Gynecologic Oncology Reports 53 (2024) 101388

Contents lists available at ScienceDirect

Gynecologic Oncology Reports

journal homepage: www.elsevier.com/locate/gynor

Research Report

Association between serum P16ink4A concentration and CIN and cervical cancer among women attending a cervical cancer clinic in western Uganda: A case control study

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ARTICLE INFO

Keywords:
Serum
P16ink4A
Cervical cancer
Cervical intraepithelial neoplasia
Mbarara Uganda

ABSTRACT

Introduction: Tissue expression of P16ink4A is correlated with cervical lesions. In this study we determined the association between serum P16ink4A concentrations and cervical lesions among women attending the cervical cancer clinic at Mbarara Regional Hospital (MRRH) South Western Uganda.
Material and Methods: We recruited 90 cervical intraepithelial neoplasia (CIN) cases, 90 cervical cancer (CC) cases before treatment and 90 controls. Clinical and demographic data were recorded. Serum P16ink4A concentrations were measured by quantitative Elisa. Cases were confirmed with cytology and/or histology. Descriptive statistics and logistic regression were done with STATA 17 and P-values of <0.05 were considered statistically significant.
Results: The mean serum P16ink4A concentration among CIN cases, CC cases and controls was 1.11(+/−0.66) ng/ml, 1.45(+/−1.11) ng/ml and 1.13(+/−0.61) ng/ml respectively ($p = 0.008$). 50 % of CIN cases and controls as well as 60 % of CC cases had P16ink4A concentration above 0.946 ng/ml. There were increased odds of CIN for serum P16ink4A though statistically insignificant (AOR: 1.11, p-value: 0.70). There was also a statistically significant reduction in odds of CC for serum P16ink4A (AOR: 0.55, p-value: 0.01).
Conclusion: Serum P16ink4A may likely be associated with cervical lesions especially CC in our study population and this may aid detection of such lesions. Diagnostic utility studies for circulating P16ink4A in detection of cervical cancer are recommended.

1. Introduction

There are over 770,828 estimated incident cases of cervical cancer worldwide (Sung, 2021) making it the second most common cancer among women aged 15 to 44 years (Sung, 2021; Arbyn, 2020; Bruni, 2019). It accounts for more than 270 000 deaths annually, mostly in developing countries (WHO, 2020), particularly in sub-Saharan Africa. Across East Africa, incident cases of cervical cancer have been reported at 43/100,000 (Sankaranarayanan, 2014). The reported age-standardized cervical cancer incidence in Uganda is higher than the global average of 56.2 per 100,000 women (WHO, 2020; WHO, 2023). Early diagnosis is key to cervical cancer elimination (Wilailak et al., 2021).

Many diagnostic modalities have been developed in relation to cervical cancer with the most popular being the cytological staining technique developed by G. Papanicolaou. In addition, population wide

Abbreviations: AOR, Adjusted Odds Ratio; COR, Crude Odds Ratio; CI, Confidence Interval; CIN, Cervical Intraepithelial neoplasia; CC, Cervical Cancer; HIV, Human Immunodeficiency Virus; HPV, Human Papilloma Virus; HrHPV, High Risk Human Papilloma Virus; LEEP, Loop Electrosurgical Excision Procedure; OR, Odds Ratio; PAP, Papanicolaou; SD, Standard Deviation; STATA, Statistical Software for Data Science; VIA, Visual Inspection with acetic acid.

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https://doi.org/10.1016/j.gore.2024.101388
Received 7 March 2024; Received in revised form 27 March 2024; Accepted 30 March 2024
Available online 31 March 2024
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screening programs have promoted early detection of preneoplastic lesions among asymptomatic patients, and thus reducing the incidence and mortality of cervical cancer in developed countries. In spite of the success of these programs, cervical cancer cases still occur, even in the developed countries, and also among screened women (Abila, 2021). Therefore, the Pap test is hampered by a number of factors thus being prone to false negative results, even in laboratories with highly effective and rigorous control procedures (Abila, 2021). In addition to that, the variance of normal epithelia or benign inflammatory conditions like reserve cell hyperplasia is capable of producing false positive results, which leads to repeated cytological testing, unnecessary treatments for patients and other related tremendous costs. Furthermore, cytology is also affected by a substantial rate of interobserver discrepancies even among a panel of expert pathologists. These factors could be some of the causes of low cervical cancer screening rates in Uganda (Sarah Maria, 2022; Isabirye et al., 2020; Wanyenze, 2022). This therefore calls for more sensitive and specific biomarkers for identification of dysplastic cells (Sankaranarayanan, 2014). These biomarkers could be user friendly and less costly tests for screening and diagnosis of cervical cancer especially in low resource settings.

