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RELATIONSHIP BETWEEN VISUAL INSPECTION WITH ACETIC ACID POSITIVITY AND CERVICAL INTRAEPITHELIAL NEOPLASIA DIAGNOSIS AMONG WOMEN IN ZIMBABWE: A RETROSPECTIVE STUDY

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ABSTRACT

Background: The burden of cervical cancer (CC) in Zimbabwe amount to 19%. Early detection is essential tools in fight against CC. Visual Inspection with Acetic Acid (VIAC) and Loop Electrosurgical Excision Procedure (LEEP) were commons screening tools for CC in Zimbabwe however their sensitivity and specificity are still questionable. At Cimas medical laboratory an increasing number of VIAC positivity was reported in recent years. **Purpose:** We tested if VIAC positivity can be a predictor of CIN (cervical intraepithelial neoplasia) diagnosis using LEEP histological analysis. **Methods:** A retrospective study was conducted, for women aged 16-70 who underwent VIAC and LEEP histological analysis. CIN diagnosis was based on LEEP histological analysis findings. A chi-square test was used to determine the relationship between VIAC positivity and CIN diagnosis. 372 participants were included using stratified sampling. **Results:** Incidence of CIN diagnosis was 61.8%. Age group 38 to 48 showed the highest number of High Grade Cervical Intra Epithelial Lesion (HSIL) of 78(34%) and Low Grade Cervical Intra Epithelial Lesion (LSIL) of 30(13%). According to geographic location the highest number of CIN cases were observed in Gweru with 86 cases. A sensitivity of 88.1%, specificity of 93.8%, Negative Predictive Value of 100%and Positive predictive value of 96.64% were observed. Human Immunodeficiency Virus (HIV) was the main risk factor in CIN and VIAC positive individuals. **Conclusion:** The incidence of CIN was higher than National rate; with HIV as major risk factors. There was a positive relationship between VIAC positivity and CIN diagnosis using LEEP histological analysis.

Keywords: VIAC, LEEP, cervical intraepithelial neoplasia, histology, cervical cancer

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INTRODUCTION

About 85% of cervical cancer global burden occurs in the less developed regions and third world countries (Bhatla *et al*., 2018). Cervical cancer accounts for about 3.3% of all female cancer deaths worldwide. According to World Health Organization (WHO), Zimbabwe has cervical cancer mortality rates of 17.6 % and the majority being poor, rural women. Zimbabwe is amongst the top five countries with high incidence of cervical cancer, with 61 of every 100 000 women being diagnosed annually. Cervical cancer is one of the few malignancies that are preventable. A 10 to 20-year lag between the pre-cancer and the invasive stages offers an opportunity to screen, detect and treat the disease before its progression to cancer. Zimbabwe implemented the "see and treat" approach in the fight against cervical cancer. In 2013 Cervical Intraepithelial Neoplasia (CIN) precedes almost all cervical cancers. CIN is still a public health problem among women in Zimbabwe due lack of screening services for detection of pre-invasive and early invasive disease (Eilu *et al*., 2020). Despite the rising incidence of cervical cancer and its corresponding CIN which always precedes Cervical cancer in Zimbabwe there is still paucity of data on CIN with regards to the incidence of CIN and its risk factor among women as well as Cervical cancer screening tools therefore leaving CIN under reported in most studies. Globally studies have revealed several risk factors associated with CIN and cervical cancer development such as in a study by Eilu *et al*. (2020) where age at first intercourse and Human Immunodeficiency Virus (HIV) were reported associated with cervical cancer. Currently, there are no detailed data that outline the burden of CIN and its associated potential risk factors with special mention to Visual Inspection with Acetic Acid (VIAC) positive women that underwent Loop Electrosurgical Excision Procedure (LEEP) histological procedure The burden of cervical cancer is still very high in Zimbabwe mainly as a result of poor screening and diagnosis facilities which is compounded by the very high HIV incidence. In 2015, 1.4 million people were estimated to be living with HIV in Zimbabwe, and HIV augments the risk of malignancy by 10% (Sachdeva *et al*., 2016), in addition to risk factors such as age and place of residency. There is a need to address reports on cervical cancer screening through targeting CIN and its associated risk factor. Several studies on cervical cancer screening revealed positive impact of VIAC as screening tool for his sensitivity and specificity as demonstrated in a study conducted in India, where VIAC as screening tools decreased the morbidity and mortality of cervical cancer by 25% and 35% respectively after a 7-year period (Bobdey *et al*., 2016). However, those evidence are still not well documented in Zimbabwe. Due to lack of screening, most women in Zimbabwe are diagnosed with cervical cancer at advanced stage as evidenced in a study conducted previously at Parirenyatwa hospital Zimbabwe by Zibako in 2022 where most of patients are in stage 2b and 3abc of cervical cancer. The screening rate in their study was 21% and was quite low due to the fact that there is no organised national screening programme as well as lack of knowledge (Zibako *et al*., 2022). In Zimbabwe routine program data showed a low cervical cancer screening positivity rate of about 3% from October 2020 to September 2021. Similarly, treatment coverage for women screening VIAC positive was suboptimal, with an overall coverage of 78% during the same period. There was paucity of data to explain the low cervical cancer screening positivity rates (Tachiwenyika *et al*., 2023) which make VIAC as screening tools to be reevaluated for its specificity and sensitivity.

