Gynecologic Oncology Reports 55 (2024) 101466

Contents lists available at ScienceDirect

Gynecologic Oncology Reports

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

FOXP3 serum concentration; a likely predictor of CIN and cervical cancer: Secondary analysis from a case control study at a clinic in South western Uganda

Frank Ssedyabane [a,*], Nixon Niyonzima [b], Joseph Ngonzi [c], Josephine Nambi Najjuma [d], Hope Mudondo [a], Christopher Okeny [a], Doreen Nuwashaba [a], Deusdedit Tusubira [e]

[a] Department of Medical Laboratory Science, Faculty of Medicine, Mbarara University of Science of Science and Technology, P.O. Box 1410, Mbarara, Uganda
[b] Research and Training Directorate, Uganda Cancer Institute, P. O. Box 3935, Kampala, Uganda
[c] Department of Obstetrics and Gynecology, Mbarara University of Science of Science and Technology, P.O. Box 1410, Mbarara, Uganda
[d] Mbarara University of Science of Science and Technology, P.O. Box 1410, Mbarara, Uganda
[e] Department of Nursing, Mbarara University of Science of Science and Technology, P.O. Box 1410, Mbarara, Uganda

ARTICLE INFO

Keywords:
FOXP3
Cervical cancer
Cervical Intraepithelial Neoplasia
Association

ABSTRACT

Biomarkers including Forkhead/winged-helix transcription factor box P3 have been proposed in immunohistochemical techniques to diagnose cervical lesions, but can be objectively quantified and measured in blood using methods that can be standardised. In this study we quantified the serum FOXP3 concentrations and assessed their association with cervical lesions at the cervical cancer clinic of Mbarara Regional Hospital (MRRH) Southwestern Uganda.

We performed secondary analysis on archived serum samples from a previous unmatched case control study in which we recruited 90 cervical cancer (CC) cases, 90 cervical intraepithelial neoplasia (CIN) cases before any form of treatment and 90 controls. Clinical and demographic data were recorded. We measured FOXP3 concentrations using quantitative ELISA. We performed descriptive statistics and logistic regression in STATA 17 and took P-values of < 0.05 as statistically significant.

The mean concentration of FOXP3 was higher in serum samples from CC cases compared with CIN cases and controls, and this difference was statistically significant (P value < 0.001). More than half (52/90,58 %) of serum samples from CC cases had FOXP3 concentrations greater than 0.0545 ng/ml (P value < 0.001). Increase serum FOXP3 expression was not associated with CIN. Increase in serum FOXP3 concentrations were observed to increase the chances of CC by 2 times (OR: 2.094, P value 0.038, 95 % CI: 1.042—4.209).

Serum FOXP3 is likely associated with cervical lesions especially CC in our study population. Serum FOXP3 testing may be useful in resource limited settings to aid detection of such lesions given the challenges associated with cytology and VIA. We recommend diagnostic utility studies for circulating FOXP3 as a biomarker for detection of cervical cancer.

1. Introduction

Worldwide incidence rate for cervical cancer is estimated to be over 770,828 cases (Sung, 2021) making cervical cancer to account for more than 270 000 annual deaths, most of which occur in low income countries (Ronco, 1996; WHO, 2019), including Uganda (Anorlu, 2008; Denny et al., 2006). The incidence rate of cervical cancer has been reported at 43/100,000 in the East African region where Uganda is located (Sankaranarayanan, 2014). Country specific statistics indicate that Uganda has a higher age-standardized cervical cancer incidence compared to global estimates (56.2 per 100,000 women) (WHO, 2019; WHO, 2023).

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; OR, Odds Ratio; PAP, Papanicolaou; SD, Standard Deviation; STATA, Statistical Software for Data Science; VIA, Visual Inspection with acetic acid.

* Corresponding author.
E-mail address: fssedyabane@must.ac.ug (F. Ssedyabane).

https://doi.org/10.1016/j.gore.2024.101466
Received 4 May 2024; Received in revised form 13 July 2024; Accepted 20 July 2024
Available online 24 July 2024

Early diagnosis is a main stay in cervical cancer elimination (Wilailak et al., 2021). Current screening and diagnostic modalities in resource limited settings mainly include conventional cytology (Pap testing) and Visual inspection with acetic acid (VIA). However, these are prone to false negative results (Abila, 2021). Furthermore, cytology is also affected by low accuracy, a substantial rate of inter-observer variability (Najib, 2020; Nkwabong et al., 2019). There is a dire need for more accurate biomarkers for detection of cervical lesions (Wilailak et al., 2021).

A number of studies have suggested that Forkhead/winged-helix transcription factor box P3 (FOXP3) expression is closely associated with existence and progression of cervical cancer (Luo, 2015; Tang, 2017). FOXP3 is not only detected in other tissues e.g. breast, lung, prostate and retinal tissue but also in cancer cells (Appelman, 2015; Arbyn, 2012; Arbyn, 2014). It is also reported that FOXP3 has antitumoral roles in the breast and prostate (Arbyn, 2020; Assoumou, 2016; Åvall-Lundqvist, 1992; Badiga, 2016).

