



Cytolytic Vaginosis in Reproductive-age Sudanese Women, Wad Medani, Gezira State, Sudan, 2023

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Abstract

Introduction: Cytolytic vaginosis is a condition that defined by the presence of a large number of lactobacilli, along with cytolysis of the intermediate cells of the vaginal epithelium.

Aims: The study aimed to investigate the occurrence of cytolytic vaginosis (CV) in cervical smears of Sudanese females at reproductive age attended to Wad Medani Obstetrics and Gynecology Teaching Hospital and some private clinics.

Methods: A total of 134 cervical Papanicolaou PAP smears were collected from Sudanese married women during the period from February 2020 to January 2023. Collected PAP smears were evaluated according to the 2014 Bethesda System for Reporting Cervical Cytology.

Result: Out of the 134 cervical smears, 78 (58.2%) had normal growth of lactobacilli, 36 (26.8%) had lactobacillus overgrowth, and 20 (14.9%) classified as cytolytic vaginosis. 50% the participants with CV were in the age group 26-36 years, 60% belonged to rural communities and vaginal discharge was the main complaint in 60% of them. And all CV smears were negative for intraepithelial lesions or malignancy (NILM).

Conclusion: The study highlights the importance of accurately diagnosing vaginal discharge, with a specific focus on considering Cytolytic Vaginosis (CV) as a potential cause. CV is an important player in cervical infection or microbial dysbiosis. Women with vaginal discharges should undergo a routine cervical screening examinations.

Key words: Cytolytic Vaginosis, Lactobacilli, Cervical smear, Sudan

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Introduction: Albert Doderlein discovered the lactobacillus-dominant configuration in 1892, when he detected a long, thick, motile, rod-shaped, gram-positive, non-spore-producing bacteria in the vaginal fluid. Originally described as Doderlein's bacillus, this bacterium was subsequently called lactobacillus due to its ability to produce lactic acid (1,2) as a result of carbohydrate metabolism(3).

Beside the formation of acid, many lactobacilli strain produce lipoteichoic acid, biosurfactants and possess fimbriae, flagella and surface layer proteins (Slps) outside the cell wall (4,5), all of which are proven to promote and improve tissue adhesion capabilities (6). Lactobacilli also produce antimicrobial compounds such as bacteriocins and hydrogen peroxide (H₂O₂) (7). All mentioned

lactobacilli properties function as crucial parts of the female mucosal genital tract's protection barriers against a wide range of microbial illnesses through an efficient natural way to reduce inflammatory responses (8). Lactobacilli colonization of the human cervix epithelial cells was reported with a range of 29-52% (9–11). The properties of such lactobacilli play an important role as defensive mechanisms against the growth of potentially harmful pathogens (7). The cervical epithelium might exhibit histological abnormalities as a result of physiochemical changes brought on by an imbalance in these defenses (12). In several cases, this might result in cervical lesions or even tumor development (13,14).

On the other side, lactobacilli overgrowth can lead to a common condition known as Cytolytic vaginosis (CV), which was originally characterized by Cibley and Cibley in 1991 (15). To sustain this balance lactobacilli increase their acid-producing capabilities, resulting in a decrease in vaginal pH (3.5-4.0) and subsequent promotion of epithelial damage(16). In the published literature, the most often used method for diagnosing CV is wet mount microscopy to reveal lactobacilli overgrowth/ cytolysis, and to exclude Candidiasis, where yeast culture is essential (17). The differences in commensal microbiota in sub-Saharan Africa are thought to contribute to the prevalence and transmission of cervicovaginal infection and dysbiosis in the region (18). Thus, health systems are in urgent need of better data most importantly, data and research specific to women's health in Sudan. This study focuses on the identification of Cytolytic Vaginosis in reproductive-age women

using the Papanicolaou (PAP) cervical smear test among a panel of Sudanese females at reproductive age.

Materials and methods: _Study design: This descriptive cross-sectional study included a total of 134 married Sudanese women, who were referred to Wad Medani Obstetrics and Gynecology Teaching Hospital and some private clinics during the period from February 2020 to January 2023.

Sample collection: After inserting a medium-sized, sterile, disposable plastic speculum that allowed complete visibility of the cervical os and ectocervix, consultant gynecologists acquired cytological samples by scraping the cervix's surface with a cervical brush. The samples were prepared into direct conventional smears, and they were then immediately fixed for 15 minutes in 95% ethyl alcohol.

