IJHOSCR

Original Article

International Journal of Hematology-Oncology and Stem Cell Research

Evaluation of Vimentin as a Marker of Cervical Carcinogenesis in Women Infected with Human Papillomavirus and *Chlamydia trachomatis* with Cervical Pathology

SJ Magaji [1], M Aminu[1], MHI Doko[1], OA Oguntayo[2], Ahmed SA[3], JD Yaro[3], MA Abubakar[3], KO Sani[3], VG Nelson[4], OE Alaba[5]

[1]Department of Microbiology, Ahmadu Bello University, Zaria, Kaduna State, Nigeria
[2]Department of Obstetrics and Gynaecology, Ahmadu Bello University, Zaria, Kaduna State, Nigeria
[3]Department of Pathology, Ahmadu Bello University, Zaria, Kaduna State, Nigeria
[4]Department of Pathology, Abubakar Tafawa Balewa University Teaching Hospital, Bauchi, Bauchi State, Nigeria
[5]Department of Pathology, University of Abuja Teaching Hospital, Abuja, FCT, Nigeria

Corresponding Author: SJ Magaji, Department of Microbiology, Ahmadu Bello University, Zaria, Kaduna State, Nigeria
E-mail: shebajoseph3@gmail.com

Received: 09, Aug, 2024
Accepted: 22, May, 2025

ABSTRACT

Background: Among the co-factors contributing to human papillomavirus (HPV)-related cervical carcinogenesis, genital chlamydial infection is considered a very strong risk factor. The molecular mechanisms by which this organism contributes to HPV-related cervical carcinogenesis remain unknown. This study aimed to evaluate the role of vimentin in cervical carcinogenesis in women infected with human papillomavirus and *Chlamydia trachomatis.*

Materials and Methods: A total of 200 formalin-fixed paraffin-embedded (FFPE) cervical tissue samples were collected from women with pre-invasive and invasive cervical disease in northern Nigeria during July 2022 to September 2023. Samples were screened for both high-risk human papillomavirus (hrHPV) and *C. trachomatis* antigen using GeneXpert® and rapid tests, respectively. Samples that were positive for hrHPV, *C. trachomatis*, and cervical cancer underwent immunohistochemistry assay for vimentin detection.

Results: The results showed that 47 (23.5%) samples had hrHPV, while 17 (8.5%) samples had *C. trachomatis* antigen. A total of 103 samples were assayed for vimentin expression, of which 16 (15.5%) samples expressed vimentin at varying degrees. It was observed that vimentin expression increased significantly with increasing tumor severity.

Conclusion: The strong statistical association between vimentin expression and hrHPV-*C. trachomatis* co-infection in cervical carcinogenesis suggests the potential application of vimentin expression assays for early detection of cervical cancer, monitoring disease progression, and implementing prompt treatment approaches. This study findings implicate epithelial-mesenchymal transition (EMT) as a possible molecular mechanism for cervical carcinogenesis in women infected with hrHPV and *C. trachomatis* and highlight vimentin as a poor prognostic indicator, given its observed correlation with tumor severity.

Keywords: hrHPV*; Chlamydia trachomatis;* Vimentin; FFPE; Cervical cancer

INTRODUCTION

Cervical cancer is characterized by the abnormal growth of cells that could invade other parts of the body. It is the second most common cancer among females aged 15-44 years and the second leading cause of death in this population group worldwide[1-2]. Persistent infection with high-risk human papillomavirus (hrHPV) is well-established as the

primary cause, accounting for over 90% of cervical cancer cases[3-6]. However, although infection with hrHPV genotypes is necessary, it is not sufficient alone to cause cervical carcinogenesis, suggesting the involvement of additional host or external factors in the disease progression[7].

Co-infection of *Chlamydia trachomatis*, a sexually-transmitted obligate intracellular bacterium, with human papillomavirus has been proposed as a significant risk factor for cervical carcinogenesis[8]. However, the molecular mechanisms by which this organism contributes to HPV-related cervical cancer remain poorly understood. Understanding these mechanisms could facilitate early confirmatory diagnosis, accurate prognosis, and timely treatment, potentially preventing the progression to invasive and metastatic cervical cancer.

Conceptually, co-factors such as *C. trachomatis* may promote the invasiveness, spread, and tumorigenicity of oncogene-transformed cervical epithelial cells by inducing specific biological processes. These processes could provide useful biomarkers for reliable early diagnosis of advancing cervical cancer. While several markers are used clinically to diagnose cervical cancer, studies on intermediate filament (IF) proteins remain limited. Vimentin, a type III intermediate filament protein, is part of the cytoskeletal network, along with microtubules and microfilaments. Increased vimentin expression and decreased E-cadherin expression are key indicators of the epithelial-mesenchymal transition (EMT) process[9-10].

Epithelial-mesenchymal transition is a crucial microRNA (miRNA)-regulated biological process that transforms normal polarized epithelial cells into fibroblastic mesenchymal cells, thereby altering epithelial integrity, functions, and tumorigenic propensity[11-12]. Mesenchymal cells exhibit enhanced motility, migratory capacity, invasive ability, higher resistance to senescence and apoptosis, and increased production of extracellular matrix (ECM) components[13].

