Original Article

Microsatellite Instability in Gastric Carcinomas in Kano, Nigeria

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Abstract

Background and Objectives: Gastric carcinoma, though relatively less prevalent in most parts of Africa and Nigeria, usually presents at an advanced stage in most of our patients. This makes the elucidation of clinical and molecular factors relating to prognosis an important avenue to explore. Gastric carcinomas exhibiting microsatellite instability (MSI) are said to have better prognosis, but there has been no study in Northern Nigeria. We, therefore, undertook this study to evaluate the proportion of MSI gastric carcinoma in our center. **Methodology:** This retrospective 5-year study was done on 48 histologically diagnosed gastric carcinomas to evaluate the immunohistochemical expression of mismatch repair DNA proteins: MLH1 and MSH2. **Results:** Forty-eight gastric cancer (GC) biopsies fulfilled the study criteria. They were from patients aged 23–80 years, with a mean of 52.1 years (standard deviation [SD] ±12.79) and male to female ratio = 3.4:1. Twenty cases (42%) had MSI, with a mean age of 51.7 years (SD ± 12.75; $P = 0.67$). The remaining 28 cases were microsatellite stable (MSS), with a mean age of 53.3 years (SD ± 12.92). Male preponderance was more marked in the MSS group (6:1) than in the MSI group (2.3:1). Intestinal carcinoma was by far the most common histologic type in both MSI (75%) and MSS (70%) groups. **Conclusion:** Forty-two percent of gastric carcinomas were harboring MSI. Although our sample size was small, it nonetheless provided useful insight and baseline data for MSI gastric carcinoma in our center. MSI GC appears to be more common in our center than an earlier Southern Nigerian study. This is consistent with the widely differing proportion of MSI gastric carcinoma across the globe, sometimes within the same country. Further studies are therefore required to make sense of this seemingly conflicting data.

Keywords: Africa, gastric cancer, microsatellite instability, Northern Nigeria

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Introduction

Globally, gastric cancer (GC) is the fifth most frequent cancer and the third (3rd) most common cause of cancer death. The highest rates are in East Asia, Eastern Europe, and Andean regions of South America, while rates are low in Western Europe, North America, and Africa.[1] There are, however, hotspots of this malignancy in parts of sub-Saharan Africa, notably in Mali where this gastrointestinal tract malignancy is the most common cancer, and the East Africa GC belt around the Great Lakes.[2]

Helicobacter pylori is the number one etiologic agent although other dietary factors are contributory.[2] These etiologic factors drive gastric carcinogenesis via a variety of molecular pathways: adenomatous polyposis coli/β-catenin, transforming growth factor beta, E-cadherin/WNT signaling, and microsatellite instability (MSI) pathways.

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MSI arises from DNA replication errors due to genetic defects in the repair of base-pair mismatch. This leads to accumulation of mutations that could result in cancer. MLH1 and MSH2 are the mismatch repair (MMR) proteins most commonly defective in MSI-related malignancies.[3]

MSI GCs are reported to have better prognosis, which might further improve with the development of MSI targeted therapy as research advances. MSI status is, therefore, an important predictive factor in the management of GC.[4]

Unfortunately, MSI testing is not available to GC patients in Nigeria. Accordingly, there has only been one published study in the country to evaluate its prevalence. That study

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was carried out in Ibadan and may not be representative of the entire country.[5]

We, therefore, conducted this study to document and evaluate the pattern of MSI GC in our center, with a view to improving clinical management of this deadly malignancy.

Methodology

This was a 5-year retrospective study (2011–2015) to evaluate the immunohistochemical expression of hMLH1/hMSH2 in gastric carcinomas at the pathology department of our hospital. The study was approved by the Hospital Research Ethics Committee. Laboratory request forms were retrieved, and relevant clinical information, age, sex, site, and histological type of tumor, was extracted. Cases with missing laboratory request forms and/or archival tissue block were excluded from the study.

Diagnoses were made in accordance with the WHO histological classification of gastric tumors.[6,7]

Statistical analysis was by means of Chi-square test to determine the correlation between MMR protein expression and clinicopathologic parameters (SPSS statistical package version 20 (Chicago, IL, USA)).