Few studies targeting CIN diagnosis through VIAC as diagnostic tool were carried out in Zimbabwe and those studies revealed discrepancy in their findings such as in a study in 2017 conducted in Zimbabwe in Kwekwe City, General Hospital where, a total of 8466 women has been screened for cervical cancer using VIAC, and out of these 444 tested positive for VIAC with 157 being suspicious of CIN hence this study suggested that not all VIAC positive cases that could turn to be CIN. While since the introduction of VIAC in Zimbabwe only 60% of those who tested VIAC positive resulted in being diagnosed with CIN (Panganai & Gono, 2017), hence the need to exploration the relationship between CIN and VIAC as diagnostics tool as evidenced again in another study conducted in 1999 in Zimbabwe which showed that VIA was a valuable tool for the detection of CIN

with a sensitivity of 76.7% However, the specificity of VIAC was lower (64.1%) than that of cytology (90.6%). The authors also found that the cervical abnormalities detected by VIA, 75% concurred with those detected by biopsy (of Zimbabwe and Project, 1999). Furthermore studies showed a pooled sensitivity of 69% (CI 95% 54–81) for VIA compared to 95% (CI 95% 84–98) for HPV testing; and a specificity of 87% (CI 95% 79–92) for VIA compared to 84% (CI 95% 72–91) for HPV hence pointing out the crucial importance of VIAC as screening tools (Sami *et al*., 2022). Another meta-analysis including 26 studies in which VIAC was performed on asymptomatic women who all underwent confirmatory testing and in which the disease threshold was CIN grade 2, it reported an 80% sensitivity (range, 79%–82%) and a 92% specificity (range, 91%–92%) for VIAC. Study region, capacity of screener, or size of the study population did not modify VIAC accuracy. The positive predictive value was 10% (range, 9%–10%). (Sauvaget *et al*., 2011). Hence it is against those backgrounds we aimed to this present study at CIMAS Medical Laboratories Zimbabwe were a large number of samples averaging about a staggering 200 samples a month which were requesting LEEP histological analysis suspecting CIN most of which whose clinical data indicated VIAC positive. The present study provided novelty by covering gap of in paucity of data to explain the low cervical cancer screening positivity rates in Zimbabwe as well the scarcity of data associated with risk factor, incidence of CIN in addition it evaluated VIAC as screening tool

The present study covered the following Specific objectives: it investigates the incidence of CIN diagnosis among study participants, evaluated the sensitivity and specificity of VIAC in predicting CIN, it identified the histological characteristics, grade of CIN lesions and risk factors associated with CIN.

METHOD

Study Design

This research was a retrospective cohort study exploring the relationship between VIAC positivity and CIN diagnosis using LEEP histological analysis on women aged 16 to 70 attending Cimas Medical Clinics in Harare from January to December 2023.

Study Setting

The study was conducted at Cimas Medical Laboratories in Harare. Cimas Medical Laboratories is situated on the ground, first and second floor of Medical Chambers in Baines Avenue. Cimas Medical Laboratories process the specimens it receives from various collection points, and these are Cimas Health Care Clinics, Avenues clinic, Highglen as well as Makoni in Chitungwiza.

Study Population

All laboratory records of LEEP histological analysis whose VIAC test was positive who attended Cimas Medical Clinics and their LEEP histological analysis done at Cimas Medical Laboratories Harare from 1 January 2023 to 31 December 2023.

Inclusion Criteria

All laboratory records of women aged 16 to 70 years with completed VIAC tests and subsequent LEEP histological analysis done at CIMAS medical laboratory in Harare from January to December 2023.

