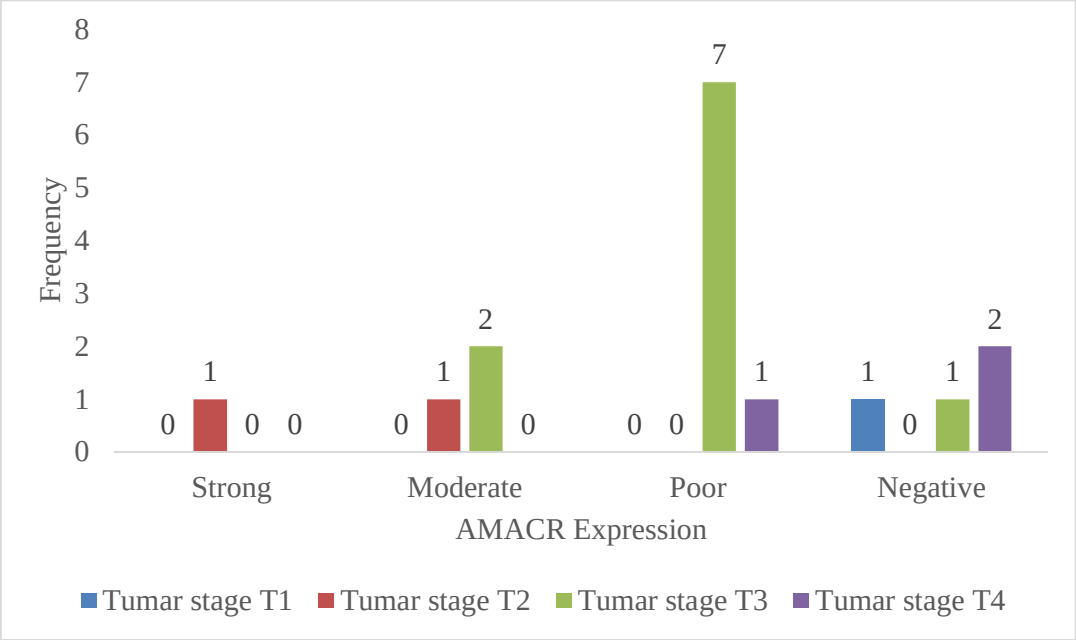

Table 12: Association of AMACR Expression with Nodal Status in Colorectal Carcinoma

AMACR Expression	Nodal status			Chi Square value (p-value)
	N0	N1	N2	
Strong	1	0	0	7.5 (0.2771)
Moderate	3	0	0	
Poor	2	3	3	
Negative	3	1	0	

There was no significant association found between AMACR expression and nodal status using the Chi-square test ($\chi^2 = 7.5$, $p = 0.2771$).

Graph 10: AMACR Expression and Nodal Status in Colorectal Carcinoma

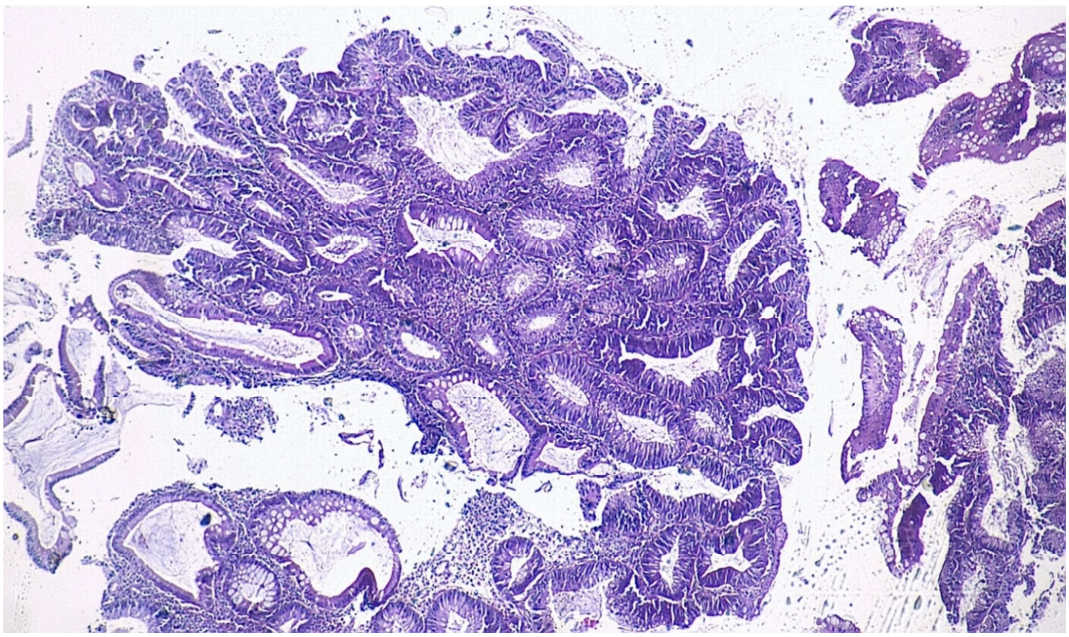

Figure 6: Tubular Adenoma (H&E 40x)