Immunohistochemistry

Sections were stained with primary monoclonal antibodies to MLH1 (clone ES05 Dako, Carpentaria, CA, USA) and MSH2 (clone 25D12 Thermofischer, Fremont, CA, USA).

Antigenicity was uncovered using heat-induced epitope retrieval by heating for 5 min at 120°C in citrate buffer. Sections were reviewed for nuclear staining with tumor infiltrating lymphocytes serving as internal control. Tumors with lack of nuclear staining for one or both antibodies were interpreted as MSI [Figures 1 and 2], while any tumor with positive nuclear staining for all the two antibodies was classified as MSS [Figure 3].

Subsequently, the cases were graded using intensity of nuclear staining of tumor cells as follows: 1 + weak intensity, 2 + moderate intensity, and 3 + strong intensity.

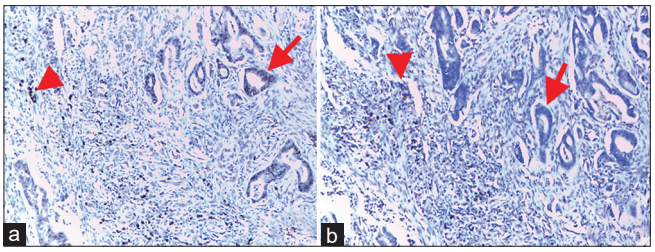

Figure 1: Microsatellite instable case of gastric carcinoma showing (a) moderate intensity nuclear staining with MLH1 (arrow) and (b) loss of nuclear staining with MSH2. Reactive lymphocytes (arrow head) as internal control (×200) (original)

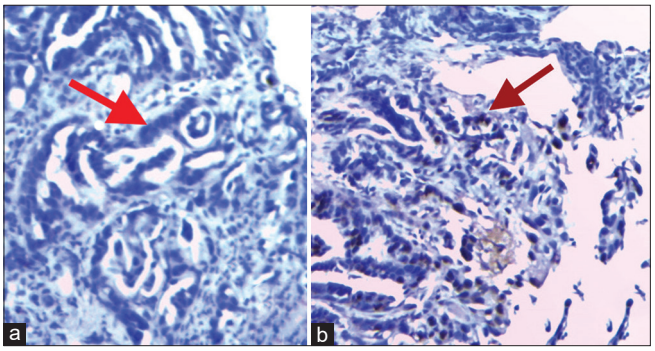

Figure 2: Microsatellite instable case of gastric carcinoma showing (a) strong intensity nuclear staining with MSH2 (arrow) and (b) loss of nuclear staining with MLH1 (arrow) (×200) (original)

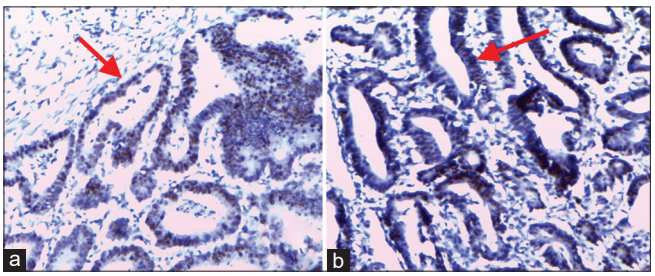

Figure 3: Microsatellite stable case showing moderate intensity nuclear staining to MLH1 (a) and strong intensity nuclear staining to MSH2 (b) (×200) (Original)

Results

Sixty-one gastric carcinomas were diagnosed during the 5-year study period, out of which 48 fulfilled the criteria for the study. Of these, 37 were male and 11 female (male: female = 3.4:1). Their ages ranged from 23 to 80 years, with a mean of 52.1 years (standard deviation [SD] ± 12.79). The mean age for MSI cases was 51.7 years (SD ± 12.75), while that of the microsatellite stable (MSS) cases was 53.3 years (SD ± 12.92; $P = 0.67$).

With 24 males and 4 females, male preponderance was more marked in the MSS group (6:1) than in the MSI group (2.3:1).