Exclusion Criteria

The study excluded patients who did not have VIAC results, aged below 16 and above 70. The study excluded records performed from other laboratories which are not CIMAS medical laboratories for years before 2023 and after 2023.

Sample Size

The sample size patients enrolled in the study was calculated according to the Fischer's equation as follows: n= Z2P(1-P)/d2, Where, n= sample size, Z= confidence interval, P= known prevalence according to literature (in this case, 38.3% was used based on a study carried out by Kaizilege *et al*. (2024), d= precision (in proportion of one; if 5%, d=0.05). Therefore, n= 1.962×0.383(0.617)/0.052=363.1, the sample size required for the study is 363.

Inclusion Criteria

Inclusion criteria were women aged 16 to 70 with a VIAC result, whose LEEP Histological analysis was assessed at CIMAS medical laboratory in Harare, Zimbabwe. The

exclusion criteria were, men, women aged younger than 16 and older than 70, whose LEEP histological analysis was done at any other lab other than CIMAS in Harare.

Sampling Procedure

The VIAC positive patients whose LEEP histological analysis was done at CIMAS medical laboratory were studied and selected through stratified sampling. Stratified sampling with 12 strata for the 12 months and a sample size of 31 records will be taken from each month's records making a total of 372 samples.

Data Collection

Data was collected from medical records from CIMAS MedLab's Laboratory Information Management System (LIMS), including VIAC test results, LEEP histological analysis findings, age, and other relevant demographic and clinical variables from 1 January to 31 December 2023. The patient's laboratory numbers were used as the patient's identifier.

Analyses and Organization of Data

Descriptive data analysis was done to calculate summary statistics for demographic and clinical variables, VIAC positivity, and CIN diagnosis. A bivariate data analysis was done to examine the relationship between VIAC positivity and CIN diagnosis using Chi square test. A subgroup analysis based on age groups was done to explore potential variations in the relationship. Sensitivity, Specificity, Positive Predictive Value, and Negative Predictive Value were done to examine the robustness of VIAC positivity as a diagnostic test. All this was done using Excel and imported into Statistical Package for the Social Sciences SPSS version 25 using a P value of 0.05 as significant and 95% confidence interval.

Ethical Clearance

The research was conducted after getting approval from the Africa University Research Ethics Committee with ethical approval number (AUREC3153/24). Number identification codes were used instead of patients' names to ensure confidentiality and privacy. The research data was kept safely in the password protected computer of the Principal Investigator. Beneficence and non-maleficence were observed as the research will be more beneficial to the community than it will be harmful. The paragraph starts with words that are indented into five digits. In the method section of this research, if there are ethical test results, they can be listed (number, location, and date of ethical testing).

RESULT

Clinico-Pathological Characteristics of The VIAC Positive Study Population

Our inclusion criteria being all VIAC positives patients admitted during study periods a total of 372 patients were enrolled in this study (Table 1) A prevalence of 230 (61.8%) in CIN cases was observed among the study population. It was followed by Carcinomas with 59(15.9%), then squamous metaplasia and chronic inflammation 45(12.1%), followed by follicular cervicitis with 19(5.1%), the schistosomiasis with 13(3.5%) and lastly no pathologic changes with 6(1.6%).

Table 1. Clinico-pathological characteristics of the study population (LEEP, N=372)

Histological Findings	Frequency	Percentage, %
CIN	230	61.8
Schistosomiasis	13	3.5
Carcinomas	59	15.9
Squamous metaplasia and chronic inflammation	45	12.1
Follicular Cervicitis	19	5.1
No pathological changes	6	1.6
Total	372	100

Incidence of CIN Among VIAC Positive Patients

Incidence is the number of new cases occurring in a defined population during a specific period. CIN Incidence = New cases in defined population/ Total population at risk over a specific period * 100= (230/ 372*1) *100=61.8%. A CIN incidence

rate of 61.8% was observed in the VIAC Positive group.

Age Stratified Prevalence and Stage of CIN Among VIAC Positive Patients

The age group 38 to 48 was observed to have the highest number of both High Grade Cervical Intra Epithelial Lesion (HSIL) of 78 (34%) and Low Grade Cervical Intra Epithelial Lesion (LSIL) of 30 (13%). Secondly, the age group 27-37 has the second highest number of cases with HSIL of 53 (23%) and LSIL of 14 (6%). These are then followed by the age group 49-59 with HSIL 27 (12%) and LSIL 4 (1.3%). The age groups with the least number of cases are 60-70 with 10 (4.3%) HSIL cases and 3 (1.3%) and the age group 16 to 26 with 4 (1.7%) HSIL cases and 7 (3%) LSIL cases (Table 2).

