Tropical Journal of Obstetrics & Gynaecology
ISSN-Print: 0189-5117
Online: 2543-148X
Official Publication of Society of Obstetrics & Gynaecology of Nigeria

■ Original Research Article

P53 Assessment in Endometrial Carcinoma: A Thirteen Year Immunohistochemical Analysis in Aminu Kano Teaching Hospital (2008-2020) Kano

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Abstract

Aims: The presence of P53 mutation in histologically diagnosed endometrial carcinoma cases in Kano.**Settings and Design:** This was a thirteen-year (2008-2020) retrospective study of endometrial carcinomas diagnosed in the histopathology department of Aminu Kano Teaching Hospital, Kano.**Methods and Material:** Relevant information on patients' biodata, clinical history, histology slides, and tissue blocks were retrieved from departmental records. P53 antibody marker testing was performed on all relevant tissue blocks. **Results:** Eight thousand one hundred and fifteen (8115) gynaecological samples were processed and analyzed during the study period. Seven hundred and eighty-eight (10%) were genital tract malignancies with endometrial carcinomas accounting for ninety-six (12.2%) of the cases. fifty five of these 96 endometrial carcinoma cases met the inclusion criteria for this study. The mean age of presentation was in the 6th decades of life with over 75% of the patients being postmenopausal. Type 1 endometrial adenocarcinoma was the commonest (76.4%) cancer type while the remaining 23.6% was type II endometrial carcinoma. A hundred percent (100%) P53 positivity was observed in type II endometrial carcinoma cases. **Conclusions:** Endometrial cancer accounted for 12.2% of gynaecological malignancies in our study and occurred by at least a decade earlier in our patients compared to cases in developed countries. The majority of our cases were the type I variants while all the type II cancers tested positive for P53 mutation, which is an important therapeutic and prognostic marker of disease progression.

Keywords: Endometrial Carcinoma, Post-Menopausal Females, P53 Mutation

INTRODUCTION

Carcinoma of the endometrium, is the third most common invasive neoplasm of the female genital tract in Sub-Saharan Africa and most common in the United States of America[1-3] Worldwide, it is the fifth most common cancer with approximately 280,000 new cases reported yearly and represents 7% of all invasive cancer in women.[1,4]In Nigeria, it is the 3rd most common cancer after the cervix and ovary.[1]

The incidence varies widely worldwide and is twice as high in whites compared to blacks. The highest rates of endometrial carcinoma occur in North America, Canada, and Eastern Europe with Japan reporting a lower incidence.[5] Studies have recorded the lowest incidence of endometrial cancer in the blacks, thus suggesting race as a determinant factor for the variation incidence.[2,5]

Primarily, endometrial carcinoma is a disease of postmenopausal women, the peak incidence being the 5th to 6th decades of life, and it is uncommon in women below 40-years of age.[1]

The exact etiology of endometrial carcinoma is not known, however, there are associated risk factors such as chronic unopposed estrogen stimulation, early menopause/late menopause, nulliparity, anovulation, and estrogen-producing tumours. Others include age greater than 50 years, hypertension, obesity, diabetes, tamoxifen usage, previous pelvic radiation therapy, family history of breast or ovarian cancer, family history of hereditary nonpolyposis colorectal cancer and other related syndromes such as Stein-Leventhal syndrome and gonadal dysgenesis.[1,2,4,5]

Based on molecular and clinicopathologic studies, endometrial carcinoma can be categorized into two groups as type 1 and type 11 cancer.[1,2,6] Type 1 cancer, also called endometrioid carcinoma or estrogen-sensitive tumour is the most common, accounting for 80% of all cases seen in the age range of 55-65 years. Recent molecular studies have given evidence that endometrial hyperplasia is a precursor to endometrioid carcinoma as well as a mutation in phosphatase and tensin homologue (PTEN), phosphoinositide 3-kinase (PIK3CA), microsatellite instability (MSI), adenomatous polyposis coli gene/beta-catenin pathway (APC/B-CATENIN) and TP53 protein.[1,2,4,5]