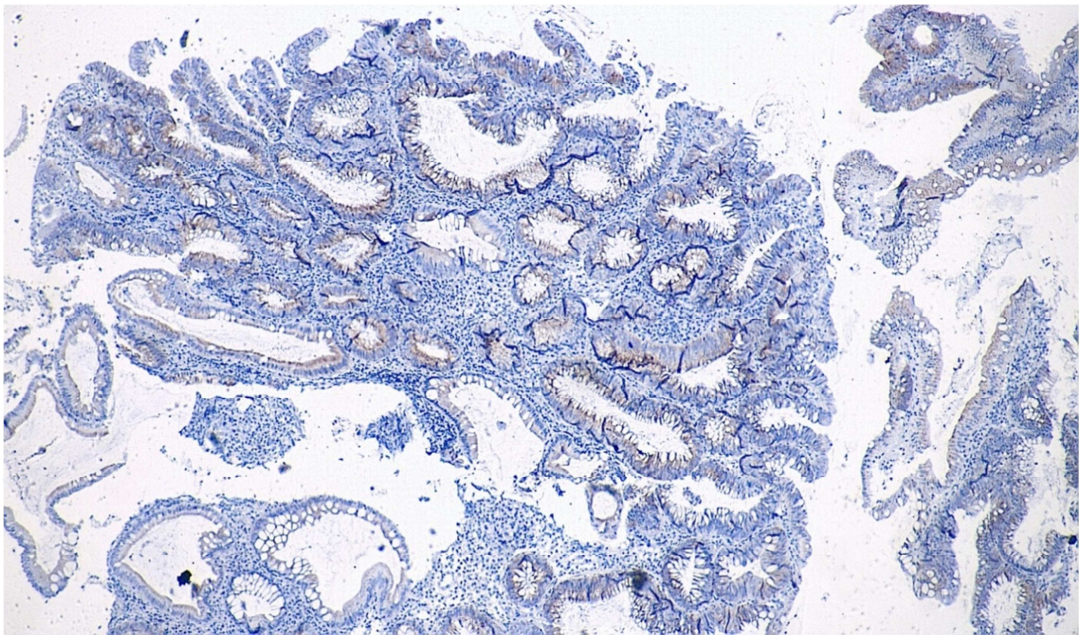

Figure 7: Tubular Adenoma (Moderate expression of AMACR IHC, 40x)

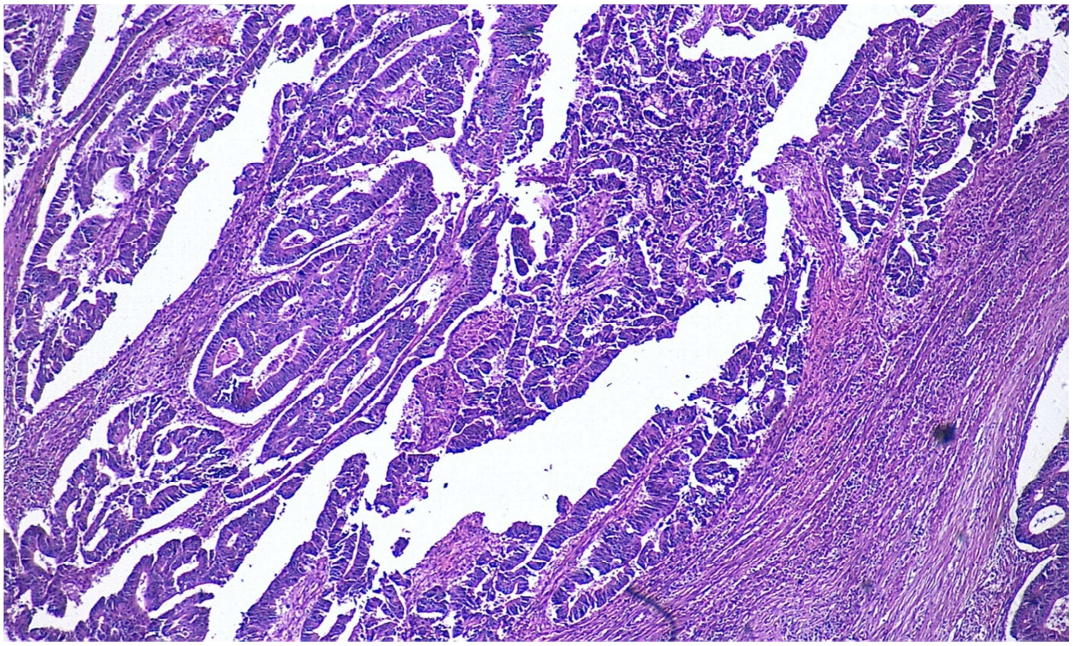

Figure 8: Moderately Differentiated Adenocarcinoma (H&E 100x)