FOXP3 is a regulator for regulatory T cells (T reg) development and function; and belongs to the Fork head protein family of transcription regulators (Luo, 2015). In normal state, it is expressed on regulatory T cells (Luo, 2015). FOXP3 is reported to play very important roles in development and functionality of regulatory T cells, in that those regulatory T cells with high FOXP3 expression may have the potential to block an immune response. (Hori and Sakaguchi, 2004; Sakaguchi, 2005; Sakaguchi, 2008; Sakaguchi, 2010; Sakaguchi, 2013).

FOXP3 tissue expression has been shown to be significantly and positively related to P16INK4A expression (Luo, 2015) whose association with cervical lesions has already been reported (Ssedyabane, 2024). There is also a positive correlation that is reported to exist between expression of FOXP3 other factors that contribute to development of cervical cancer for example lyonhangiogenesis (Tang, 2017). In cervical cancer, FOXP3 expression is reported to increase with grades (Baker, 1991; Al-Daghri, 2015).

FOXP3 can be objectively demonstrated in blood using methods like ELISA. In this secondary analysis, we therefore aimed to describe the potential association between serum FOXP3 concentration and cervical lesions among women in South western Uganda.

2. Materials and methods

2.1. Study design

We performed secondary analysis (from October 2023 to November 2023) on archived serum samples collected from our previous unmatched case control study (Ssedyabane, 2024). The previous study had purposively sampled all women who sought cervical cancer care at the Mbarara Regional Referral Hospital (MRRH) cervical cancer clinic, between April 2022 and June 2023. Case groups comprised women with a confirmed diagnosis of cervical intraepithelial neoplasia (CIN) or cervical cancer (CC) before treatment while the control group comprised those women negative for intraepithelial lesion or malignancy. In this secondary analysis, our outcomes of interest were cervical intraepithelial neoplasia or cervical cancer whereas the exposure was serum FOXP3 concentration.

2.2. Study setting

Located in a rural area of southwestern Uganda, MRRH is a tertiary hospital serving roughly four million people (Black et al., 2019) across the entire region as well as neighbouring countries such as Tanzania, Rwanda, Burundi, and the Democratic Republic of the Congo. The clinic sees 15 women a day on average and is open five days a week. Numerous nursing officers, senior residents, and gynecologists, who are led by a gynecologic oncologist, work at the clinic. The screening procedures that are regularly performed at the clinic include visual inspection with acetic acid, colposcopy and conventional cytology. Other tests including HPV DNA are being introduced into routing practice but not yet popular given resource limited nature of the setting. Hence we did not ascertain participants' HPV status. For women diagnosed with high grade dysplasia, biopsies are collected for histology to confirm the presence of cervical lesions. Women who have been diagnosed with cervical cancer are either treated with total abdominal hysterectomy or they are referred to Uganda cancer institute for chemotherapy or radiotherapy. Those with premalignant lesions are treated with either cryotherapy or thermocoagulation.

2.3. Sampling method

We identified all archived serum samples used in the previous study (Ssedyabane, 2024). From the previous study, cases had been selected through purposive sampling and corresponding controls enlisted based on the incidence density sampling method.

2.4. Sample size determination

In this secondary analysis, we used a total of 270 serum samples collected from 90 CIN cases, 90 CC cases and 90 unmatched controls as calculated and used previously using OpenEpi, Version 3, Open source calculator-SSCC. OpenEpi – Sample Size for Unmatched Case-Control Studies (Ssedyabane, 2024). The calculation considered a two-sided confidence level (1-alpha) of 95 %, a study power of 80 % and a case to control ratio of 1.

2.5. Data collection

2.5.1. Demographic data

We obtained demographic information from our earlier study (Ssedyabane, 2024). These factors, which included age, residential region, family planning practices and methods, HIV status, educational attainment, marital status, history of blood pressure, and history of diabetes, had been collected through the use of a validated questionnaire.

2.5.2. Serum sample preparation, measurement and interpretation of FOXP3 concentration

Frozen serum samples were thawed and left to reach room temperature and mixed gently thoroughly. The Human FOXP3 (Forkhead/winged-helix transcription factor box P3) ELISA Kit from Elabscience Biotechnology Inc. was used to assess the quantity of FOXP3 quantitatively. The sensitivity and detection range of this kit are 0.19 ng/mL and 0.31–20 ng/mL, respectively. Following the manufacturer's recommendations, actual measurements with this ELISA kit were likewise based on the Sandwich-ELISA concept. 450 nm ± 2 nm was the wavelength at which a microplate reader was used to determine the optical density (OD). The OD value correlated with the FOXP3 concentration. Every sample was examined in tandem with reference standards. Using Phil Clayton's cutpt, which establishes a cut-off point (0.0545) for FOXP3 on the ROC curve that is closest to the point with ideal diagnostic values for sensitivity, we produced two categories of concentrations. These were Low FOXP3 concentration (≤0.0545 ng/ml) and increased concentration (0.0545 ng/ml).