Histologically, 15 (75%) of MSI tumors were intestinal type and 5 (25%) diffuse type. About similar proportion was obtained for the MSS tumors – 22 intestinal type (70%) to 6 diffuse type (21%).

Within the MSI group, 22 (78.6%) cases occurred in the proximal stomach (fundus and body), while 6 (21.4%) cases occurred in the distal stomach (pylorus and antrum). Sixteen (80%) cases within the MSS group occurred in the proximal stomach, while 4 (20%) cases occurred in the distal stomach [Table 1].

Out of the total 48 cases studied, 28 (58%) cases retained expression of all the two proteins (MSS), while 20 (42%) cases had loss of expression of at least one protein (MSI)

Among the 20 (42%) cases with loss of protein expression, 9 (45%) had loss of both hMSH2 and hMLH1, while 8 (40%) were only hMSH2 negative and 3 (15%) were only hMLH1 negative [Tables 2 and 3].

The mean age for these MSI cases was 51.7 years (SD ± 12.75), while that of the MSS cases was 53.3 years (SD ± 12.92; $P = 0.67$) [Table 2].

Discussion

Forty-two percent of gastric carcinomas in this study had MSI, as evident from loss of expression of either or both MLH1 and MSH2. This is comparable to 43% in South Korea by Lee *et al*. and to a lesser extent 33% in Japan (1994), but much higher than 25.5% in Ibadan (2014), 24% in South Africa (2011), and 3.7% in Iran (2009).[5-11]

These significantly differing relative frequencies in MSI gastric carcinomas across the globe (from as little as 3.7% in Iran to as much as 50% in another South Korean study) can be partly ascribed to differences in methodology.[10,11] Some studies use polymerase chain reaction (PCR) to detect new microsatellites in the tumor, while others use immunohistochemistry to detect absence of MMR proteins (MLH1, MSH2).

The Ebili *et al.* Ibadan study (2014) used PCR rather than immunohistochemistry used in this study. In the same vein, different studies from South Korea variously reported the relative frequency of MSI gastric carcinoma to be 9.5%, 10.9%, 43%, and 50%.[5,8,11,12]

Even with immunohistochemistry and PCR, differences in monoclonal antibodies and microsatellite markers used can influence the proportion of reported MSI cancer.[13] For instance, the choice of microsatellite markers used in PCR influences the frequency of detected and reported unstable microsatellites. Furthermore, Sepulveda *et al*. noted that microsatellite markers differ among populations.[13] BAT 26 and BAT 40 markers were more prevalent in U.S., while D13S170 and TP53 were more common in South Korea.[13]

With regard to MSI immunohistochemistry, the absence of a standardized reporting format may also partly explain widely differing reports on the frequency of MSI tumors. Most published MSI immunohistochemistry studies consider any nuclear MLH/MSH immunostaining to be positive, however slight and/or few cells. In other words, there is no gradation of MSI immunostaining (weak, strong, or moderate), which does not make much sense given that PCR distinguishes between high and low MSI.

This unsatisfactory all-or-none reporting format of MSI immunohistochemistry leaves much room for subjective interobserver error. What one pathologist considers light or scanty staining, another may perceive as negative.

However, differences in laboratory methodology alone do not adequately explain the widely differing proportions of MSI gastric carcinomas across the globe. An international study using the same PCR technique in three different countries on three different continents found wide disparities in MSI GC – 7% in USA, 15% in Columbia, and 50% in South Korea.[13]

Some of the reported global MSI GC disparities reflect differences in genetics and dietary/other environmental etiological factors among different populations, which, in turn, lead to activation of different molecular pathways (MSI, non-MSI) in gastric carcinogenesis.[13] Similar to GC, colorectal cancer (CRC) cases in Africa have been found to present few decades earlier and with more advanced disease at presentation. This has led to the postulation of the hypothesis that these cancers likely have different tumor biology and by extension different molecular signature in native Africans.[14,15] For example, the frequency of MSI in CRC in Caucasians has been reported as 10%–15%.[16] This figure is significantly lower than the 23% and 34.5% reported in Ibadan and Ife.[17,18] A study by Raskin *et al*. in Ghana reported a frequency of 41% MSI in CRC.[19] The relatively high MSI frequency observed in the two cancers in Africans, as compared to Caucasians, serves to strengthen the hypothesis of a unique tumour biology in black population.

