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From Pap Smear to Dual Testing: Assessing Co-infection of HPV and Chlamydia trachomatis in the Cervical Screening Programme of Khartoum State- Sudan

Alaa O Ahmed [1], Mubarak S Karsany[2], Mohamed Elfatih Abdelwadoud[3], Elryah I Ali[4], Ogail Yousif Dawod[5], Rasha Babiker[6,7], Husham T. Alshejlawe[8], Samah.B. HHamad[9], Saadalnour Abusail Mustafa[10], Rabab. H. Elshaikh[11], Salah Eldin Omar Hussein[12], Salma Elnour[12], Ayman H. Alfeel[12]

[1]Department of Histopathology and Cytology, Faculty of Medical Laboratory Sciences, Karrary University, Khartoum, Sudan. alaaa_777@hotmail.com
[2]Department of pathology, Faculty of Medicine, Karrary University, Khartoum, Sudan. Mubarak88@gmail.com
[3]Department of Histopathology and Cytology, Faculty of Medical Laboratory Sciences, Nile University, Khartoum, Sudan. Mohdsanhori79@gmail.com
[4]Department of Medical Laboratory Technology, College of Applied Medical Sciences, Northern Border University, Arar, Saudi Arabia.
[5]Physical Therapy, College of Nursing and Health Sciences, Jazan University, Jazan, 45142, Saudi Arabia. odawod@jazanu.edu.sa
[6]Department of Physiology, RAK College of Medical Sciences, Ras Al Khaimah Medical and Health Sciences University, Ras Al Khaimah P.O. Box 11172, United Arab Emirates.
[7]Department of Physiology, Faculty of Medicine and Surgery, National University - Sudan (NUSU), Khartoum, Sudan.
[8]RAK College of Medical Sciences, RAK Medical and Health Sciences University, Ras Al Khaimah 11172, United Arab Emirates. husham@rakmhsu.ac.ae.
[9]RAKCOMS, RAK Medical and Health Sciences University, Ras Alkahimah, United Arab Emirates. Samah@rakmhsu.ac.ae.
[10]Assistant professor of Histopathology and cytology, Department of Clinlcal Laboratory Sciences, Faculty of Applied Medical Sciences, Najran University, KSA saadosail@yahoo.com
[11]Assistant professor at College of Health and Applied Sciences, Medical Laboratory Sciences department, A' Sharqiyah University/ Ibra/Oman E-mail: rababhassan1982@gmail.com
[12]Department of Medical Laboratory Sciences, College of Health Sciences, Gulf Medical University, Ajman, United Arab Emirates.

Correspondance :
Salma Elnour Email: dr.salmaelnour@gmu.ac.ae
Rabab. H. Elshaikh rababhassan1982@gmail.com.

ABSTRACT

Background: Cervical cancer remains a leading public health concern in Sudan. Beyond high-risk human papillomavirus (HPV), co-infection with Chlamydia trachomatis (CT) may influence disease progression. This study evaluated the prevalence of CT–HPV co-infection and its association with cytological abnormalities among women in Khartoum State.

Methods: In a cross-sectional design, 236 sexually active women aged ≥18 were recruited at the Omdurman Cervical Cancer Prevention Centre (June–December 2024). Cervical samples underwent Pap smear and PCR-based detection of high-risk HPV genotypes and CT. Associations with cytological outcomes were assessed via Chi-square tests.

Results: The mean participant age was 38 ± 10.2 years. Abnormal cytology was observed in 57.2% (n = 135). The prevalence of high-risk HPV was 41.9% (99/236), CT prevalence was 12.3% (29/236), and co-infection prevalence was 7.6% (18/236). HPV and CT positivity correlated significantly with lesion severity (χ^2 = 148.7 and 21.9, respectively; $p < 0.001$).

Conclusions: Integrated CT–HPV co-screening could enhance early detection of at-risk women in Sudan. Further, longitudinal studies are warranted to clarify causal pathways and inform cervical cancer prevention programs.