Sample processing: Ethyl alcohol fixed smears were hydrated by using descending concentrations of 95% alcohol through 70% alcohol to distilled water. Smears were then stained with Papanicolaou stain using standard PAP stain procedure and evaluated according to the 2014 Bethesda System for Reporting Cervical Cytology. Lactobacilli growth on smears was classified into three categories. Group one had a few lactobacilli in the background and an aberrantly normal growth pattern. Group 2 was presented with an overgrowth of lactobacilli (a large number of lactobacilli in the background). Group 3 displayed additional characteristics of epithelial cell cytolysis; mainly the presence of fragmented epithelial cells, bare nucleoli with prevalent lactobacilli overgrowth in the background. Group 3 represents the CV criteria

as described by The International Society for the Vulvovaginal Disease (ISSVD).

Data analysis: Descriptive statistics, such as frequency distributions, were examined using IBM Statistical Package for Social Sciences (SPSS) version 25 (data were presented as percentages).

Ethical consideration: Each participant was asked to sign a written informed consent before specimen collection. The study was approved by the Ministry of Health in Gezira State, Sudan.

Results: In the present study 134, randomly selected Sudanese married women at reproductive age were enrolled. The socio-demographic characteristics of the participating women are shown in Table 1. The ages of the participants ranged from 15 to 49 years. The predominant population of the subjects in this study falls into the age group 26-36 years (47.8%) with more than half (58.2%) residing in rural areas. The majority (70.1%) of participants were undergraduates, 27.6% were graduates and only 2.2% were postgraduates (Table 1).

Cytomorphologically, 78 (58.2%) of smears appeared to be having normal growth of lactobacilli, 36 (26.8%) had lactobacillus overgrowth, and 20 (14.9) had noticeable Cytolytic vaginosis (Table 1, Figure 1).

Half of the participants with CV were between the ages of 26 and 36 years (50%, n = 10/20), the majority (60%) belonged to rural communities, and more than half of them were undergraduates (55%)

(Table 1).

The smears with CV displayed marked epithelial cytolysis with prominent bare nuclei, such as the presence of fragmented epithelial cells, bare nucleoli due to the prominent lysis of the cytoplasm and lactobacillus overgrowth without pathognomonic findings of bacterial vaginosis, trichomonas vaginalis or candida infection (Figure 1 (C)).

Cytopathological diagnosis was also used for identifying infectious microorganisms and cytomorphological changes associated with various infections. Data regarding some other infection was available for comparison in our previous study in which reported microorganisms in the studied cases were *Gardnerella vaginalis* (n=11), *Candida* species (n=9), *Trichomonas vaginalis* (n=2) and Koilocytic changes of Human Papilloma Virus in only one sample. The presence of these pathogens tend to associate with a decrease or absence of Lactobacilli.

The cytology findings for the smears of study participants showed that 92% of them were negative for intraepithelial lesions or malignancy (NILM), 2.2% of them had atrophy, 2.2% had atypical squamous cells of undetermined significance (ASC-US), 0.7% had atypical glandular cells (AGC), and 0.7% had adenocarcinomas. All CV smears were detected in the NILM group.