Biomarkers including P16ink4A have been used severally, though in immunohistochemical studies. A number of studies have suggested that P16ink4A may be used for the identification of abnormal cells in cytologic cervical specimens. For instance, Yang et al (Yang, 2016) innovatively demonstrated the importance of P16ink4A in detecting cervical lesions using a piezoelectric immunosensor in liquid based preparations. P16ink4A is a cyclin dependent kinase inhibitor whose over expression is closely associated with high risk HPV infection and Cervical Intraepithelial Neoplasia (CIN) (Pientong, 2004). This therefore suggests that P16ink4A could be used in cervical cancer screening and this can reduce the ambiguity from other diagnostic techniques (Pientong, 2004; Anufrieva, 2022; Chuerduangphui, 2018). It is hypothesized that, the outgrowth of dysplastic lesions is enhanced by increasing expression of viral oncogenes E6 and E7 whose interaction with the various cell cycle regulating proteins like the retinoblastoma gene product, inhibit transcription of P16ink4A. This creates a direct proportionality, where by, increase in expression of viral oncogenes in dysplastic cervical cells reflects an increased expression of P16ink4A. A significant linear relationship is thought to exist between lesion grade and intensity of P16ink4A immunohistochemical staining, ($p = 0.0001$) with the expression of P16ink4A reported to have a 100 % specificity for CIN and 83.5 % sensitivity in detecting HR- HPV. Although P16ink4A can be objectively quantified and measured in blood using methods that can be standardised, this has never been demonstrated in serum samples. This study therefore aimed at describing the association between serum P16ink4A concentration and cervical lesions among women attending the cervical cancer clinic of Mbarara Regional Referral Hospital, South western Uganda.

2. Materials and methods

2.1. Study design

We conducted an unmatched case control study that purposively sampled all women seeking cervical cancer care at the cervical cancer clinic of Mbarara Regional Referral Hospital (MRRH) between April 2022 and June 2023. Cases were defined as women with a confirmed diagnosis of CIN or cervical cancer prior to treatment and controls were all those negative for intraepithelial lesions or malignancy. Our outcomes of interest were cervical intraepithelial lesions/cervical cancer while the exposure was serum concentration of P16ink4A.

2.2. Study setting

MRRH, is a tertiary hospital located in rural south western Uganda, whose catchment area is approximately four million people (Uganda Ministry of Health., 2016) across 13 districts of south western Uganda plus neighbouring countries including Rwanda, Burundi, Tanzania and the Democratic Republic of Congo. The clinic operates five days a week and receives an average of 15 women per day. Staff at the clinic include several nursing officers, senior residents and gynaecologists, who are headed by a gynaecologic oncologist. Visual inspection with acetic acid, colposcopy, conventional cytology and HPV DNA (for HIV positive women) are the screening tests routinely done at the clinic while confirmation of cervical lesions is achieved on histology (Fig. 1**)**. Women with pre-malignant lesions are treated with cryotherapy and thermocoagulation; those with confirmed cervical cancer either undergo gynecologic cancer surgery or are referred to Uganda Cancer Institute for radiotherapy and chemotherapy, according to their cancer stage and clinical findings.

2.3. Sampling method

Cases were selected through purposive sampling and controls enlisted based on the incidence density sampling method; for each identified case, a corresponding control was recruited in real-time. This prospective process continued until we achieved the necessary sample size.

2.4. Sample size determination

This sample size was calculated using an online software, OpenEpi, Version 3, Open source calculator-SSCC. OpenEpi - Sample Size for Unmatched Case-Control Studies. We considered a two-sided confidence level (1-alpha) of 95 %, a study power of 80 %, a case to control ratio of 1, an average proportion of cervical cancer or cervical intraepithelial neoplasia cases with P16ink4A expression of 30.0 and a proportion of controls with P16ink4A expression of 10. We also used the least extreme odds ratio of 3.86. Considering the module of Fleiss with continuity correction, the calculated sample size came to 72 participants in each of the case and control groups. Factoring in an expected 25 % attrition rate, this gave a total of 270 participants (90 CIN cases, 90 CC cases and 90 unmatched controls).