Due to the asymptomatic nature of early HPV infection, many women present to the hospital late when cervical cancer has advanced; thus, it may be too late for effective treatment, and most of them may end up not surviving. This necessitates the need for early prognosis, which will be instrumental in prompt commencement of treatment. This study aimed to elucidate the role of vimentin in cervical carcinogenesis among women co-infected with HPV and *C. trachomatis* with cervical pathology. By identifying the contribution of vimentin to cervical cancer progression, this research sought to advance early detection, improve monitoring, and inform treatment strategies.

MATERIALS AND METHODS

This hospital-based cross-sectional study was conducted on women who were histopathologically diagnosed with pre-invasive and invasive cervical disease in northern Nigeria during July 2022 to September 2023. A total of 200 women participated in the study, of whom 111 and 89 were diagnosed with benign cervical conditions and cervical cancer, respectively. Cervical tissue samples were collected from these women and processed into formalin-fixed paraffin-embedded (FFPE) specimens. These samples were subsequently assessed for hrHPV infection, *C. trachomatis* infection, and vimentin expression to investigate their roles in the development and progression of cervical cancer.

Sample Processing for High-Risk Human Papillomaviruses and *C. trachomatis* Assay

Approximately 10-µm sections were prepared from formalin-fixed paraffin-embedded (FFPE) samples and deparaffinized in two xylene changes (1 mL each) for 5 min each. The samples were then serially hydrated in decreasing concentrations of alcohol: 1 mL each of absolute ethanol, 80% ethanol, and 70% ethanol. Following hydration, the tissues were transferred to distilled water for 10 min, rinsed in phosphate-buffered saline (PBS) for 2 min, and finally suspended in 2 mL of PBS[14].

The suspended tissues were centrifuged at 10,000 rpm for 2 min, after which the supernatant was discarded. Tissue digestion was performed by adding 180 µL of DNase/RNase-free water, 15 µL of Tween 20, and 25 µL of Proteinase K. The samples were then spun at 10,000 rpm for 1 min and incubated overnight in a water bath at 56 °C. Enzyme inactivation was achieved by raising the water bath temperature to 90 °C and maintaining it for 30 min.

The samples were then aliquoted into two portions: the first portion was used for hrHPV detection, and the second portion was stored at -20 °C for *C. trachomatis* antigen detection.

Detection of high-risk human papillomavirus genotypes in tissue samples

All cervical tissue samples were analyzed using the GeneXpert system (GeneXpert® Cepheid®, Sunnyvale, CA, USA) to detect the presence of high-risk HPV (hrHPV) genotypes. Approximately 1.2 mL of PBS was added to each digested tissue sample, and 1 mL of this dilution was then aspirated and transferred to a pre-labelled Xpert® HPV test cartridge. The cartridge was inserted into the GeneXpert system, which initiated the reaction. The assay ran for 58 min, after which the results were read. The hrHPV test results were displayed in real time and recorded in an Excel spreadsheet.

Detection of *C. trachomatis* Antigen

The cervical tissue samples were brought to room temperature and analyzed for bacterial antigens using a rapid test kit (Atlas Link Technology CO., Ltd, China) following the manufacturer's instructions. Briefly, 300 µL of extraction solution A was added to an extraction tube, followed by the addition of a portion of the homogenized cervical tissue sample. The mixture was thoroughly mixed and left at room temperature for 2 min. Then 300 µL of solution B was added to the tube, mixed thoroughly, and kept on the bench.

For the assay, a test cassette was removed from a sealed pouch and placed on a clean, dry surface. Approximately 150 µL of the extracted sample was dropped into the sample well on the test cassette and allowed to run for 10-15 min. A positive result was indicated by the appearance of two colored lines within 10 min: one in the C region and the other in the T region. A negative result was indicated by the appearance of a single line in the C region within 15 min. The test was considered invalid if no line appeared in the C region after 15 min.

Immunohistochemistry Assay for Vimentin Detection

Formalin-fixed paraffin-embedded (FFPE) cervical tissue samples were sectioned at 4 µm thickness and mounted onto glass slides. The slides were deparaffinized in xylene, rehydrated through a graded ethanol series (100, 90, and 70%), and then rinsed in deionized water. Vimentin expression was evaluated using immunohistochemistry (IHC) staining with mouse monoclonal antibodies against vimentin (dilution 1:200; Bio SB, Europe).

Antigen retrieval was achieved by steam heating the slides for 20 min in 0.01 M trisodium citrate buffer (pH 6.0). To block endogenous peroxidase activity, the slides were immersed in ChemMate peroxidase-blocking solution for 5 min at room temperature. The samples were then incubated with primary antibodies for 90 min, followed by HRP-labeled anti-mouse or anti-rabbit secondary antibodies. After washing in PBS for 10 min, immunoreactive proteins were visualized using 3,3-diaminobenzidine (Sigma-Aldrich) as a chromogen, and nuclei were counterstained with Mayer's hematoxylin.

The slides were dehydrated through an ethanol series (70, 90, and 100%), mounted with dibutylphthalate polystyrene xylene (DPX), and independently evaluated by two pathologists. The pathologists were blinded to clinical data and other immunohistochemical findings. Any discrepancies in scoring were resolved through discussion between the two pathologists.

Scoring

The expression levels and sub-cellular localization of vimentin were assessed using both positive and negative controls as references. In each section of the tumor tissue samples, at least 1,000 cells from five randomly-selected areas were analyzed at ×400 magnification to determine labelling indices. The final immunohistochemistry score was calculated using a semi-quantitative approach based on the staining intensity and the percentage of positively-stained cells. Vimentin expression was considered positive if cytoplasmic or nuclear staining was observed in more than 10% of tumor cells[15-17].

