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TABLE OF CONTENTS

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ORIGINAL ARTICLE

Immunohistochemical Study and Clinicopathologic Correlation of Cox-2 and Her-2 Expression in Colorectal Carcinoma: A 5-Year Retrospective Study

Étude Immunohistochimique et Corrélation Clinicopathologique de l'Expression de COX-2 et de Her-2 dans le Carcinome Colorectal : Étude Rétrospective Sur 5 Ans

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ABSTRACT

BACKGROUND: Colorectal cancer (CRC) is the fourth most common cancer in Nigeria, and it affects mostly persons in their middle age. In a bid to gain some insight into the molecular characteristics of CRC in our environment, we set out to investigate the expression of COX-2 and HER-2 among Nigerian subjects.

OBJECTIVES: To evaluate the expression of COX-2 and HER-2 and determine their correlation with clinicopathologic parameters in surgically resected histologically diagnosed cases of colorectal cancer.

METHODS: Fifty-three paraffin-embedded tissue blocks of colorectal resections and corresponding patient information were retrieved from the archives of the Anatomic and Molecular Pathology Department of Lagos University Teaching Hospital. A 4-micron slide section was obtained from each specimen and immunohistochemistry for COX-2 and HER-2 expression was performed.

RESULTS: Mean age of cases was 53.9years with an almost equal M:F ratio of 1.12:1. Half of the cases were moderately differentiated adenocarcinoma and 17% were high grade tumors. Eighty three percent of the tumours showed positive cytoplasmic COX-2 expression and extremely low membranous HER-2 positivity was observed in 2%. There was no significant correlation between COX-2 expression and age, gender, tumour location, tumour size, depth of invasion or lymph node status. However, COX-2 expression revealed a significant correlation with tumour grade (p= 0.013).

CONCLUSION: This study detects a high COX-2 and low HER-2 expression in colorectal cancer using immunohistochemistry, suggesting a possible role for COX-2 in CRC pathogenesis. This report should trigger further investigations of both markers vis-à-vis the management of CRC in our environment. **WAJM 2022; 39(11): 1134–1140.**

Keywords: Colorectal cancer, COX-2, HER-2, Adenocarcinoma, tumour, Immunohistochemistry.

RÉSUMÉ

CONTEXTE: Le cancer colorectal (CCR) est le quatrième cancer le plus fréquent au Nigeria et il touche surtout les personnes d'âge moyen. Dans le but de mieux comprendre les caractéristiques moléculaires du CCR dans notre environnement, nous avons entrepris d'étudier l'expression de COX-2 et de HER-2 chez les sujets nigérians. Objectifs : Évaluer l'expression de COX-2 et HER-2 et déterminer leur corrélation avec les paramètres clinicopathologiques dans les cas de cancer colorectal diagnostiqués histologiquement et réséqués chirurgicalement.

MÉTHODES: Cinquante-trois blocs de tissus inclus en paraffine provenant de résections colorectales et les informations correspondantes sur les patients ont été récupérés dans les archives du département de pathologie anatomique et moléculaire du Lagos University Teaching Hospital. Une section de lame de 4 microns a été obtenue de chaque spécimen et une immunohistochimie pour l'expression de COX-2 et HER-2 a été réalisée.

RÉSULTATS: L'âge moyen des cas était de 53,9 ans avec un rapport M:F presque égal de 1,12:1. La moitié des cas étaient des adénocarcinomes modérément différenciés et 17% des tumeurs de haut grade. Quatre-vingt-trois pour cent des tumeurs présentaient une expression cytoplasmique positive de la COX-2 et une positivité HER-2 membranaire extrêmement faible a été observée dans 2 % des cas. Il n'y avait pas de corrélation significative entre l'expression de la COX-2 et l'âge, le sexe, la localisation de la tumeur, la taille de la tumeur, la profondeur de l'invasion ou le statut des ganglions lymphatiques. Cependant, l'expression du COX-2 a révélé une corrélation significative avec le grade de la tumeur (p= 0,013).