Table 2. CIN Categories stratified by age (N=230)

Age group	HSIL (%)	LSIL (%)
16-26	4 (1.7)	7 (3)
27-37	53 (23)	14 (6)
38-48	78 (34)	30 (13)
49-59	27 (12)	4 (1.7)
60-70	10 (4.3)	3 (1.3)
Total	172 (75)	58 (25)

Prevalence of CIN Stratified by Geographic Location

The highest number of CIN cases are observed in Gweru with 86 cases, followed by Mutare with 46 cases, then Masvingo with 45 cases and Kwekwe with 21 cases. The lowest number of CIN cases are observed in Gokwe with 10 positive CIN cases, Chitungwiza with 8 positive cases, Chiredzi with seven cases, Birchenough with five positive cases and lastly Chipinge with one positive case (Figure 1).

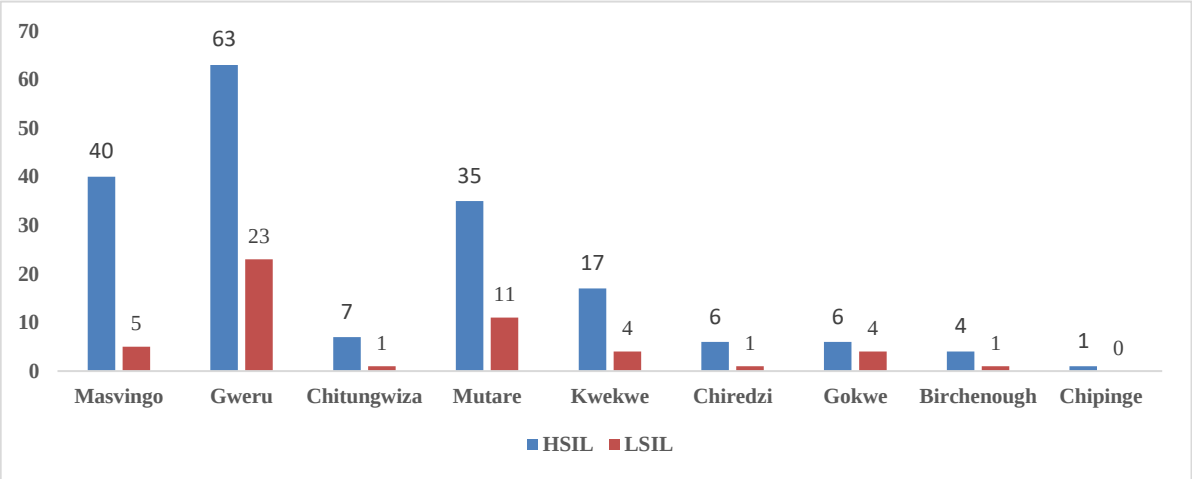

Figure 1. Stratified prevalence of all subtypes of CIN by location

Association between VIAC Positivity and CIN Diagnosis

Since the obtained chi-squared test statistic (38.574) (obtained from Table 3) is greater than the critical value (3.841), the null hypothesis is rejected. Therefore, it is concluded there is evidence of an association between VIAC positivity and CIN diagnosis at a significant level of 95%.

Table 3. Contingency table with observed and expected frequencies

VIAC Status	CIN Positive	CIN Negative	Total
VIAC Positive	230(194.3)	142(177.6)	372
VIAC Negative	31(66.6)	97(61.36)	128
Total	261	239	500

Chi-squared = Σ ((observed frequency - expected frequency) ^2 / expected frequency) = 38.574.

The Accuracy and Effectiveness of VIAC as a Diagnostic Tool

Specificity and Sensitivity

Sensitivity = TP / (TP+FN), Sensitivity = 230 / (230+31) = 88.1%
Specificity = TN / (TN+FP), Specificity = 120/ (120+8) = 120/128 =93.8%

A sensitivity of 88.1% means that the VIAC (Visual Inspection with Acetic Acid) test correctly identifies 88.1% of the cases that have cervical intraepithelial neoplasia (CIN) positive. In other words, when the VIAC test is positive, it detects CIN in 88.1% of the cases. A specificity of 93.8% means that the VIAC test correctly identifies 93.8% of the cases that do not have CIN positive. In other words, when the VIAC test is negative, it correctly excludes CIN in 93.8% of the cases.