KEYWORDS: human papillomavirus; Chlamydia trachomatis; cervical screening; co-infection; Sudan

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INTRODUCTION

Cervical cancer remains a critical public health challenge in low- and middle-income countries, with incidence and mortality rates in sub-Saharan Africa—and particularly in Sudan among the highest worldwide (Small et al., 2017). Although persistent infection

with high-risk human papillomavirus (HPV) genotypes is the principal etiological factor, epidemiological evidence suggests that co-infection with other sexually transmitted pathogens, notably Chlamydia trachomatis (CT), may exacerbate disease progression through chronic inflammation and immune modulation (Araldi et al., 2018; Silva et al., 2014). In Sudan, limited surveillance of CT and HPV co-infection has hindered the development of integrated prevention strategies.

Understanding the population-level prevalence of CT–HPV co-infection and its association with cervical cytological abnormalities is essential to inform resource allocation, optimize screening programs, and guide vaccine policy. Integrating CT testing with existing cervical cancer screening could enhance early detection of at-risk women and reduce the burden of cervical disease.

In this context, we conducted a cross-sectional, supportive molecular study among women attending cervical cancer screening in Khartoum State to estimate the prevalence of CT and high-risk HPV co-infection and assess their association with cytological outcomes. Molecular assays (PCR-based) were employed to ensure sensitive detection, but their role was primarily to generate accurate epidemiological estimates that underpin public health interventions, including combined STI screening, targeted health education, and expansion of HPV vaccination and CT treatment services in Sudan.

The findings from this study will fill a critical gap in local epidemiological data and support the design of integrated cervical health programs tailored to the Sudanese context.

MATERIALS AND METHODS

Study design and setting: A cross-sectional study was conducted between June and December 2024 at the Omdurman Cervical Cancer Prevention Centre, Khartoum State, following approval from the University of Krari Institutional Ethical Committee (Approval No. 431, 20/10/2022) and the Omdurman Military Hospital administration. The centre provides routine cervical cancer screening services to urban and peri-urban populations.

Study population and sampling: Consecutive, sexually active women aged ≥18 years presenting for screening or reporting symptoms (e.g. discharge, bleeding, itching) were invited to participate. Exclusion criteria were pregnancy, antibiotic or intravaginal medication use within 15 days, and current menstruation. The target sample size of 236 participants was calculated to detect a minimum CT–HPV co infection prevalence of 8% with 95% confidence and 5% precision.

Ethical considerations and data collection: All participants provided written informed consent. A structured questionnaire captured sociodemographic data, sexual and reproductive history, contraceptive use, smoking habits, and prior screening. A standardized case record form guided clinical examination and specimen collection.

Cytological evaluation: Two cervical specimens were collected per participant using an Ayre's spatula and cytobrush. One sample was processed immediately for conventional Pap smear, stained by the Papanicolaou method, and reported according to the Bethesda System by a cytopathologist blinded to clinical data.

Molecular analysis: The second specimen was placed in 5 mL phosphate buffered saline (PBS), transported on ice, and stored at –70 °C. For DNA extraction, thawed samples were centrifuged at 13 000 g for 10 min; pellets were lysed in Tris–EDTA buffer with 1% SDS and proteinase K (20 µg/mL) at 56 °C for 1 h, then subjected to phenol–chloroform extraction and ethanol precipitation (Sambrook & Russell, 2001). DNA was resuspended in 50 µL nuclease free water; purity (A260/A280) was measured spectrophotometrically.
HPV detection and genotyping: High risk HPV genotypes (16, 18, 33, 45, 58) were identified by multiplex PCR targeting the L1 region. PCR conditions and reagent concentrations were as previously described (Bhatla et al., 2006). Amplicons were separated on a 2.5% agarose gel stained with ethidium bromide and visualized under UV light.

Chlamydia trachomatis detection: CT was detected by singleplex PCR amplifying a 207 bp segment of the cryptic plasmid. Reaction conditions followed the manufacturer's instructions for the CT PCR kit (Paavonen, 2012). Products were resolved on a 2% agarose gel and verified against a 100 bp ladder.

Quality control: Each PCR run included no template and positive controls (HPV16 positive SiHa DNA; CT reference strain). Ten percent of samples were randomly re tested to confirm reproducibility.