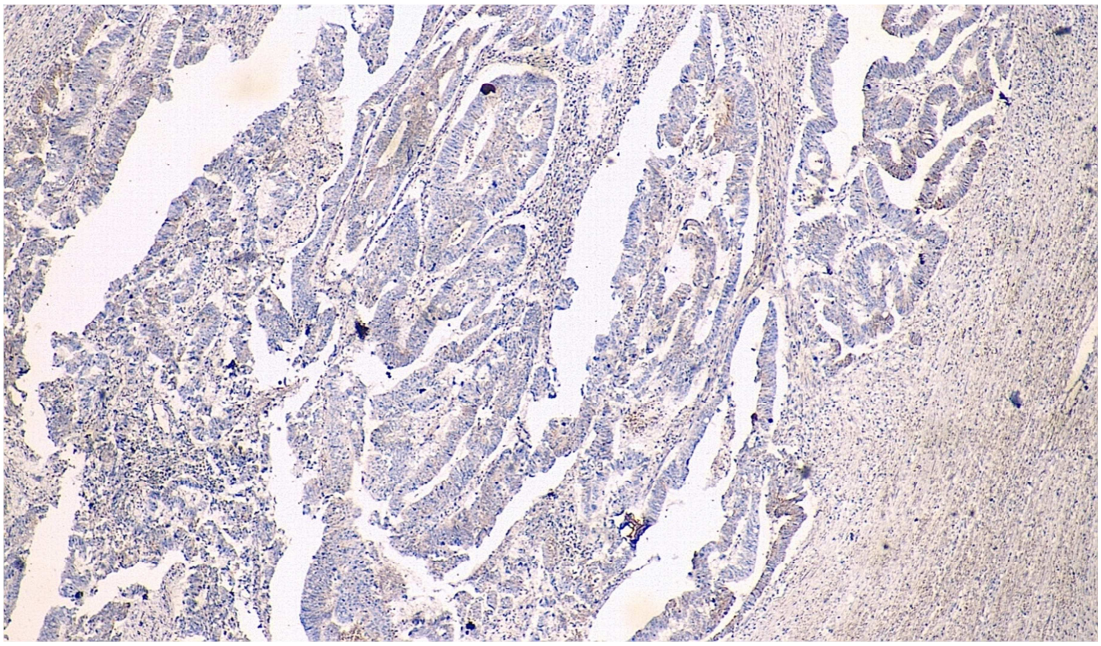

Figure 9: Moderately Differentiated Adenocarcinoma (Strong expression of AMACR IHC, 100x)

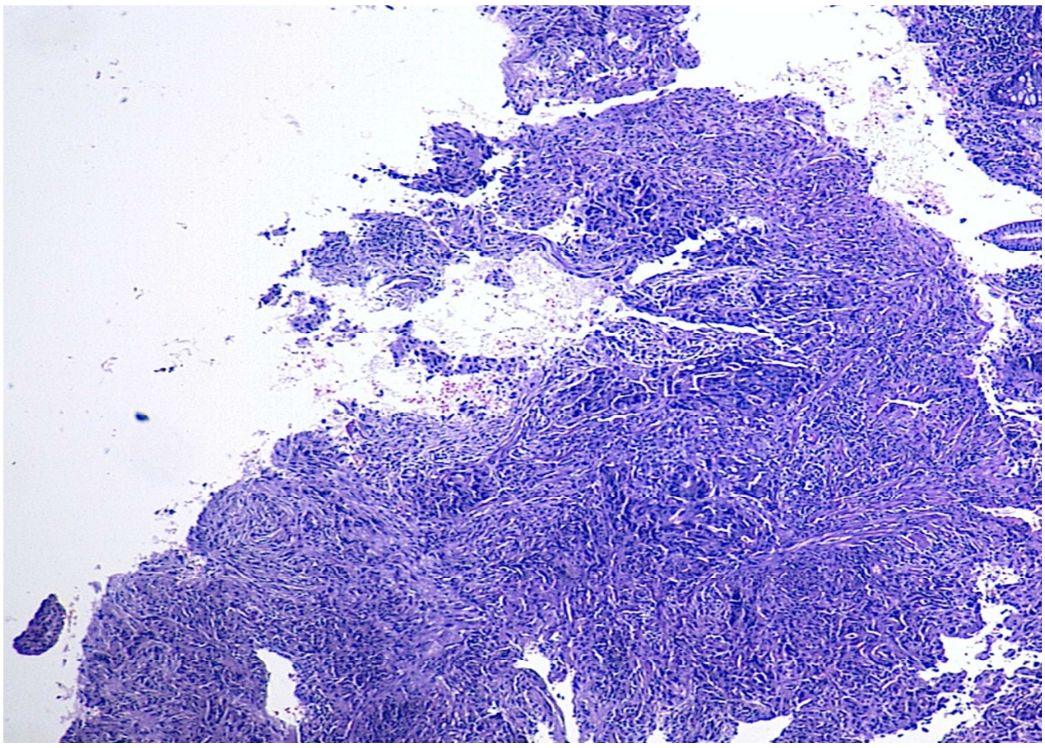

Figure 10: Poorly Differentiated Adenocarcinoma (H&E 40x)

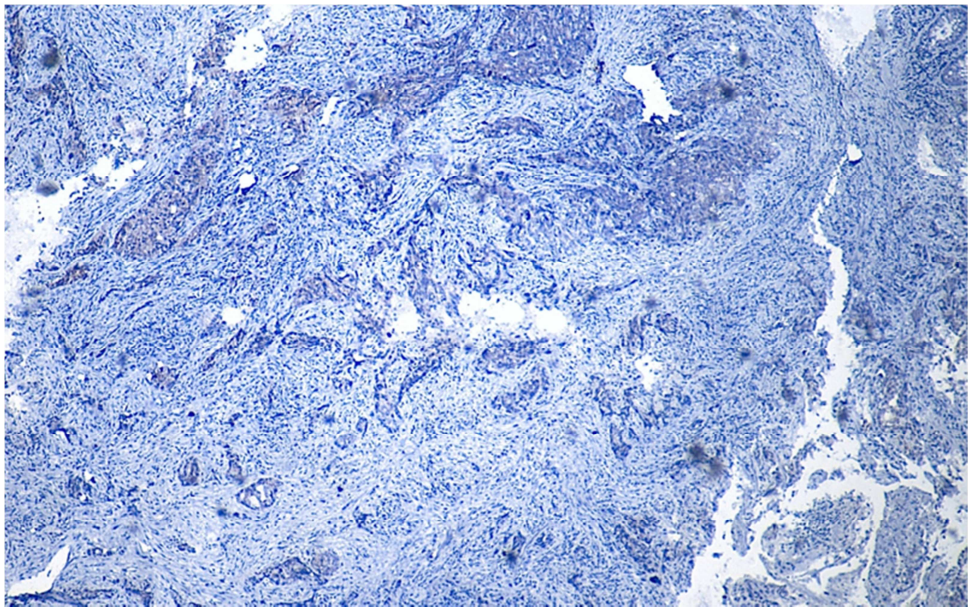

Figure 11: Poorly Differentiated Adenocarcinoma

(Poor expression of AMACR IHC, 40x)

