Original Article

Immunohistochemical Analysis Of Human Papilloma Virus On Cervical Biopsy In Patients Attending Aminu Kano Teaching Hospital, Kano

***Riruwai GY [1*], Eze GI [2], Umar AB [3], Mohammed AZ [3]**

[1]*Medical Laboratory Science Department Faculty of Allied Health Sciences Bayero University Kano,*
[2]*Anatomy Department Faculty of Basic Medical Sciences University of Benin, Benin City,*
[3]*Pathology Department College of Health Sciences, Bayero University Kano,*

Corresponding author: *garbayahaya@gmail.com, +2347030666204.*

ABSTRACT

Background: *Epidemiological study has shown that human papilloma virus infections play a major role in cervical pre-invasive and invasive lesions.*
Aim: *Application of immunological methods can detect Human Papilloma Virus genetic material in almost 100% of the premalignant and malignant tissues of the uterine cervix.*
Materials and Methods: *One hundred and twenty five blocks previously prepared cervical biopsy block from January, 2015 to December, 2015 were retrieved and re-evaluated to confirm the nature of specimen microscopically. Clinical data such as the age, sex and routine diagnosis were extracted from the record in the Histopathology Department of Aminu Kano Teaching Hospital Kano.*
Result: *Statistically one hundred and twenty five blocks were identified and used for the study. Out of which, 32(25.6%) were age group 20-30 years, 32(25.6%) were 31-40 years, 29(23.2%) were 41-50 years and 32(25.6%) were 50 years and above. Relationship of age and different stages of dysplastic lesions shows women of 20-30 years have 2(6.3%) of inflammation, 1(3.1%) CIN1, 4(12.5%) CIN2, 0(0%) CIN3 and 25(78.1%) are cancer. Thirty one to fourty years were 0 (0%) inflammations, 5(15.6%) CIN1, 1(3.1%) CIN2, 3(9.4%) CIN3, 25(78.1%) were cancers. Fourty one to fifty years has 0 (0%) inflammations, 5(15.6%) CIN1, 1(3.1%) CIN1, 3(9.4%) CIN3, 23(71.9%) are cancers. Fifty one years and above were found to have no inflammation, CIN1, CIN2, and CIN3 while 32(100%) were cancers. This indicate that ($p<0.01$.) using Anova Statistical package.*
Conclusion: *Immunological staining using P^{16} can be used to differentiate High Risk Human Papilloma Virus from Low Risk Human Papilloma Virus in cervical lesions. P^{16} antibody is found to be useful as a dysplasia associated antigen in differentiating High Risk Human Papilloma Virus from Low Risk Human Papilloma Virus.*

Key words: CIN, HPV, BIOPSY.

BACKGROUND OF THE STUDY

Cervical cancer is found to be the second-most common type of cancer among women, worldwide. In India, an estimated 1, 32,082 fresh cases are reported annually.[1] The causative agent of the disease is HPV, a sexually transmitted virus.[2] Risk factors are immune suppression, genital infection with Chlamydia and trachomonal.[3,4] In 100 HPV genotypes, 30 were shown to infect the uterine cervical epithelium.[5] the subsets for inducing cervical cancers are low-, intermediate-, and high-risk.[6] The HPV infection is asymptomatic in the benign stage and it clinically manifests as a neoplastic transformation.[7,8]

The initial method for HPV detection is the Pap smear test. Human error is probably the main threat to accurate interpretation.[9] To overcome these limitations, high sensitivity and specificity for demonstration of truly dysplastic cells, in the form of an objective biomarker with polymer-based

methods, is adopted. However, these methods have less specificity in differentiating the types. Hence, application of molecular methods can detect HPV genetic material in almost 100% of the premalignant and malignant tissues of the uterine cervix.[8]

MATERIALS AND METHOD

This Retrospective descriptive stratified study conducted in Aminu Kano Teaching Hospital of Kano city. An approval for the study was obtained from the Ethical Committee of Aminu Kano Teaching Hospital Kano.All cervical biopsies paraffin blocks for the year 2015 are included.
A total of one hundred and twenty five (125) cervical biopsies paraffin blocks that are processed in year 2015 were selected using Histology register.
Using 2015 histology register, cervical blocks biopsies were selected and categorized according the diagnosis as cancer, CIN1, CIN2, CIN3, and inflammation. The slides were made from the blocks and then stain using standard immuno-histochemical techniques

Microscopy

P16 antibody (surrogate for HPV) is monoclonal and stains the cytoplasm (cytoplasmic stain). It stains dark brown coloration in the cytoplasm.
The slides where screened under high x10 objectives lens and reported accordingly as positive or negative using positive control slide as a guide.
The finding was recorded and analyzed using standard statistical package (Anova).

Data Analysis

Data obtained was analyzed using SPSS (version 20.0) statistical package and presented in tabular form.

