

Clinical Implications of Tissue Carcinoembryonic Antigen Expression in Ugandan in the Colorectal Cancer Patients

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ABSTRACT

Introduction: The global incidence of colorectal cancer (CRC) is projected to rise up to 60% in 2030. In Uganda and other resource-deprived countries in Sub-Saharan Africa, colonoscopy is not easily accessible for screening. Therefore, Carcinoembryonic Antigen (CEA) may have a larger role as a marker for CRC development in these resource-limited settings. The aim of this study was to investigate the incidence of positive CEA in CRC tissue and compare it to clinicopathological features in Ugandan patients. **Methods:** Participants were consecutively recruited from hospitals in central Uganda between 2019-2021, with corresponding formalin-fixed and paraffin-embedded (FFPE) tissue blocks that were included for CEA analysis. A data extraction form was used to obtain data regarding age, sex, stage, grade of colorectal cancer and topography. Haematoxylin and eosin sections from FFPE CRC blocks were prepared to establish the grade, lymphovascular invasion (LVI) and histopathological subtype. The CEA molecular marker was analysed using the ABC method for immunohistochemistry. The Pearson chi-square test was used to assess the association between CEA expression and demographic and histopathological variables. The correlation between the CEA biomarker and grade was determined using the Spearman's rank correlation. A p-value of ≤ 0.05 was considered statistically significant. **Results:** Out of 118 participants there were 67 male participants and 52 female participants and the median age (SD) for all the participants was 59.9 (15.3) years. Compared to stage I, there were 31.4% CRC tissues that stained positively for CEA in stage IV disease and this reached statistical significance ($p=0.0101$). There were 73.4% CRC tissues with grade II CRC that stained positively for CEA compared to 15.2% CRC tissues with grade I CRC ($p=0.0000$). There were more AC (85%) participants that stained positively for CEA compared to MAC (5.8%) and SRCC (9.2%) participants ($p=0.0000$). A positive correlation between tumour grade and CEA expression was found ($r=+0.2204$) ($p=0.0232$). **Conclusions:** In Uganda, increasing CEA intensity in CRC tissues is associated with a poorer grade. High grade CRC has a higher incidence of lymph node metastasis and a higher CRC CEA tissue expression. Therefore, in Uganda, there is a poorer prognosis for patients expressing high levels of CEA in their colorectal cancer tissue.

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Keywords: colorectal cancer; CEA; topography; lymphovascular invasion; histopathological subtype

Introduction

Globally colorectal cancer (CRC) is the third most common malignancy (1). By 2030, the global incidence of CRC is projected to rise up to 60% (1-3). Every year, 530,000 deaths and more than one million new cases are diagnosed. Four distinct pathways are responsible for the evolution of CRC: the cytosine-phosphate-guanine (CpG) methylator pathway-1 and pathway-2; microsatellite instability pathway and the chromosomal instability pathway (4). Early screening to reduce both the mortality and incidence of the disease is required due to the slow growth of CRC. Detection of CRC at an early stage is the objective of screening. In Uganda, and other resource-deprived countries in Sub-Saharan Africa, colonoscopy is not easily accessible, yet it is the most reliable diagnostic tool. Worldwide, the most widely used tumour marker is carcinoembryonic antigen (CEA).

Carcinogenic antigen was discovered in 1965 by Gold and Freedman and they observed it in colon cancers and faetal colon (5). It is a high molecular weight glycoprotein found in colorectal malignancy and embryonic tissues (6). The content of CEA tends to be higher in colorectal cancer tissue than in normal colorectal mucosa (7,8). The concentration of CEA in CRC tumour tissue has been found to be 60x greater than in healthy tissues ~~(Boucher et al., 1989)~~. ~~Recent~~ Previous research has shown that in colorectal cancer patients, CEA is a glycoprotein with changing glycosylation patterns. These glycan variants are responsible for early detection of CRC (9).

