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Immunohistochemical Expression of MSH2 and MLH1 and Their Correlation with Histological Grades of Colorectal Carcinoma at Uganda Cancer Institute

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ABSTRACT

Background: Colorectal Cancer (CRC) is a rising health challenge in Uganda. The mismatch repair (MMR) system, particularly MSH2 and MLH1, plays a critical role in maintaining genomic stability; defects contribute to CRC development. Immunohistochemistry (IHC) is a cost-effective method for detecting MMR protein expression in CRC tissues and aids diagnosis and treatment.

Study design

The study was a retrospective cross-sectional design

Objectives: To determine the immunohistochemical expression of MSH2 and MLH1 in archived CRC tissue blocks at the Uganda Cancer Institute (UCI) and to assess their correlation with tumor histological grades.

Methods: A retrospective analysis of 41 CRC tissue blocks collected from August 2017 to June 2023 was conducted using IHC. Histologically confirmed tissue sections were immunostained with MLH1 and MSH2 antibodies.

Histological grading and IHC scoring were blinded performed on deferent dates and with different identifiers to avoid observers bias Spearman's correlation assessed relationships between protein expression and tumor

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differentiation.

Results: MSH2 was expressed in 95% of tissue blocks and MLH1 in 92.7%. Loss of MSH2 and MLH1 occurred in 5% and 7.3% of cases, respectively. Most tumors were well differentiated (58.5%), followed by moderately (24.4%) and poorly differentiated (17.1%). There was no significant correlation between MSH2 expression and CRC grades (p = 0.151). MLH1 expression showed a positive correlation with poorly differentiated tumors (rho = 0.414, p = 0.007).

Conclusion: MSH2 and MLH1 expression was largely retained in this cohort of colorectal cancer cases at UCI, suggesting that most tumours were MMR proficient. Although MLH1 expression demonstrated a statistically significant correlation with poor differentiation, this pattern contradicts the loss of function biology of MLH1 and should therefore be interpreted with caution. The observed relationship is preliminary and requires validation using larger cohorts, molecular MSI testing, and more rigorous pathological review processes.

Keywords: Colorectal cancer, Mismatch repair, Immunohistochemistry, MSH2 protein, MLH1 protein, Tumor differentiation.

INTRODUCTION

Colorectal cancer (CRC), affecting the colon and rectum, is a major global health concern. It develops slowly over several years, often beginning as benign polyps that may progress to malignancy. Worldwide, CRC is the third most commonly diagnosed cancer and the fourth leading cause of cancer-related deaths, reflecting its significant impact. In Africa, although only 3.4% of the 1.93 million global CRC cases are reported, the continent faces a growing burden due to limited screening and late-stage diagnoses, which contribute to high mortality rates. CRC ranks as the sixth most prevalent cancer in Sub-Saharan Africa, highlighting its contribution to the regional cancer burden.

Recent studies (2016-2021) have highlighted the clinical importance of mismatch repair status in CRC, particularly in determining prognosis, Lynch syndrome screening, and response to immune checkpoint inhibitors (Wismayer et al., 2025) African data remain limited, but emerging research from Ethiopia, Kenya, and South Africa reports varying MSI-H prevalence and emphasizes the need for region specific molecular profiling. Incorporating updated evidence strengthens the rationale for studying MMR protein expression in Ugandan CRC patients

In East Africa, CRC is associated with high morbidity and mortality, largely due to late presentation. Over the past two decades, the incidence has steadily increased. In Uganda, for instance, the age-standardized incidence rate among females rose from 6.8 per 100,000 (1991–1995) to 11.0 per 100,000 (2011–2015), reflecting a 2.2% annual increase. A retrospective review at Mulago National Referral Hospital reported a 9.3% prevalence among hospitalized patients between 2010 and 2020. These trends indicate a rising public health challenge, with an increasing number of Ugandan patients being diagnosed with CRC, underscoring the need for improved awareness, screening, and treatment strategies.

At the molecular level, CRC development is strongly influenced by genetic factors, particularly mutations or altered expression of the MSH2 and MLH1 genes. These genes play critical roles in DNA repair, and their dysfunction is linked to Lynch syndrome, which significantly increases the risk of CRC and other cancers. Low expression of MSH2 and MLH1 leads to the accumulation of genetic mutations that drive tumorigenesis, often resulting from genetic or epigenetic alterations. Clinically, assessing MSH2 and MLH1 expression is essential for diagnosing microsatellite instability, guiding treatment decisions, and identifying patients who may benefit from targeted therapies addressing these molecular pathways.