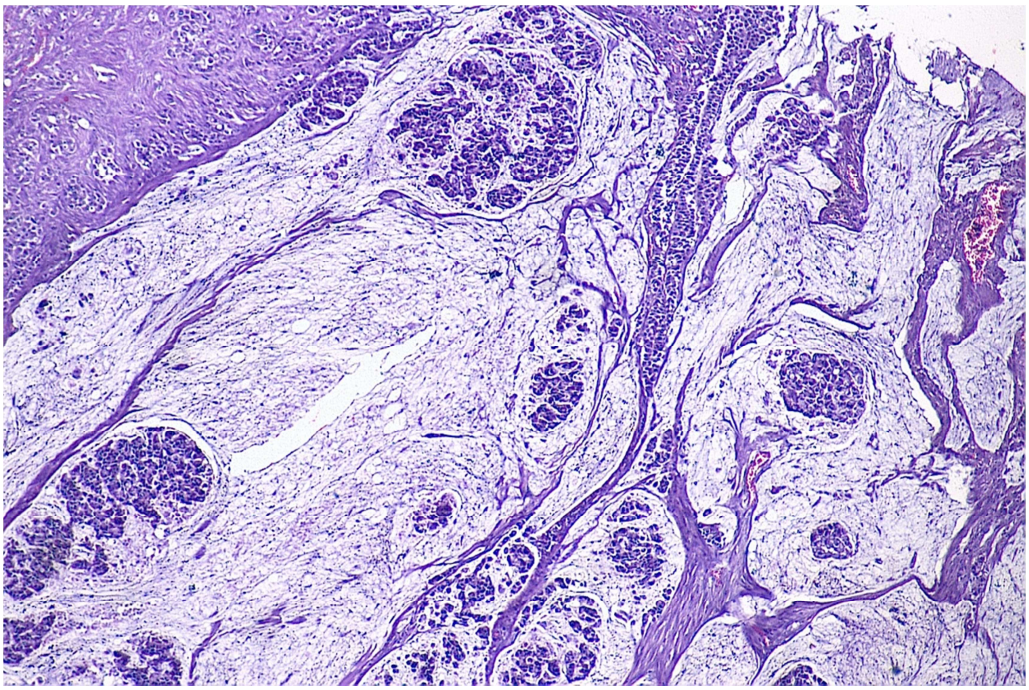

Figure 12: Mucinous Carcinoma (Negative expression of AMACR IHC, 100x)

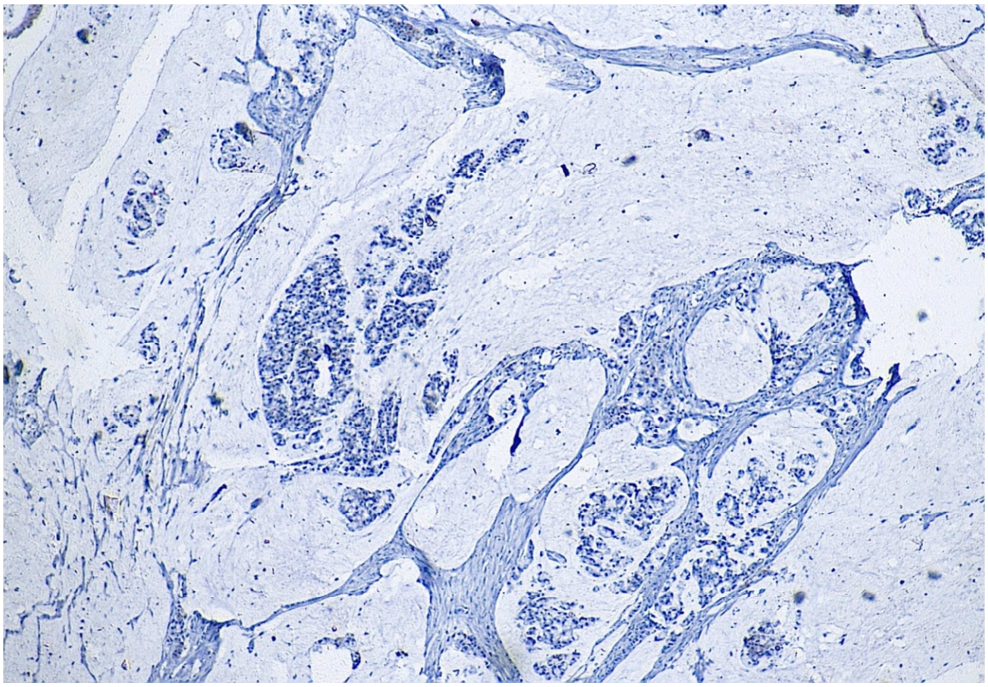

Figure 13: Mucinous Carcinoma (H&E 100x)