Positive Predictive Value (PPV) and Negative Predictive Value (NPV)

PPV = TP / (TP+FP), NPV = TN / (TN+FN), True positives (TP) = 230, False positives (FP) = 8, True negatives (TN) = 120. To calculate the false negatives (FN), true positives (TP) are subtracted from the total number of cases with CIN positive:

FN = CIN Positive – TP, FN = 230 - 230 = 0,
PPV = TP / (TP+FP)
PPV = 230/ (230+8), PPV = 230/238 ≈ 0.9664
NPV = TN / (TN+FN), NPV = 120 / (120+0),
NPV = 120/120 = 1.0

Therefore, the Positive Predictive Value (PPV) is approximately 0.9664, or 96.64%, which means that when the VIAC (Visual Inspection with Acetic Acid) test is positive, there is a 96.64% probability that the individual has cervical intraepithelial neoplasia (CIN) positive. The Negative Predictive Value (NPV) is 1.0 or 100%, indicating that when the VIAC test is negative, there is a 100% probability that the individual does not have CIN positive.

Association between CIN and Other Co-morbidities

HIV is seen as a risk factor in CIN Positive and VIAC Positive Individuals. The highest number of HIV-positive individuals was observed in the age group 38-48 and more CIN cases were observed in this age group, followed by the age group 27-37. (Table 4) Conversely, the lowest number of HIV-positive individuals was observed in the age group 60-70 and 16 to 26, and the lowest number of CIN cases was observed in these age groups.

Table 4. HIV as a risk factor in CIN Positive and VIAC Positive Individual, N=372

Age Groups	HIV Positive	HIV Negative	Total
16-26	5	6	11
27-37	40	27	67
38-48	63	45	108
49-59	11	20	31
60-70	7	6	13
Total	126	104	230

DISCUSSION

In this study, the LEEP histological analysis in the VIAC positive individuals showed that 230 (61.8%) were CIN positive This study was dissimilar from the study carried out in Bungando Tanzania whereby, there were 203 (61.7%) patients with benign lesions, including four patients with schistosomiasis and two patients with cervical tuberculosis (Kaizilege *et al*., 2024). In the subgroup analysis, the age group 38 to 48 was observed to have the highest number of both HSIL of 78 (34%) and LSIL of 30 (13%). One possible explanation for this is that women in the 38-48 age group may have had more exposure to risk factors for cervical intraepithelial neoplasia (CIN), such as multiple sexual partners, smoking, or a weakened immune system. This group of women may have had fewer regular screenings for cervical cancer compared to the other groups, leading to a higher prevalence of CIN in this age group. Secondly, the age group 27-37 has the second highest number of cases with HSIL of 53 (23%) and LSIL of 14 (6%). These are then followed by the age group 49-59 with HSIL 27 (12%) and LSIL 4 (1.3%). The age groups with the least number of cases are 60-70 with 10 (4.3%) HSIL cases and 3

(1.3%) and the age group 16 to 26 with 4 (1.7%) HSIL cases and 7 (3%) LSIL cases. In the younger age group (16-26), the immune system is typically stronger and more efficient at clearing HPV infections, which are the primary cause of CIN. These results were similar to a study done in eastern Ghana in 2020 which showed that the age of the patient was another factor that showed a significant association with the presence of a precancerous lesion. With a p-value of 0.042, patients aged 31–40 years have significantly more precancerous lesions than other age groups (Eilu *et al*., 2020).

These disparities in CIN high grades in this study by region could be due to various reasons. Differences in healthcare access and availability, is another reason that may impact the differences in the number of cases in the different geographical areas, whereby areas like Gweru, Masvingo, Mutare and Kwekwe have better access to healthcare compared to their counterparts. These results were dissimilar from a retrospective cross-sectional study carried using routine VIAC and CC management data for period October 2020 to September 2021 in Zimbabwe, which showed that Manicaland and Midlands provinces recorded low VIAC positivity of 3% (target 5–25%) and treatment coverage of 78% (target = 90%) between October 2020 and September 2021 (Tachiwenyika *et al*., 2023)

In line with previous study conducted in Zimbabwe in 2013, HIV is found to be a risk factor for CIN (Michael, 2013); our finding revealed that, in addition, we found the highest number of HIV-positive individuals was observed in the age group 38-48 and more CIN cases were observed in this age group, followed by the age group 27-37. Conversely, the lowest number of HIV-positive individuals was observed in the age group 60-70. These results were similar to the ones obtained in a study in Zimbabwe by Thistle *et al*, (2020) whereby HIV status and patient age (31–40 years) had a significant influence on the LEEP outcome, HIV-positive patients were more likely to have a cervical precancerous lesion than HIV-negative patients. These results indicate that there might be differences in the odds of being HIV positive across different age groups. Individuals with HIV are at a higher risk of developing CIN, which is a premalignant condition of the cervix that can progress to cervical cancer if left untreated. This increased risk is due to the immunosuppressive effects of HIV, which can weaken the body's ability to fight off HPV infections, the primary cause of CIN. Based on the available data, there appears to be an association between age and HIV status.