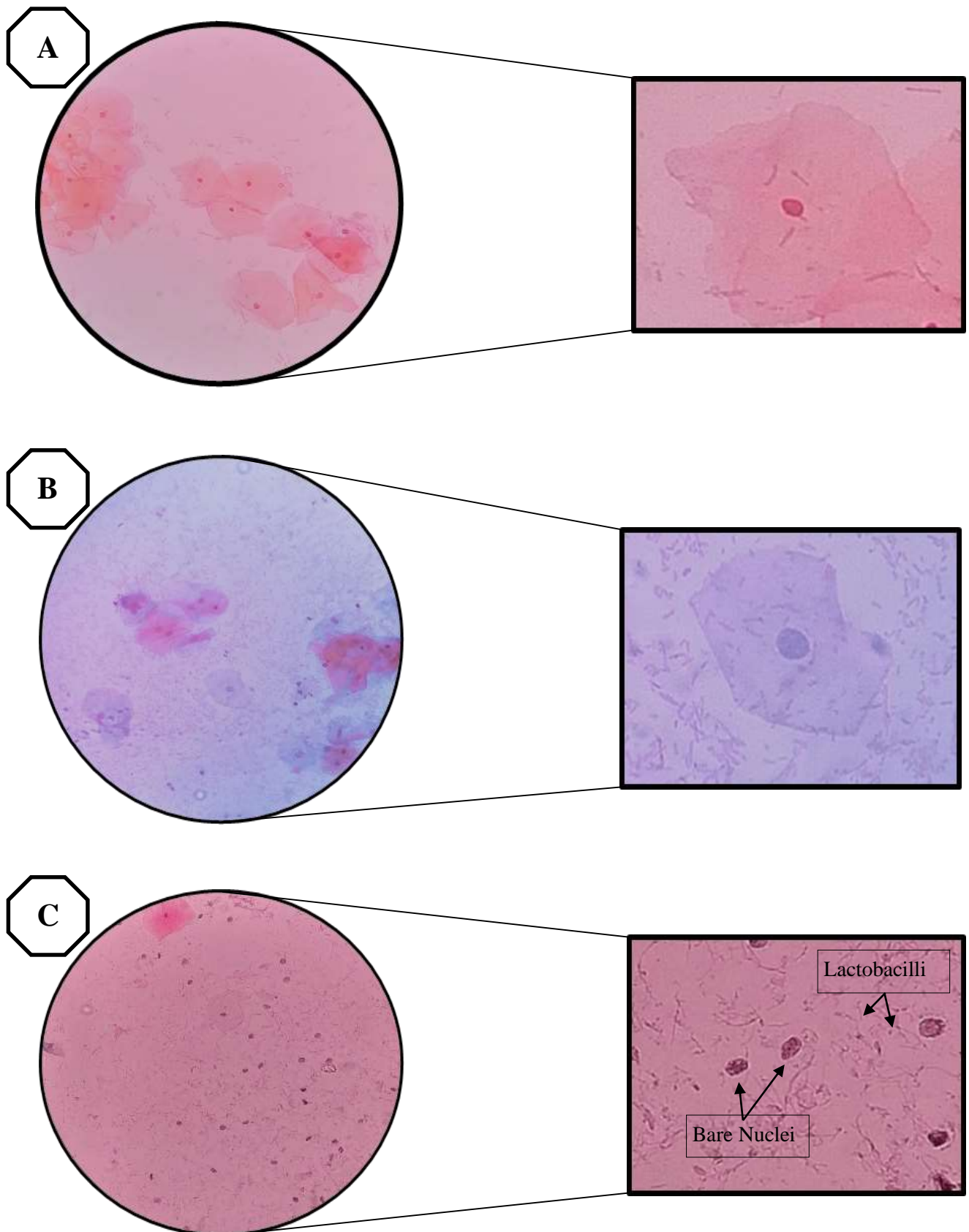


Figure 1: Microscopic observation of the cervical PAP smears (lactobacilli distribution). A, Apparently normal growth of lactobacilli (presence of normal growth of lactobacillus). B, Overgrowth of lactobacilli (presence of a large number of lactobacilli and normal epithelial cells). C, Cytolytic vaginosis (presence of a large number of lactobacilli, and naked nuclei of intermediate cell).

Table 1. Demographic and cytomorphological presentation of study participants

Characteristics	Apparently normal lactobacillus growth (n = 78)	lactobacillus overgrowth (n = 36)	Cytolytic vaginosis (n = 20)	Total (n = 134)
Age at presentation				
15 – 25 years	16 (11.9%)	3 (2.2%)	4 (3.0%)	23 (17.2%)
26 – 36 years	37 (27.6%)	17 (12.7%)	10 (7.5%)	64 (47.8%)
37 – 49 years	25 (18.7%)	16 (11.9%)	6 (4.5%)	47 (35.1%)
Residency				
Rural	46 (34.0%)	20 (14.9%)	12 (9.0%)	78 (58.2%)
Urban	32 (23.9%)	16 (11.9%)	8 (6.0%)	56 (41.8%)
Level of education				
Undergraduate	56 (41.8%)	27 (20.1%)	11 (8.2%)	94 (70.1%)
Graduate	19 (14.2%)	9 (6.7%)	9 (6.7%)	37 (27.6%)
Postgraduate	3 (2.2%)	0 (0.0%)	0 (0.0%)	3 (2.2%)

Discussion: A balanced microbiota shields healthy women against illnesses and premature birth. Among women who are of reproductive age, a small percentage may develop lactobacilli overgrowth leading to Cytolytic vaginosis (CV), which refers to vaginal intermediate epithelium destruction by lactobacilli or in combination with other bacteria. The prevalence and incidence of CV is unclear due to frequent misdiagnosis with candidiasis, as indicated by their lack of response to repeated antifungal medication (15).