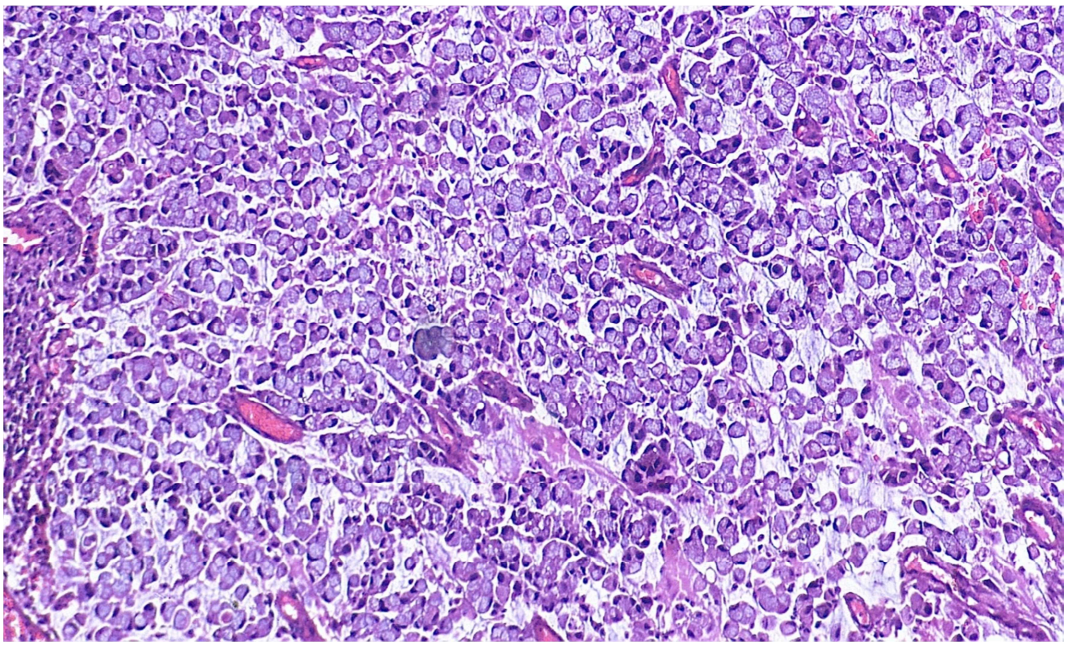

Figure 14: Signet Ring Cell Carcinoma (H&E 100x)

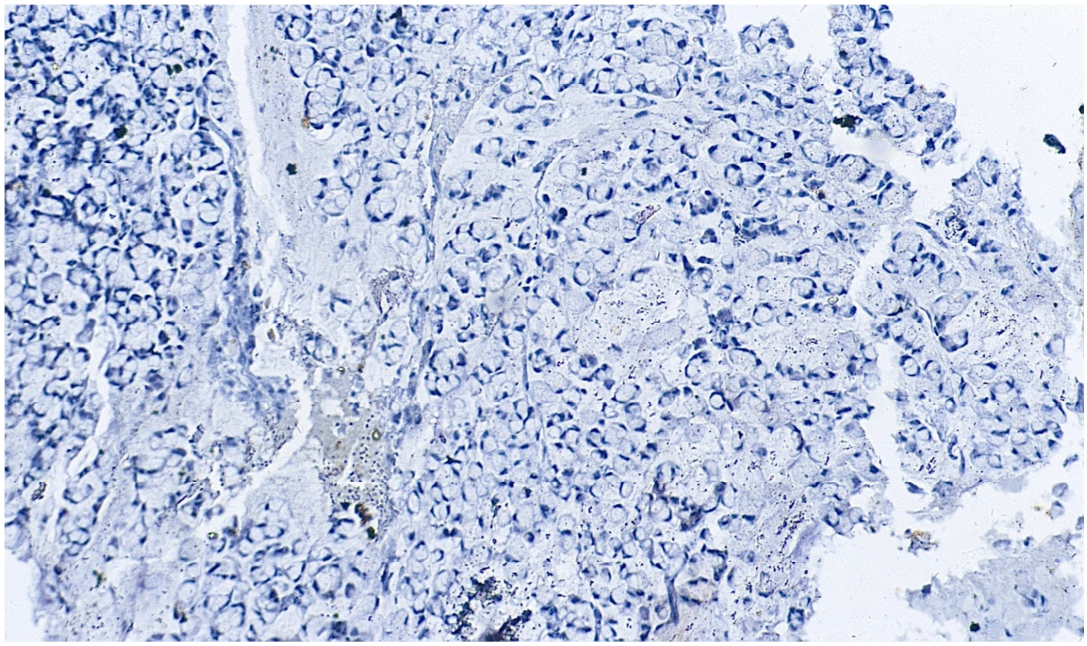

Figure 15: Signet ring cell Carcinoma

(Negative expression of AMACR IHC, 100x)

DISCUSSION

The significance of AMACR overexpression in carcinogenesis of multiple organ systems has been noted in the literature and has been linked to beta-oxidation of branched chain fatty acids.[85]

The role of AMACR expression in colorectal carcinoma have been described by very few studies and the reports are confined to assessing the occurrence of AMACR expression in normal mucosal epithelium, adenomas, and colorectal carcinomas[86-88].

The results of our study showed increased expression of AMACR in cases of adenomas, with 100% (11/11) adenoma cases showing positive expression. Amongst the positive cases,36% (4) showed poor expression and 64% (7) showed moderate expression. No cases of adenoma exhibited either strong or negative expression.

These findings were similar to the ones by Lakis S et al. and Jiang Z et al.[86,89]. Subsequently adenomas are known to be precursor lesions of CRC and they also showed increased AMACR expression, suggesting role of AMACR in colorectal carcinogenesis. Consequently, suggesting role of AMACR as a potential marker for diagnosis of neoplastic changes.

A total of 30% (12/40) of the carcinoma cases were negative for AMACR; 45% (18/40) showed poor; 20% (8/40) showed moderate; and 5% (2/40) showed strong expression.