Data analyses

Data were analyzed using the Epi-Info statistical package (Version 7.2.5) with a significance level of 0.05 and a confidence interval of 95%. The association between hrHPV and *C. trachomatis* co-infection and other clinicopathologic factors was examined using Pearson Chi-square or Fisher's exact test (FET) where appropriate. The degree of association was evaluated using odds ratio (OR) and relative risk (RR). Categorical variables were summarized using frequencies and percentages.

RESULT

Of the 200 cervical tissue samples analyzed, 47 (23.5%) were positive for hrHPV (Figure 1). Following the study by Guerendiain et al. (2016)[18], which demonstrated 92% agreement between the Cepheid GeneXpert® assay and a sensitive Luminex®-based assay, any invalid hrHPV sample was classified as negative. For *C. trachomatis*, 17 (8.5%) samples tested positive for the bacterial antigen (Figure 2).

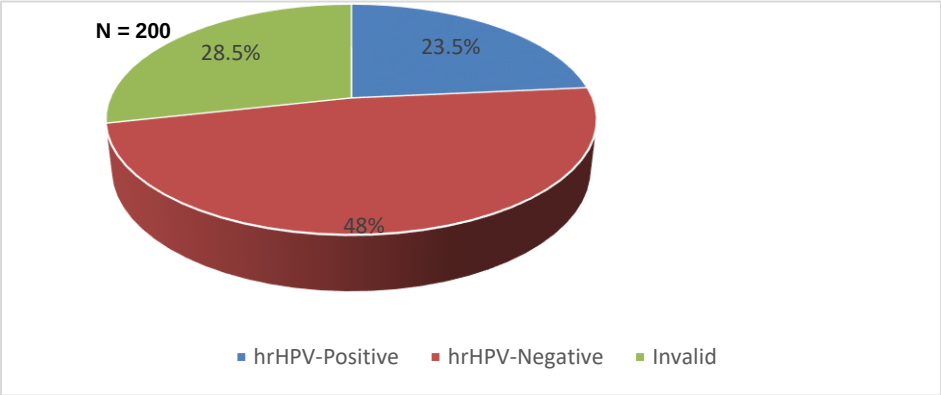

Figure 1: High-risk human papillomavirus genotypes detected in formalin-fixed paraffin- embedded cervical tissues

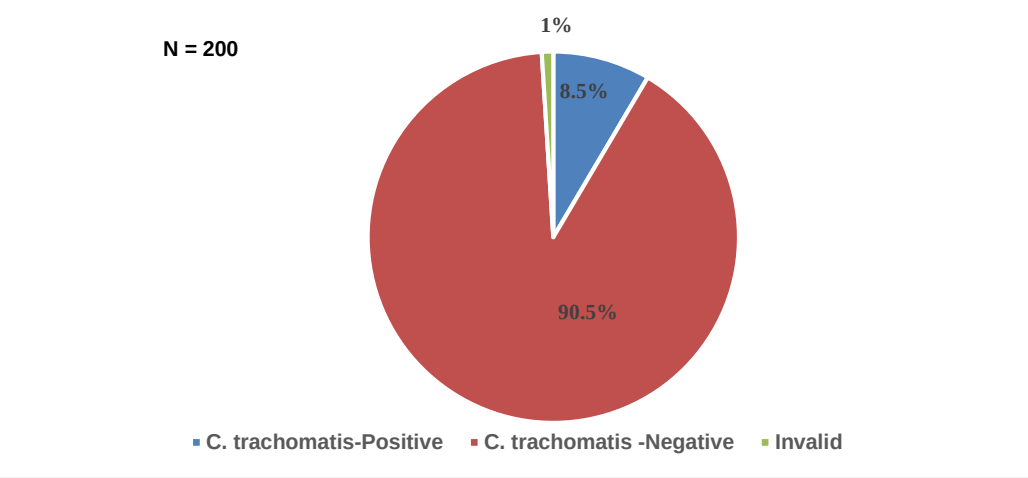

Figure 2. Prevalence of *Chlamydia trachomatis* detected in formalin-fixed paraffin- embedded cervical tissues

The study examined co-infection rate and its association with cervical cancer. Of the total cohort, 10 (5.0%) women were co-infected with both hrHPV and *C. trachomatis* and diagnosed with cervical cancer (Figure 3).

Vimentin expression was assessed in 103 samples, including those positive for hrHPV, *C. trachomatis*, and cervical cancer (Plate I). The results indicated that 16 (15.5%) samples exhibited varying degrees of vimentin expression. Specifically, 84.5% (87 of 103) were negative for vimentin (Plate II), while 10.7% (11 of 103) showed weak vimentin expression (Plate III), and 4.9% (5 of 103) demonstrated strong vimentin expression (Plate IV).

Association of Vimentin Expression with hrHPV and *C. trachomatis* Infections

The association between vimentin expression and hrHPV infection was not significant (FET: *df* = 1, *p* = 0.1092, OR = 3.11, RR = 2.62) (Table 1).

Also, there was no significant association between vimentin expression and *C. trachomatis* infection (FET: *df* = 1, *p* = 0.1728, OR = 2.84, RR = 2.30) (Table 2).