2.5.3. Data management and analysis

The Principal Investigator and research assistants gathered data and entered it into an excel spreadsheet (Microsoft Office Professional Plus 2013, version 15.0.4675.1003, Microsoft Inc., USA) before importing it into the STATA 17 programme (StataCorp LLC, College Station, TX, USA). Using frequencies, means ± standard deviations (SDs), or median values for continuous variables and frequencies and proportions for categorical variables, descriptive statistics were employed to characterise the populations. Bivariate and multivariate logistic regression analysis was employed to determine the associations between cervical lesions and serum FOXP3. After adjusting for age, family planning type

and usage, HIV status, marital status, history of blood pressure, history of diabetes, and smoking status, multivariate logistic regression analysis was performed. Odd ratios and 95 % confidence intervals are used to display associations using P-values of < 0.05 as statistically significant.

2.5.4. Eligibility criteria

We considered all serum samples of more than 1 ml in volume, those that had not been damaged, with proper labels and with adequate corresponding demographic information. We planned to exclude all those samples that showed signs of multiple freeze–thaw cycles as well as those that has been frozen for more than 6 months.

3. Results

3.1. Population characteristics

270 serum samples were used for this study and these represent 270 participants. 90 of these were CIN cases, 90 were CC cases and the rest (90) were unmatched controls. The mean age of the study participants was 38.6(+/- 8.7) for the controls, 51.1(+/-13.1) for the CC cases and 34.9(+/- 7.8) for the CIN cases. There was a statistically significant difference in the mean age of the control and both case groups (CC and CIN) with a P value < 0.001. Majority of our study participants were married 56 %(50/90), 60 %(54/90) and 57 %(51/90) for the unmatched controls, CIN cases and CC cases respectively and this observation was statistically significant with a P value < 0.001. Almost all our study participants were non-smokers with a proportion of 99 %(89/90) among controls, 94 %(84/90) among CIN cases, and 100 % among CC cases. Majority of participants in the CIN case group used contraceptives and of these, 76 % were hormonal based as shown in Table 1.

3.2. Distribution of serum FOXP3 concentrations across study groups

The mean concentration of FOXP3 was higher among serum samples from CC cases compared with CIN cases and controls, and this difference was statistically significant (P value < 0.001). More than half (52/90,58 %) of serum samples from CC cases had FOXP3 concentrations greater than 0.0545 ng/ml, and this proportion differed significantly from CIN cases and controls (P value < 0.001) as shown in Table 2.

The mean concentration of FOXP3 was higher among serum samples from HIV positive CC cases compared with CIN cases and controls, and this difference was statistically significant (P value < 0.001). More than half (45/68,66.2 %) of serum samples from HIV positive CC cases had FOXP3 concentrations greater than 0.0545 ng/ml, and this proportion differed significantly from CIN cases and controls (P value < 0.001) as shown in Table 3.

3.3. Association between serum FOXP3 concentrations and cervical lesions

After controlling for other factors which included smoking, HIV status, History of BP and Diabetes, age, presenting complaint, usage of contraceptives, type of contraceptive used and marital status, we observed that serum FOXP3 concentrations were significantly associated with cervical cancer, with a P value < 0.001. Increase in serum FOXP3 concentrations increases the chances of CC by 2 times (OR: 2.094, P value 0.038, 95 % CI: 1.042——4.209). However, there was no statistically significant association between CIN and raised serum FOXP3 concentration (OR: 1.131, P value 0.719, 95 % CI: 0.579——2.205), hence as shown in Table 4.

4. Discussion

This study presents a statistically significant association between serum FOXP3 concentration and cervical cancer. We present a two-fold likelihood of cervical cancer with an increase in serum FOXP3 concentration. This is in agreement with prior studies that have found increased FOXP3 expression in cervical cancer. For instance, Li et al found that FOXP3 imunohistochemical expression was significantly higher in cervical cancer compared with CIN and cervicitis (Wanyenze, 2022). In the same regard, Vattai et al also demonstrated that FOXP3 expression is significantly higher in higher CIN grades (Black et al., 2019). However, their studies were conducted on formalin fixed paraffin embedded cervical tissues. In a related study Xu et al studied the circulating autoantibody to FOXP3, in a case control study. They observed that the anti FOXP3 circulating autoantibody was significantly raised in cervical cancer group compared to the control group (Yeo, 2018).

A case control study that included HPV-infected and HPV-uninfected women diagnosed with or without low or high-grade intraepithelial lesions of cervix revealed that increased FOXP3 expression was independently associated with the HPV infection (Baseman and Koutsky, 2005). A similar study that investigated the clinical significance of FOXP3 in cervical cancer also showed that Foxp3 had increased expression in cervical cancer cells and it was significantly associated with FIGO stage (Bast, 2005). These studies were also conducted on formalin fixed paraffin embedded cervical tissues, unlike ours which was conducted on serum.