Statistical evaluation revealed a significant difference in AMACR expression in cases of adenoma and carcinoma as compared to a study by Shukla N et al. which

did not show any significant difference in expression in adenomas versus carcinomas.[84]

It has been suggested that AMACR expression reduced with progression of cancer at transcriptional level as well as protein level. As per some studies, AMACR showed reduced expression in prostatic carcinoma metastases.[90,91]

In the present study, 80.6% cases of moderately differentiated carcinomas showed positive AMACR expression, which was almost 2-fold higher than that of the poorly differentiated carcinomas (33.4%). There was no expression of AMACR in normal colon; and significant expression in adenomas and cases of moderately differentiated adenocarcinomas. Poorly differentiated carcinomas showed poor or negative expression of AMACR. Thus, it can be deduced that expression of AMACR is associated significantly with tumor differentiation, implying its likely role in carcinogenesis of CRC.[84]

These findings are in concordance with other studies in the literature.[83,86,92] Hence, this strongly suggests a close relation of AMACR expression with histologic differentiation of CRC.

In the current study, high tumor stage was significantly associated with decreased AMACR expression. The significant findings in our study ($p=0.048$) indicate a potential relationship between AMACR expression and tumor stage. However, the small sample size ($n=16$) limits the generalizability of these results. A small sample may not capture the full variability present in a larger population, raising concerns about external validity.

The observed association between AMACR expression and tumor stage aligns with another research, by Marx A et al., which reported that AMACR expression tends to decrease in tumors with higher tumor stage.[83]

The result showed no significant difference in AMACR expression with nodal status (pN stage) in our study. This is similar to the findings by other researchers published in the literature.[84,86] However, the absence of significant association between AMACR expression with nodal status in the current study indicates that while AMACR is a potential marker for tumor differentiation, its utility in predicting lymphatic metastasis is not reliable.

Comparison with results of other studies: (Table 13)

Study	AMACR Expression in Adenomas	AMACR Expression in Carcinomas	Association with Grade	Association with Stage	Association with Nodal Status
Lin A et al[92]	Positive Expression in 64% cases	Positive Expression in 75% cases	Significant association	No correlation with overall AJCC Stage	Significant association
Jiang Z et al[86]	Positive Expression in 79% cases	Positive Expression in 69% cases	Significant association	No correlation with overall AJCC Stage	No correlation with Nodal status
Marx A et al[83]	Adenomas not included in study	Positive Expression in 81.7% cases	Significant association	Decreased AMACR expression with higher tumor stage	-
Shukla N et al[84]	Positive Expression in 75% cases	Positive Expression in 66% cases	No significant association	No correlation with overall AJCC Stage	No association found
Present study	Positive Expression in 100% cases	Positive Expression in 70% cases	Significant association	Decreased AMACR expression with higher tumor stage	No association found

LIMITATIONS:

- The major limitation of this study is small sample size owing to the time constraint.
- Follow up of cases to correlate AMACR expression with prognosis and survival is necessary.
- A larger number of controls can aid in confirmation of these findings.

CONCLUSION

The results of this study revealed increased AMACR expression in colorectal neoplasms when compared with normal colonic epithelium, indicating a potential role of AMACR in early neoplastic changes.

There was significant variation of AMACR expression in moderately and poorly differentiated carcinomas reinforcing AMACR as a marker of tumor differentiation.

In this study, expression of AMACR was found to be associated with tumor stage but there was no such association found with nodal status.

However, further large-scale studies are required for validation of these findings.

FUTURE PROSPECTS:

Further research with follow up periods is necessary to establish role of AMACR in tumor progression and potential clinical implications.

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ANNEXURE – I - INFORMED CONSENT FORM

"CLINICOPATHOLOGICAL CORRELATION OF ALPHA-METHYLACYL - COENZYME A RACEMASE EXPRESSION IN COLORECTAL NEOPLASIA- A CROSS-SECTIONAL STUDY IN A TERTIARY CARE HOSPITAL"

Objective: To find clinicopathological correlation of alpha methylacyl-coenzyme- a recemase expression with colorectal neoplasia.

Introduction: Research reveal that nutrition, alcohol consumption, smoking are the major environmental risk factor for Colorectal carcinoma. Red meat and dairy products are the major sources of branched chain fatty acids. AMACR is an enzyme which has an important role in branched chain fatty acid beta oxidation.

Explanation of procedure: Biopsy specimens of colorectal neoplasia will be collected and immediately fixed in 10% formalin and processed. Paraffin blocks will be prepared. Blocks will be used for haemotoxylin and eosin stain for histological evaluation then AMACR staining.

Withdrawal from participation in the study: Participation in this study is voluntary. You will be free to decide whether to participate in this study or continue participation once enrolled. In case you decide to withdraw your participation, you are free to do so. However, please convey the decision to the principal investigator.

Possible benefits from participating in the study: You will/will not have nor get any benefits by participating in this study. The data gathered will help the population at large.

Possible risks from participating in the study: There are no risks involved in participating in this study.

Privacy and confidentiality: The information collected from you will be coded, to prevent any person from identifying you. Your identity will never be revealed. The data collected from you will be kept confidential and only processed or aggregated data will be used for publication. No information about you or information provided by you during research will be disclosed to other without your written permission except -

1. In emergency to protect your rights and welfare.
2. If required by law.

Financial incentives: You will not receive any payment for participating in this study.

Authorization for publication of aggregated data: Results obtained after processing of the aggregated data will be published for scientific purposes and or presented to scientific groups. However, your identity will never be revealed.

Questions: In case of any questions with regard to this study, you are free to contact:

If you have any question or complaints with regard to your right as study participant you may contact: Dr Harsha Hegde, Chairperson, Ethical Committee for Human Subject's Research of JNMC, 0831-2473777 Extension 4052.

Legal rights: By signing this consent form, we are not waving any of your legal rights.