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Carcinoembryonic antigen was initially thought to be a tumour specific antigen for colorectal carcinoma, however subsequently many studies showed that plasma CEA levels were elevated in many benign diseases such as inflammatory bowel disease, pancreatitis and hepatobiliary disorders (10-13). Malignancies outside the gastrointestinal tract such as lung, breast, ovary and uterine cancers have also shown elevated plasma CEA levels (14-16).

Studies have shown that CEA promotes tumour progression and blocks cell differentiation (17,18). Intracellular adhesion, protection against apoptosis which is associated with cell detachments from the extracellular matrix, and cellular migration result in the increased metastatic potential which has been associated with CEA in colon cancer (19,20). Therefore, CEA tends to be associated with the progression of colorectal metastasis.

Some studies have reported strong staining intensity in well-differentiated adenocarcinoma. A low CEA content and weak staining intensity have been found in poorly differentiated adenocarcinoma (21,22). In well-differentiated colorectal adenocarcinoma, the CEA is primarily localized at the apical surface of cancer glands. Whilst in moderately differentiated adenocarcinoma, the CEA is localized to the intracytoplasm and the entire surface of the cancer cells (23).

CEA is localized to the luminal surface and has been found to be associated with lower serum CEA levels than CEA localized to the basolateral membrane or stromal tissue (19). With these findings, one may conclude that CEA which flows into the ductal lumen contributes lower serum CEA than CEA flowing from interstitial tissue into the blood vessels. Vascular and lymphatic drainage of colon cancer contributes to a high serum CEA level (24).

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Plasma CEA is not a sensitive marker for colorectal carcinoma and lacks specificity. The diagnostic value of preoperative plasma CEA showed positive (≥ 2.5 ng/ml CEA) results in only 62% of the patients (25). Another study by Livingstone ~~AS~~ et al., has shown that only 65% of patients have elevated preoperative plasma CEA levels with surgically proven colorectal carcinoma (26). Using immunoperoxidase and immunofluorescence techniques, CEA has been detected on the surface of colorectal carcinoma (27,28). Add the significant statement

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The purpose of this study was to investigate the incidence of positive cellular CEA in colorectal cancer tissue and compare it to clinicopathological features in Ugandan patients.

Methodology

From 16th September 2019 to 16th September 2021, How many? samples of colorectal biopsy specimens and resected colorectal carcinoma specimens were obtained from patients in Masaka Regional Referral Hospital, Mulago National Referral Hospital, Uganda Martyrs' Hospital Lubaga and Mengo Hospital. There were 119 consecutively recruited participants with corresponding formalin-fixed and paraffin-embedded (FFPE) tissue blocks that were included for FFPE tissue CEA analysis. Using a standard pretested data extraction form, data for all tissue samples was extracted ~~from~~ the clinical patients' files in the respective hospitals. The data included age, sex, stage, grade of colorectal cancer and topography. For those patients that had more than one FFPE tissue block following a colorectal resection all the blocks were examined and the one which represented more than 50% tumour, with not much mesenchymal tissue and with no necrosis was selected for the study.

The FFPE tissue block samples came from patients with histologically confirmed colorectal adenocarcinoma and who fulfilled the following selection criteria: Inclusion criteria included index histologically diagnosed colorectal adenocarcinoma samples taken from patients after having had chemotherapy or radiotherapy treatment, poor quality tissue block samples and tissue samples with incomplete or unavailable data.