FOXP3 is a transcriptional factor belonging to the forkhead/winged-helix family and it is widely recognized for its ability regulate T CD4 + CD25 + cells, hence fostering immunological tolerance and maintaining homeostasis in normal and non-diseased cells. About 700 genes and miRNAs linked to the TCR pathway, cell communication, and transcriptional control are activated or repressed transcriptionally to create these functions (Nelson, 2010). With those roles, FOXP3 is able to control the lymphoid lineage effectively especially in malignancies including cervical cancer (Adams, 2006). It acts as a master regulator that modulates the genetic functional programming of regulatory T cells (Treg) and this promotes immunological tolerance (Ahn, 2015). Stable FOXP3 expression is hence necessary for Tregs to exert their suppressive effects (Ahn, 2015; Aitken, 2019; Ajah, 2015). Specific isoforms of FOXP3 stimulate migration, cell division, and proliferation in non-tumorigenic keratinocytes by transduction and also regulate critical pathways associated with the immunological response and the production of several proto-oncogenes (Akindele and Useh, 2021).

FOXP3 is thought to be a distinct marker of T cells and is crucial to the growth, development and function of T cells (Allan, 2008). It is suggested that FOXP3 facilitates the proliferation of cervical cancer cells through promoting cell cycle progression, promoting over proliferation and invasion of the cells and intensifying the malignant potential of cancer cells (Al-Daghri, 2015). In addition, FOXP3 activates CD4 + CD25 + Treg cells in some malignancies thus inhibiting the development and functional roles of Treg cells and this promotes tumor proliferation in the long run (Wanyenze, 2022; Allegra, 2012).

This study highlights the functional role of FOXP3 in cervical carcinogenesis. Research studies have revealed that FOXP3 has a number of isoforms. Notable is the isoform FOXP3Δ2Δ7 which is said to stimulate cell proliferation, migration and division in keratinocytes, hence exhibiting a protumorigenic activity (Adams, 2006). In the presence of the isoform FOXP3Δ2Δ7, there is increased expression of SATB1 gene, which itself is involved in epithelial-mesenchymal transition, one of the early steps in carcinogenesis (Bansil, 2015; Barrow-Laing et al., 2010). This isoform also induces the hypoxia signalling pathway, a very important pathway for carcinogenesis, through proliferation, survival, tumor invasion and then metastasis (Bartel, 2009; Bartholomew, 2011; Bartel, 2004).

In the current study, we included participants who were mostly HIV positive and most likely having HPV infections. The distribution of FOXP3 serum concentrations significantly varied across CC, CIN and controls, highlighting the role of HIV in cervical carcinogenesis. There has been no clear assertion on the link between FOXP3 + Tregs and HrHPV (Anindo and Yaqinuddin, 2012). However, it is important to note

Table 1
Demographic characteristics of participants at Mbarara Regional Referral Hospital between April 2022 and June 2024.

Variable	Category	CONTROL N=90	CASES CIN N=90	CC N=90	Test	p-value
Age		38.6(8.7)	34.9(7.8)	51.1(13.1)	ANOVA	<0.001
Age group	21–29	18 (20 %)	23 (26 %)	2 (2 %)	Fisher's exact	<0.001
	30–39	26 (29 %)	37 (41 %)	18 (20 %)		
	40–49	39 (43 %)	29 (32 %)	21 (23 %)		
	50–59	7 (8 %)	1 (1 %)	20 (22 %)		
	60-max	0 (0 %)	0 (0 %)	29 (32 %)		
Region	Central	2 (2 %)	2 (2 %)	6 (7 %)	Fisher's exact	<0.001
	Other districts	43 (48 %)	50 (56 %)	69 (77 %)		
	Mbarara	45 (50 %)	38 (42 %)	15 (17 %)		
History of high BP	No	71 (79 %)	68 (76 %)	71 (79 %)	Chi-square	0.82
	Yes	19 (21 %)	22 (24 %)	19 (21 %)		
History of Diabetes	No	75 (83 %)	78 (87 %)	70 (78 %)	Chi-square	0.28
	Yes	15 (17 %)	12 (13 %)	20 (22 %)		
Marital status	Divorced	19 (21 %)	20 (22 %)	4 (4 %)	Fisher's exact	<0.001
	Married	50 (56 %)	54 (60 %)	51 (57 %)		
	Single	20 (22 %)	16 (18 %)	28 (31 %)		
	Widowed	0 (0 %)	0 (0 %)	7 (8 %)		
Highest level of education	Never studied	11 (12 %)	5 (6 %)	32 (36 %)	Fisher's exact	<0.001
	Pre-primary	6 (7 %)	3 (3 %)	48 (53 %)		
	Primary	40 (44 %)	45 (51 %)	10 (11 %)		
	Secondary	29 (32 %)	23 (26 %)	0 (0 %)		
	Tertiary institution	2 (2 %)	6 (7 %)	0 (0 %)		
	University	2 (2 %)	7 (8 %)	0 (0 %)		
HIV status	Negative	52 (58 %)	42 (47 %)	22 (24 %)	Fisher's exact	<0.001
	Positive	38 (42 %)	47 (52 %)	68 (76 %)		
	Unknown	0 (0 %)	1 (1 %)	0 (0 %)		
Smoking	No	89 (99 %)	84 (94 %)	90 (100 %)	Fisher's exact	0.027
	Yes	1 (1 %)	5 (6 %)	0 (0 %)		
Presenting complaint	Vaginal discharge	12 (13 %)	7 (8 %)	14 (16 %)	Fisher's exact	<0.001
	Back pain	2 (2 %)	3 (3 %)	0 (0 %)		
	Cervicitis	59 (66 %)	64 (71 %)	52 (58 %)		
	Candidiasis	5 (6 %)	6 (7 %)	0 (0 %)		
	Painful micturation	2 (2 %)	0 (0 %)	10 (11 %)		
	Vulvar warts	6 (7 %)	6 (7 %)	14 (16 %)		
	Syphilis	2 (2 %)	1 (1 %)	0 (0 %)		
	Trichomoniasis	2 (2 %)	0 (0 %)	0 (0 %)		
	Others	0 (0 %)	3 (3 %)	0 (0 %)		
Contraceptive use	No	56 (64 %)	38 (44 %)	57 (63 %)	Chi-square	0.009
	Yes	32 (36 %)	49 (56 %)	33 (37 %)		
Type of contraceptive	IUD	4 (13 %)	9 (18 %)	17 (45 %)	Fisher's exact	0.007
	Hormonal	26 (81 %)	39 (76 %)	21 (55 %)		
	BTL	2 (6 %)	3 (6 %)	0 (0 %)		