Determining MSI status is not just an academic exercise, but is relevant to patient management as MSI is said to be a good prognostic factor, thus requiring less aggressive treatment.[4,20]

Table 1: Comparison of hMLH1/hMSH2 immunoprofile with clinicopathologic parameters

Variables	MSI ($n=20$)	MSS ($n=28$)	P
Age (years)	53.3±12.92	51.7±12.89	0.677
Gender			
Male	24 (85.7)	13 (65)	0.092
Female	4 (14.3)	7 (35)	
Lauren's classification			
Intestinal	22 (78.6)	15 (75)	0.772
Diffuse	6 (21.4)	5 (25)	
Location			
Proximal	22 (78.6)	16 (80)	0.904
Distal	6 (21.4)	4 (20)	

MSI: Microsatellite instability, MSS: Microsatellite stable

Table 2: Pattern of immunohistochemical expression of hMLH1/hMSH2

Expression pattern	MMR protein			Total
	hMLH1 and hMSH2	hMLH1	hMSH2	
Intact expression (MSS)	28 (100)	-	-	28 (100)
Loss of expression (MSI)	9 (45)	3 (15)	8 (40)	20 (100)

MMR: Mismatch repair, MSS: Microsatellite stable, MSI: Microsatellite instability

Table 3: Staining intensity pattern for the 48 cases

Antibodies	Staining intensity			Negative
	1+	2+	3+	
MLH1	17	13	6	12
MSH2	19	8	4	17

Given the poor long-term follow-up of patients in our center, as in most other parts of the country, we could not evaluate the relationship of MSI to GC survival. Hence, we could not confirm or refute it as a good prognostic indicator in our center.

A Japanese study, however, contradicts MSI as good prognosticator as it associated MSI with advanced GC.[21] Hence, they posited that MSI is a late consequence of gastric carcinoma tumor progression.

This suggests that MSI patients should on the average be older than non-MSI GC patients, which is not supported by this study. The mean age of MSI GC patients in this review (53.3 years) was not significantly different from non-MSI GC patients (51.7 years). Not surprisingly, an American study by Strickler et al. published the same year as the study by Chong JM et al, came to the opposite conclusion-that MSI occurs early in tumor progression of GC.[21,22]

With regard to histology, three quarters of MSI GC s in this study were intestinal (tubular). This is consistent with most published reports.[8,21] A notable exception was a German study that reported slightly more diffuse carcinomas in MSI GCs.[23,24]

Conclusion

Although the sample size of this study is small, it nonetheless provides useful insight and baseline data on MSI gastric carcinoma in Kano. MSI comprised four out of ten of gastric carcinomas in Kano, which should imply good prognosis for the affected patients. Unfortunately, because of poor follow-up, this is difficult to ascertain. Given the widely disparate data on the relative frequency of MSI GC, there are still many unanswered questions. Further studies are therefore required; particularly, as such in depth MSI studies could reveal avenues for targeted therapy with improved survival.

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Conflicts of interest

There are no conflicts of interest.