Haematoxylin and eosin sections were prepared to establish the histopathological subtype, degree of differentiation of the tumour (grade), and lymphovascular invasion (LVI) status. The histopathological examination established the histopathological subtype of colorectal adenocarcinoma (AC, MAC or SRCC), LVI status and the main features of the tumours and then the ABC method for immunohistochemistry was used for the CEA marker. 4µm thick sections from 10% formalin-fixed paraffin-embedded tissue were taken to perform immunohistochemistry for CEA. The procedure used was first deparaffinization in xylene, rehydration in alcohol series, and brought to distilled water. Heat-induced epitope retrieval in alkaline novolink epitope retrieval buffer (pH9) was done. Then there was incubation with peroxide block, washing with Tris-buffer saline (RBS), incubation for 30 minutes with primary antibody which included 1:100 CEA antibody standard Novolink post-primary antibody, and Novolink Polymer and washing in Tris-buffer saline (TBS) then development in DAB chromogen and counter-stained with Mayer's haematoxylin. The CEA antibody dilution was 1:100, the source was DAKO Agilent USA, clone I17 and reference IR622.

Free peroxide splits the diaminobenzidine from the ABC complex and this localizes the antigen precisely in the nuclei of the cytoplasm by providing a brown precipitate. The nuclei which were counterstained with Mayer's haematoxylin followed by dehydration were then mounted in an organic medium. The specimen slides were mounted and then photographed on a Nikon eclipse microscope. The immunohistochemical technique applied on the paraffin-embedded tumoral samples for CEA used a scale for staining intensity, adopted by Vrabie et al., 2008 which included: negative (0) (none); weak (+1) CEA immunoreactivity in 1-10% of tumour cells; moderate (+2): CEA immunoreactivity in 10-50% of tumour cells; strong (+3): CEA

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immunoreactivity in >50% tumour cells. The CEA molecular marker was correlated with the grade and stage of the tumour. The histological grade and LVI status was determined by two experienced consultant pathologists who reviewed all the histological slides as soon as they were received and prepared by the laboratory technicians. Internal quality control of the immunohistochemical experiments was carried out and screened by two independent pathologists to ensure the reliability of the experimental study.

Left-sided tumours were splenic flexure tumours or tumours located distal to this site including the rectal tumours (29,30). Right-sided tumours included tumours in the caecum, ascending colon, hepatic flexure, and transverse colon (29,30).

Continuous numeric data were summarized by mean (standard deviation) and categorical data were summarized as frequencies and percentages. The distribution of the intensities of CEA was determined and compared by age ($\leq$ median age and above median age), sex (male and female), grade (I-III), stage, LVI status, histopathological subtypes and topography. Pearson chi-square test was used to assess the association between CEA expression and demographic and histopathological variables. Spearman's rank correlation coefficients were used to determine and compare correlations between the CEA biomarker and grade. A p-value of ≤ 0.05 was considered statistically significant.

Results

Baseline characteristics for CEA participants

In this study, there were 119 CEA participants with CRC including 67 male participants and 52 female participants. The median age (SD) of all participants was 59.9(15.3) years. Among 119 participants, 56(50.9%) had colon cancer; 54(49.1%) had rectal cancer; 26(23.6%) had right-sided colon cancer and 30(27.3%) had left-sided colon cancer (Table 1).

A significant proportion of participants, 29.4% (n=35) had stage III CRC, whereas 33(27.7%) had stage IV disease. Early stage CRC consisted of 17(14.3%) participants with stage I CRC and 30(25.2%) participants with stage II disease. The stage was missing for 4(3.4%) participants.

The histological subtypes for all CEA participants included 99(85.3%) participants with classical adenocarcinoma, 8(6.9%) participants with MAC and 9(7.8%) participants with SRCC.

Based on the grade of differentiation, grade II was most commonly seen in 77(72.6%) participants, 16(15.1%) had grade III, and 13(12.3%) had grade I tumours. There were 79(66.4%) participants that had positive lymphovascular invasion (LVI), whilst 1(0.8%)

participant had no lymphovascular invasion (LVI). The LVI status was unknown in 39(32.8%) participants.