Type II cancer also called non-endometrioid carcinoma or poorly differentiated tumour, occurs in the 6th and 7th decade in the setting of endometrial intraepithelial carcinoma because of endometrial atrophy.[1] These cancers represent 15% of the disease presentation with a poor prognosis. The commonest histologic subtype is serous endometrial carcinoma, others include clear cell carcinoma, mixed cell adenocarcinoma, transitional cell carcinoma, and undifferentiated carcinoma. The P53 mutation (missense mutation) represents 90% of the serous endometrial carcinoma mutation.[1,2,5]

TP53 mutation has been found to be twice as common in serous carcinoma than endometrioid endometrial carcinoma and patients having p53 gene mutation and overexpression of p53 have a higher mortality rate than those without p53 gene changes thus justifying studies worldwide that have classified P53 expressivity as a prognostic indicator. P53 immunohistochemistry has been helpful in differentiating a serous carcinoma from a high-grade endometrioid carcinoma in a subset of cases.[1,2,4-12]

This study seeks to determine the P53 immunohistochemical profile, frequency, histologic type, and variants using 2016 WHO criteria of all endometrial carcinoma cases diagnosed in Aminu Kano Teaching Hospital over a thirteen and compares these findings with other part of the country and the world in general.

MATERIALS AND METHODS

Hematoxylin and eosin-stained histology slides of all diagnosed endometrial cancer cases were retrieved from departmental records, bench books, and patient case cards. Tissue blocks were retrieved for cases with faded or missing slides and new sections were made for confirmation of diagnosis. Patients' clinical data were obtained from the case cards accompanying the specimen. The inclusion criteria were all diagnosed cases of endometrial carcinoma within the study period of 2008-2020. Excluded from this study were cases where both slides and blocks could not be found or inadequate clinical details.

Representative tissue blocks of confirmed endometrial cancer cases were selected for immunohistochemistry using TP53 antibody. P53 expressivities were assessed using the immunoperoxidase method (Thermo Scientific). Thin sections were cut onto a glass slide, slides were deparaffinized in xylene, using graded alcohol, then hydrated and immersed in 3% hydrogen peroxide to block endogenous peroxidase activity. To enhance antigen detection, the slide was microwaved for two cycles of 5minute at 460'c in a citrate buffer at PH 6.5. After incubation overnight with antibody to P53(clone DO-7, Thermo Scientific), it was rinsed and counterstained with hematoxylin. The anti-p53

antibody DO-7 assesses both wild and mutant p53. Both positive and negative controls were run with this test to detect the P53 gene.

Scoring for positivity using the nuclear P53 staining was categorised as negative (score 0); focally positive, with less than 5% tumour cells positive in one or multiple foci (score 1+); low positive, with 5-40% of tumour cell positive (score 2+); or high positive, with 40-100% of tumour cells positive (score 3+). A score of 1+ and above was considered positive.

The histologic classification and the clinical staging using the FIGO criteria was done using the World Health Organisation Classification of Tumour Pathology and Genetics Tumour of The Breast and Female Genital Organs 2016.[2]

RESULT

Eight thousand one hundred and fifteen (8115) gynaecological samples were processed and analyzed in the department during the thirteen-year study period,

Table 1. Age Distribution Frequency for All Endometrial Cancers Analysed

Age (Years)	Number of cases	Percentage (%)
<20	0	0
20-29	1	1.8
30-39	4	7.2
40-49	8	14.6
50-59	15	27.3
60-69	14	25.5
70-79	8	14.6
80-89	5	9.1
Total	55	100

while seven hundred and eighty-eight (10%) of these were genital tract malignancies. Ninety-six (12.2%) of the genital tract malignancies were endometrial cancers.