CONSENT STATEMENT

I am making a voluntary decision to participate in the study "CLINICOPATHOLOGICAL CORRELATION OF ALPHA-METHYLACYL - COENZYME A RACEMASE EXPRESSION IN COLORECTAL NEOPLASIA- A CROSS-SECTIONAL STUDY IN A TERTIARY CARE HOSPITAL". My signature below indicates that I have decided to participate and I have read the information provided above or the information provided above has been read to me in the language that I understand best. I was given the opportunity to ask questions and that they have been answered to my satisfaction.

Name of the participant:

Signature or left thumb impression of the participant:

Name of the witness:

Signature or left thumb impression of the witness:

Name of the investigator:

ANNEXURE II- PROFORMA

Name :

Age :

Sex:

IP no. :

History :

Clinical diagnosis with stage :

Surgery Performed:

Type of sample: Biopsy/Resected specimen

Location:

Lymph node involvement:

Microscopy:

- Histopathological diagnosis and grade:
- IHC staining score for AMACR:

Signature of the investigator:

ANNEXURE III- WHO Classification of Colorectal Neoplasia

Benign Epithelial Tumors and Precursors

1. Serrated Dysplasia

- Low-grade serrated dysplasia
- High-grade serrated dysplasia
- Hyperplastic polyp – microvesicular type
- Hyperplastic polyp – goblet cell type

2. Adenomatous Polyps

- Low-grade dysplasia
- High-grade dysplasia

3. Adenomas

- Tubular adenoma (low grade)
- Tubular adenoma (high grade)
- Villous adenoma (low grade)
- Villous adenoma (high grade)
- Tubulovillous adenoma (low grade)
- Tubulovillous adenoma (high grade)
- Advanced adenoma

4. Glandular Intraepithelial Neoplasia

- Low grade
- High grade

Malignant Epithelial Tumors

1. Adenocarcinoma (Not Otherwise Specified - NOS)

- Adenoma-like adenocarcinoma
- Micropapillary adenocarcinoma
- Mucinous adenocarcinoma
- Poorly cohesive carcinoma
- Signet ring cell carcinoma
- Medullary adenocarcinoma
- Adenosquamous carcinoma
- Undifferentiated carcinoma (NOS)
- Carcinoma with sarcomatous features

2. Neuroendocrine Tumors (NOS)

- Grade 1 neuroendocrine tumor
- Grade 2 neuroendocrine tumor
- Grade 3 neuroendocrine tumor
- L-cell tumor
- Glucagon-like peptide-secreting tumor
- PP / PYY-producing tumor
- Enterochromaffin cell carcinoid
- Serotonin-secreting carcinoid

3. Neuroendocrine Carcinoma (NOS)

- Large cell neuroendocrine carcinoma
- Small cell neuroendocrine carcinoma

4. Mixed Neuroendocrine-Nonneuroendocrine Neoplasm (MiNEN)

ANNEXURE IV- AJCC (8TH EDITION) TNM CLASSIFICATION OF COLORECTAL CANCER

Primary Tumor (T)

- TX – Primary tumor cannot be assessed.
- T0 – No evidence of primary tumor.
- Tis – Carcinoma in situ: intraepithelial or invasion of lamina propria.
- T1 – Tumor invades submucosa.
- T2 – Tumor invades muscularis propria.
- T3 – Tumor invades through the muscularis propria into pericolorectal tissues.
- T4a – Tumor penetrates to the surface of the visceral peritoneum.
- T4b – Tumor directly invades or is adherent to other organs or structures.

Regional Lymph Nodes (N)

- NX – Regional lymph nodes cannot be assessed.
- N0 – No regional lymph node metastasis.
- N1 – Metastasis in 1-3 regional lymph node(s).
    - N1a – Metastasis in 1 regional lymph node.
    - N1b – Metastasis in 2-3 regional lymph nodes.
    - N1c – Tumor deposit(s) in the subserosa, mesentery, or non-peritonealized pericolic or perirectal tissues without regional nodal metastasis.
- N2 – Metastasis in 4 or more regional lymph nodes.
    - N2a – Metastasis in 4-6 regional lymph nodes.
    - N2b – Metastasis in 7 or more regional lymph nodes.

Distant Metastasis (M)

- M0 – No distant metastasis.
- M1 – Distant metastasis.
    - M1a – Metastasis confined to one organ or site (e.g., liver, lung, ovary, nonregional node).
    - M1b – Metastasis in more than one organ/site.
    - M1c – Metastasis to peritoneum.

ANNEXURE V- MASTER CHART

S. NO	HP No	AGE	SEX	Specimen	Histopathological diagnosis	Grade	Tumor Stage	Nodal status	Intensity(I)	Percentage of positive tumor cells(P)	I x P	Final Score
1.	2146	52	F	Anterior Resection-Rectum	Adenocarcinoma	Moderately differentiated	T3	N0	1	3	3	Poor
2.	941	66	M	Hepatic flexure	Adenocarcinoma	Moderately Differentiated	-	-	2	3	6	Moderate
3.	3849	72	F	Ascending Colon Biopsy	Adenocarcinoma	Moderately Differentiated	-	-	2	2	4	Poor
4.	3777	36	M	Low Anterior Resection	Adenocarcinoma	Moderately differentiated	T4	N2	1	4	4	Poor
5.	69	65	F	Rectum	Adenocarcinoma	Moderately differentiated	T3	N2	1	4	4	Poor
6.	2927	71	M	Anterior Resection-Sigmoid	Adenocarcinoma	Moderately differentiated	T2	N0	3	5	15	Strong
7.	4800	34	F	Rectal biopsy	Tubular adenoma	-	-	-	2	4	8	Moderate
8.	1594	64	M	Rectal Biopsy	Adenocarcinoma	Moderately differentiated	-	-	1	4	4	Poor
9.	4879	70	M	Rectal Biopsy	Adenocarcinoma	Moderately differentiated	-	-	2	4	8	Moderate
10.	3072	34	F	Right hemicolectomy-Hepatic Flexure	Mucinous Carcinoma	Poorly differentiated	T3	N1	1	3	3	Poor
11.	1706	44	M	Rectal biopsy	Tubular Adenoma	-	-	-	2	4	8	Moderate

