Microsatellite Instability Is Common in Colorectal Cancer in Native Nigerians

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Abstract. *Background: Colorectal cancer (CRC) is the fifth most common cancer in Africa, with significant differences in incidence, biology and clinical behavior from other populations. Materials and Methods: We studied prevalence and clinicopathological features of microsatellite instability (MSI) and young onset CRC in 83 archival samples from the University of Ibadan, Nigeria. Results: Nigerian cases of CRC were MSI-high in 43% and MSI-high CRC had significantly lower histological heterogeneity than microsatellite-stable CRC (20% vs. 55% respectively, p=0.046). Presence of signet ring cell differentiation (10-50% of tumor) was significantly higher in younger patients with CRC (<50 years) (odds ratio(OR)=5.93, 95% confidence interval(CI)=1.17-29.95, p=0.038). Poor differentiation (34%), invasive growth (96%), and high prevalence of mucinous (10%) and signet ring cell adenocarcinomas (4%) were among distinct features of Nigerian patients with CRC. Conclusion: MSI-high CRC is more common in West Africa and more detailed molecular and genetic analysis is warranted as CRC incidence and mortality continue to increase in the Sub-Saharan Africa.*

Once a rare cancer type, colorectal cancer (CRC) is now the fifth most common cancer in Africa (1). However, there are

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significant differences in incidence rates, tumor biology, and clinical behavior in comparison to other populations. While the total incidence rates are about one-tenth of those in the developed world, CRC occurs at a younger age in Nigerians and rapidly metastasizes (2). The mortality from CRC in Sub-Saharan Africa may be considered as very high, since many patients are diagnosed at late stages and up to 50% of them do not return for surgical treatment (3). Molecular pathology and genetics of CRC in Africa have not been comprehensively studied. It has been demonstrated that some molecular characteristics of CRC in patients of African descent are different from those in other populations. For example, we have shown distinctive clinicopathological pattern and high prevalence of CRC with high microsatellite instability (MSI-high) (41%) in Ghanaian patients (4) compared to 15% in Whites (5). In addition, a unique set of somatic mutations in CRC has been also identified in African–American patients (6). Analysis of genetic and molecular biomarkers, including MSI, is aimed at better understanding CRC biology, as well as improving diagnosis, treatment, and prognosis of CRC in Africa. Our study performed analysis of pathological features and MSI in a series of archival paraffin-embedded CRC samples from the Department of Pathology at the University of Ibadan in Nigeria. The goal of the study was to investigate prevalence and clinicopathological features of MSI-high and young-onset CRC in Nigeria.

Materials and Methods

Study samples. Archived CRC paraffin-embedded tissue blocks (n=114) were obtained from the Pathology Department of the College of Medicine at the University of Ibadan and private pathology laboratories in the city of Ibadan. The study was

Table I. *Demographic and clinicopathological characteristics of Nigerian colorectal cancer samples by age and microsatellite status.*