Association of Vimentin Expression with Cervical Cancer

The association between vimentin expression and cervical cancer was not significant, indicating an independent relationship (FET: *df* = 1, *p* = 1.0000, OR = 1.12, RR = 1.10) (Table 3).

When examining hrHPV-*C. trachomatis* co-infection in relation to vimentin expression in cervical cancer, a very strong statistically significant association was found (FET: *df* = 1, *p* = 0.0082, OR = 12.45, RR = 5.58) (Table 4).

Vimentin Expression in Cancer Types

Vimentin expression varied across different cancer types, peaking at 19.0% (8 of 42) in poorly differentiated squamous cell carcinoma. However, no significant association was found between vimentin expression and cancer type (χ^2 = 0.5144, *df* = 5, *p* = 0.9157) (Table 5). Overall, 15.7% (14 of 89) of women with cervical cancer exhibited vimentin expression.

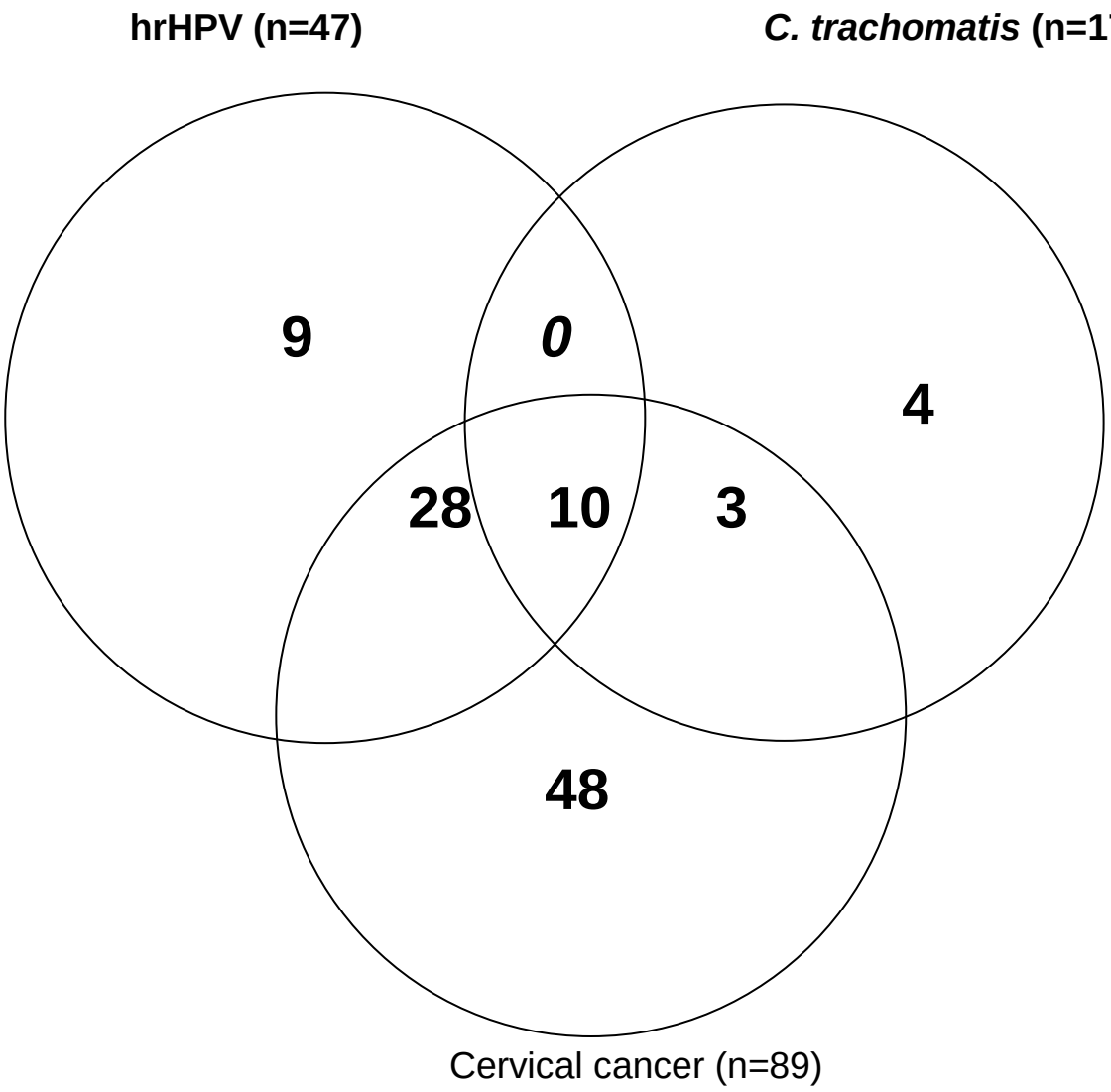

Figure 3. Venn diagram illustrating the prevalence of high-risk human papilloma viruses, *Chlamydia trachomatis*, and co-infections in women with cervical pathology, and how these infections are related to the occurrence of cervical cancer in this group