2.5. Informed consent statement

We sought written informed consent from every participant before taking part in the study. We also used study numbers, not names, on all data collection tools as well as on serum specimens. During data analysis, we delinked all participants' identifiable information. All participant interaction with research assistants including consenting and specimen collection took place in a private and comfortable side room in the clinic, free from disturbances; and only accessible to one participant at a time.

2.6. Institutional review board statement

For this study we secured ethical approval from the Mbarara University of Science and Technology Research Ethics Committee (MUST-REC) (MUST-2022–612). Our study was also registered by the Uganda National Council for Science and Technology (UNCST) (HS2722ES). We also sought administrative clearance from the Hospital Director, Mbarara Regional Referral Hospital, before commencing the study. All women diagnosed with cervical lesions received the standard package of care, following national guidelines at the cervical cancer clinic.

2.7. Data collection

2.7.1. Demographic data collection

Demographic data were gathered using a previously validated questionnaire, administered by a proficient research assistant who was a qualified midwife stationed at the cervical cancer clinic. The data

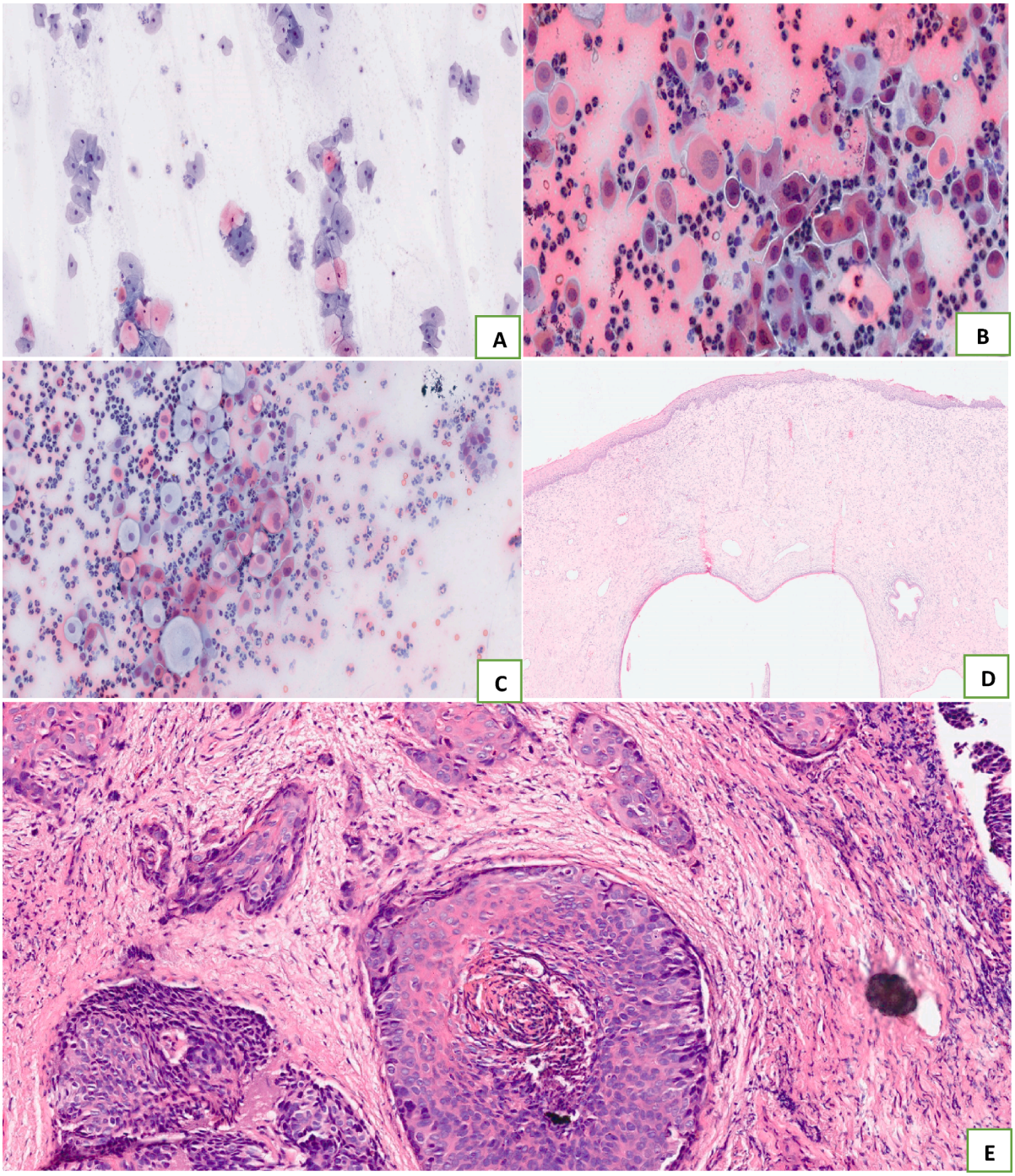


Fig. 1. Photomicrographs. A-Pap smear negative of intraepithelial lesion or malignancy ×400; B- Pap Smear Atypical squamous cells of undetermined significance ×400; C- Pap Smear Low grade squamous intraepithelial lesion ×400; D- Benign cervical tissue (Haematoxylin and Eosin) ×400; E- Squamous cell carcinoma (Haematoxylin and Eosin) ×400. Fig. 1: A box plot showing serum concentration of P16ink4A across different groups of study participants. CC: Cervical Cancer; CIN: Cervical Intraepithelial Neoplasia. Fig. 1: A-Pap smear negative of intraepithelial lesion or malignancy ×400; B- Pap Smear Atypical squamous cells of undetermined significance ×400; C- Pap Smear Low grade squamous intraepithelial lesion ×400; D- Benign cervical tissue (Haematoxylin and Eosin) ×400; E- Squamous cell carcinoma (Haematoxylin and Eosin) ×400.

encompassed key variables such as age, residential region, family planning practices and methods, HIV status, educational attainment, marital status, history of blood pressure, and history of diabetes. Following standard care procedures and upon obtaining written informed consent, each participant was guided by the research assistants in completing the questionnaire.

2.7.2. Blood collection

Four millilitres (4 mL) of venous blood were aseptically drawn from the mid-cubital vein by venepuncture and collected into plain-vacutainers. Each specimen was meticulously labelled with a unique identification number (code) and left to clot at room temperature for two hours. Subsequently the specimens were transported to the laboratory, where centrifugation was performed at 1000 × g for 15 min at 2 ~ 8°C to separate serum from blood cells. The resultant serum was then