Our study indicated that VIAC positivity is a valuable tool in identifying individuals at risk for CIN and is useful in guiding further diagnostic and treatment decisions. The study concluded that there is evidence of an association between VIAC positivity and CIN diagnosis at a significant level of 95% using chi-squared test statistics whereby, a value of 38.574 was obtained which is greater than the critical value of 3.841. In the study, sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated as measures of the accuracy and effectiveness of a VIAC as screening and diagnostic tool for CIN. The sensitivity of 88.1% indicated that VIAC has a relatively high ability to correctly identify individuals who were CIN positive. However, it also suggests that there was a possibility of false negatives, where some CIN-positive individuals may have been incorrectly classified as CIN negative. With a specificity of 93.8%, VIAC demonstrated a relatively high ability to correctly identify individuals who were CIN negative. However, it implied that there was a chance of false positives, where some CIN-negative individuals may have been incorrectly classified as CIN positive.

In line with previous study in Zambia where women with HIV had a greater risk of screen positivity than women with HIV negative status (Pry *et al*., 2021), our finding found a Positive Predictive Value (PPV) of 96.64% which suggested that, when VIAC yielded a positive result, there was a high probability that the individual was truly CIN positive. This indicated that the test has a high accuracy in identifying CIN-positive cases among those who tested positive. A Negative Predictive Value (NPV) of 100% implied that, when the test yielded a negative result, there was a very high probability that the individual is truly CIN negative. This indicated that the test has a high accuracy in ruling out CIN-negative cases among those who tested negative. Overall, these metrics suggested

VIAC has a high sensitivity, specificity, PPV, and NPV, indicating that it is reliable and accurate in both identifying individuals with CIN and excluding individuals without CIN. VIAC abnormal and these finding were further supported by a study assessing the Clinical performance of cervical cancer screening algorithms with HPV testing and VIAC, for CIN2+ detection among 2,668 women in which VIAC had shown less false negative rate; and good outstanding sensitivity, specificity, among others candidates testing screening tools (Wang *et al*., 2019)

The study provides a geographical limitation, as, since the study is being carried out at CIMAS Medical laboratory in Harare Zimbabwe, the results may not be representative of the whole population of Zimbabwe. Additionally, with CIMAS being a private laboratory, not everyone will be able to afford LEEP histological analysis after having a VIAC positive thus making the data not representative of the real issue at hand and limiting the generalizability of the findings

The study is also limited to a specific group of women within the age range 16 to 70 and this limitation helps in providing more focused insights into the relationship between VIAC positivity and CIN LEEP abnormalities within the target group. As well the sample representativeness

CONCLUSION

This study found a statistically significant association between VIAC positivity and CIN diagnosis, indicating that VIAC positivity can be a reliable indicator of CIN in the specified age range. It identified variations in the relationship between VIAC positivity and CIN diagnosis across different age groups, highlighting the importance of considering age as a factor when interpreting VIAC results and diagnosing CIN. HIV was also seen as a risk factor for CIN diagnosis. The study highlighted the significance of VIAC positivity as a screening tool for detecting CIN and the importance of further histological analysis through LEEP to confirm diagnosis and guide treatment could not be generalized to a broader population. The accuracy and reliability of VIAC and LEEP histological analysis techniques can vary depending on factors such as operator skill, equipment quality, and interpretation consistency.

SUGGESTION

The present study suggested a causal link between VIAC positivity and CIN diagnosis using LEEP histological analysis however large a large-scale study is needed to further support our findings and draw robust conclusions.

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CONFLICT OF INTEREST

The authors declare they have no conflict of interest.

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AUTHOR CONTRIBUTION

Tanyaradzwa Chipenzi and Maibouge Tanko Mahamane Salissou Conceptualize the work. Tanyaradzwa Chipenzi wrote the first draft of research, Maibouge Tanko Mahamane Salissou revised it for its critical contents and supervised the research. Maibouge Tanko Mahamane Salissou and Tanyaradzwa Chipenzi analyzed the data and prepared the final draft of the manuscript.

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