Relation of CEA expression with baseline parameters

The relationship of CEA expression with baseline demographic and histopathological parameters is shown in Table 1. The expression of CEA increased with the increasing stage of CRC. In stage I, there were 12(15.1%) participants whose tumours stained positively for CEA, compared to 23(26.7%) for stage II and stage III and this increased to 27(31.4%) for stage IV. However, compared to those that lacked CEA staining this did not reach statistical significance (p=0.480). There were 58(73.4%) colorectal tumours that were moderately differentiated and 9(11.4%) that were poorly differentiated with CEA-positive tissue tumours (Table 1). Representative images of CEA staining at different magnifications are shown in Figures 1-5. [add the significant values](#)

Table 1: Relationship of CEA with baseline and pathological parameters

Variables	Categories	Positive expression CEA	Negative expression CEA	p-value
Age	≤54 years	42(47.2)	15(50.0)	0.790
	≥55 years	47(52.8)	15(50.0)	
Gender	Male	47(52.8)	20(66.7)	0.186
	Female	42(47.2)	10(33.3)	
Stage	I	12(15.1)	4(13.8)	0.480
	II	23(26.7)	7(24.1)	
	III	23(26.7)	12(41.4)	
	IV	27(31.4)	6(20.7)	
Site of Tumour	Colon	38(46.9)	18(62.1)	0.161
	Rectum	43(53.1)	11(37.9)	
Grade	I	12(15.2)	1(3.7)	0.082
	II	58(73.4)	19(70.4)	
	III	9(11.4)	7(25.9)	
Histological subtypes	AC	74(85.0)	25(86.2)	0.450
	MAC	5(5.8)	3(10.3)	
	SRCC	8(9.2)	1(3.5)	
LVI Lymphovascular invasion	Positive	1(1.7)	0(0.0)	0.737*
	Negative	58(98.3)	21(100.0)	

*Fischers exact test

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There were 58(98.3%) participants that had a positive tissue CEA, however were associated with no lymphovascular invasion. All participants whose tissues lacked CEA, 21(100%) were associated with no lymphovascular invasion. There was no difference between the presence or absence of CEA in the colorectal tissues for lymphovascular invasion ($p=0.737$).

CEA positive colorectal tumours

Among the positive CEA participants, the proportion that stained positively was 26.7% for stage III versus 15.1% for stage I and this was borderline statistically significant ($p=0.057$). Compared to stage I, there were 31.4% CRC tissues that stained positively for CEA in stage IV and this reached statistical significance ($p=0.0101$) (Table 2). Compared to 15.2% CRC tissues with grade I CRC that stained positively for CEA there were 73.4% with grade II CRC which stained positively for CEA and this reached statistical significance ($p=0.0000$). There were more participants with AC(85%) compared to MAC(5.8%) and SRCC(9.2%) that stained positively for CEA and this reached statistical significance ($p=0.0000$) (Table 2).

Table 2: Relation of the presence of CEA in CRC tissue with baseline parameters

Variable	Categories	Proportions	95% intervals	Confidence	p-value
Age	≤54 years	47.2	37.0-57.0		0.455
	≥55 years	52.8	42.0-63.0		
Sex	Male	52.8	42.0-63.0		0.455
	Female	47.2	37.0-58.0		
Stage	I	15.1	7.7-22.5		0.057
	III	26.7	17.5-35.9		
	I	15.1	7.7-22.5		0.0101
	IV	31.4	21.7-41.0		
Topography	Rectum	46.9	36-57.8		0.43
	Colon	53.1	42.2-63.9		
Grade	I	15.2	7.3-23.1		0.00
	II	73.4	63.7-83.1		
	I	15.2	7.3-23.1		0.4819
	III	11.4	4.4-18.4		
LVI	Positive	1.7	-1.6-4.9		0.00
	Negative	98.3	95-100		
Histological subtypes	AC	85.0	77.5-92.5		0.00
	MAC	5.8	0.88-10.7		

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	AC	85	77.5-92.5	0.00
	SRCC	9.2	3.13-15.3	

A positive correlation between grade of colorectal cancer and CRC tissue CEA was found ($r=+0.2204$) and this was statistically significant ($p=0.0232$) using the Spearman correlation.