		All samples (n=83)	Age at diagnosis		p-Value	MSS/MSI-low (n=20)	MSI-high (n=15)	p-Value
			>50 Years (n=45)	≤50 Years (n=37)				
Age at diagnosis, mean±SD, years		56±14.6	64±8.1	39±7.7	<0.0001	60±16.2	53±15.3	0.2438
Age at diagnosis, median, years		53 (16-85)	63 (51-85)	40 (16-50)	-	64 (30-85)	55 (19-79)	-
MSI	MSS/MSI-L	20 (57%)	15 (65%)	5 (45%)	0.2733	-	-	-
	MSI-H	15 (43%)	8 (35%)	6 (55%)				
Sex	Female	43 (52%)	20 (44%)	23 (62%)	0.1099	10 (50%)	5 (33%)	0.4916
	Male	40 (48%)	25 (56%)	14 (38%)		10 (50%)	10 (67%)	
Tumor location	Proximal colon	15 (23%)	11 (32%)	5 (15%)	0.2912	4 (27%)	4 (27%)	1.0000
	Distal colon	7 (11%)	3 (9%)	4 (13%)		3 (20%)	2 (13%)	
	Rectum	43 (66%)	20 (59%)	23 (72%)		8 (53%)	9 (60%)	
	Colon, unspecified	16	11	5		5	-	
Stage	Stage I	1 (4%)	1 (7%)	-	0.1531	-	-	1.0000
	Stage II	10 (37%)	6 (40%)	4 (33%)		4 (57%)	1 (100%)	
	Stage III	8 (30%)	6 (40%)	2 (17%)		3 (43%)	-	
	Stage IV	8 (30%)	2 (13%)	6 (50%)		-	-	
	Unknown	56	30	25		13	14	
Specimen type	Resection	54 (65%)	15 (34%)	11 (31%)	0.8025	14 (70%)	8 (62%)	0.3126
	Biopsy	26 (31%)	29 (66%)	24 (69%)		6 (30%)	7 (58%)	
	Unknown	3 (4%)	-	-		-	-	
Histological type	Adenocarcinoma, NOS	63 (76%)	36 (82%)	26 (70%)	0.4123	15 (75%)	11 (73%)	1.0000
	Medullary adenocarcinoma	2 (2%)	-	2 (5%)		1 (5%)	-	
	Mucinous adenocarcinoma	8 (10%)	5 (11%)	3 (8%)		3 (15%)	2 (13%)	
	Signet ring cell carcinoma	3 (4%)	1 (2%)	2 (5%)		1 (5%)	1 (7%)	
	Other	7 (8%)	3 (7%)	4 (12%)		-	1 (7%)	
Tumor grade	Well-differentiated	4 (5%)	3 (6%)	1 (3%)	0.2771	11 (55%)	8 (53%)	0.7704
	Moderately differentiated	51 (61%)	30 (67%)	20 (54%)		8 (40%)	5 (33%)	
	Poorly differentiated	28 (34%)	12 (27%)	16 (43%)		1 (5%)	2 (13%)	
Mucinous differentiation	No	56 (68%)	31 (69%)	25 (68%)	0.8982	12 (60%)	10 (67%)	0.7372
	Yes	27 (32%)	14 (31%)	12 (32%)		8 (40%)	5 (33%)	
Signet ring cells	No	73 (88%)	43 (96%)	29 (78%)	0.0375	17 (85%)	13 (87%)	1.0000
	Yes	10 (12%)	2 (4%)	8 (22%)		3 (15%)	2 (13%)	
Medullary differentiation	No	81 (86%)	44 (98%)	35 (95%)	0.5864	19 (95%)	15 (100%)	1.0000
	Yes	3 (4%)	1 (2%)	2 (5%)		1 (5%)	-	
Micropapillary component	No	76 (94%)	42 (96%)	34 (92%)	0.6557	18 (95%)	14 (93%)	1.0000
	Yes	5 (6%)	2 (4%)	3 (8%)		1 (5%)	1 (7%)	
	Unknown	1	1	-		1	-	
Crohn-like response	None	39 (56%)	20 (54%)	19 (59%)	0.5416	10 (59%)	5 (38%)	0.2685
	Mild to moderate	30 (42%)	17 (46%)	12 (38%)		6 (35%)	8 (62%)	
	Marked	1 (1%)	-	1 (3%)		1 (6%)	-	
	Unknown	13	8	5		3	2	
Histological heterogeneity	No	56 (68%)	33 (73%)	22 (61%)	0.2417	9 (45%)	12 (80%)	0.0461
	Yes	26 (32%)	12 (27%)	14 (59%)		11 (55%)	3 (20%)	
	Unknown	1	-	-		-	-	
Garland-like necrosis	No	43 (52%)	26 (58%)	17 (46%)	0.5044	10 (50%)	8 (53%)	0.1077
	Yes	20 (24%)	9 (20%)	11 (30%)		2 (10%)	5 (33%)	
	Focal	20 (24%)	10 (22%)	9 (24%)		8 (40%)	2 (14%)	
Growth pattern	Invasive	52 (75%)	28 (74%)	23 (74%)	1.0000	15 (79%)	10 (72%)	0.8367
	Expansive and invasive	15 (21%)	9 (24%)	7 (23%)		3 (16%)	3 (21%)	
	Expansive	3 (4%)	1 (2%)	1 (3%)		1 (5%)	1 (7%)	
	Unknown	13	7	6		1	1	
TILs	None	66 (80%)	36 (80%)	30 (81%)	0.8417	15 (75%)	10 (67%)	0.8628
	Mild to moderate (2/HPF)	7 (8%)	4 (9%)	2 (5%)		2 (10%)	3 (20%)	
	Marked (3/HPF)	10 (12%)	5 (11%)	5 (14%)		3 (15%)	2 (13%)	
LVI	No	51 (65%)	28 (65%)	23 (66%)	0.9560	11 (58%)	10 (67%)	0.7282
	Yes	28 (35%)	15 (35%)	12 (34%)		8 (42%)	5 (33%)	
	Unknown	4	2	2		1	-	