CONCLUSION: Cette étude détecte une forte expression de COX-2 et une faible expression de HER-2 dans le cancer colorectal en utilisant l'immunohistochimie, suggérant un rôle possible de COX-2 dans la pathogenèse du CCR. Ce rapport devrait déclencher des investigations plus poussées des deux marqueurs vis-à-vis de la gestion du CRC dans notre environnement. **WAJM 2022; 39(11): 1134–1140.**

Mots clés: Cancer colorectal, COX-2, HER-2, adénocarcinome, tumeur, immunohistochimie.

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Abbreviations: CRC, Colorectal cancer.

INTRODUCTION

Colorectal cancer (CRC) is the third commonest cancer among men and the second most common cancer in women worldwide.[1] Approximately 1.8 million new cases of colorectal cancers, and 881,000 associated deaths, were documented worldwide according to the 2018 International Agency for Research on Cancer (IARC) reports.[1] As a disease, colorectal cancer is considered a marker of socioeconomic progress, because countries undergoing major economic breakthroughs tend to experience increasing incidence rates, which matches their rising human development index (HDI). Highest rates in the world are recorded in Australia/New Zealand and the lowest rates are found in Western Africa.[1]

In Nigeria, studies have reported increasing incidence of colorectal cancer over the past few decades.[2,3] Overall, colorectal cancer now ranks as the 4th most common cancer in Nigeria. It is the second most incident cancer of men and third most frequent in women in Nigeria.[4] Thus, colorectal cancer is fast becoming a serious disease of significant economic burden, as some researchers have reported a younger age of occurrence in Nigerian patients.[2,5] The molecular and premalignant factors responsible for development of CRCs in Africans, and by extension Nigerians are still largely inconclusive.[5] Cyclooxygenase-2 (COX-2) acting as a superoxidant, transforms arachidonic acid into prostaglandins - PGG2, and then into PGH2. Recent clinical and epidemiological studies have demonstrated the preventive effect of COX inhibitors on cancer, especially on colorectal cancer. Cellular and animal experimental studies have also indicated its relevance to tumour invasion, metastasis, apoptosis, cell cycle, immunity, and its role in the development of colorectal cancer.[6] HER-2 protein (also known as HER-2/neu, ErbB 2) is an 185 kDa transmembrane receptor tyrosine kinase that belongs to the four member family of epidermal growth factor receptors (EGFRs). High HER-2 overexpression correlates with poor survival, a phenomenon that has equally been well documented in breast and gastric cancers.[7] HER-2 has gained prominence as a therapeutic target in cancer treatment including the treatment of colorectal cancers, while COX-2 inhibitors are undergoing various stages of trials as potential anticancer agents. Due to their interaction at the molecular level, a potential synergistic effect has also been suggested for HER-2 and COX-2 inhibitors in the treatment of colorectal cancer.[8, 9]

Although many studies have been carried out on colorectal cancers in Nigeria, the frequency of COX-2 and HER-2 expression, as far as we know, are yet to be investigated among Nigerian patients with CRC. There is therefore a growing need to further our understanding of colorectal cancer development, especially its biologic behavior in relation to various clinicopathologic parameters of patients in Nigeria. The hope is that this will stimulate further research into their potential benefits in the management of patients.

MATERIALS AND METHODS

CRC cases identified from our departmental archival files between January 2014 and December 2018 constituted the data framework for the study. Tissue blocks were retrieved, and new H&E sections were cut to ascertain suitability of cases for the study. The immunohistochemistry was done in the Knapp Center for Biomedical Discovery at the University of Chicago, Illinois, United States. In the 5-year study, 93 CRC resection cases were identified but only 58 cases were suitable for the study. The remaining thirty-five cases were excluded either due to missing or damaged tissue blocks and incomplete clinical information.

Ethical approval for this study was obtained from the Health and Research Ethics Committee of Lagos University Teaching Hospital, Idi-Araba. Lagos.