carefully transferred into cryovial tubes using a micropipette. All serum samples were stored at −80℃ until analysis.

2.7.3. Measurement and interpretation of serum P16INK4A concentration

P16ink4A was measured using Human CDKN2A (Cyclin Dependent Kinase Inhibitor 2A) ELISA Kit, Elabscience Biotechnology Inc. This kit is designed for in vitro quantitative determination of Human CDKN2A concentrations in serum, plasma and other biological fluids with a sensitivity of 0.38 ng/mL and a detection range of 0.63–40 ng/mL. Actual measurements using this ELISA kit were based on the Sandwich-ELISA principle. The micro ELISA plates were pre-coated with an antibody specific to Human CDKN2A. Standards or samples were added to the micro ELISA plate wells and combined with the specific antibody. Then a biotinylated detection antibody specific for Human CDKN2A and Avidin-Horseradish Peroxidase (HRP) conjugate were added successively to each micro plate well and incubated. Free components were washed away. The substrate solution was added to each well. Only those wells that contained Human CDKN2A, biotinylated detection antibody and Avidin-HRP conjugate appeared blue in colour. The enzyme-substrate reaction was terminated by the addition of stop solution and the colour turned yellow. The optical density (OD) was measured using a microplate reader at a wavelength of 450 nm ± 2 nm. The OD value was proportional to the concentration of Human CDKN2A. Prior to running, all samples were diluted 1:3 and this was considered while making the final calculations for concentration. We ran samples along with reference standards. We created two categories of P16ink4A concentrations as calculated using the cutpt by Phil Clayton which determines a cut-off point (0.946) that maximises sensitivity and specificity. This method finds cutpoint on the ROC curve closest to the point with perfect sensitivity and specificity. The first category was named low P16ink4A concentration (≤0.946 ng/ml) while the second category was named raised concentration (0.946 < ng/ml).

2.7.4. Data management and analysis

Data was collected by the Principal Investigator together with research assistants into an excel spread sheet (Microsoft Office Professional Plus 2013, version 15.0.4675.1003, Microsoft Inc, USA) and then imported into STATA 17 software (StataCorp LLC, College Station, Texas, United States). Descriptive statistics were used to describe the populations using frequencies, means ± standard deviations (SDs) or median values for continuous variables as well as frequencies and proportions to describe categorical variables. We used bivariate and multivariate logistic regression analysis to derive associations between serum P16INK4A and cervical lesions. Multivariate logistic regression analysis was done after controlling for age, family planning usage and type, HIV status, marital status, history of blood pressure, history of diabetes and smoking status. Associations are presented as odds ratios and 95 % confidence intervals and a P-value of < 0.05 was considered statistically significant.