CC: Cervical Cancer; CIN: Cervical Intraepithelial Neoplasia; IUD: Intra Uterine Device; BTL: Bilateral Tubal Ligation
Age is a continuous variable and was presented as mean (standard deviation).

that increased FOXP3 expression is dependent on HrHPV genotypes especially HPV 16 (Adams, 2006). The virus uses the FOXP3 increased expression to evade and control the immune system and hence persistence of the HPV infection (Baker, 1991). In the current study, we did not test for HPV and hence cannot make any assertion on this link.

We report that more than half of the cervical cancer participants were using contraceptives (hormonal contraceptives). Studies have associated long term use of hormonal contraceptives to development of cervical cancer especially among HPV positive women (Asthana et al., 2020; Basu, 2015; Appleby et al., 2007). Therefore, use of hormonal based contraceptives is likely to have resulted in increased expression of serum FOXP3 among these CC participants, though indirectly.

The association between serum FOXP3 concentrations and CIN was not statistically significant both at bivariate and multivariate analysis. These results are in agreement with those from earlier studies which also found that CIN had a weaker expression of FOXP3 and its association wasn't statistically significant (Al-Daghri, 2015). Some studies have reported a positive correlation between FOXP3 cellular expression and CIN. For instance, total FOXP3 expression was reported higher in more advanced CIN grades (Black et al., 2019). Hou et al also found higher proportions of FOXP3 expressing T cells among CIN patients (Rao, 2012). However, we did not stratify our analysis according to grades of cervical lesions.

When serum FOXP3 was used as one of the predictors for CIN, the prediction rate for CIN was 94.4 % and this was suggestive of good and high prediction accuracy for FOXP3 (Yu Yang, et al., 2018). FOXP3 expression levels were found to be high in the cytoplasm, nucleus, and cancer interstitium in cervical cancer, but low in cervical intraepithelial neoplasia (CIN). This suggests that FOXP3 may not play a role in the initiation of the malignancy, but rather promoting the growth of the tumor (Li, et al., 2020). We acknowledge that most of the studies in which there was a statistical significant association between FOXP3 and CIN, they categorised the CIN into different grades (that is to say CIN stage 1, 2 and three) whereas ours wasn't categorised. In addition to that, some of these studies that found out an association between serum FOXP3 and CIN used FOXP3 alongside other markers like glucocorticoid receptor (한관희, 2022), VISTA (Wanyenze, 2022) Th17 cells (Rao, 2012), ZAP70 (Li, et al., 2020) among others.

We acknowledge that in this study we did not determine HPV infection. Though being recommended as part of cervical cancer screening, HPV DNA is not yet fully available in resource limited settings. Therefore, we could not determine any associations between

Table 2
Distribution of serum FOXP3 concentrations between cervical intraepithelial neoplasia cases, cervical cancer cases and controls at Mbarara Regional Referral Hospital between April 2022 and June 2023.

Variable	Categories	CONTROL N=90	CASES CIN	CC	Test	p-value
			N=90	N=90		
FOXP3 Concentration (ng/ml)		0.058(0.038)	0.055(0.019)	0.132(0.145)	ANOVA	<0.001
FOXP3 Categories	≤0.0545 ng/ml 0.0545 < ng/ml	62 (69 %) 28 (31 %)	61 (68 %) 29 (32 %)	38 (42 %) 52 (58 %)	Chi-square	<0.001

FOXP3 concentration (ng/ml) is presented as a continuous variable with mean (standard deviation).

Table 3
Distribution of serum FOXP3 concentrations between HIV positive cervical intraepithelial neoplasia cases, cervical cancer cases and controls at Mbarara Regional Referral Hospital between April 2022 and June 2023.

	Categories	Controls	CIN	CANCER	Test	p-value
		N=38	N=47	N=68		
FOXP3 Concentration (ng/ml)		0.057(0.046)	0.054(0.017)	0.144(0.152)	ANOVA	<0.001
FOXP3 Categories	≤0.0545 ng/ml 0.0545 < ng/ml	30 (78.9 %) 08 (21.1 %)	33 (70.2 %) 14 (29.8 %)	23 (33.8 %) 45 (66.2 %)	Chi-square	<0.001

FOXP3 concentration (ng/ml) is presented as a continuous variable with mean (standard deviation).