Table I. *Continued*

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		All samples (n=83)	Age at diagnosis		*p*-Value	MSS/ MSI-low (n=20)	MSI- high (n=15)	*p*-Value
			>50 Years (n=45)	≤50 Years (n=37)				
PNI	No	74 (94%)	40 (93%)	33 (94%)	1.0000	17 (89%)	14 (93%)	1.0000
	Yes	5 (6%)	3 (7%)	2 (6%)		2 (11%)	1 (7%)	
	Unknown	4	2	2		1	-	
Residual adenoma	No	42 (75%)	20 (89%)	21 (62%)	0.5889	17 (89%)	8 (62%)	0.4434
	Yes	14 (25%)	8 (11%)	6 (38%)		2 (11%)	5 (38%)	
	Unknown	27	17	10		1	2	
Tumor budding	No	52 (70%)	25 (63%)	26 (78%)	0.1312	12 (67%)	9 (60%)	0.6918
	Yes	22 (30%)	15 (37%)	7 (22%)		6 (33%)	6 (40%)	
	Unknown	9	5	4		2	-	
EMT-like response	No	51 (69%)	27 (66%)	23 (72%)	0.5827	12 (67%)	10 (67%)	1.0000
	Yes	23 (31%)	14 (34%)	9 (28%)		6 (33%)	5 (33%)	
	Unknown	9	5	4		2	-	
Treatment response	No	80 (98%)	44 (98%)	35 (97%)	1.0000	20 (100%)	15 (100%)	-
	Yes	2 (2%)	1 (2%)	1 (3%)		-	-	
	Unknown	1	-	1		-	-	
Angiolymphatic invasion	No	52 (67%)	28 (67%)	23 (66%)	0.9299	11 (58%)	11 (79%)	0.2783
	Yes	26 (33%)	14 (33%)	12 (34%)		8 (42%)	3 (21%)	
	Unknown	5	3	2		1	1	

TILs: Tumor-infiltrating lymphocytes, HPF: high-power field, LVI: lymphovascular invasion, PNI: perineural invasion, EMT: epithelial–mesenchymal transition.

approved by Institutional Review Boards at both the University of Ibadan and Vanderbilt University (IRB# 140219). The blocks were made from biospecimens collected from 2007 to 2014. Following pathology review, we removed mislabeled samples (no CRC), duplicate samples, and samples that did not have enough material for analysis. The remaining 83 blocks were used for pathological and molecular analysis. These biospecimens originated from resection in 54 (65%) cases, biopsy in 26 (31%), and unknown source in three (4%).

Pathology review. Pathology review was performed at the University of Ibadan and Vanderbilt University and all discrepancies were resolved. Histological slides were reviewed at Vanderbilt for histological type, tumor grade, Crohn-like response, histological heterogeneity, garland-like necrosis, growth pattern, tumor Infiltrating Lymphocytes (TILs), lymphovascular invasion (LVI), residual adenoma, and treatment response. Histological type was determined based on the WHO criteria (7). For tumors with certain differentiation but not meeting the WHO criteria, the percentage of the differentiation was recorded. Histological heterogeneity was determined by significant presence of two histological types (>5%). Quality of hematoxylin and eosin (H&E) staining was also assessed.