In Uganda, CRC diagnosis primarily relies on histological evaluation, which has limitations in sensitivity, specificity, and prognostic value, and cannot detect the majority of genetic defects in MSH2 and MLH1. Histology is also prone to inter-observer variability and may fail to identify early-stage tumors. Integrating molecular diagnostic techniques, such as immunohistochemistry (IHC) for MSH2 and MLH1, alongside traditional histology, could significantly improve early detection, prognostic accuracy, and personalized treatment of colorectal cancer in Uganda.

METHODS

Study Design and Site

This cross-sectional retrospective study was conducted at the Uganda Cancer Institute (UCI), Mulago, a national referral centre with well-equipped pathology laboratories and a comprehensive tissue block archive.

Sample Selection

Archived formalin-fixed paraffin-embedded (FFPE) CRC tissue blocks from August 2017 to June 2023 were identified. A purposive sampling method yielded 41 histologically confirmed blocks with complete patient data and adequate tissue quality. Specimens included both endoscopic biopsies and resections.

Tissue Processing and Staining

Three 2 micron sections per block were prepared using a manual rotary microtome. One section underwent hematoxylin and eosin (H&E) staining for histological confirmation and grading by a pathologist. Two sections were used for IHC staining.

Immunohistochemistry Procedure

IHC employed VENTANA ready-to-use monoclonal antibodies against MSH2 (clone G219-1129) and MLH1 (clone M1). Sections were deparaffinized, rehydrated, and subjected to antigen retrieval by steaming in Cell Conditioning 1 solution at 100°C for 40 minutes. Primary antibody incubation was followed by sequential washing, application of secondary antibody, endogeneous enzyme blocking, polymer amplification, and chromogen development using diaminobenzidine (DAB). Slides were counterstained with Mayer's hematoxylin, dehydrated, cleared, and mounted.

Interpretation and Scoring

MSH2 and MLH1 expression was assessed semi-quantitatively, based on nuclear staining intensity and percentage of positive tumor cells. Scores ranged from 0 (no staining) to 3 (51–100% positive cells). Tumors with complete nuclear loss of MSH2 or MLH1 were classified as negative. Scoring was performed independently by the principal investigator and a pathologist following established criteria (Fedchenko & Reifenrath, 2014).

DATA ANALYSIS

Demographic and clinicopathologic data were summarized with descriptive statistics. The relationship between MSH2 and MLH1 expression and histological grades (well, moderately, poorly differentiated) was assessed using Spearman's rank correlation coefficients. A p-value <0.05 was considered significant. STATA v17 software was used.

RESULTS

Of the 41 CRC cases, 78.1% were from males, 22.9% females with mean age of 57.2 ± 12.2 years. Well-differentiated tumors comprised 58.5%, moderately differentiated 24.4%, and poorly differentiated 17.1%. MSH2 expression was retained in 95.1% of cases, with

Table 1: Expression of MLH1 & MSH2 in colorectal cancer blocks at Cancer Institute Mulago.

Variable	Frequency (n=41)	Percentage (%)
	MLH1	
MLH1 Expression		
Negative (<5%)	3	7.32
Score 1(6- 25%)	1	2.44
Score 2 (26- 50%)	**15**	**39.02**
Score 3 (51- 100%)	**22**	**53.66**
	MSH2	
Variable	**Frequency (n=41)**	**Percentage (%)**
MSH2 Expression		
Negative (<5%)	2	4.88
Score 1(6- 25%)	2	4.88
Score 2 (26- 50%)	**18**	**43.90**
Score 3 (51- 100%)	**19**	**46.34**

loss in 4.9%. MLH1 was expressed in 92.7%, with 7.3% loss. No significant correlation was found between MSH2 expression and tumor grade (rho = -0.229, p = 0.151). MLH1 expression showed a moderate positive correlation with poor differentiation (rho = 0.414, p = 0.007).

The distribution of MLH1 and MSH2 expression scores is summarized in Table 1 above. The majority of tumors retained expression of both proteins, with loss observed in only 7.3% of MLH1 cases and 4.9% of MSH2 cases. Both proteins showed high retention; MSH2/MLH1 showed no statistically significant association with tumor grade tumors.