Immunostaining Technique

Rabbit anti-human COX-2 (SP21) monoclonal antibody from Sigma Aldrich, USA, and rabbit anti-human HER-2 monoclonal antibody (Invitrogen) from Thermo Fisher Scientific, USA were used as primary antibodies. MACH 3 rabbit HRP polymer detection kit (Biocare Medical, USA) and DAB (diaminobenzidene) liquid chromogen (Biocare Medical, USA) were employed as colour development kit.

Representative sections were cut at 4 µm from each tissue block. These sections were preheated at 57°C for 45 minutes each was subsequently deparaffinized in two exchanges of xylene lasting 10 minutes each and then rehydrated in graded alcohol solution (100%, 90%, 90% & 70%). This was followed by heat induced epitope retrieval (HIER) with slides immersed in Diva Universal Decloaker solution (Biocare Medical USA) in a pressure cooker for 20 minutes. The slides were allowed to cool at room temperature for 20 minutes and then rinsed in Tris buffered saline (TBS). This step was followed by endogenous peroxidase activity blockade by applying hydrogen peroxide (H_2O_2) for 10 minutes. Protein blocking was done with background sniper (Biocare medical, USA) and followed by rinsing with TBS twice. The primary antibody (monoclonal anti-COX-2 or anti-HER-2 as the case may be) was then applied at dilution ratio of 1:300 and sections were incubated in a humidified chamber at 4°C overnight. The sections were washed in TBS 2–3 times for 3–5 minutes. Secondary antibody (MACH3 Rabbit probe, Biocare Medical USA) was applied, and sections were incubated at room temperature for 10 minutes. Each section was then washed in TBS 2 times for 3 minutes. This was followed by incubation with MACH3 (Rabbit) HRP-polymer for 10 minutes. A TBS wash step (2x for 3minutes) followed and Diaminobenzidine (DAB) chromogen was applied for 5minutes to allow for colour development. The slides were rinsed in distilled water once for 3 minutes and then immersed in 50% Gill's haematoxylin for 1 minute. A distilled water step followed for 5minutes. Slides were then subjected to bluing solution for 1minute, and distilled water rinse step followed for another 3minutes. This was followed by rinsing with distilled water and blueing after which sections were then subjected to dehydrations steps (in Ethanol 70%, 95%, 95%, and 100%) followed by Xylene (2x for 3minutes each).

Sections of normal renal tissue were used as positive external control for COX-

2 using the same dilution ratio of 1:300. In the case of HER-2, sections of confirmed HER-2 positive breast cancer case were used as a positive control. To ensure biopsy tissue were antigen viable, we carried out vimentin IHC on the cases using the same protocol described. Cases evaluated as negative for vimentin, COX-2 and HER-2 were considered not antigen viable and therefore not eligible for the study. Five cases out of the initial 58 were therefore excluded from the study.

Immunostaining Evaluation

COX-2 Immunoreactivity Score

Cytoplasmic staining was examined under light microscope in five random high-power fields (x40 magnification). We employed the method used by Masunaga et al. to assign immunoreactivity scores (IRS) to COX-2 stained cases.[10] The method employed a semi-quantitative scoring system by estimating the average percentage of stained cells and the most predominant staining intensity score (from 1, 2 and 3) to calculate combined COX-2-IRS. Intensity was graded as: 1 = low, 2 = intermediate, 3 = high. The percentage of cells stained was scored as follows: 0 = 0%, 1 = 1–25%, 2 = 26–50%, 3 = 51–75%, 4 = 76–100%.

A combined COX-2-IRS score was calculated as the sum of staining intensity and percentage of stained cells, and immunostaining was regarded as negative (–), combined score = 0–1; weakly positive (+), combined score = 2–3; moderately positive (++), combined score = 4–5; strongly positive (+++), combined score = 6–7. Overall, the scores were further categorized into two groups; scores of between 0–1 as negative and 2–7 as positive for ease of interpretation and statistical analysis.