In the present study, a total of 134 samples of cervical smears were evaluated. The result of the cytomorphological PAP smears indicated CV in 14.9% of smears out of 134 smears evaluated as satisfactory according to the 2014 Bethesda System for Reporting Cervical Cytology. Lower frequencies were reported by Cerikcioglu and Beksac (19), who described that CV was identified in 7.1% of 210 women with vaginal signs or symptoms indicative of vulvovaginal candidiasis (VVC). Only 3.9% of those in a different study's

sample of 1,152 people with vulvovaginal symptoms had CV (20). Contrarily, Puri (21) reported a relevant CV prevalence of 16.3% in 190 women who underwent a year of outpatient gynecological care and had cervical smears that showed signs of inflammation. Higher incidences were observed in other studies, such as Yang *et al* (22) finding that 143 (26.7%) women were diagnosed with CV and had vaginal discharge.

The most common complaint among women of reproductive age is vaginal discharge, which is a clinical manifestation of cytolytic vaginosis(23). 60% of participants with CV in the current study experienced vaginal discharge, while all other CV subjects reported other problems or none at all. Indicating that a wide range of ineffective antifungal and antibacterial medications would be used to treat a great majority of women with CV (i.e., 10% of all individuals with vaginal discharge in the total group). These treatments have the potential to change the pH balance in the vagina, leading to an excessive proliferation of lactobacilli

(24).

Numerous factors can affect the risk factors of vaginal infections. In contrast to non-tropical regions, the hot climate and its impact on specific hygiene practices may have an impact on instances in Sudan. Sociodemographic traits, other clinical conditions, inadequate access to healthcare, inadequate health education, and a lack of attention to other prevalent vaginal diseases like bacterial vaginosis and Sexually transmitted diseases (STDs) are also contributing factors (25,26). Finding the cause of any vaginal discharge is crucial since it accounts for a significant portion of gynecological consultations, which in turn contributes significantly to health care costs.

Cytolytic vaginosis (CV) is a little-understood gynaecological condition. The cervicovaginal microbiome encompasses a wide variety of microorganisms found in the cervix, such as bacteria, viruses, fungi, and other microbes. Conversely, women who have a dominant presence of *Lactobacillus* species (except for *L. iners-dominant*) can facilitate the clearance of human papillomavirus (HPV) and prevent cervical lesions (27). Several studies propose key factors in the diagnosing of CV, which include the absence of *Trichomonas* spp., *Gardnerella vaginalis*, *Atopobium vaginae*, *Megasphaera* spp., *Sneathia* spp., and *Prevotella* spp., as well as an increase in *Lactobacillus* spp. (19,20,28). This correlates with a decrease in the number of *Lactobacilli*, when other pathogenic organisms were identified in our previous study (29).

The prevalence of cervical cancer in sub-Saharan Africa was supposed to partially attribute to

increased cervical inflammation resulting from higher likelihood of cervical infection and/or microbial dysbiosis. According to cervicovaginal microbiome reports, major health concerns like human immunodeficiency virus (HIV) and human papillomavirus (HPV) alter the microenvironment of the cervical epithelium, which in turn puts some selective pressure on the bacterial populations that live there (18). Therefore, it is important to carefully consider the intricate and dynamic interactions in the vaginal microbiota as well as the crucial elements maintaining balance. Cervical disorders can be caused by a number of different factors, any of which that significantly affect the vaginal microbiota and upset this equilibrium.

To improve the prevention, diagnosis, and treatment of serious health concerns, it is imperative to have a better understanding of the particular elements specific to the region that cause cervical disorders. CV development at the moment.

Conclusion: The study highlights the importance of accurately diagnosing vaginal discharge, with a specific focus on considering Cytolytic Vaginosis (CV) as a potential cause. CV is less prevalent than candidiasis or bacterial vaginosis (BV), yet it might be confused with both. Women experiencing CV constitute significant portion of gynecological problems affecting female's health. CV is an important player in cervical infection or microbial dysbiosis. Women with vaginal discharges should undergo a routine cervical screening examinations.

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Conflict of interest: Authors have nothing to declare.

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