Table 1: Primer sequences

Target	Primer	Sequence (5'-3')
HPV L1 F	MY09	CGTCCMARRGGAWACTGATC
HPV L1 R	MY11	GCMCAGGGWCATAAYATGG
Chlamydia trachomatis cryptic	CT-F	GAGCATGCCCGAAGCAAAC
Chlamydia trachomatis cryptic	CT-R	CAATCTGCTGCTGCTGGTT

RESULTS

Participant demographics and cytological outcomes of the 236 enrolled women showed a mean age of 38.0 years (SD 10.2; range 21–72 years). Most participants were aged 20–40 years (65.3%, n = 154), with the remainder over 40 years (34.7%, n = 82). Cytological evaluation classified 101 (42.8%) as NILM and 135 (57.2%) as abnormal: ASCUS (n = 8; 3.4%), LSIL (n = 67; 28.4%), HSIL (n = 48; 20.3%), SCC (n = 6; 2.5%), and adenocarcinoma (n = 6; 2.5%) (Figure 1).

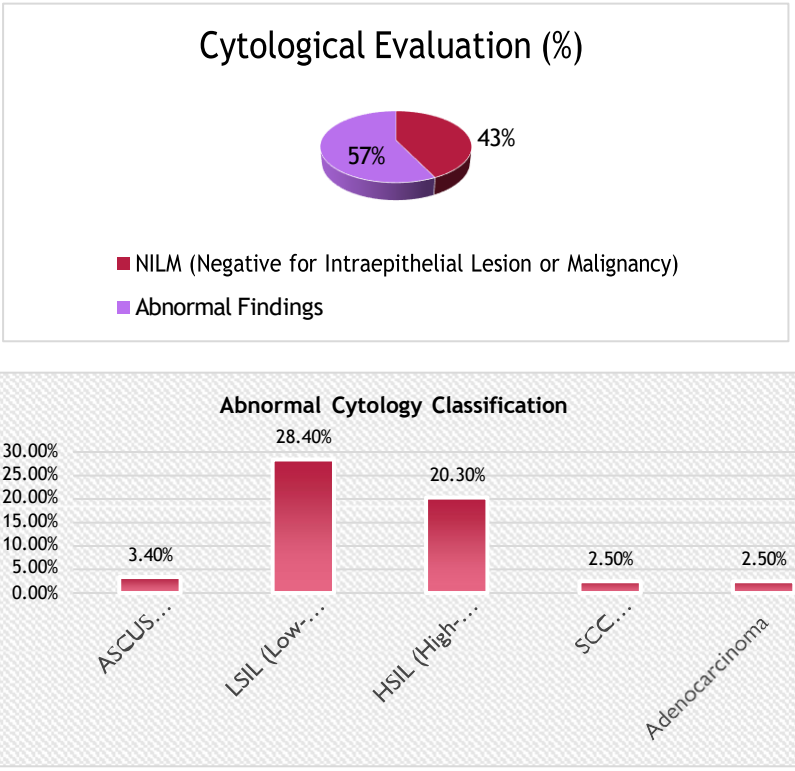
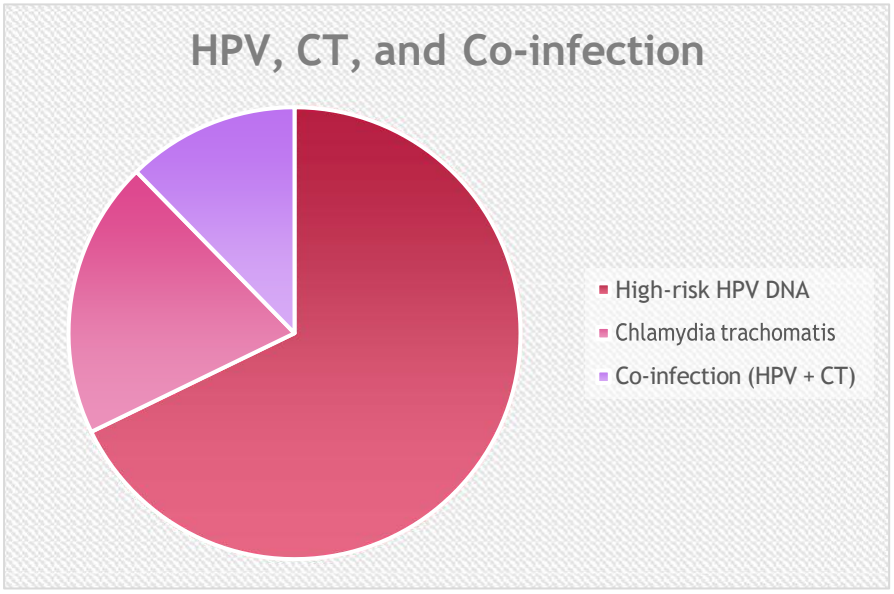