The distribution of CEA antibody staining intensity with tumour grade, tumour depth, stage, topography, histopathological subtype and lymphovascular invasion is shown in tables 3-8.

Table 3: CEA antibody distribution regarding CRC tumour grade

Grade	CEA intensity	Intensity(%)
I(well differentiated)	+1	2(15.4)
	+2	7(53.9)
	+3	3(23.1)
	Negative	1(7.7)
II(moderately differentiated)	+1	21(27.3)
	+2	23(29.9)
	+3	14(18.2)
	Negative	19(24.7)
III(Poorly differentiated)	+1	1(6.3)
	+2	3(18.8)
	+3	5(31.3)
	Negative	7(43.8)

Table 3: [eC](#) Compares the CEA intensity of expression in CRC with the tumour grade. It shows [tat](#) the highest CEA expression (+3) was more commonly associated with poorly differentiated (G3) tumours in 31.3% of CRC compared to 23.1% of well-differentiated (G1) CRC.

Table 4: CEA antibody distribution regarding CRC tumour depth (T)

Tumour depth (T)	CEA intensity	Intensity(%)
T1	+1	3(50.0)
	+2	0(0.0)
	+3	1(16.7)
	Negative	3(33.3)
T2	+1	5(27.8)
	+2	7(38.9)
	+3	3(16.7)
	Negative	3(16.7)

T3	+1	12(24.0)
	+2	17(34.0)
	+3	7(14.0)
	Negative	14(28.0)
T4	+1	6(14.6)
	+2	12(29.3)
	+3	13(31.7)
	Negative	10(24.4)

Table 4: eCompares the CEA intensity of expression in CRC with the tumour depth (T). T1 and T2 tumours were associated with a 16.7% CEA intensity (+3). However, T4 tumours were associated with a 31.7% CEA intensity (+3) and T3 tumours were associated with a 14% CEA intensity (+3).

Table 5: CEA antibody distribution regarding the CRC stage

CRC Tumour stage	CEA intensity	Intensity(%)
I	+1	8(47.1)
	+2	4(23.5)
	+3	1(5.9)
	Negative	4(23.5)
II	+1	5(16.7)
	+2	13(43.3)
	+3	5(16.7)
	Negative	7(23.3)
III	+1	6(17.1)
	+2	10(28.6)
	+3	7(20.0)
	Negative	12(34.3)
IV	+1	7(21.2)
	+2	9(27.3)
	+3	11(33.3)
	Negative	6(18.2)

Table 5: eCompares the CEA intensity of expression in CRC with the stage of CRC. Stage II CRC was associated with a 16.7% CEA intensity (+3) compared to stage I CRC which was associated with a 5.9% CEA intensity (+3). Stage IV CRC was associated with a 33.3% CEA intensity (+3) compared to 20% CEA intensity (+3) for stage III disease.

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Table 6: CEA antibody distribution regarding CRC tumour topography

Topography	CEA intensity	Intensity(%)
Rectum	+1	14(25.9)
	+2	19(25.2)
	+3	10(18.5)
	Negative	11(20.4)
Right-sided colon	+1	3(11.5)
	+2	6(23.1)
	+3	6(23.1)
	Negative	11(42.3)
Left-sided colon	+1	7(23.3)
	+2	10(33.3)
	+3	6(20.0)
	Negative	7(23.3)

Table 6: [eC](#) Compares the CEA intensity of expression in CRC with the location of the tumour. Right-sided colon tumours were associated with a 23.1% CEA intensity (+3) and left-sided colon tumours were associated with a comparable 20% CEA intensity (+3). Rectal tumours were also associated with a comparable 18.5% CEA intensity (+3).