12.	2579	52	M	Anterior Resection Rectum	Adenocarcinoma	Moderately differentiated	T2	N0	2	4	8	Moderate
13.	5268	62	F	Rectal biopsy	Adenocarcinoma	Moderately differentiated	-	-	0	0	0	Negative
14.	1971	60	F	APR-Rectum	Adenocarcinoma	Poorly differentiated	-	-	1	0	0	Negative
15.	71	55	M	Rectal Biopsy	Tubular Adenoma	-	-	-	1	1	1	Poor
16	718	68	M	Sigmoid biopsy	Adenocarcinoma	Moderately differentiated	-	-	1	1	1	Poor
17	2627	42	F	Sigmoid biopsy	Villous adenoma	-	-	-	2	5	10	Moderate
18	2263	52	M	Descending colon Biopsy	Adenocarcinoma	Moderately differentiated	-	-	1	2	2	Poor
19	1908	30	F	Anterior Resection-Carcinoma Rectosigmoid junction	Adenocarcinoma	Moderately differentiated	T3	N0	2	3	6	Moderate
20	359	46	M	Ascending colon	Adenocarcinoma	Moderately differentiated	T4	N0	1	0	0	Negative
21	1974	40	M	Rectal biopsy	Villous adenoma	-	-	-	1	3	3	Poor
22	5016	34	M	Rectal biopsy	Tubular Adenoma	-	-	-	2	4	8	Moderate
23	3772	40	M	Rectum biopsy	Adenocarcinoma	Moderately differentiated	-	-	0	0	0	Negative
24	736	49	F	Splenic flexure	Adenocarcinoma	Moderately differentiated	T3	N0	0	0	0	Negative
25	3945	62	M	Descending colon	Adenocarcinoma	Moderately differentiated	T3	N1	1	4	4	Poor

26	3018	51	M	Anterior Resection-Rectum	Adenocarcinoma	Moderately differentiated	T4	N1	0	0	0	Negative
27	2122	59	F	Small biopsy - Sigmoid colon	Adenocarcinoma	Moderately differentiated	-	-	1	1	1	Poor
28	5404	48	M	Transverse Colon biopsy	Adenocarcinoma	Moderately differentiated	-	-	1	2	2	Poor
29	5046	33	M	Rectal biopsy	Adenocarcinoma	Poorly differentiated	-	-	0	0	0	Negative
30	3940	38	F	Right hemicolectomy-Ascending colon	Adenocarcinoma	Moderately differentiated	T3	N1	1	1	1	Poor
31	806	62	M	Sigmoid colon	Adenocarcinoma	Moderately differentiated	T3	N2	1	2	2	Poor
32	2370	54	M	Anterior resection-Sigmoid colon	Adenocarcinoma	Moderately differentiated	T3	N0	2	4	8	Moderate
33	5186	48	M	Right hemicolectomy Caecum	Adenocarcinoma	Moderately differentiated	T3	N0	1	1	1	Poor
34	5195	44	M	Rectal biopsy	Signet Ring cell carcinoma	Poorly differentiated	-	-	0	0	0	Negative
35	4487	48	M	Sigmoid biopsy	Adenocarcinoma	Moderately differentiated	-	-	1	1	1	Poor
36	457	31	F	Sigmoid biopsy	Tubular adenoma	-	-	-	1	1	1	Poor
37	1578	48	F	Rectal biopsy	Villous adenoma	-	-	-	2	4	8	Moderate
38	4513	39	F	Rectal biopsy	Adenocarcinoma	Moderately differentiated	-	-	2	3	6	Moderate
39	2418	48	M	Sigmoid biopsy	Adenocarcinoma	Moderately differentiated	-	-	3	5	15	Strong

40	2225	46	M	Rectal biopsy	Adenocarcinoma	Poorly differentiated	-	-	1	2	2	Poor
41	1571	52	M	Abdominoperineal resection-Rectum	Adenocarcinoma	Poorly differentiated	T1	N0	0	0	0	Negative
42	3344	51	F	Rectal biopsy	Mucinous Carcinoma	Poorly differentiated	-	-	1	1	1	Poor
43	2842	45	M	Sigmoid biopsy	Adenocarcinoma	Poorly differentiated	-	-	0	0	0	Negative
44	5950	35	F	LAR-Rectum	Adenocarcinoma	Poorly differentiated			1	0	0	Negative
45	123	53	M	Rectum biopsy	Adenocarcinoma	Moderately differentiated	-	-	1	1	1	Poor
46	6002	44	F	Rectum biopsy	Adenocarcinoma	Moderately differentiated	-	-	2	3	6	Moderate
47	6220 B	42	M	Caecum	Tubulovillous Adenoma	-	-	-	1	4	4	Poor
48	6319	52	F	Rectum biopsy	Tubular Adenoma	-	-	-	2	3	6	Moderate
49	6220 A	42	M	Rectal biopsy	Tubular Adenoma	-	-	-	2	4	8	Moderate
50	154	44	F	Sigmoid colon	Adenocarcinoma	Moderately differentiated	-	-	3	3	9	Moderate
51	113	48	F	Biopsy	Adenocarcinoma	Moderately Differentiated	-	-	0	0	0	Negative