Only fifty-five of the 96 endometrial cancer cases met the inclusion criteria for this study. Of the fifty-five cases, thirty-seven were from total abdominal hysterectomy, while eighteen cases were from endometrial biopsy samples.

The ages of the affected patients ranged from 20-86 years with a mean of 59 years. The peak age distributions were in the 6th decade (50-59 years) with 15 cases representing 27%. Table 1.

Table 2 shows the distribution by age of all analyzed endometrial cancer cases using the 2016 WHO classification. The type I endometrial carcinoma comprising of endometrioid carcinoma and mucinous carcinoma variants accounted for 76.4%

Table 2. All Endometrial Cancer Cases Analysed Using 2016 Who Classification

Histologic type	Age distribution(years)								Total number of cases	(%)
	<20	20-29	30-39	40-49	50-59	60-69	70-79	80-89		
Type I Endometrioid carcinoma	0	1	3	7	12	9	4	5	41	74.6
Mucinous carcinoma	0	0	0	0	0	1	0	0	1	1.8
	0	0	1	1	3	4	3	0	12	21.8
Type II serous carcinoma										
Clear cell carcinoma	0	0	0	0	0	0	1	0	1	1.8
Grand total	0	1	4	8	15	14	8	5	55	100

Table 3: Grading of Type I Endometrial Carcinoma Cases

Grade	Number of cases	Percentage (%)
Grade I: less than 5% solid growth pattern without nuclear atypia	26	62
Grade II: 6 to 50% solid growth pattern with mild to moderate nuclear atypia	10	24
Grade III:more than 50% solid growth pattern with severe nuclear atypia	6	14
Total	**42**	**100**

Table 4. Type II Endometrial Carcinoma (P53 Testing)

Histologic subtype	Number of cases	Intensity			Distribution	
		1	2	3	Widespread	Focal
Serous carcinoma	12	0	3	9	11	1
Clear cell carcinoma	1	0	0	1	1	0
Total	**13**	**0**	**3**	**10**	**12**	**1**

(42 cases) overall. They were morphologically characterized by well-formed glands, with some exhibiting villoglandular architecture lined by simple to stratified columnar cells having mild to moderate nuclear atypia and eosinophilic to granular cytoplasm in the endometrioid variant while intracytoplasmic mucin was present in the solitary mucinous carcinoma variant (Figures 1). Type II endometrial carcinoma comprised the serous and clear cell carcinoma variants and accounted for 13 cases (23.6%). The serous variants were morphologically characterized by complex papillary and or glandular structures with diffuse marked nuclear pleomorphism while the clear cell variant showed polygonal cells with clear cytoplasm (Figure 2). The peak age distribution for type I endometrial carcinoma was in the 6th decades of life (50-59years) while for type II

endometrial carcinoma, it was in the 7th decade of life(60-69years) as in Table 1.

Grading was done for the type I endometrial carcinoma variants in this study according to the 2016 WHO established criteria of architectural pattern and nuclear features (Table 3).

Grade I tumours accounted for twenty-six (62%) cases, and histologically exhibited less than 5% solid growth pattern and malignant cells without nuclear atypia (Figure 3). The grade 2 tumours accounted for ten (24%) cases and morphologically showed a 6 to 50% solid growth pattern with lesional cells having mild to moderate atypical nuclear features (Figure 4**),** while the grade 3 tumours were only six (14%) cases. These grade 3 tumours had more than 50% solid growth histologically with tumour cells showing severe atypical nuclear features. (Figure 5)

Grading was done for the type I endometrialcarcinoma variants in this study according to the 2016 WHO established criteria of architectural pattern and nuclear features (Table 3).

Grade 1 tumours accounted for twenty-six (62%) cases, and histologically exhibited less than 5% solid growth pattern and malignant cells without

The thirteen (13) cases of type II endometrial carcinoma analyzed in this study were subjected to P53 testing for confirmation of histological diagnosis as recommended in documented literature. It is noteworthy that WHO gave no grading requirements for this type II group. Table 4 shows the distribution of P53 of immunoreactivity of varying distribution and intensity with 10 cases having intense staining and wide distribution of staining of the tumour cells (Figure 6).