MSI testing. MSI was tested using the protocol developed in our laboratory and described in detail elsewhere (8). Briefly, we compared the length and pattern of 10 amplified, fluorescently labeled mono- and dinucleotide microsatellite markers (BAT26, BAT25, β-catenin, D10S197, BAT40, D18S58, D2S123, TGFβ, D5S346, and D17S250) in tumor and normal adjacent tissue. Only tumors with a minimum of three available markers including at least one mononucleotide

marker were considered for analysis. Tumors with ≥30% unstable markers were considered MSI-high, <30% as MSI-low, and tumors with all stable markers as microsatellite stable (MSS).

Statistical analysis. All statistical analyses were performed using SAS 9.4 (SAS Institute, Cary, NC, USA). Univariate analysis of continuous variables was performed using *t*-test, and categorical variables were analyzed using chi-square and Fisher's exact tests. Pearson correlation coefficient was used to measure linear relationship between two variables. In all analyses, *p*-values of less than 0.05 were considered statistically significant.

Results

Our Nigerian sample had almost equal numbers of males and females (48% and 52%) with a median age at diagnosis of 53 years (from 16 to 85 years) (Table I). Tumor location distribution in our unselected sample was significantly different from that observed in the USA. The Nigerian sample had an almost four-fold number of rectal cancer cases [66% *vs*. 18% in the USA (9)], while the number of distal colon adenocarcinomas was three times lower in Nigerian patients [11% *vs*. 35% in the US (9)]. Stage information was only available for 27 patients, since many patients had no data on metastases, probably due to lack of follow-up. Close to 60% of the patients had stage III or IV CRC. Almost 34% (28/83) of CRC in Nigerians were poorly differentiated. Mucinous adenocarcinomas were also more common in Nigerians

Table II. *Frequency of tumors according to microsatellite instability status in Native Africans and African–Americans from a number of studies.*

MSI status	Ethnicity						
	Nigeria*	Ghana (4)	African-Americans (19)	African-Americans (20)	African-Americans (21)	African-Americans (31)	African-Americans (22)
MSS/MSI-low	20 (57%)	41 (59%)	12 (55%)	29 (57%)	178 (81.2%)	66 (69%)	170 (85%)
MSI-high	15 (43%)	29 (41%)	10 (45%)	22 (43%)	44 (19.8%)	29 (31%)	29 (15%)

MSI: Microsatellite instability; MSS: microsatellite stable; *Present study.

(10%) compared to the US populations (6.8% in Whites and 5.4% in Blacks) (10). Signet ring cell carcinomas were found in 4% (3/76) of all histological types. Overall, 96% of CRCs had an invasive or invasive/expansive growth pattern, including all mucinous and signet ring carcinomas. Tumor-infiltrating lymphocytes (TILs) were found in 20% of CRCs and Crohn-like response (CLR) was observed in 44% of the cases. LVI was observed in 35% of all CRCs.

Analysis of patients with CRC diagnosed at 50 years or younger showed higher prevalence of stage IV disease (50% *vs*. 13%) and poorly differentiated tumors (43% *vs*. 27%), although overall differences were not statistically significant (Table I). Presence of signet ring cell differentiation (10-50% of tumor) was significantly higher in CRC diagnosed before age 50 years (odds ratio=5.93, 95% confidence interval=1.17-29.95, p=0.038), although these cases could not be classified as signet ring cell carcinomas, which requires >50% of signet ring cells present. Garland-like necrosis occurred in 24% of all CRCs; 20% in those patients diagnosed after 50 years, and 30% in those diagnosed below 50 years.

We found MSI-high in 43% (15/35) of all CRCs in Nigerians, which is comparable with our previous findings in Ghana (41%) (4) (Table II). MSI-high CRC had significantly lower histological heterogeneity than MSS CRC (20% *vs*. 55% respectively, p=0.046) (Table I). TILs and CLR were more common in MSI-high compared to MSS CRCs (33% and 62% *vs*. 25% and 41%, respectively), although not statistically significantly. There were no other significant differences in clinicopathological features, including tumor location, between MSI-high and MSS CRCs. Although not statistically significant, we observed more MSI-high CRC in patients younger than 50 years (55% *vs*. 35% in older patients).

Discussion

One of the most prominent characteristics of CRC in native Nigerians is that the majority of the patients present with advanced disease (11). Some researchers have declared that this is not necessarily due to late presentation, but rather more to rapid progression of an aggressive tumor type (12). Higher prevalence of younger patients and scanty knowledge of the molecular biomarkers of CRC in Sub-Saharan Africans also contribute to the challenges in clinical management of CRC in Nigeria (13).