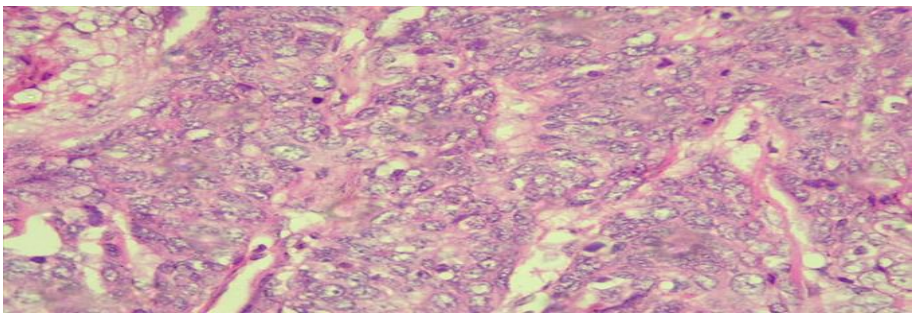

Plate I: Squamous cell carcinoma with foci of abnormal mitoses (H&E staining, Mag.: x400)

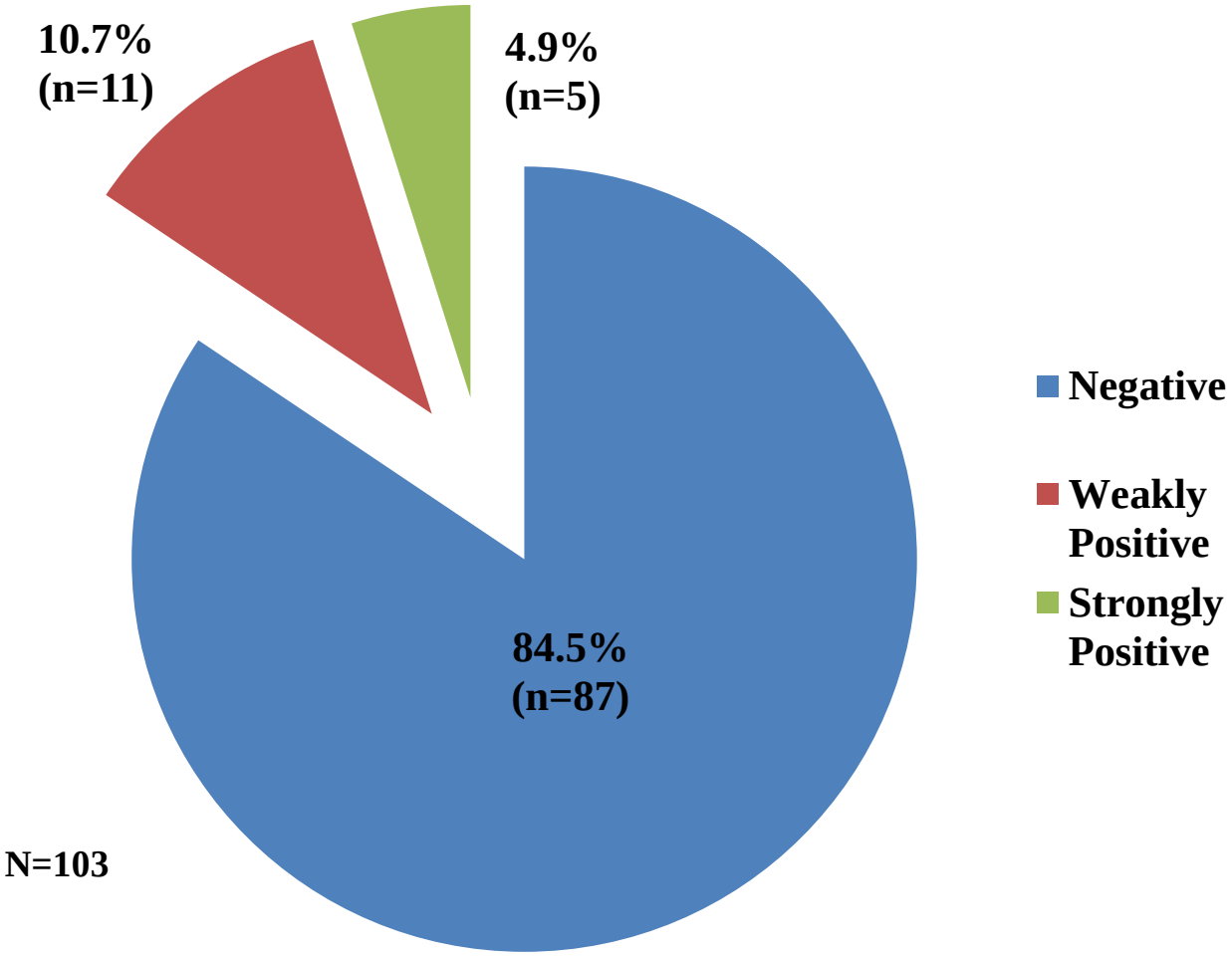

Figure 4. Expression percentage of vimentin in formalin-fixed paraffin-embedded cervical tissues. Negative: vimentin expression of 0 to <10% (Score 0-1), weakly positive: vimentin expression of ≥10 to 50% (Score 2), strongly positive: vimentin expression of >50% (Score 3)

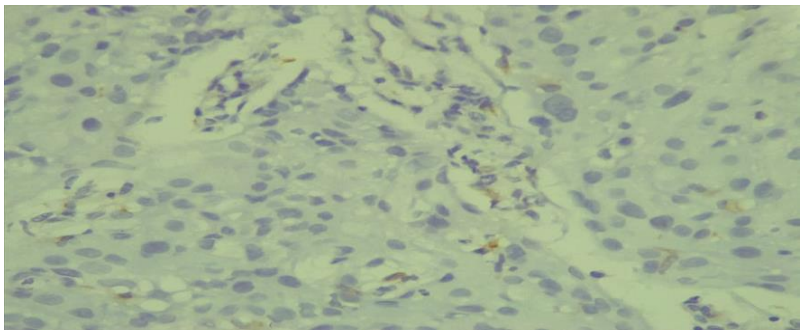

Plate II: Immunohistochemistry staining, negative for vimentin (Mag.: x400)