DISCUSSION

Endometrial carcinoma is the most common female genital tract malignancy, particularly in the developed countries. [13]

It accounted for 12.2% of all genital tract malignancies during our thirteen-year study period. This finding is comparable with 11.5% reported by Yakassai et al in their clinical study in the same center as this study and the 13% reported by Sanni et al in Jos, North- central Nigeria.[14,15] However, our frequency rate is higher than reports by Keshinro et al (4.9%) in Lagos Southwest Nigeria, Okeke et al (9%) in Enugu, Southeast Nigeria, Kyari et al (8.5%) in Maiduguri North-east Nigeria and Briggs et al (8%) in Port Harcourt, South-south Nigeria.[16-19]

Studies from some African countries showed varying frequency rates as seen in reports by Nkyekyer et al in Ghana (7.4%), Kasule et al in Zimbabwe (8%), and Meye et al in Gabon (5.3%).[20-22] Several studies on endometrial cancer in these African countries attributed the low- frequency rates of endometrial carcinoma to the (high) prevalence of endometrial hyperplasia without atypia which has minimal tendency (1%) of evolving into endometrial carcinoma.[15]

Reports from Asia are relatively low (2%) from documents by Globocan2012, and a study done by Chhabra et al in India.[23,24] Whereas, the highest incidence of 19.1 per 100000, 12.8 -15.6%, and 16% were reported in North America, Northern and Western Europe, and Ejeckan et al in Qatar respectively in keeping with our findings in this study.[23, 25]

Over 60% of the endometrial carcinoma in this study were diagnosed from hysterectomy specimens with endometrial curettages and biopsies accounting for approximately 33% of cases. Accuracy of diagnosis is also increased in hysterectomy specimens as compared to endometrial curettage or biopsy from documented reports.[15]

The mean age at presentation of endometrial cancer in this study was 59 years with a broad age range of 20 years to 86 years. This mean age is like reports from Ghana (59 years), Gabon (56 years), India (53 years), and Pakistan (56.6 years).[20, 22,24,26] Our mean age is however lower by more than a decade when compared with data from the United Kingdom (83 years) and United States of America (89 years).[3]

The majority (75%) of our patients in this study were postmenopausal with the highest age-specific rate in the 6th (50-59 years) decades of life. Similar findings were reported in Port Harcourt, South-South, Nigeria, and Botswana.[27, 28] These findings are supported by literature that endometrial carcinoma predominantly develops after menopause.[15] In general, cancers tend to develop at least a decade earlier in people of African descent to several documented studies.

Overall, there were forty-two of type I endometrial cancer and thirteen of type II endometrial cancer cases. This report is comparable to a previous study by Lax et al, Malik et al and Zajac et al. [8,26,29]

The type I endometrial carcinoma cases had a mean age presentation of 58years, whereas the type II endometrial carcinoma had a mean age presentation of 60years in this study, as reported

study from Pakistan.[26] The proportion of type II endometrial cancer in this study is however low when compared with the 76% reported in Poland.[29]

Type I endometrial carcinoma is the most common histologic type and is often associated with a good prognosis. It accounted for 76% of the cases in this study while the type II endometrial carcinoma accounted for approximately 24%. These type II tumours are associated with poor prognosis. [30, 31] Our distribution pattern of this cancer is comparable with reports by Muhammad et al, Meye et al, Jemal et al and Hamilton et al .[3,22],[30,31]

Also, over 50% of our endometrioid carcinoma type I (62%) cases were well differentiated. This finding is in keeping with a study done in the United Kingdom by Sudha et al.[32] It has been documented that well-differentiated endometrioid carcinoma is associated with a better prognosis for most patients than was previously reported. [3]