HER-2 Immunoreactivity Score

HER-2 IR scoring was performed according to the CAP guidelines for breast cancer.[11]

Data Analysis/Statistical Methods

Collected data were entered into Microsoft Excel spreadsheets and data analysis was performed using STATA IC 15.1 version (TX, USA). Data were summarized using descriptive statistics, using mean, median, and standard deviation for numerical variables and frequency and percentage for categorical variables. Chi-square test was used to detect association between COX-2 and HER-2 expression. Chi-square and fisher's exact test were also used to test relationship between both markers and clinicopathologic parameters (age, sex, tumor size, tumor grade, depth of invasion (pT stage) & lymph node involvement) to obtain a 2-tailed p-value. $P < 0.05$ was considered statistically significant.

RESULTS

Clinicopathologic Characteristics of CRC

Fifty-three cases of resected colorectal cancer that met the inclusion criteria were included in the study. The mean age of all cases was 53.9 years (Median = 56.0 years, range = 17–82 years, SD = 15.4 years). More than a quarter (37.3%) of all the cases were 50years or younger. The male to female ratio was almost equal at 1.12:1.

Majority (30, 56.6%) of the colorectal cancer cases were right-sided tumours, left sided tumors accounted for 28.3% of the cases with only about 11.3% involving the rectum. Most of the cancers (25/46, 54.4%) were larger than 5cm in their greatest dimensions.

Fifty-one percent of the colorectal cancers were moderately differentiated while well and poorly differentiated tumours constituted 32% and 17% of all cases, respectively. Thirteen percent of the 53 specimens were mucinous carcinomas and all but one was located on the right side.

About 62% of CRCs were pT3 tumours, with pT4 tumours constituting 18% of cases. The depth of tumor invasion was not reported in nine (9) cases. There was lymph node involvement in 58% of cases. Lymph node status was not reported in 15 cases.