Figure 1: Cytological Evaluation

Prevalence of HPV, CT, and co-infection High-risk HPV DNA was detected in 99 of 236 women (41.9%), while Chlamydia trachomatis was identified in 29 of 236 (12.3%). Co-infection occurred in 18 participants (7.6%) (Figure 2).



Association with cytological abnormality High-risk HPV detection was significantly associated with abnormal cytology (χ^2 = 148.7; $p < 0.001$), with positivity rates rising from 0% in NILM to 100% in HSIL, SCC, and adenocarcinoma categories. CT infection also correlated with cytological abnormalities ($\chi^2 = 21.9$; $p < 0.001$), which were detected in 2.0% of NILM cases versus 20.0% of abnormal cases. HPV/CT co-infection was markedly higher among women with lesion severity: LSIL (11.9%), HSIL (8.3%), SCC (50.0%), and adenocarcinoma (50.0%) (Table 4).

Table 2: Prevalence of HPV, CT, and co-infection

Infection status	Normal cytology (n=101)	Abnormal cytology (n=135)	χ^2	p-value
High-risk HPV positive	0 (0.0)	99 (73.3)	148.7	<0.001
Chlamydia trachomatis	2 (2.0)	27 (20.0)	21.9	<0.001
HPV/CT co-infection	0 (0.0)	18 (13.3)	13.5	<0.001

These findings underscore the high prevalence of high risk HPV and the contributory role of CT as well as their synergistic impact on cervical cytological abnormalities in this Sudanese population.

DISCUSSION

This study provides epidemiological data on CT–HPV co infection among women undergoing cervical cancer screening in Khartoum State. We observed that 41.9% of participants harbored high risk HPV and 12.3% had CT, with 7.6% co infection prevalence. The consistent stepwise increase in co infection rates from low grade to invasive lesions raises important public health considerations.

However, the cross-sectional design restricts causal inference: while CT and HPV co infection appear more frequent in advanced lesions, we cannot determine whether CT predisposes to HPV persistence or lesion progression. This limitation cautions against overinterpreting synergistic mechanisms; our data should be viewed as hypothesis-generating, warranting longitudinal follow-up studies to establish temporal relationships.

From a public health standpoint, integrating CT detection into existing cervical cancer screening programs could substantially improve the early identification of women at increased risk. Co-screening allows for simultaneous detection of HPV and CT, enabling prompt treatment of CT—often asymptomatic—and close monitoring of HPV-positive women. Training healthcare workers on dual testing protocols, community awareness campaigns about the benefits of comprehensive STI screening, and the use of risk-strategized referral pathways are key components to operationalize co screening at scale.

Additionally, co screening data can inform targeted health education interventions, emphasizing safe sexual practices and adherence to screening schedules. Building partnerships with primary care centers and leveraging mobile health platforms can further extend the reach of integrated screening services, particularly in underserved rural areas.

Conclusions and recommendations Our findings underscore the value of integrated CT–HPV co screening within routine cervical health programs. We recommend pilot implementation of dual testing protocols in Khartoum State, training for laboratory and clinical staff, and enhanced patient education. Further research should include prospective cohort studies to clarify the temporal relationship between CT and HPV infections and evaluate the impact of co screening on cervical cancer outcomes.

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