The p53 immunohistochemistry profile of the thirteen cases of endometrial carcinoma (type II) in this study showed an 100% positivity, supporting the morphologic criteria used in the diagnosis of the tumours. This finding is comparable to several reports suggesting a strong association between p53 expression and high histologic-grade endometrial carcinoma, particularly those with advanced-stage disease and lympho-vascular invasion. [6 -12, 15]

CONCLUSION

Endometrial cancer accounted for 12.2% of gynaecological malignancies in our study and occurred by at least earlier in our patients compared to cases in developed countries. The majority of cases were the type I variants while all the type II cancers tested positive for P53 mutation, which is an important therapeutic and prognostic marker of disease progression.

Reference

1. Kumar V, Abbas AK, Aster JC. The Female Genital Tract. In: Kumar V, Abbas AK, Aster JC, editors. Robbins and Cotran Pathologic Basis of Disease. 9th ed. Canada: Saunders Elsevier; 2015. p. 1013-1019.
2. Tavassoli FA, Devilee P. Tumour of the uterine corpus. Pathology and genetics of tumours of the breast and female genital organs. *Lyon: IARC Press 2014;* 123-133.
3. Muhammad AA, Adekunle OO, Modupeola SO, Muhammad U, Zulaiha S, Abdullahi A. A diary of endometrial malignancies in Zaria, Northern Nigeria. *Sub-Saharan Afr J Med*2017; 4:43-6.
4. Dragomirescu M, Stephan AE, Margaritescu C, Simionescu CE. The immunoexpression of p53 and Snail in endometrioid endometrial carcinoma. *Rom J Morphol Embryo* 2018;59(1):131-137.
5. Ronnett BM, Zaino RJ, Ellenson LH, Kurman RJ. Endometrial carcinoma. InKurman RJ editor. Blaustein's Pathology of the female genital tract. 5th Edition. New Delhi: Springer; 2002. p. 501-538.
6. Dragomirescu M, Stephan AE, Margaritescu C, Simionescu CE. The immunoexpression of p53 and Snail in endometrioid endometrial carcinoma. *Rom J Morphol Embryo* 2018;59(1):131-137.
7. Monika MZ, Jacek RW, Marta K, Kinga K-L, Marek N, Grzegorz S, et al. The significance of markers in the diagnosis of endometrial cancer.2016;15(3):176-185.
8. Lax SF, Kendall B, Tashiro H, Slebos RJ, Hedrick L. The frequency of p53, K-ras mutations and microsatellite instability differs in uterine endometrioid and serous carcinoma: evidence of distinct molecular genetic pathways, *Cancer* 2000;88(4):814-24.
9. Wang H, Bao W, Jiang F, Che Q, Chen Z, Wang F et al. Mutant p53(p53-R248Q) function as an oncogene in promoting endometrial cancer by up-regulating REGy. *Cancer Lett* 2015;360(2):269-279.
10. Jia L, Liu Y, Yi X, Miron A, Crum CP, Kong B, Zheng W. Endometrial glandular dysplasia with frequent p53 gene mutation:A genetic evidence supporting its precancer nature for endometrial serous carcinoma. *Clin Cancer Res* 2008;14(8):2263-2269.
11. Kaku T, Kamura T, Hirakawa T, Sakai K, Amada S, Kobayashi H, Nakawo H. Endometrial carcinoma associated with hyperplasia--Immunohistochemical study of angiogenesis and p53 expression. *GynecolOncol* 1999;72(1):51-55
12. Ito K, Watanabe K, Nasim S, Sasamo H, Sato S, Yajima A, Silverberg SG, Garrett CT. Prognostic significance of p53 overexpression in endometrial cancer. *Cancer Res* 1994.2001;54(17):4667-70.
13. Yeole BB. Trends in cancer incidence in females breast,cervixuteri,corpus uteri and ovary in India. Asia Pac J Cancer Prev. 2007;9(1):119-112.
14. Yakassai IA, Ugwu EA, Otubu J. Gynaecological cancer in Aminu Kano Teaching Hospital Kano: A 3-year review. *Niger J ClinPract* 2013;16(10):63-66.
15. Sanni WO, Ocheke AN, Oyebode T, Jonah M, Nyango DD, Silas OA, Sugay AS. Pattern of gynecologic malignancy in Jos. Trop J ObstetGynaecol 2013;30(1):97-102.
16. Kehinro SO, Nwafor CC, Osun P. Histological analysis of gynaecologic lesions in Nigeria. *East Afri Med J*. 2015;92(5):245-252.
17. Umeobika JC, Ikeako LC, Ezenyeaku CT, Ezebialu UI, Ojiyi EC. Gynaecologic malignant tumour at Imo State University Teaching Hospital Orlu Southeastern Nigeria. *East Afr Med* 2018;6(1):52-58.
18. Kyari O, Nggada H, Mairiga A. Maliganattumour of female genital tract in Northeastern Nigeria. *East Afri Med J*2004; 81:142-5
19. Briggs ND, Katchy KC. Pattern of primary gynaecological malignancies as seen in a tertiary hospital situated in the Rivers State of Nigeria. *Int J GynaecolObstet* 1990; 31(2):157-61.