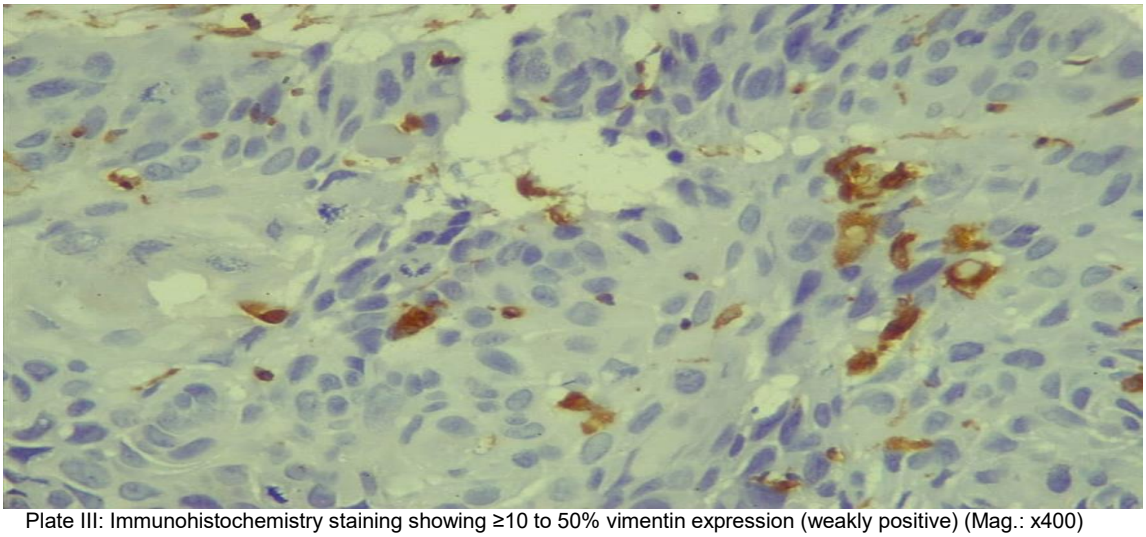

Plate III: Immunohistochemistry staining showing ≥10 to 50% vimentin expression (weakly positive) (Mag.: x400)

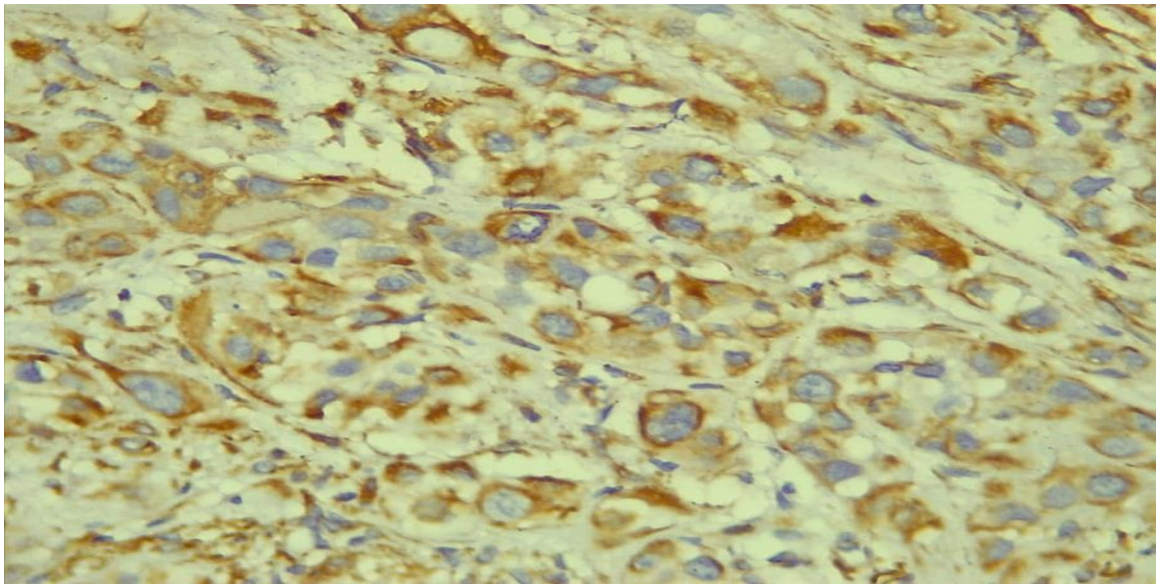

Plate IV: Immunohistochemistry staining showing >50% vimentin expression (strongly positive) (Mag.: x400)

Table 1. Correlation of vimentin expression with high-risk human papillomavirus infection

Presence of hrHPV	Numbers Screened	Vimentin Positive (%)	Vimentin Negative (%)	*P*	OR	RR
Yes	47	11 (23.4)	36 (76.6)	0.1092	3.11	2.62
No	56	5 (8.9)	51 (91.1)			
Total	103	16 (15.5)	87 (84.5)			