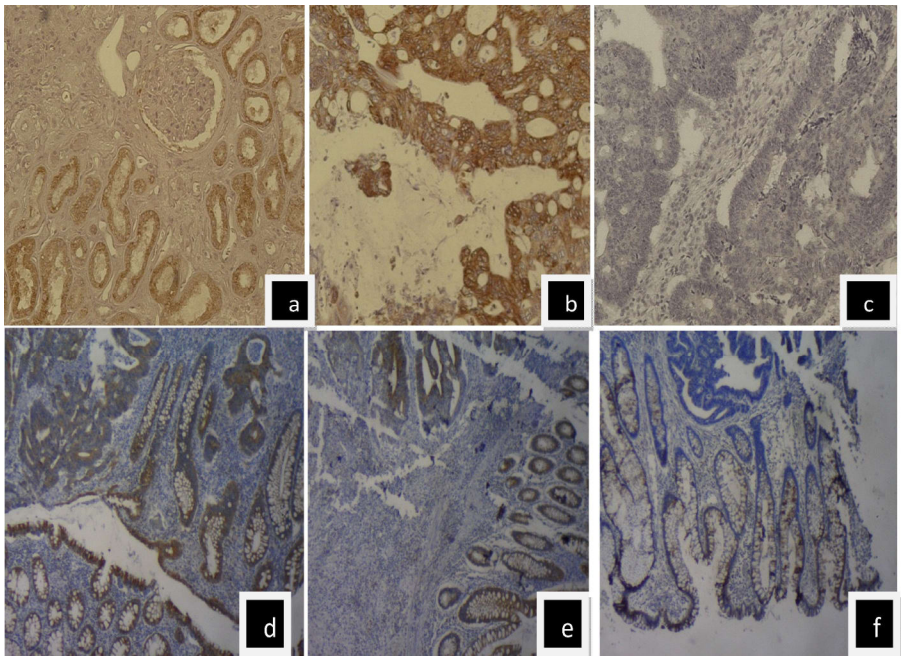

Fig. 2: Photomicrographs of COX-2 immunohistochemistry [a]COX-2 positive normal renal tubular cells; positive control at x400 magnification, [b] a COX-2 positive colorectal cancer; strong diffuse cytoplasmic staining at x400 magnification, [c] a COX-2 negative colorectal cancer at x400 magnification, [d] a case of colorectal cancer showing strong diffuse COX-2 cytoplasmic stain in both tumour cells and adjacent normal appearing colonic epithelial cells at x400 magnification [e]a case of colorectal cancer showing moderate diffuse COX-2 cytoplasmic stain in both tumour cells and strong cytoplasmic stain in adjacent normal appearing colonic epithelial cells at x400 magnification [f]a COX-2 negative colorectal cancer case with diffuse moderate cytoplasmic stain in adjacent normal appearing colonic epithelial cells at x400 magnification.

Table 1: Age and Gender Distribution of Colorectal Cancer seen in LUTH

Characteristics	Number of Cases (%)
Age(years)	
10 – 20	1(1.96)
21 – 30	6(11.8)
31 – 40	4(7.8)
41 – 50	8(15.7)
51 – 60	16(31.4)
61 – 70	10(19.6)
71 – 80	5(9.8)
>80	1(1.96)
Not recorded	2
Mean age (years) 53.9 (SD= 15.4, Median =56.0)	
Sex	
Male	28(52.8)
Female	25(47.2)

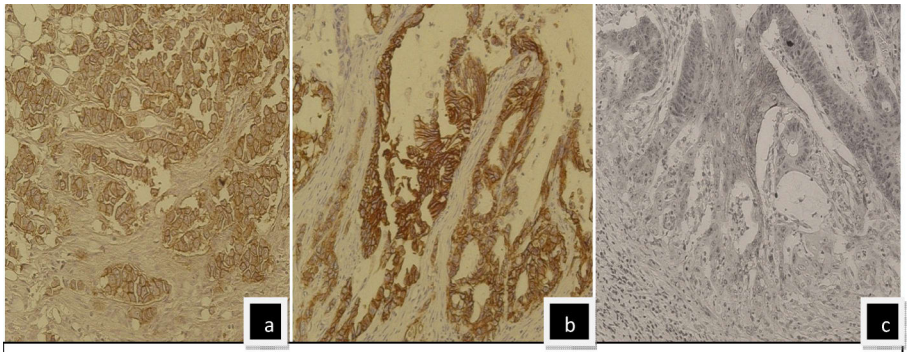

Fig. 2: Photomicrographs of a HER-2 immunohistochemistry [a] HER-2 positive Invasive ductal carcinoma; positive control at x400 magnification, [b] a HER-2 positive colorectal cancer; strong membranous (and some cytoplasmic) staining at x400 magnification, [c] a HER-2 negative colorectal cancer at x400 magnification.

Table 2: Pathologic Characteristics of Colorectal Cancers

Characteristics	Number of Cases (%)
Tumour Site	
Right colon cancer (RCC)	30(56.6)
Left colon cancer (LCC)	15(28.3)
Rectal Cancer (RecC)	6(11.3)
Not recorded	2
Specific Anatomic Location	
Caecum	10(21.3)
Ascending colon	8(17.0)
Transverse colon	10(21.3)
Descending colon	4(8.5)
Sigmoid	9(19.2)
Rectum	6(12.8)
Not recorded	6
Tumuor Size (cm)	
≤5cm	21(45.7)
>5cm	25(54.4)
Not recorded	7
Tumour Grade	
Well differentiated Adenocarcinoma	17(32.1)
Moderately differentiated adenocarcinoma	27(50.9)
Poorly differentiated/ high grade adenocarcinoma	9(17.0)
Depth of Invasion (pT Stage)	
pT1	2(4.6)
pT2	7(15.9)
pT3	27(61.4)
pT4	8(18.2)
Not recorded	9
Lymph Node Involvement	
Positive	22(57.9)
Negative	16(42.1)
Not recorded	15

RCC, Right Colon Cancer; LCC, Left Colon Cancer; RecC, Rectal Cancer.