Almost 34% (28/83) of CRCs were poorly differentiated adenocarcinomas and more significantly, supporting the aggressive nature of these tumors, 96% of them had an invasive growth pattern. This rate is much higher than the 18.5% recorded in the USA. This probably explains the twice more common incidence of distant metastases (stage IV) in Nigerian patients (30% *vs*. 15.4% in the USA) (9). High prevalence of poorly differentiated adenocarcinoma in Nigerian patients is probably also facilitated by the absence of CLR and TILs in 56% and 80% of CRCs, respectively. Studies have shown that lymphocytic reactions to tumor (TILs and CLR) are associated with improved prognosis among patients with CRC, regardless of lymph node involvement and other clinical, pathological, and molecular characteristics (14, 15). In addition, LVI in 35% of CRCs in Nigerians correlated with poor differentiation of 34% of CRCs (Pearson p<0.0001) and is considered to be a poor prognostic factor leading to lymph node and systemic metastases (16).

A few molecular studies on CRC in native West Africans are available. A pilot study in Ibadan, Nigeria found two MSI-high tumors among five randomly selected CRCs (40%) (17). Immunohistochemical studies in 26 CRCs in Nigerians showed loss of MMR proteins MLH1 and MSH2 in 23% of cases (18). Our previous study of 90 CRCs from Ghana (4), a West African country neighboring Nigeria, found 41% MSI-high CRCs and no *BRAF* mutations, which is similar to the 43% MSI-high tumors in our present study in Nigeria and several other studies of MSI in CRC in African–Americans (19-22) (Table II). These findings are in a stark contrast to 10-15% MSI-high CRC in whites (5). Abdulkareem *et al*. demonstrated a lower frequency of *KRAS* and *BRAF* activating mutations (21% and 4.5% respectively) in Nigerian patients with CRC (23). A recent study identified genes exclusively mutated in African–Americans with CRC (6), which further confirms the

hypothesis that some aspects of cancer biology, whether due to environmental or genetic factors, are different between African and Caucasian patients with CRC (2).

The rate of signet ring cell carcinomas found here as 4% of all adenocarcinomas is greater than the 1.2% found in White and 0.9% in Black populations in the USA (10, 24). It appears that a higher prevalence of signet ring cell carcinomas is associated with the younger age at diagnosis of CRC as observed in Nigerians (53 years) in our study in comparison to older age (70 years) in the USA (2). It is known that signet ring cell carcinomas are more common in younger patients with CRC: at 2.44% before age 50 years *vs.* 0.93% after age 50 years ($p<0.0001$) in the US (9). The presence of signet ring cell differentiation (10-50%) was significantly higher in Nigerian patients diagnosed with CRC before the age of 50 years. Mucinous carcinoma and signet ring cell carcinoma are more difficult in diagnosis and have a poor prognosis (25), with frequent metastases in multiple sites including the peritoneum and liver (26). Higher prevalence of signet ring cell carcinomas may contribute to the high mortality from CRC in Nigeria.

The overwhelming rectal location of CRC in native Africans, similar to our unselected sample (66%), has been reported before with no plausible explanation (27-29). It is unusual, considering that our sample had increased prevalence of MSI-high CRC that is typically associated with right-sided location. Among features associated with MSI-high CRC, we found increased TILs and CLR, expansive growth pattern, mucinous tumors, poor differentiation, and younger age; however, we did not see prevalent right-sided location and absence of dirty necrosis (30). Moreover, we observed less histological heterogeneity in MSI-high colorectal cancer in Nigerians.

We identified unique pathological and molecular features of CRC in Nigeria and found a high prevalence of MSI-high tumors. These findings may have a direct translational importance because patients with MSI-high CRC appear to have ineffectual or even detrimental responses to 5-fluorouracil-based adjuvant chemotherapy (16), the mainstay of many chemotherapy regimens for CRC (13). More detailed prospective molecular and genetic analyses are warranted as CRC incidence and mortality continue to increase in Sub-Saharan Africa.

Funding

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