2.7.5. Eligibility criteria

All women aged 21 years and above, who presented at the MRRH cervical cancer clinic during the study period, and provided written informed consent for participation in the study were included. We excluded all those women that were too ill and those currently undergoing treatment for cervical lesions.

3. Results

3.1. Population characteristics

A total of 270 participants were included in the study out of whom 90 were positive for cervical cancer (CC), 90 positive for cervical intraepithelial neoplasia (CIN) and 90 negative for any cervical lesion (controls). The mean age of CC cases was significantly (p < 0.001) higher (51.1+/-13.1) than that of CIN cases (34.9+/-7.8) and that of controls (38.6+/-8.7). Majority of participants, 23 %(21/90) of CC cases, 32 % (29/90) of CIN cases and 43 %(39/90) of controls, belonged to the 40–49 age bracket. This difference in distribution was also statistically significant (p < 0.001). More than half of study participants in each group, i.e. 57 %(51/90) of CC cases, 60 %(54/90) of CIN cases and 56 % (50/90), were married. However, 31 %(28/90) of CC cases, 18 %(16/90) of CIN cases and 22 %(20/90) of controls were single (p < 0.001). The highest level of education among study participants was generally primary level and below, with 53 %(48/90) of CC cases having attained a maximum of preprimary, and 51 %(45/90) CIN cases and 44 %(40/90) controls also reporting to have attained a maximum of primary school education. Positivity with HIV was reported in 76 %(68/90) CC cases, 52 %(47/90) of CIN cases and 42 %(38/90) of controls and this difference in distribution was statistically significant (p < 0.001). The most common presenting complaint among study participants was cervicitis with a proportion of 58 %(52/90) among CC cases, 71 %(64/90) among CIN cases and 66 %(59/90) among controls and this difference in distribution was also statistically significant (p < 0.001) as shown in Table 1.

3.2. Distribution of serum P16ink4A concentrations between cervical intraepithelial neoplasia, cervical cancer and controls

Fig. 2 shows the distribution of serum P16ink4A across participant groups. CC cases had a higher serum P16ink4A concentration compared to CIN cases and controls. The mean serum P16ink4A concentration among CIN cases was 1.11(+/−0.66) ng/ml and 1.13(+/−0.61) ng/ml among controls, and 1.45(+/−1.11) ng/ml among CC cases and this difference in means was statistically significant (p = 0.008). Half of CIN cases (50 %, 45/90) and controls (50 %, 45/90) had P16ink4A concentration above 0.946 ng/ml. Majority of CC cases (60 %, 54/90) and 50 % of controls (45/90) had P16ink4A concentration above 0.946 ng/ml. This difference was also statistically significant as shown in Table 2.

3.3. Association between serum P16ink4A concentrations and cervical lesions

From univariate analysis, there was increased odds of CIN (COR: 1.07, p-value: 0.78) and reduced odds of CC for serum P16ink4A (COR: 0.66, p-value: 0.03). After adjusting for smoking status, HIV status, History of high blood pressure, History of diabetes, age, presenting complaint, contraceptive use, type of contraceptive and marital status, multivariate logistic regression analysis showed increased odds of CIN for serum P16ink4A though not statistically significant (AOR: 1.11, p-value: 0.70). There was also a statistically significant reduction in odds of CC for serum P16ink4A (AOR: 0.55, p-value: 0.01) as shown in Table 3.

4. Discussion

This study reveals a compelling association between serum P16INK4A concentrations and both cervical cancer and cervical intraepithelial neoplasia within our study population. Notably, our investigation marks a significant contribution to the existing literature as one of the pioneering studies in Uganda to explore the potential link between circulating P16INK4A concentrations and cervical lesions. Our findings align with prior research demonstrating a consistent association between P16INK4A immunoexpression and cervical lesions, as documented in previous studies (Zuberi, 2021; Sarwath, 2017; Omran and Alsheeha, 2015). Other studies have demonstrated that overexpression of P16ink4A increases with severity of lesions (Kanthiya, 2016; Cheah, 2016; Ding, 2020; Dixon, 2017; Peres, 2016). This explains the extensive usage of P16ink4A immunohistochemical staining in staging of cervical cancer due to its perceived diagnostic and prognostic potential (Ding, 2020; Peres, 2016; Shi, 2019; Pandey, 2018) especially among HPV infected women (Bergeron, 2015).