RESULTS

All of the selected cases were from patients aged 20 years above with age group 41-50 years having a slightly lower frequency accounting for 23.2% of cases (Table 1). The H&E diagnosis of Inflammation and CIN II were commonest in the 20-30 years age group, while CIN III was commonest in the 31-40 years age group. CIN I was commonest in the age groups 31-40 years and 41-50 years with both age groups together accounting for 10 out of the 11 cases of CIN II (Table II). About 70% of cases were positive for p16 immunostain, with 34% having focal positivity and 36% having diffuse positivity (Tables III).

Table 1: Frequency distribution in different age group.

Age group	Frequency	Percent
20 - 30 Years	32	25.6
31 - 40 Years	32	25.6
41 - 50 Years	29	23.2
51Years & Above	32	25.6
Total	125	100.0

Table 2: Shows relationship between age groups and different stages of cervical dysplasia diagnosed by H&E diagnosis

Age group Years.	H&E Diagnosis					
	Inflammation n(%)	CINI n(%)	CIN II n(%)	CIN III n(%)	Cancer n(%)	Total
20 - 30	2(6.3)	1(3.1)	4(12.5)	0(0)	25 (78.1)	32
31 - 40	0(0)	5(15.6)	1(3.1)	3(9.4)	23(71.9)	32
41 - 50	0(0)	5(17.2)	2(6.9)	1(3.4)	21(72.4)	29
>50	0(0)	0(0)	0(0)	0(0)	32(100)	32
Total	2(1.6)	11(8.8)	7(5.6)	4(3.2)	101(80.8)	125

Keys: H&E – haematoxylin and eosin; CIN – cervical intraepithelial neoplasia. $X^2 = 26.714$, *p*-value =0.008 using Anova Statistical package.

Table 3: Frequency of immunological diagnosis.

Immuno Diagnosis	Frequency	Percent
Positive (focal)	43	34.4
Negative	37	29.6
Positive (diffuse)	45	36.0
Total	125	100.0

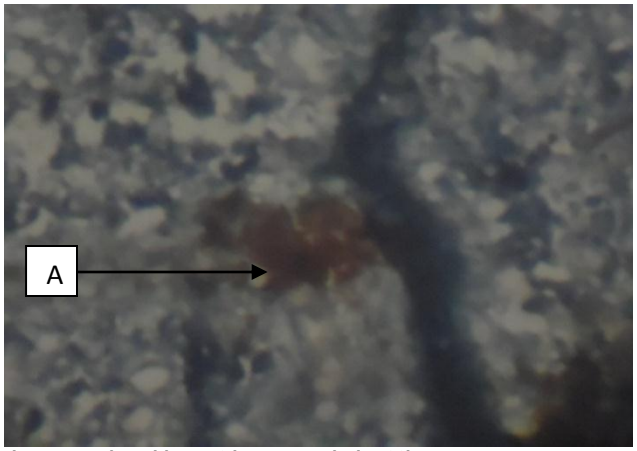

Fig. 1: Focal positive p16 immunostain (x 100)
FIG 1: Shows clear focal staining to P^{16} antibody A demonstrating focused staining on cytoplasm. This shows possible infection with only low risk Human Papilloma Virus.

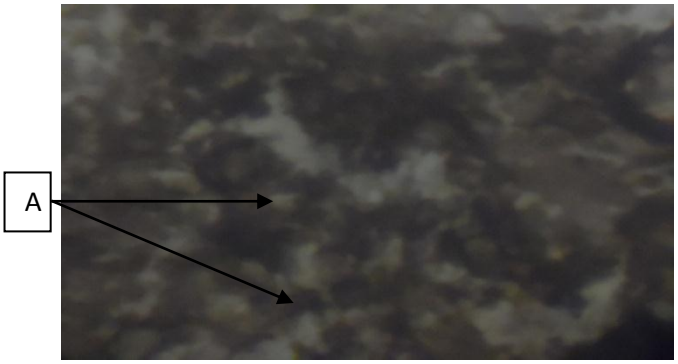

Fig. 2: A, diffuse positive p16 immunostain (x 100)

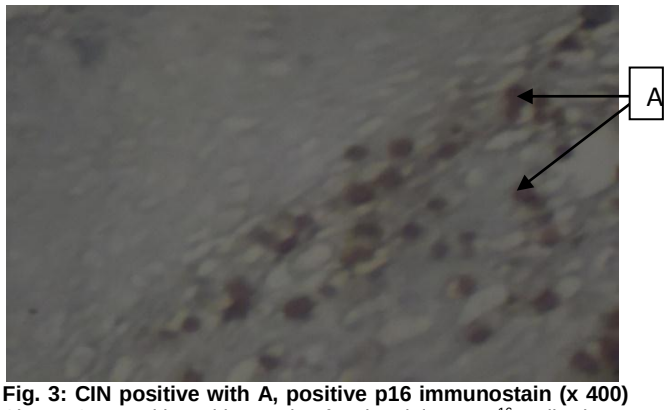

Fig. 3: CIN positive with A, positive p16 immunostain (x 400)
Shows CIN positive with reactive focal staining to P^{16} antibody on connective tissue, Basal, Para basal and superficial cells.