Correlation between expression of MSH2 and MLH1 proteins and histological grades of colorectal cancer tissue blocks at the UCI pathology laboratory

No statistically significant correlation existed between MSH2 expression and tumor grade (rho = -0.2285, p = 0.151). MLH1 showed a significant moderate positive correlation with poorly differentiated tumors (rho = 0.414, p = 0.007). Weak and non-significant correlations were detected between MLH1 and well- or moderately differentiated tumors.

MLH1 (MutL homolog 1), MSH2 (MutS homolog 2) * statistically significan

Table 2 presents Spearman's rank correlations between the immunohistochemical expression scores of MSH2 and MLH1 proteins and histological grades of colorectal cancer (well, moderately, and poorly differentiated) in tissue blocks from the UCI pathology lab.

The correlations for MSH2 expression with all three grades were weak and statistically non-significant, indicating no clear relationship between MSH2 expression levels and tumor differentiation.

MLH1 expression showed some significant correlations: a moderate negative correlation with moderately differentiated tumors at score 2 expression (rho = -0.314, p = 0.046), and a moderate positive correlation with poorly differentiated tumors at score 3 expression (rho = 0.414, p = 0.007). These findings suggest that MLH1 expression is somewhat associated with tumor grade, particularly showing increased expression correlation with poorer differentiation. However, given the small sample size (n-41) that was available, this doesn't give the study power to generalise the findings

In contrast, the positive correlation observed between high MLH1 expression (Score 3) and poorly differentiated tumors is biologically unexpected, as loss of MLH1 is typically associated with aggressive phenotypes and MSI-high status. This paradox could be due to prolonged fixation, variable antigen preservation in older archival tissue, or uneven tissue processing which can affect staining intensity and lead

Table 2: Correlation between expression of MSH2 and MLH1 proteins and histological grades of colorectal cancer tissue blocks at the UCI pathology laboratory.

	Expression	Well differentiated	Moderately differentiated	Poorly differentiated
		Spearman's rho (Prob >t)	Spearman's rho (Prob >t)	Spearman's rho (Prob >t)
MSH2	Negative	0.237 (0.137)	-0.159 (0.319)	-0.128 (0.427)
	Score 1	-0.039 (0.808)	-0.129 (0.423)	0.198 (0.214)
	Score 2	0.105 (0.512)	-0.132 (0.410)	0.013 (0.937)
	Sore 3	-0.211 (0.186)	0.269 (0.0884)	0.032 (0.844)
MLH1	Negative	0.237 (0.137)	-0.1596 (0.3189)	-0.1275 (0.4270)
	Score 1	0.133 (0.407)	-0.0898 (0.5766)	-0.0717 (0.6558)
	Score 2	0.125 (0.435)	**-0.314 (0.046)***	0.194(0.2251)
	Sore 3	-0.286(0.701)	**0.414 (0.007)***	-0.0982 (0.5410)

to artificially elevated scores. secondly, it is possible that these tumors represent biologically distinct subtypes in which MLH1 overexpression reflects compensatory upregulation or alternative tumorigenic pathways. Future studies integrating MSI testing and next-generation sequencing are highly recommended to clarify whether the correlation represents true biology or a technical artifact

Overall, MLH1 protein expression does not correlate with histological grade of colorectal cancer. For MSH2, the correlations with well, moderately, and poorly differentiated tumors were weak and not statistically significant, indicating no clear link between MSH2 expression levels and tumor grade.

DISCUSSION

Expression of MSH2 and MLH1 in Colorectal Cancer at Uganda Cancer Institute

A study conducted at the Uganda Cancer Institute (UCI) Pathology Laboratory investigated the expression of mismatch repair (MMR) proteins MSH2 and MLH1 in colorectal cancer (CRC) tissue samples and their correlation with tumor grading. Among 41 tissue blocks analysed, 39 (95%) were immunoreactive for MSH2, with only 4.9% showing negative expression. This high retention rate indicates that the majority of tumors in this population remain MMR-proficient, suggesting mechanisms other than MSH2 deficiency drive tumorigenesis. The 95% positivity is comparable to other studies, though slightly lower than reports showing 100% expression, likely due to differences in sample size, tumor heterogeneity, and the prevalence of microsatellite instability (MSI) in Uganda. The 4.9% of MSH2-negative cases may represent sporadic MSI-high (MSI-H) tumors, consistent with global observations linking MSH2 loss to MSI-H status. However, not all genetic alterations necessarily result in protein loss, emphasizing the need for combined immunohistochemistry (IHC) and molecular testing for accurate MMR assessment. Geographic, ethnic, and technical factors, including antibody specificity and scoring criteria, also contribute to variability in MSH2 expression across studies.