Table 1
Demographic characteristics of participants at Mbarara Regional Referral Hospital between April 2022 and June 2023.

Variable	Category	CC N = 90 f(%)	CIN N = 90 f(%)	Controls N = 90 f(%)	Test	p-value
Age		51.1 (13.1)	34.9 (7.8)	38.6 (8.7)	ANOVA	<0.001
Age Group	19–29	2 (2 %)	23 (26 %)	18 (20 %)	Fisher's exact	<0.001
	30–39	18 (20 %)	37 (41 %)	26 (29 %)		
	40–49	21 (23 %)	29 (32 %)	39 (43 %)		
	50–59	20 (22 %)	1 (1 %)	7 (8 %)		
	60-max	29 (32 %)	0 (0 %)	0 (0 %)		
History of high BP	No	71 (79 %)	68 (76 %)	71 (79 %)	Chi-square	0.78
	Yes	19 (21 %)	22 (24 %)	19 (21 %)		
History of Diabetes	No	70 (78 %)	78 (87 %)	75 (83 %)	Chi-square	0.22
	Yes	20 (22 %)	12 (13 %)	15 (17 %)		
Marital status	Divorced	4 (4 %)	20 (22 %)	19 (21 %)	Fisher's exact	<0.001
	Married	51 (57 %)	54 (60 %)	50 (56 %)		
	Single	28 (31 %)	16 (18 %)	20 (22 %)		
	Widowed	7 (8 %)	0 (0 %)	0 (0 %)		
Highest level of education	Never studied	32 (36 %)	5 (6 %)	11 (12 %)	Fisher's exact	<0.001
	Pre-primary	48 (53 %)	3 (3 %)	6 (7 %)		
	Primary school	10 (11 %)	45 (51 %)	40 (44 %)		
	Secondary school	0 (0 %)	23 (26 %)	29 (32 %)		
	Tertiary institution	0 (0 %)	6 (7 %)	2 (2 %)		
	University	0 (0 %)	7 (8 %)	2 (2 %)		
HIV status	Negative	22 (24 %)	42 (47 %)	52 (58 %)	Fisher's exact	<0.001
	Positive	68 (76 %)	47 (52 %)	38 (42 %)		
	Unknown	0 (0 %)	1 (1 %)	0 (0 %)		
Smoking	No	90 (100 %)	84 (94 %)	89 (99 %)	Fisher's exact	0.029
	Yes	0 (0 %)	5 (6 %)	1 (1 %)		
Contraceptive use	No	57 (63 %)	38 (44 %)	56 (64 %)	Chi-square	0.02
	Yes	33 (37 %)	49 (56 %)	32 (36 %)		
Type of contraceptive	IUD	17 (45 %)	9 (18 %)	4 (13 %)	Fisher's exact	0.002
	Hormonal	21 (55 %)	39 (76 %)	26 (81 %)		
	BTL	0 (0 %)	3 (6 %)	2 (6 %)		

CC: Cervical Cancer; CIN: Cervical Intraepithelial Neoplasia; IUD: Intra Uterine Device; BTL: Bilateral Tubal Ligation
Age is a continuous variable and it is presented as mean (standard deviation).
All other variables are categorical and are presented as frequency (proportion).