Table 4
Logistic regression analysis for association between serum FOXP3 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	1.053	0.873	0.562—1.973	1.131	0.719	0.579—2.205
CC	3.031	<0.001	1.644—5.586	2.094	0.038	1.042—4.209

CI: Confidence interval, COR: Crude Odds Ratio, AOR: Adjusted Odds Ratio, CIN: Cervical Intraepithelial Neoplasia, CC: Cervical Cancer.
All values were got after controlling for factors including smoking, HIV, History of high BP and diabetes, age, presenting complaint, usage of contraceptives, contraceptive type as well as marital status.

FOXP3 and HPV infection as seen in other studies. Also, we did not determine presence of specific FOXP3 isoforms. This is a missed opportunity for determination of the exact FOXP3 isoforms that could be associated with cervical lesions in our study population. Another limitation to this study is the fact that a large proportion of study participants, especially the cases, were HIV positive. This selection bias could have led to persistence of HPV and hence increased expression of FOXP3. During the presentation of our data, we put emphasis on the overall diagnosis rather than specific CIN grading. We did not provide exact classifications based on CIN grades. This was because some categories would have very few observations and this would hinder meaningful statistical analysis. Therefore, this study did not show differences between CIN I and CIN II/III which would be clinically impactful.

A major strength of this study lies in the novel usage of serum FOXP3 concentration rather than immunohistochemistry in the diagnosis of cervical lesions. We also take note of statistical power of this study, which we considered right from sample size calculation. All laboratory experiments were performed following internationally acceptable standards. These boosts our confidence in statistical results.

5. Conclusion

After adjusting for age and other factors, serum FOXP3 concentrations may likely be associated with cervical lesions especially cervical cancer, among our study population. Quantitative measurement of circulating FOXP3 may be advantageous in diagnosis or even monitoring prognosis of cervical lesions. We recommend prospective studies to assess the diagnostic utility of circulating FOXP3 in diagnosis of cervical lesions based on the fact that it is less costly and user friendly during measurement compared to HPV DNA.

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. **Nixon Niyonzima:** Writing – review & editing, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Joseph Ngonzi:** Writing – review & editing, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Josephine Nambi Najjuma:** Writing – review & editing, Supervision, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Hope Mudondo:** Writing – review & editing, Resources, Methodology, Investigation, Formal analysis, Conceptualization. **Christopher Okeny:** Writing – review & editing, Supervision, Methodology, Investigation, Formal analysis, Conceptualization. **Doreen Nuwashaba:** Writing – review & editing, Resources, Methodology, Investigation, Formal analysis, Conceptualization. **Deusdedit Tusubira:** Writing – review & editing, Visualization, Validation,

Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

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.

Acknowledgements

We thank Ms. Annah Kitibwa and the Makerere University Centre for Biosecurity and Global Health for their assistance in determining the concentrations of FOXP3. We also thank the MRRH cervical cancer clinic's administration, employees, research assistants, and patients for the respective roles played in this study.

Informed Consent Statement

Prior to participation in the study, we obtained each participant's signed informed consent. Additionally, we identified all data gathering instruments and serum specimens with research numbers rather than names. We disentangled all participant personal information throughout data analysis. The process of consenting and collection of data and all interactions with research assistants took place in a quiet, pleasant side room in the clinic that was free from interruptions, and taking in one person at a time.

Institutional Review Board Statement

The Mbarara University of Science and Technology Research Ethics Committee (MUST-REC) granted us ethical approval for this study (MUST-2022-612). The Uganda National Council for Science and Technology (UNCST) has also registered our study (HS2722ES). Prior to starting the trial, we also obtained administrative approval from the hospital director of Mbarara Regional Referral Hospital. At the cervical cancer clinic, all women who were diagnosed with cervical lesions were given the standard package of care, adhering to national recommendations.

Consent for publication

Not applicable.

Author contribution

Data curation, FRANK SSEDYABANE and Joseph Ngonzi; Formal analysis, FRANK SSEDYABANE; Funding acquisition, FRANK SSEDYABANE; Investigation, FRANK SSEDYABANE, Joseph Ngonzi, Deusdedit Tusubira, Nixon Niyonzima, Josephine Najjuma, Christopher Okeny, Doreen Nuwashaba and Hope Mudondo; Methodology, FRANK SSEDYABANE, Joseph Ngonzi, Deusdedit Tusubira, Nixon Niyonzima, Josephine Najjuma, Christopher Okeny and Doreen Nuwashaba; Project administration, FRANK SSEDYABANE, Josephine Najjuma, Christopher Okeny and Doreen Nuwashaba; Resources, FRANK SSEDYABANE; Supervision, Joseph Ngonzi and Deusdedit Tusubira; Validation, Deusdedit Tusubira; Visualization, FRANK SSEDYABANE, Deusdedit Tusubira and Josephine Najjuma; Writing – original draft, FRANK SSEDYABANE; Writing – review & editing, FRANK SSEDYABANE, Joseph Ngonzi, Deusdedit Tusubira, Nixon Niyonzima, Josephine Najjuma, Christopher Okeny, Doreen Nuwashaba and Hope Mudondo. All authors are accountable for all aspects of this manuscript.