Table 7: CEA antibody distribution regarding histopathological subtype

Histopathoogical subtype	CEA intensity	Intensity(%)
AC	+1	23(23.3)
	+2	32(32.3)
	+3	19(19.2)
	Negative	25(25.3)
MAC	+1	2(25.0)
	+2	1(12.5)
	+3	2(25.0)
	Negative	3(37.5)
SRCC	+1	1(11.1)
	+2	2(22.2)
	+3	5(55.6)
	Negative	1(11.1)

Table 7 compares CEA intensity with the histopathological subtype. There were more SRCC tumours with a higher 55.6% CEA intensity (+3) compared to 25% CEA intensity (+3) for MAC and 19.2% CEA intensity (+3) for AC.

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Table 8: CEA antibody distribution regarding lymphovascular invasion (LVI) status

Lymphovascular invasion (LVI)	CEA intensity	Intensity(%)
LVI Present	+1	15(19.0)
	+2	26(32.9)
	+3	17(21.5)
	Negative	21(26.6)
LVI Absent	+1	1(100.0)
	+2	0(0.0)
	+3	0(0.0)
	Never	0(0.0)

Table 8: demonstrates the CEA antibody distribution with the presence or absence of lymphovascular invasion. The presence of LVI in CRC was more commonly associated with the presence of CEA at (+2) and (+3) CEA intensities. The presence of LVI was associated with a 32.9% CEA intensity (+2) compared to lack of LVI which was associated with a 0% CEA intensity (+2). The presence of LVI was associated with a 21.5% CEA intensity (+3) compared to lack of LVI which was associated with a 0% CEA intensity (+3).

Discussion

The present study evaluated the CRC tissue CEA and the correlation of the tissue staining intensity with the grade, stage, LVI status, topography and histopathological subtype of CRC. CEA may be detected in the blood of CRC patients, however, also in the CRC tumour tissue and hence is a widely used tumour marker globally (31). Recent research has shown the role of CEA in the development of CRC and the changing glycosylation patterns which highlight the importance of glycan variants on CEA for staging and early detection of CRC (32). [This reference on 2018, it cannot be recent](#)

There is a known association between CEA levels in CRC and the tumour extent preoperatively, recurrence and tumour outcomes (33). Although, some studies investigated palliative chemotherapy in the efficacy of CEA monitoring for the evaluation of tumour responses, no consensus has been achieved regarding the role of CRC tumour response to chemotherapy (34-38). Previous studies showed that compared to poorly differentiated CRC specimens, well-differentiated CRCs produced more CEA in the primary tissues and the serum (39,40). This was

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possibly because it was not defined whether cellular CEA or free CEA protein or both take an effect in cellular differentiation (41). In this study there was no correlation found between pT stage, stage, location of CRC, LVI status and histopathological subtype of the CRC when compared to CEA tumour intensity. However, the intensity of CEA expression was significantly correlated with the degree of tumour differentiation (grade). The tumour tissue CEA expression increased with a poorer CRC differentiation.

There are various cellular functions associated with CEA in colon cancer, which includes adhesion in both CEA-matrix interactions and intracellularly, cellular migration following signal transduction and cellular migration which suggests that CEA plays a role in tumour metastasis and invasion (42-48).

~~The findings from this study are in keeping with findings from a study by Vrabie et al., which also demonstrated a positive correlation between the intensity of tissue CEA with the grade of CRC (49).~~ Our The results demonstrated that CRC tissue CEA was not significantly correlated with the size and depth of tumour invasion. ~~The findings from this study are in keeping with findings from a study by Vrabie et al., which also demonstrated a positive correlation between the intensity of tissue CEA with the grade of CRC (49).~~ These findings are consistent with those of Vrabie et al., which showed that irrespective of tumour stage there was the same CEA tissue intensity in most CRC cases (49).