MLH1 expression was observed in 38 out of 41 tissue blocks (92.7%), with 7.3% showing no expression. This is higher than some prior studies, which reported lower MLH1 expression due to missense mutations or promoter hypermethylation. The current study did not differentiate between colon and rectal tumors or perform sequencing but focused on overall MLH1 protein expression. MLH1 loss is generally more prevalent than MSH2 loss in CRC, making its assessment critical for evaluating MSI status and identifying patients who may benefit from immunotherapy. IHC using MSH2 and MLH1 antibodies provides a reliable and cost-effective alternative to molecular testing such as PCR or DNA sequencing, with reported sensitivity and specificity of 92.3% and 100%, respectively.

The notably high retention of MMR proteins in our cohort resembles findings from other low-resource settings, where MSI-high prevalence tends to be lower than in Western populations. This could be attributed to a number of factors like a different spectrum of CRC molecular subtypes in African populations, referral bias toward more advanced-stage tumors with chromosomal instability pathways, and limited routine MSI testing leading to under-recognition of Lynch syndrome. Comparative analysis with recent African studies is therefore essential to contextualize our findings

Correlation analyses between MMR protein expression and CRC grading showed no statistically significant associations. For MSH2, Spearman's correlation indicated a weak positive trend with well-differentiated tumors (rho = 0.2363, p = 0.137), but overall, no significant correlation with tumor grade was found. Similar findings have been reported in other cohorts, suggesting that MSH2 expression may not reliably predict histological differentiation.

The positive correlation observed between high MLH1 expression and poorly differentiated tumors is an aspect that calls for more study, as loss of MLH1 is typically associated with aggressive phenotypes and MSI-high status. Several explanations may account for this paradox. First, pre analytical factors including prolonged fixation, variable antigen preservation in older archival tissue, or uneven tissue processing may affect staining intensity and lead to artificially

elevated scores. It is possible that these tumors represent biologically distinct subtypes in which MLH1 overexpression reflects compensatory upregulation or alternative tumorigenic pathways. Future studies integrating MSI testing and next-generation sequencing will be essential to clarify whether the correlation represents true biology or a technical artifact

CONCLUSION

MSH2 and MLH1 immunoexpression is largely preserved in colorectal cancer at Uganda cancer institute cohort. although there was MLH1 loss correlation with poor tumour differentiation, a post hoc power analysis indicates that the study was underpowered to detect small to moderate correlations, reinforcing the need to interpret the findings as preliminary

IHC can serves as a practical screening approach in resource-limited contexts and lays the foundation for integrated molecular diagnostics in CRC care in Uganda.

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STRENGTHS AND LIMITATIONS

Strength

This study provides important insights into the immunohistochemical expression of MSH2 and MLH1 mismatch repair proteins in a Ugandan colorectal cancer cohort, an underrepresented population in molecular oncology research. Utilizing archived FFPE tissue spanning six years increases the study's external validity in real-world diagnostic settings. The use of validated IHC protocols and independent slide scoring by two observers strengthens the reliability of protein expression assessments, supporting the potential of IHC as a cost-effective screening tool in resource-limited environments.

LIMITATIONS

This study is limited by its small sample size (n=41), which reduces statistical power and increases the probability of type II error. Its retrospective design introduces selection bias, as only archived tissue with adequate morphology was included. The absence of MSI testing or molecular sequencing prevents definitive validation of IHC results. The semi-quantitative scoring system is subject to inter-observer variability, and although two observers scored slides independently, the level of agreement (e.g., Cohen's κ) was not measured. These limitations necessitate cautious interpretation of the observed correlations.

RECOMMENDATION

Future studies should incorporate MSI testing, digital image analysis for objective scoring, and larger multi-center sample sizes to more accurately define the MMR deficiency landscape in Ugandan CRC patients.

ETHICAL CONSIDERATIONS

Approval was obtained from Mbarara University Research Ethics Committee (REC) and administrative clearance from Uganda cancer institute. Patient confidentiality was maintained by anonymizing samples with unique identifiers.

INFORMED CONSENT

The study didn't involve human participants therefore getting consent was not considered

PATIENT AND PUBLIC INVOLVEMENT STATEMENT

It was impossible to involve the patient and the public in this study because it did not involve human participants and because it was an academic study in partial fulfilment of the requirements for the award of master's degree in Medical Laboratory science of Mbarara University of Science and Technology.

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CONFLICTS OF INTEREST

The authors declare no competing interests.

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