COX-2 Expression in Colorectal Cancer

COX-2 positive colorectal carcinoma cells showed brownish cytoplasmic granules (Figure 2). Overall, COX-2 expression was observed in 83.0% (44/53) of CRC specimens. Of the 44 positive tumours, 59.1% (26/44) showed strong intense cytoplasmic staining, 26.4% showed moderate positivity and 14.5% were weakly positive. About 17.0% were negative for COX-2. Twenty-four of the 53 cases had adjacent non-cancerous colonic epithelial cells, 95% (23/24) showed some cytoplasmic COX-2 expression. Twelve (50%) of the 23 showed strong cytoplasmic COX-2 staining and nine out of these equally showed strong cytoplasmic COX-2 expression in tumour cells (Figure 1). Two cases had weak expression of COX-2 in tumour cells and strong expression in adjacent normal appearing colonic epithelial cells.

COX-2 Expression and Clinicopathologic Parameters

Correlation of COX-2 with clinicopathologic parameters showed that only tumour grade/differentiation showed significant association with COX-2 overexpression (χ^2 = 6.947, p = 0.031, Fisher's exact p = 0.019). There was no evidence of association with tumour size observed (χ^2=1.665, p=0.197). There was no significant association between COX-2 expression in colorectal cancers cells and patients age (χ^2= 0.610, p=0.435), sex (χ^2=0.833, p=0.361), tumour location (χ^2=1.350, p=0.509), pT stage (χ^2=0.195 p=0.659) and lymph node involvement (χ^2=0.225, p=0.635). The relationship

between COX-2 expression and clinicopathologic parameters are shown in Table 3.

HER-2 Expression in Colorectal Cancer

HER-2 positive colorectal cells showed brownish membranous staining (Figure 2). HER-2 overexpression was observed in only 1.89% (1/53) of all cases with strong membranous positivity. The case was a right-sided $pT_2N_2M_x$ moderately differentiated adenocarcinoma in a 29-year-old female. This case was also strongly positive for COX-2 protein.

younger than or aged 50 years. The youngest patient in the present study was a 17-year-old with mucinous carcinoma. Irabor, *et al* in Ibadan reported that about 48% of patients with rectal cancer were younger than 50 years.[5] Studies done in Nigeria have reported a male preponderance.[2,13,14] The present study found an almost equal male to female ratio of 1.12:1.1 This is contrast to the male to female ratio of 1.3:1, 1.5:1 and 1.64:1.04 reported by Rotimi, *et al,* Ibrahim, *et al* and Saluja, *et al* but is similar to the 1:1.14 in a report by Irabor, *et al* in 2014.[2,5,13,14]

high cytoplasmic COX-2 in cancerous cells and also found variable levels of expressions in adjacent noncancerous colonic cells. Wu QB, *et al* similarly reported that about 12% of 50 CRC cases exhibited COX-2 over-expression in normal colonic epithelium adjacent to tumour cells.[8] Whether this signifies a subcellular premalignant alteration in these normal appearing cells cannot be explained in the present study. That this is likely the case is attested to in the results of some experimental studies that reported upregulation of COX-2 gene in