A study by Tong G et al., showed that tumour topography, size and TNM stage were not significantly associated with CRC tissue CEA expression (50), ~~similar to the findings in this study.~~ Tong G et al., also showed a significant correlation between a higher CEA CRC tissue expression and grade of CRC similar to this study. Poorly differentiated CRC has a higher incidence of lymph node metastasis and a higher CRC tissue CEA expression. Poor CRC differentiation (high grade CRC) and increasing numbers of positive lymph nodes are associated with a high tissue CEA expression (50).

The guideline used for staging CRC is the American Joint Cancer Commission/tumour node metastasis (AJCC/TNM) classification and represents a good prognostic indicator of outcome in CRC patients (51,52). The outcome of patients with CRC has also been found to be dependent on the histopathological subtype of colorectal adenocarcinoma (53). Histopathological analysis and accurate AJCC/TNM staging are dependent on postoperative pathological examination of

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colorectal cancer tissue. In our study, the more aggressive histopathological subtypes of CRC particularly signet ring colorectal carcinoma and mucinous adenocarcinoma had a higher staining intensity than AC. However, overall there were more classical adenocarcinoma cases that stained for CEA compared to signet ring colorectal carcinoma and mucinous adenocarcinoma and this reached statistical significance. Possible reasons are that many patients with classical adenocarcinoma which is a less aggressive histopathological subtype that presented with late stage disease. Compared to stage I disease, there were more patients with stage IV disease that presented with positive CEA in colorectal cancer tissue. This is consistent with the results from other studies which have shown that tissue CEA expression was significantly associated with CRC prognosis in stages I to III (54-56).

Conclusions

The findings from this study showed that increasing CEA intensity in CRC tissue is associated with a poorer grade in Ugandan patients. Poorly differentiated CRC also have a higher incidence of lymph node metastasis and a higher CRC CEA tissue expression. These findings suggest a poorer prognosis for patients expressing high levels of CEA in their colorectal cancer tissue in Uganda.

Limitations

For this study, the storage of the specimens was kept for a short period of time in 10% formalin for up to 24 hours with biopsy specimens and up to 72 hours for resection specimens however, there was no control over the quality of fixation. High standards of laboratory testing were also followed. High standard of laboratory testing means using procedures based on sound quality control and quality assurance. Quality control referred to measures that were taken to ensure that the results and interpretation meets a specified standard of quality. Quality assurance refers to measures that were taken to monitor, verify, and document performance by the laboratory.

~~Other limitations were inclusion of a heterogenous population of colon and rectal tumours. The number of rectal cancer patients excluded due to neoadjuvant chemoradiotherapy was small and therefore it is unlikely that selection bias was introduced. However, the effect of neoadjuvant chemoradiotherapy on tissue CEA expression should be explored in future studies. Instead of tissue microarray, the use of whole tissue sections were used which although labour intensive, avoided false negative results.~~

Declarations

Ethical approval

~~This study was part of the PhD work, which was approved by the Doctoral Committee and Higher Degrees Research and Ethics Committee of the School of Biomedical Sciences, College of Health Sciences, Makerere University for the corresponding author (SBS-HDREC-630).~~ Final approval of this research study was obtained from the Uganda National Council for Science and Technology (HS-2574). Written informed consent was obtained from prospective participants included in the study before completing the Data Extraction Form. All the data and specimens pertaining to the research were kept confidential. The ethical standards that apply to research were applied according to the Helsinki Declaration.

Consent for publication

Consent was obtained from all the participants enrolled in this study.

Competing interests

The authors declare that they have no competing interests.

Funding

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Authors' contributions

Richard Wismayer conceived the concept and proposal, collected data, performed data analysis and wrote the first draft. Julius Kiwanuka performed data analysis and provided statistical support. Michael Odida and Henry Wabinga interpreted all the immunohistochemical slides. Michael Odida, Henry Wabinga, Josephat Jombwe and Emmanuel Elobu performed critical reviews of the manuscript for intellectual content. All authors approved the final manuscript for publication.