OR: Odds ratio
RR: Relative risk

Table 2. Correlation of vimentin expression with *Chlamydia trachomatis* infection

Presence of *C. trachomatis*	Numbers Screened	Vimentin Positive (%)	Vimentin Negative (%)	*P*	OR	RR
Yes	17	5 (29.4)	12 (70.6)	0.1728	2.84	2.30
No	86	11 (12.8)	75 (87.2)			
Total	103	16 (15.5)	87 (84.4)			

OR: Odds ratio
RR: Relative risk

Table 3. Correlation of vimentin expression with cervical cancer cases

Presence of Cervical Cancer	Numbers Screened	Vimentin Positive (%)	Vimentin Negative (%)	*P*	OR	RR
Yes	89	14 (15.7)	75 (84.3)	1.0000	1.12	1.10
No	14	2 (14.3)	12 (85.7)			
Total	103	16 (15.5)	87 (84.5)			

OR: Odds ratio
RR: Relative risk

Table 4. Correlation of vimentin expression with co-infection of high-risk human papillomaviruses and *Chlamydia trachomatis* in cervical cancer cases

Presence of hrHPV, *C.* trachomatis, and Cervical Cancer	Numbers Screened	Vimentin Positive (%)	Vimentin Negative (%)	*P*	OR	RR
Yes	10	6 (60.0)	4 (40.0)	0.0082	12.45	5.58
No	93	10 (10.8)	83 (89.2)			
Total	103	16 (15.5%)	87 (84.5)			

OR: Odds ratio
RR: Relative risk

Table 5. Expression pattern of vimentin in cervical carcinoma cases

Cervical Cancer	Total Cases	Vimentin Positive Cases (%)	P
Highly Differentiated SCC	24	3 (12.5)	
Poorly Differentiated SCC	42	8 (19.0)	0.9157
Unclassified SCC	9	1 (11.1)	
Adenocarcinoma	10	2 (20.0)	
Leiomyosarcoma	1	0 (0)	
Carcinoma *in situ*	3	0 (0)	
Total	89	14 (15.7)	

SCC: Squamous cell carcinoma

DISCUSSION

The GeneXpert® assay detected hrHPV in 23.5% of cervical tissue samples, indicating significant circulation of hrHPV in the study area. This prevalence is consistent with the known association between hrHPV and cervical cancer, as hrHPV is responsible for approximately 90% of cervical cancer cases[3].

C. trachomatis was identified in 8.5% of tissue samples, reflecting its considerable prevalence among the study population. This may be attributed to limited awareness and screening for this common sexually-transmitted bacterial infection[19]. Notably, *C. trachomatis* co-infection with hrHPV has been shown to accelerate cervical carcinogenesis[8].

The findings revealed that all women with hrHPV-*C. trachomatis* co-infection also had cervical cancer, suggesting a potential synergistic effect between these pathogens in cervical carcinogenesis. This finding aligns with those of previous studies by Koskela et al. (2000)[20] and Arnheim et al. (2011)[8].

Immunohistochemical analysis showed that 15.5% of women exhibited vimentin expression, with 10.7% showing weak expression and 4.9% demonstrating strong expression. Despite the high incidence of hrHPV, vimentin expression was not significantly correlated with hrHPV infection, highlighting that hrHPV, although crucial, is not sufficient alone to induce cervical cancer[7,21]. Similarly, vimentin expression was not significantly associated with *C. trachomatis* infection. Although *C. trachomatis* disrupts epithelial integrity and induces inflammation, these factors alone are not sufficient

to cause cancer as this bacterium does not carry oncogenes[22-23].

However, a strong association was found between vimentin expression and co-infection with hrHPV and *C. trachomatis* in cervical cancer. Co-infection with these pathogens may facilitate cervical carcinogenesis through several mechanisms. *C. trachomatis* could compromise the cervical epithelium, increasing susceptibility to hrHPV infection[23]. Both hrHPV and *C. trachomatis* are intracellular and could significantly alter gene expression and protein production, leading to chromosomal instability and cell transformation[24-26]. *C. trachomatis* infection has been shown to be linked to centrosome amplification and spindle defects, which contribute to chromosomal instability and eventually cell transformation[22,27]. Additionally, *C. trachomatis*-induced secretion of local immune mediators could elevate the production of reactive oxygen species, causing persistent damage to mucosal barriers and impairing immune surveillance. This environment allows hrHPV-infected cells to evade immune detection and proliferate uncontrollably, with further implications for tumor progression[28-29].

According to Franchini et al. (2022)[30], *C. trachomatis* induces cervical metaplasia, which creates target cells for hrHPV infection and interferes with immune responses, leading to persistent infection. Chronic inflammation caused by *C. trachomatis* may promote cellular proliferation, inhibit apoptosis, and create a favorable microenvironment for hrHPV-associated carcinogenesis. The synergy between hrHPV and *C. trachomatis* likely exacerbates cellular damage and enhances the risk of cervical dysplasia and cancer[22,27].

The findings suggest that co-infection with high-risk human papillomavirus (hrHPV) and *C. trachomatis* may trigger a physiological process, likely epithelial-mesenchymal transition (EMT), as evidenced by upregulation of vimentin. Overexpression of vimentin is known to facilitate EMT, characterized by decreased expression of E-cadherin and acquisition of stemness-related traits[31-32]. This study proposes that hrHPV-*C. trachomatis* co-infection acts as an EMT mediator by activating mesenchymal genes, including vimentin, which regulates cell motility[33-34]. Women with hrHPV and *C. trachomatis* co-infection exhibited a six-fold increased risk of cervical carcinogenesis compared to those without this co-infection.