Table 3: Relationship between COX-2 Expression and Clinicopathologic Parameters

Variables	COX-2			Pearson χ^2	P-value	Fisher's Exact (2-sided)
	N	Positive n (%)	Negative n (%)			
Age (years)				0.610	0.435	0.694
≤50	19	17(89.5)	2(10.5)			
>50	32	26(81.8)	6(18.8)			
Sex				0.833	0.361	0.474
Male	28	22(78.6)	6(21.4)			
Female	25	22(88.0)	3(12.0)			
Tumor Size (cm)				1.665	0.197	0.260
<5	21	19(90.5)	2(9.5)			
≥5	25	19(76.0)	5(24.0)			
Tumor Site				1.350	0.509	0.742
RCC	30	25(83.3)	5(16.7)			
LCC	15	12(80.0)	3(20.0)			
RecC	6	6(100.0)	0(0.0)			
Tumor Grade/Differentiation				6.947	0.031	0.019
Well Differentiated	17	26(96.30)	5(29.4)			
Moderate differentiated	27	12(70.6)	1(3.70)			
Poorly differentiated	9	6(66.7)	3(33.3)			
pT Stage				0.195	0.659	1.000
pT1/ pT2	9	8(89.0)	1(11.1)			
pT3/ pT4	35	29(82.9)	6(17.1)			
Lymph Node Involvement				0.225	0.635	1.000
Positive	22	18(81.8)	4(18.2)			
Negative	16	14(87.5)	2(12.5)			

DISCUSSION

The incidence of CRC has been on the rise in Nigeria and it appears to be more common amongst middle aged young persons.[2,5,12,13] The present study concurs with most previous works in Nigeria since it reports the mean age of occurrence of CRC to be 53.8 years and a median of 56 years.[2,13,14] There is significant involvement of young patients as more than a third of the cases (37%) were

Several studies have reported that COX-2 is overexpressed in colorectal cancer tumour tissue compared to the non-neoplastic colonic mucosa.[6,15–17] Negi, *et al* used immunohistochemistry, and real time qPCR to quantify COX-2 mRNA in colorectal cancer cells and adjacent normal colonic cells. With both methods, they demonstrated a higher expression in colonic cancer cells.[18] However; the current study observed a

normal mucosa appearing cells in APC^{min}mice.[19] In the same study, normal appearing colonic epithelial cells in human colorectal cancer cases following resection were found to have COX-2 gene upregulation irrespective of distance from the primary tumour. The study reported that COX-2 gene was one of several genes that were upregulated to levels more than 200-folds compared to normal colonic epithelial cells from matched patients without CRC.[19]

Despite the availability of numerous studies on this subject, research examining the role of COX-2 in CRC is somewhat limited in Nigeria. COX-2 expression (cytoplasmic) was recorded in 83.0% of colorectal cancers in this study. This level of expression is largely in consonance with most of the previous studies in which very high expression of COX-2 were reported in CRC.[6,8,20–22] In two separate studies carried out by Fux et al and Wu, *et al* more than a decade ago, Fux and colleagues examined 747 cases while Wu and colleagues investigated 170 cases of CRC; both groups reported COX-2 expression levels to be as high as 85%.[6,20] Venkatachala, *et al* in a recent study in 2016 involving 65 cases in India reported expression levels of 87% in colorectal carcinoma.[21]

There is variation in the frequency of expressions reported amongst authors in different studies. For instance, Tomozawa, *et al* reported that only 20.6% of the sixty-three cases they evaluated had COX-2 expression.[23] The differences in the proportion reported might be due to variations in sample size, use of different antibody clones and diversity of IHC scoring methods. Despite these variations, what remains constant however as evident in a meta-analysis of 24 publications by Peng et al is that many of the studies recorded COX-2 expression levels of greater than 50% in CRC.[17] Similar to the finding of Venkatachala, *et al* who reported that of their 65 cases, about 68% showed very strong expression of COX-2; nearly 50% of our cases had very strong expression of the protein.[21] The result of our study would imply that in terms of pathways involving COX-2 in colorectal cancer biology, our cases might not be too different from colorectal tumours from other regions of the world.