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Abbreviations

CRC – colorectal cancer

LVI – lymphovascular invasion

AC – classical adenocarcinoma

CEA – carcinoembryonic antigen

MAC – mucinous adenocarcinoma

SRCC – signet ring colorectal carcinoma

DAB chromogen – 3,3' – Diaminobenzidine chromogen

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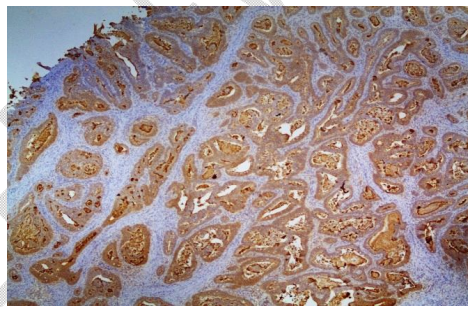
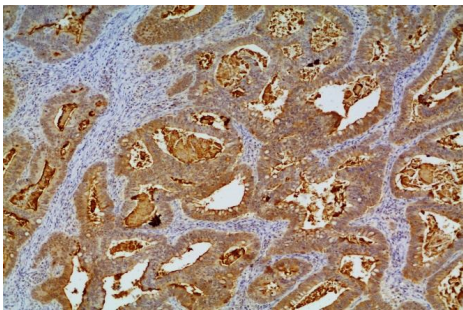
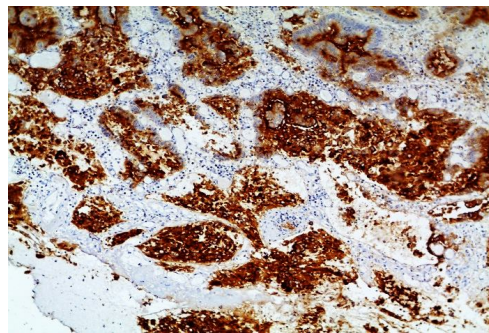
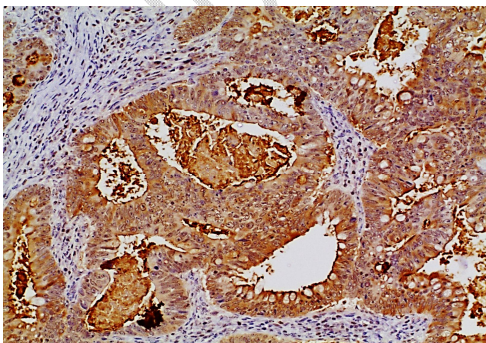


Figure 1: Carcinoembryonic antigen (CEA) distribution in colorectal cancer. Diffuse-cytoplasmic pattern of CEA with low intensity. Magnification x10.

Figure 2: Carcinoembryonic antigen (CEA) distribution in colorectal cancer. Diffuse-cytoplasmic pattern of CEA with low intensity. Magnification x4.



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Figure 3: Carcinoembryonic antigen (CEA) distribution in colorectal cancer. Diffuse-cytoplasmic pattern of CEA with low intensity. Magnification x20.

Figure 4: Carcinoembryonic antigen (CEA) distribution in colorectal cancer. Diffuse-cytoplasmic pattern of CEA with low intensity. Magnification x20.

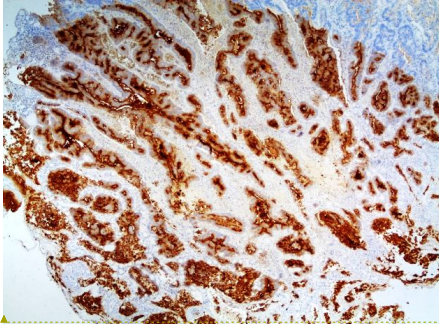


Figure 5: Carcinoembryonic antigen (CEA) distribution in colorectal cancer. Diffuse-cytoplasmic pattern of CEA with low intensity. Magnification x4.

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