References

1. Bray F, Ferley J, Forman D. Global cancer. CA Cancer J Clin 2011;61:65-90.
2. Asombang AW, Rahman R, Ibdah JA. Gastric cancer in Africa: Current management and outcomes. World J Gastroenterol 2014;20:3875-9.
3. Ionov Y, Peinado MA, Malkhosyan S, Shibata D, Perucho M. Ubiquitous somatic mutations in simple repeated sequences reveal a new mechanism for colonic carcinogenesis. Nature 1993;363:558-61.
4. dos Santos NR, Seruca R, Constância M, Seixas M, Sobrinho-Simões M. Microsatellite instability at multiple loci in gastric carcinoma: Clinicopathologic implications and prognosis. Gastroenterology 1996;110:38-44.
5. Ebili HO, Oluwasola AO, Akang EE. Microsatellite instability status of gastric carcinoma from patients in the University College Hospital, Ibadan. Paper presented at: World Cancer Congress. Melbourne, Australia; 3-6 December 2014.
6. Hamilton SR, Aaltonen LA, editors. World Health Organization Classification of Tumours. Pathology and Genetics of Tumours of the Digestive System. Lyon: IARC Press; 2000.
7. Lauren P. The two histological main types of gastric carcinoma: Diffuse and so-called intestinal-type carcinoma. An attempt at a histo-clinical classification. Acta Pathol Microbiol Scand 1965;64:31-49.
8. Lee HS, Choi SI, Lee HK, Kim HS, Yang HK, Kang GH, *et al*. Distinct clinical features and outcomes of gastric cancers with microsatellite instability. Mod Pathol 2002;15:632-40.
9. Buffart TE, Louw M, van Grieken NC, Tijssen M, Carvalho B, Ylstra B, *et al*. Gastric cancers of Western European and African patients show different patterns of genomic instability. BMC Med Genomics 2011;4:7.
10. Molaei M, Yadollahzadeh M, Mansoori B. Immunohistochemistry stain assessment of DNA Mismatch repair protein in Gastric cancer. Govaresh 2009;14:148-52.
11. Lee HJ, Jang YJ, Lee EJ, Kim JH, Park SS, Park SH, *et al*. The significance of mismatch repair genes in gastric cancer. J Cancer Res Ther 2013;9:80-3.
12. Bae YS, Kim H, Noh SH, Kim H. Usefulness of Immunohistochemistry for microsatellite instability screening in gastric cancer. Gut Liver 2015;9:629-35.
13. Sepulveda AR, Santos AC, Yamaoka Y, Wu L, Gutierrez O, Kim JG, *et al*. Marked differences in the frequency of micro- satellite instability in gastric cancer from different countries. Am J Gastroenterol 1999;94:3034-8.
14. Irabor DO, Afuwope OO, Ayandipo OO. The present state of management of colon and rectal cancer in Nigeria. J Cancer Res 2014;190:1-7.
15. van't Hof A, Gilissen K, Cohen RJ, Taylor L, Haffajee Z, Thornley AL, *et al*: Colonic cell proliferation in two different ethnic groups with contrasting incidence of colon cancer: Is there a difference in carcinogenesis? Gut 1995;36:691-5.
16. Vilar E, Gruber SB. Microsatellite instability in colorectal cancer-the stable evidence. Nat Rev Clin Oncol 2010;7:153-62.
17. Duduyemi BM, Akang EE, Adegboyega PA, Thomas JO. Significance of DNA mismatch repair genes and microsatellite instability in colorectal carcinoma in Ibadan, Nigeria. Am J Med Biol Res 2013;1:145-8.
18. Adegoke OO, Komolafe AO, Ojo OS. Microsatellite instability statuses and clinicopathological characteristics of colorectal carcinomas in a Sub Saharan African population. Gastroenterol Hepatol Int J 2017;2:1-6.
19. Raskin L, Dakubo JC, Palaski N, Greenson JK, Gruber SB. Distinct molecular features of colorectal cancer in Ghana. Cancer Epidemiol 2013;37:556-61.
20. Fink D, Aebi S, Howell SB. The role of DNA mismatch repair in drug resistance. Clin Cancer Res 1998;4:1-6.
21. Chong JM, Fukayama M, Hayashi Y, Takizawa T, Koike M, Konishi M, *et al*. Microsatellite instability in the progression of gastric carcinoma. Cancer Res 1994;54:4595-7.
22. Strickler JG, Zheng J, Shu Q. p53 mutations and microsatellite instability in sporadic gastric carcinomas: When guardians fail. Cancer Res 1994;54:4750-5.
23. Jiricny J. The multifaceted mismatch-repair system. Nat Rev Mol Cell Biol 2006;7:335-46.
24. Keller G, Rotter M, Vogelsang H, Bischoff P, Becker KF, Mueller J, *et al*. Microsatellite instability in adenocarcinomas of the upper gastrointestinal tract. Relation to clinicopathological data and family history. Am J Pathol 1995;147:593-600.