Both hrHPV and *C. trachomatis* infections trigger inflammatory responses within the cervical microenvironment. Vimentin has been shown to be implicated in modulating these inflammatory responses by influencing cytokine production and immune cell migration. In cases of co-infection, vimentin expression may exacerbate inflammation, creating a microenvironment conducive to tumor growth and progression[35]. These findings suggest that vimentin could serve as a direct diagnostic marker for cervical carcinogenesis, aiding in early detection and timely intervention. Previous studies have noted that vimentin promoter methylation occurs early in carcinogenesis, in stages such as CIN I and CIN II, highlighting its potential role as an early biomarker for cervical cancer[36].

The present study results showed that 15.7% of women with cervical cancer exhibited vimentin expression, with the highest expression observed in those with adenocarcinoma and high-grade tumors. Adenocarcinoma (ADC), arising from the mucus-producing glands of the endocervix, and squamous cell carcinoma (SCC), originating from the exocervix, are the predominant histological types of cervical cancer[37]. ADC and SCC account for over 70% and up to 20% of cervical cancer cases, respectively. Studies have shown that ADC often presents with a poorer prognosis compared to SCC[38-41]. For instance, the Japan Society of Obstetrics and Gynecology revealed that the 5-year overall survival rate was significantly higher in SCC patients (80.4%) compared to ADC patients (75.5%)[42]. These observations likely explain the higher vimentin expression in ADC compared to SCC.

The lower prevalence of vimentin expression (15.7%) in this study compared to those reported in previous research, including 40% by Nazik et al. (2016)[43] and 75.4% by Yu et al. (2015)[16], may be due to variations in tumor differentiation and proliferation rates. High vimentin expression is associated with high tumor grade and metastatic progression, supporting the theory that vimentin expression is associated with advanced disease stages[44-46,16,10]. However, the

findings are in contrast to those of Cheng et al. (2012)[47], who reported an inverse relationship between vimentin upregulation and histological differentiation, metastasis, and recurrence. The discrepancy in vimentin expression patterns among different grades of cervical cancer is not fully understood, but it is known that vimentin promotes cell migration, adhesion, and metastasis[48-51]. Differences in prevalent rates could also be due to variations in the populations studied or methodologies used in different studies. Although crucial in embryonic development and wound healing, EMT is a key factor in cancer metastasis[52,9].
In summary, this study indicates that vimentin expression is significantly associated with hrHPV and *C. trachomatis* co-infection and cervical cancer severity. Elevated vimentin levels are linked to tumor migration, invasion, and metastasis, supporting the hypothesis that hrHPV and *C. trachomatis* co-infection induces EMT, a critical mechanism in cervical carcinogenesis. Vimentin could serve as a valuable prognostic marker, aiding in early detection and treatment of cervical cancer. Targeting vimentin or its associated pathways may offer a therapeutic strategy for managing cervical cancer, particularly in patients with hrHPV and *C. trachomatis* co-infection.

CONCLUSION

This study establishes vimentin as a promising biomarker for early detection of cervical carcinogenesis, particularly in the context of hrHPV and *C. trachomatis* co-infection. A strong statistical association was observed between vimentin expression and hrHPV-*C. trachomatis* co-infection (p= 0.0082), supporting the role of vimentin upregulation in cervical cancer development. This finding highlights the potential utility of vimentin in early diagnosis, guiding timely intervention, and tracking disease progression. The study also suggests that epithelial-mesenchymal transition (EMT) may underlie the carcinogenic process induced by these infections, further emphasizing the role of vimentin as a critical prognostic marker, given its correlation with tumor severity. The findings of this study support the future integration of vimentin expression assays into routine diagnostic frameworks for better risk stratification.

Limitation

A limitation of this study was that the HPV assay used (GeneXpert®) could only detect 14 high-risk HPV types, which were the most prevalent.

Recommendation

Vimentin expression assays should be considered as both a diagnostic and prognostic tool in cervical carcinogenesis. Incorporating vimentin assays into routine diagnostic protocols could enhance early detection and improve management strategies for patients at risk of or diagnosed with cervical cancer.

ACKNOWLEDGMENTS

We extend our gratitude to the doctors, nurses, and laboratory personnel of the Obstetrics & Gynecology Department and Histopathology Department of Ahmadu Bello University Teaching Hospital (ABUTH) in Zaria, Abubakar Tafawa Balewa Teaching Hospital in Bauchi, and Abuja University Teaching Hospital (UATH) in Abuja for their invaluable assistance in sample collection and support throughout this research.

Ethical considerations

Before the commencement of the study, ethical approval was obtained from the Research Ethics Committee of the selected hospitals: Ahmadu Bello University Teaching Hospital (ABUTH) in Zaria, Kaduna State; Abubakar Tafawa Balewa Teaching Hospital in Bauchi, Bauchi State; and Abuja University Teaching Hospital (UATH) in Abuja, FCT**.**

Funding

This research was partly sponsored by the Tertiary Education Trust Fund (TETFund) of the Federal Government of Nigeria. Grant No: TETF/DR&D/UNI/ZARIA/IBR/2024/BATCH 8/12.

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