DISCUSSION

One hundred and twenty five (125)
archival tissue blocks were used for the study. Out of these, 32(25.6%) were age group 21-30 years, 32(25.6%) were 31-40 years, 29(23.2%) were 41-50 years and 32(25.6%) were 50 years and above.

Relationship of age and different stages of dysplastic lesions is presented in Table 3. It shows women of ages 20-30 years have 2(6.3%) of inflammation, 1(3.1%) CIN1, 4(12.5%) CIN2, 0(0%) CIN3 and 25(78.1%) are cancer. Thirty one to forty (31-40) years had 0 (0%) inflammations, 5(15.6%) CIN1, 1(3.1%) CIN2, 3(9.4%) CIN3, 25(78.1%) were cancers. Forty one to fifty (41-50) years had 0 (0%) inflammations, 5(15.6%) CIN1, 1(3.1%) CIN1, 3(9.4%) CIN3, 23(71.9%) are cancers. Fifty one years and above were found to have no inflammation, CIN1, CIN2, CIN3 and 32(100%) were cancers. This clearly indicate that ($p<0.01$.) by Anova Statistical package.

Immunohistochemical technique shows that 43(34.4%) focal positive, 45(36%) positive diffuse and 37(29.6%) were negative.

Relationship of immunohistochemical technique and age show that woman of 20-30 years have 14(43.8) Focal positive, 10(31.3%) negative, 8(25%) diffuse positive. Thirty one to forty (31-40) years were 7(21.9%) positive focal, 14(43.8%) negative, 11(34.4%) positive diffuse. Forty one to fifty years showed 10(34.5%) positive focal, 5(17.2%) negative and 14(48.3%) positive diffuse. Fifty years and above have 12(37.5%) positive focal, 8(25%) negative and 12(37.5%) were positive diffuse. This shows that ($p>0.05$.) by Anova.

Immunohistochemical demonstration of Human Papilloma virus on cervical biopsy using P^{16}antibody is a good diagnostic tool for differentiating HR-HPV from LR-HPV from colposcopy specimen.

Human papilloma virus was found to be the most significant risk factor for cervical cancer.[1] HPV is recognized to be the public health problem for its

role as a co-factor in the pathogenesis of cervical cancer.[8]

In the present study histochemical staining of cervical biopsies showed women of 30 years and below and those of 50 years and above has the highest number of cervical lesions. This finding is in agreement with Berkowitz (1979) who work on invasive cervical carcinoma in young women.

More over in this study it was found that carcinoma in situ has the highest percentage101(80.8%), among cervical biopsy collected CIN1 11(8.8%), CIN2 7(5.6%), CIN3 4(3.2%) and inflammation the lowest percentage with 2(1.6%) which is in agreement with the work of Wang, Sherman on cervical adenocarcinoma and incidence trends among white and black women in U.S.A.[10]

P16 antibody staining technique showed that (45%) of slide subjected to immuno staining shows diffuse positive staining which is an indication that the lesions are either due to HR-HPV or deregulation of viral oncogene (E7) and (43%) shows focal positive staining with P^{16} antibody which is due to LR-HPV or regulated viral oncogene (E7). This is in agreement with work of Sano, Oyama that showed expression status of P^{16} protein is associated with HPV oncogenic potential in cervical and genital lesions.[11]

Statistical analysis of age distribution of P^{16}reactivity showed that there is no statistical significance ($P > 0.05$). This correlates with finding of Kurman, Schiffman who worked on P^{16} immunochemistry inter-observer agreement in the diagnosis of cervical intraepithelial neoplasia.[12]

Immunochemistry staining using P^{16} antibody also indicate that there is no significant relationship between P16 and H&E staining diagnosis ($p = 0.662$). This work correlates with the finding of Negri, Vittadello who work on P^{16} expression and progression risk of low grade intraepithelial dysplasia of the cervix.[13]

CONCLUSION

It is therefore of high importance to take appropriate precautions to prevent an invasive carcinoma from existing once the diagnosis of dysplastic lesions is made by assessing all the risk factors using appropriate diagnostic tools. The use of P16 as neoplastic antigen is very useful in differentiation of HR-HPV and LR-HPV or in other word reversible and irreversible lesions.

Use of P^{16} antibody will also expose regulation and deregulation of viral oncogene (E^7) by demonstrating either focal or diffuse staining on the stained slide. This study is limited to the demonstration of Human Papilloma Virus using P^{16} antibody.

However p16 is recommended to be the gold standard immunological techniques for differentiating HR-HPV from LR-HPV.

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