Support

A small portion of the funding for this study came from Mbarara University of Science and Technology's Faculty of Medicine. The bigger portion was raised individually by the corresponding author.

References

Abila, D.B., et al., 2021. Burden of risk factors for cervical cancer among women living in East Africa: an analysis of the latest demographic health surveys conducted between 2014 and 2017. JCO Global Oncology 7, 1116–1128.
Adams, K.F., et al., 2006. Overweight, obesity, and mortality in a large prospective cohort of persons 50 to 71 years old. N. Engl. J. Med. 355 (8), 763–778.
Ahn, H.K., et al., 2015. Metabolic components and recurrence in early-stage cervical cancer. Tumor Biol. 36 (3), 2201–2207.
Aitken, C.A., et al., 2019. Introduction of primary screening using high-risk HPV DNA detection in the Dutch cervical cancer screening programme: a population-based cohort study. BMC Med. 17 (1), 228.
Ajah, L.O., et al., 2015. A five year review of cervical cytology in Abakaliki. *Nigeria.* Am J Cancer Prev 3 (2), 23–26.
Akindele, M.O., Useh, U., 2021. Multimorbidity of chronic diseases of lifestyle among South African adults. Pan Afr. Med. J. 38.
Al-Daghri, N.M., et al., 2015. Association of Type 2 Diabetes Mellitus related SNP genotypes with altered serum adipokine levels and metabolic syndrome phenotypes. Int. J. Clin. Exp. Med. 8 (3), 4464.
Allan, B., et al., 2008. Cervical human papillomavirus (HPV) infection in South African women: implications for HPV screening and vaccine strategies. J. Clin. Microbiol. 46 (2), 740–742.
Allegra, A., et al., 2012. Circulating microRNAs: new biomarkers in diagnosis, prognosis and treatment of cancer. Int. J. Oncol. 41 (6), 1897–1912.
Anindo, M.I.K., Yaqinuddin, A., 2012. Insights into the potential use of microRNAs as biomarker in cancer. Int. J. Surg. 10 (9), 443–449.
Anorlu, R.I., 2008. Cervical cancer: the sub-Saharan African perspective. Reprod. Health Matters 16 (32), 41–49.
Appelman, Y., et al., 2015. Sex differences in cardiovascular risk factors and disease prevention. Atherosclerosis 241 (1), 211–218.
Arbyn, M., et al., 2012. Evidence regarding human papillomavirus testing in secondary prevention of cervical cancer. Vaccine 30, F88–F99.
Arbyn, M., et al., 2014. Accuracy of human papillomavirus testing on self-collected versus clinician-collected samples: a meta-analysis. Lancet Oncol. 15 (2), 172–183.
Arbyn, M., et al., 2020. Estimates of incidence and mortality of cervical cancer in 2018: a worldwide analysis. Lancet Glob. Health 8 (2), e191–e203.
Assoumou, S.Z., et al., 2016. Human papillomavirus genotypes distribution among Gabonese women with normal cytology and cervical abnormalities. Infectious Agents and Cancer 11 (1), 1–8.
Asthana, S., Busa, V., Labani, S., 2020. Oral contraceptives use and risk of cervical cancer—A systematic review & meta-analysis. European Journal of Obstetrics & Gynecology and Reproductive Biology 247, 163–175.
Åvall-Lundqvist, E.H., et al., 1992. Prognostic significance of pretreatment serum levels of squamous cell carcinoma antigen and CA 125 in cervical carcinoma. Eur. J. Cancer 28 (10), 1695–1702.
Badiga, S., et al., 2016. Expression of p16INK4A in cervical precancerous lesions is unlikely to be preventable by human papillomavirus vaccines. Cancer 122 (23), 3615–3623.
Baker, T., et al., 1991. Structures of bovine and human papillomaviruses. Analysis by cryoelectron microscopy and three-dimensional image reconstruction. Biophys. J . 60 (6), 1445–1456.
Bansil, P., et al., 2015. Performance of cervical cancer screening techniques in HIV-infected women in Uganda. J. Low. Genit. Tract Dis. 19 (3), 215–219.
Barrow-Laing, L., Chen, W., Roman, A., 2010. Low-and high-risk human papillomavirus E7 proteins regulate p130 differently. Virology 400 (2), 233–239.
Bartel, D.P., 2004. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 116 (2), 281–297.
Bartel, D.P., 2009. MicroRNAs: target recognition and regulatory functions. Cell 136 (2), 215–233.
Bartholomew, D.A., et al., 2011. Analytical performance of Cervista® HPV 16/18 genotyping test for cervical cytology samples. J. Clin. Virol. 51 (1), 38–43.
Baseman, J.G., Koutsky, L.A., 2005. The epidemiology of human papillomavirus infections. J. Clin. Virol. 32, 16–24.
Bast, R.C., et al., 2005. Translational crossroads for biomarkers. Clin. Cancer Res. 11 (17), 6103–6108.
Basu, P., et al., 2015. Diagnostic accuracy of VIA and HPV detection as primary and sequential screening tests in a cervical cancer screening demonstration project in I ndia. Int. J. Cancer 137 (4), 859–867.
Black, E., Hyslop, F., Richmond, R., 2019. Barriers and facilitators to uptake of cervical cancer screening among women in Uganda: a systematic review. BMC Womens Health 19 (1), 1–12.
Denny, L., Quinn, M., Sankaranarayanan, R., 2006. Screening for cervical cancer in developing countries. Vaccine 24, S71–S77.
Hori, S., Sakaguchi, S., 2004. Foxp3: a critical regulator of the development and function of regulatory T cells. Microbes Infect. 6 (8), 745–751.
International Collaboration of Epidemiological Studies of Cervical Cancer; Appleby P, Beral V, Berrington de González A, Colin D, Franceschi S, Goodhill A, Green J, Peto J, Plummer M, Sweetland S. Cervical cancer and hormonal contraceptives:

collaborative reanalysis of individual data for 16,573 women with cervical cancer and 35,509 women without cervical cancer from 24 epidemiological studies. Lancet. 2007 Nov 10;370(9599):1609-21. doi: 10.1016/S0140-6736(07)61684-5. PMID: 17993361.

Li, L., et al., *A riskscore-based nomogram for the prediction of overall survival in cervical squamous cell carcinoma.* 2020.

Luo, Q., et al., 2015. Roles of Foxp3 in the occurrence and development of cervical cancer. Int. J. Clin. Exp. Path. 8 (8), 8717.

Najib, F.S., et al., 2020. Diagnostic accuracy of cervical pap smear and colposcopy in detecting premalignant and malignant lesions of cervix. Indian Journal of Surgical Oncology 11, 453–458.

Nelson, J.A., et al., 2010. Healers in a non-traditional role; a focus group study of Sangoma's knowledge of and attitudes to cervical cancer prevention and screening in Johannesburg. *South Africa.* Sexual & Reproductive Healthcare 1 (4), 195–196.

Nkwabong, E., Laure Bessi Badjan, I., Sando, Z., 2019. Pap smear accuracy for the diagnosis of cervical precancerous lesions. Trop. Doct. 49 (1), 34–39.

Rao, Q., et al., 2012. Aberrant microRNA expression in human cervical carcinomas. Med. Oncol. 29 (2), 1242–1248.

Ronco, G., et al., 1996. Estimating the sensitivity of cervical cytology: errors of interpretation and test limitations. Cytopathology 7 (3), 151–158.

Sakaguchi, S., 2005. Naturally arising Foxp3-expressing CD25+ CD4+ regulatory T cells in immunological tolerance to self and non-self. Nat. Immunol. 6 (4), 345–352.

Sakaguchi, S., et al., 2008. Regulatory T cells and immune tolerance. Cell 133 (5), 775–787.

Sakaguchi, S., et al., 2010. FOXP3+ regulatory T cells in the human immune system. Nat. Rev. Immunol. 10 (7), 490–500.

Sakaguchi, S., et al., 2013. The plasticity and stability of regulatory T cells. Nat. Rev. Immunol. 13 (6), 461–467.

Sankaranarayanan, R., 2014. Screening for cancer in low-and middle-income countries. Ann. Glob. Health 80 (5), 412–417.

Ssedyabane, F., et al., 2024. Association between serum P16ink4A concentration and CIN and cervical cancer among women attending a cervical cancer clinic in western Uganda: A case control study. Gynecologic Oncology Reports 53, 101388.

Sung, H., et al., 2021. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J. Clin. 71 (3), 209–249.

Tang, J., et al., 2017. Foxp3 is correlated with VEGF-C expression and lymphangiogenesis in cervical cancer. World J. Surg. Oncol. 15 (1), 1–5.

Wanyenze, R.K., et al., 2022. Social network-based group intervention to promote uptake of cervical cancer screening in Uganda: study protocol for a pilot randomized controlled trial. Pilot and Feasibility Studies 8 (1), 247.

Wilailak, S., Kengsakul, M., Kehoe, S., 2021. Worldwide initiatives to eliminate cervical cancer. Int. J. Gynecol. Obstet. 155, 102–106.

World Health Organization, n.d.. *Assessing national capacity for the prevention and control of noncommunicable diseases: report of the 2019 global survey*. p. 2020.

Yeo, C., et al., 2018. Factors affecting Pap smear uptake in a maternity hospital: A descriptive cross-sectional study. J. Adv. Nurs. 74 (11), 2533–2543.

World Health Organization, *Strategic framework for the comprehensive prevention and control of cervical cancer in the Western Pacific Region 2023-2030.* 2023.

Yu Yang, Y.Y., et al., *Predicting progression of cervical cancer by high risk human papillomavirus load and cellular immunologic indexes.* 2018.

한관희, et al., 2022. Glucocorticoid receptor promotes the progression of cervical cancer in combination with FoxP3 and induces cisplatin resistance through p38 MAP kinase. 대한부인종양학회 학술대회지 37, 273.