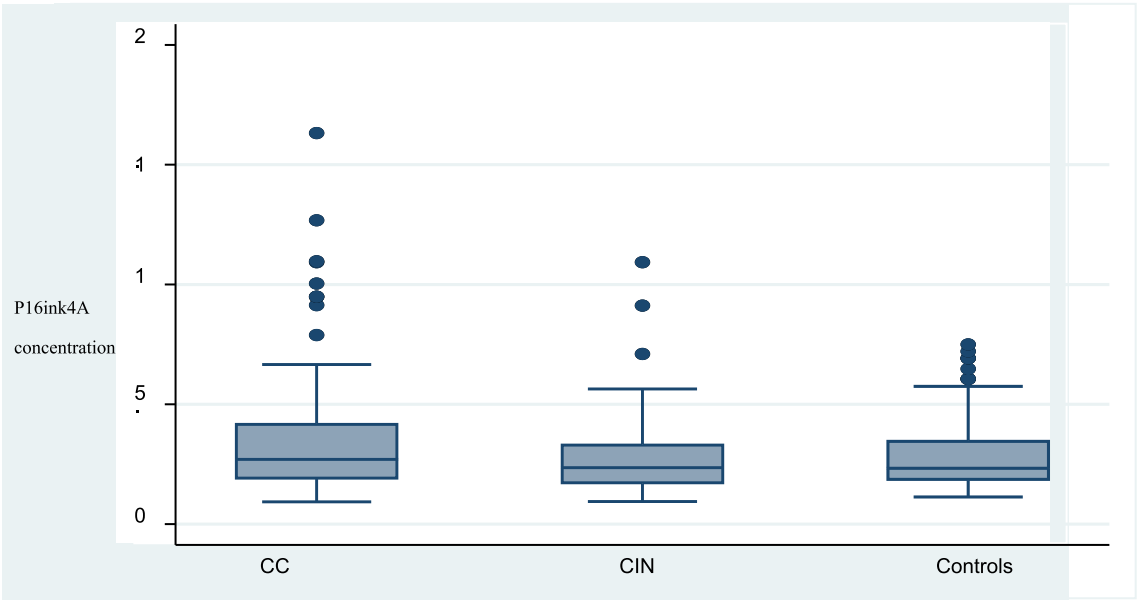


Fig. 2. A box plot showing serum concentration of P16ink4A across different groups of study participants. CC: Cervical Cancer; CIN: Cervical Intraepithelial Neoplasia.

The significantly increased P16ink4A serum concentrations in cervical cancer could be as a result of inactivation of retinoblastoma protein in HPV infected cervical cancer cells unlike in non-cancerous and normal cells. Inactivation of retinoblastoma protein results from over expression and binding by viral oncoprotein E7, which eventually leads to increased expression of P16ink4A (Hellman, 2014). It has been demonstrated that P16ink4A exhibits oncogenic potential in HPV transformed cervical cells (Li, 2020) as well as cell lines (McLaughlin-Drubin et al., 2013).

We also observed a statistically significant difference in age among

Table 2
Distribution of serum P16ink4A concentrations between cervical intraepithelial neoplasia cases, cervical cancer cases and controls at Mbarara Regional Referral Hospital between April 2022 and June 2023.

	Category	Cancer N = 90 f(%)	CIN N = 90 f(%)	Control N = 90 f(%)	Test	p-value
Baseline p16 (ng/ml)		1.45 (1.11)	1.11 (0.66)	1.13 (0.61)	ANOVA	0.008
Baseline p16 categories (ng/ml)	≤0.946	36 (40 %)	45 (50 %)	51 (57 %)	Chi-square	0.079
	0.946<	54 (60 %)	45 (50 %)	39 (43 %)		

Baseline p16 (ng/ml) is presented as a continuous variable with mean (standard deviation).

Table 3
Logistic regression analysis for association between serum P16ink4A concentrations and cervical lesions among study participants at Mbarara Regional Referral Hospital between April 2022 and June 2023.

	Bivariate analysis			Multivariate analysis		
	COR	P Value	95 % CI	AOR	P Value	95 % CI
CIN	1.07	0.78	0.67–1.71	1.11	0.70	0.66–1.87
CC	0.66	0.03	0.46–0.97	0.55	0.01	0.34–0.88

COR: Crude Odds Ratio; AOR: Adjusted Odds Ratio; CI: Confidence Interval; CIN: Cervical Intraepithelial Neoplasia; CC: Cervical Cancer.
All values were got after adjusting for smoking status, HIV status, History pf high blood pressure, History of diabetes, age, presenting complaint, contraceptive use, type of contraceptive and marital status.

our study groups. Among CC cases, the mean age was 51.1 (13.1), which was statistically different compared to controls. This collaborates well with previous studies in Tanzania (Zuberi, 2021) that showed increased expression of P16ink4A in older women (40–49) years who are considered a high risk group for cervical lesions. This is likely due to the fact that this group of women could have low screening rates for cervical cancer (Weng, 2020) as well as low cervical cancer awareness (Isabirye et al., 2020; Black et al., 2019) which translate into increasing incidence of cervical cancer in older women (Weng, 2020).