20. Nkyekyer K, Pattern of gynecological cancer in Ghana *East Afr Med J* 2000;77:534-538.
21. Kasule J. The pattern of gynecologic malignancy in Zimbabwe. *East Afr Med J* 1989;66(6):393-399.
22. Meye JF, Mabicka BM, Belembaogo E, Minko-Mi-Etoua DI, Engongah-Beka T, Minko-Mi-Etoua D. Endometrial carcinomas in Gabon. A study of 34 cases in 11years:1988-1998. Santé 2000;10(1):43-5.
23. Bray F, Ferlay J, Soerjomatara I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018, GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries *CA Cancer J Clin.* 2018;0:1-31.
24. Chhabra S, Sonak M, Prem V, Sharma S. Gynaecological malignancies in a rural institute in India. J ObstetGynaecol. 2002;22(4):426-9.
25. Ejeckan GC, Abdulla F, El-Sakka M, Danleh W, HaseebF. Gynaecological malignancies in Qatar. East Afr Med J 1994;71(12):777-81.
26. Malik TY, Chishti U, Aziz AB, Sheikh I. comparison of risk factors and survival of type I and type II endometrial cancer. Pak J Med Sci. 2016;32(4):886-890.
27. Nwosu SO, Anya S E. Malignancies of the female genital tract at the university of port Harcourt Teaching Hospital:A ten year review-1990-1999.Nig Postgrad Med J 2004;11:107-109.
28. Tanko MN, KayembeMA, Cainelli F, Vento S. Malignant tumours of the genital tract among Botswana women. Ghana Med J 2012; 46:142-146.
29. Zajac A, Stachowiak G, Stetkiewicz T, Wilczynski JR. TP53 and p53 proteins vs endometrial carcinoma.*MenopauseReview/PrzegladMenopauzalny*2013;12(3)228-230.
30. Jemal A, Siegel R, Ward E, Murray T, Xu J, Smigal C et al. Cancer statistics 2006. CA Cancer J Clin 2006; 56:106-130.
31. Hamilton CA, Cheung MK, Osann K, Chen L, Teng NN, Longacre TA et al. Uterine papillary serous and clear cell carcinoma predicts for poorer survival compared to grade 3 endometrioid corpus cancer. Br J Cancer 2006;94(5):642-646.
32. Sudha SS, Suhail A. Malignant disease of the uterus. In: Shaw RW, LuesleyD, MongaA, Editors. Gynecology. 4th ed. London: Churchill livingstone; 2011. p. 629-30