In this study, it was shown that tumour size was not associated with COX-2 expression in colorectal carcinoma. Thus, this finding corroborates the report of other studies that equally found no relationship between tumor size and COX-2 overexpression.[21–23] Also, there was no correlation between tumour location and COX-2 expression. The present study showed that majority of the tumours (30 cases) were in the right colon and 90% of these expressed COX-2. Ninety percent of the remaining twenty-one cases of CRC were positive for COX-2, and all six tumours located in the rectum were amongst them. COX-2 expression was associated with tumour grade or degree of tumour differentiation. This is a common trend in most reports reviewed regarding COX-2 expression in CRC. In line with this observation, Wu, and colleagues, observed a significant relationship between COX-2 positivity and degree of differentiation.[8] About half (27/53, 51%) of the colorectal cancers examined in the present study were grade 2 tumours, that exhibited moderate differentiation and 96% of them had COX-2 overexpression. Roelofs *et al* and Al-Maghrabi, *et al* made similar observations in their studies.[24,25] Whether there is statistical association between COX-2 expression and tumour grade or not, one constant finding in all these studies is that moderately differentiated tumours consistently showed the highest proportion of COX-2 positivity. Despite these findings, it is important to acknowledge that COX-2 overexpression has been shown to confer a more aggressive behavior in colorectal cancer.[26] In this study, all mucinous carcinomas; (7/53, 13.2%), were classified as high-grade tumours and they all showed moderate to strong cytoplasmic overexpression of COX-2. This agrees with the reports of Ventakachala and colleagues, who reported that all five cases of mucinous carcinomas in their study showed high cytoplasmic expression of COX-2.[21]

Regarding depth of tumour invasion (pT), our study revealed no significant correlation between depth of invasion and COX-2 positivity. Tumour invasiveness and metastasis are believed to be induced by COX-2 via its ability to upregulate the expression of matrix metalloproteinases (MMPs), especially MMP-2. Tsujii and colleagues found in an *in-vivo* experiment that COX-2 expressing human colon cancer cells were six times more invasive compared to COX-2 negative cells.[27] In the same vein, bearing in mind that depth of invasion, lymph node status and metastases are the three factors considered in staging; several studies have therefore reported higher COX-2 expression with advanced stage CRC.[8,10,21,25] The same studies found significant relationship between lymph node involvement and COX-2 positivity. The lack of association between depth of invasion and COX-2 expression in the present study contrasts greatly with observations in some earlier studies.[8,10] Our report was limited by sample size, which was further complicated by the fact that of the 53 cases; depth of invasion was only reported in 38 cases. A similar situation holds for lymph node involvement in the index study. This could explain the deviation from the reports of other studies.

Molecular interactions between COX-2 and HER-2 have been demonstrated in studies of CRCs.[9,28] HER-2 is known to be overexpressed in a subset of colorectal cancers; 0–15% on the average, when membranous expression is evaluated and 0–66% if cytoplasmic expression is considered. However, like in breast cancer, membranous expression is believed to be responsible for its contribution in colorectal carcinogenesis.[29]

The current study could not establish a correlation between the two markers since only a single case had HER-2 overexpression. Studies have demonstrated that only a small proportion of CRCs express HER-2. In a recent study, Richman and colleagues observed membranous HER-2 expression in 1.3% of 1914 stage II-III tumours and in 2.2% of 1342 stage IV tumours.[30] A review article by Blok *et al* on the topic of HER-2 expression in colorectal cancers also confirms that a small proportion of CRCs express membranous HER-2.[29] HER-2 expression has implications for patient management and outcome. For example, the five-year survival rate of patients with HER-2 expression was lower compared to those with HER-2 negative tumours.[8] The proportion in our study though small, represents a sizeable number in a disease group with increasing incidence in Nigeria. Moreover, since results of clinical trials have only shown the benefits of anti-HER-2 in metastatic or advanced CRCs; it therefore means that not all patients with CRC will require screening for HER-2 positivity.

In conclusion, the present study found a high frequency of COX-2 expression and a low proportion of HER-2 expression in colorectal carcinomas. Of all the evaluated clinicopathologic characteristics, only tumour grade had a significant relationship with COX-2 expression. Overall, our finding though limited constitutes a significant step towards understanding the molecular biology of colorectal cancers in our environment.

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Conflict of Interest

None declared.

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