We observed a big proportion of cervical cancer and cervical intraepithelial neoplasia cases having P16ink4A above 0.946 ng/ml. This is in agreement with previous studies which show increased expression of P16ink4A in cervical intraepithelial neoplasia and cervical cancer (Cheah, 2016). However, a similarly big proportion of cases also showed raised P16ink4A serum concentration. This can be explained by the fact that big proportions of our study population reported numerous gynaecological conditions, mostly cervicitis, vaginal discharge and valvular warts; moreover, with significant distribution across CC, CIN and control groups. These are conditions that can be easily associated with low literacy, poor hygiene, limited cervical cancer knowledge and may lead to persistent genitourinary tract infections including HPV, which increases expression of P16ink4A (Dahiya, 2017). Previous HPV infections have also been reported to increase the chances of acquiring another HPV infection. Most of our participants, especially cases, could have been having transient HPV infections which are known to be very prevalent though most of them get cleared without causing any lesions (Shanmugasundaram and You, 2017).

We report an increased number of participants, especially cases who were married and with increase P16ink4A serum concentration. Being married is thought to increase one's chances of acquiring hrHPV infections which results in increased chances of developing cervical lesions and hence expression of P16ink4A (Diouf, 2020) although Yetimalar et al (Yetimalar, 2012) reports otherwise.

Other factors including HIV, smoking, contraceptive use and level of education varied significantly across cases and control groups. This emphasizes their known role as factors associated with acquisition or persistence of HPV and hence cervical lesions (Yu, 2016; Zhang, 2020; Ghebre, 2017; Stelzle, 2021; Asthana et al., 2020). For instance, smoking is itself said to increase HPV viral load in infected cells (Xi, 2009). Other genetic and life style factors can increase chances of HPV infection (Haukioja, 2014). HIV has been categorically proven to be a risk factor for HPV persistence (Bowden, 2023) which in turn increases expression of P16ink4A. This further explains the observation of raised P16ink4A serum concentrations in control groups, who were mostly HIV positive, compared to controls. This confirms reports that P16ink4A is strongly associated with cervical pathology (Diouf, 2020).

Considering the fact that P16ink4A, a tumor suppressor protein, can become an oncogene, and accumulates in the nucleus and cytoplasm of HPV infected cells (Pandey, 2018; Rokitka, 2021), studies have reported its potential application in early diagnosis of cervical cancer (Ding, 2020; Weng, 2020). Also considering the fact that a non-subsidized HPV DNA single test can cost as much as 20 dollars in Uganda, P16ink4A quantification in serum presents an opportunity for a cheaper alternative. P16ink4A ELISA test can cost as low as 8 dollars for a single test and this is likely to improve cervical cancer screening uptake and eventually reduce cervical cancer mortality and morbidity. Basing on this, P16ink4A ELISA could be evaluated more for usage along with routine tests such as VIA, HPV DNA, or Pap cytology, in an algorithm. The observed significant association between serum P16ink4A concentration and cervical lesions points to its likely clinical utility. P16ink4A ELISA could as well be studied more for its application in triage of CIN, monitoring prognosis of cervical cancer after treatment and monitoring risk of recurrence of cervical lesions.

A major strength of this study is the adequate statistical power which is reflected in the sample size that was calculated using scientifically accepted methods. We also measured P16ink4A using scientifically accepted methods. A major limitation to this study is that we did not test for HPV. Therefore, we could not exactly ascertain whether increased serum P16ink4A concentrations arise from HPV positivity, though it is known that HPV leads to increased expression of P16ink4A. Also, this being a case control study, we think there must have been selection bias while recruiting participants, and hence some variables may not have been distributed randomly across the study population. We did not stratify our analyses based on grades of lesions for cases (CIN and CC). This could have masked statistically significant associations between P16ink4A concentrations and specific grades of cervical lesions.

5. Conclusion

Serum P16ink4A concentrations may likely be associated with cervical lesions especially cervical cancer, among our study population. Quantitative measurement of circulating P16ink4A may be beneficial in diagnosis of cervical cancer. Prospective studies are recommended to evaluate the diagnostic utility of circulating P16ink4A in diagnosis of cervical cancer.

Availability of data and materials

All data from which this article was generated is available from the corresponding author upon reasonable request.

Consent for publication

Not applicable.

Support

This study was funded not funded by any agency.

CRediT authorship contribution statement

Frank Ssedyabane: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Joseph Ngonzi:** Writing –

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

We acknowledge the staff, research assistants and patients of the cervical cancer clinic at Mbarara Regional Referral Hospital.

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