



# Microbes and Infectious Diseases

Journal homepage: <https://mid.journals.ekb.eg/>

## Original article

### The role of cytological smears in identification of bacterial infection and abnormalities in vaginal epithelium cells among apparently healthy married Sudanese women

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#### ARTICLE INFO

##### Article history:

Received 3 September 2021

Received in revised form 17 October 2021

Accepted 19 October 2021

##### Keywords:

Cytological Atypia

HPV

Keratinization

PAP

Inflammatory changes

#### ABSTRACT

**Background:** Vaginal infection represents a major health problem in most part of the world. Many factors have been identified as important causative agents responsible for the development of vaginal precancerous and cancerous lesions. In Sudan, infection has been identified as a major cause. **Aim:** To determine the role of cytological smears in identification of bacterial infection and abnormalities in vaginal epithelium cells among apparently health married Sudanese women. **Methods:** In this study 100 apparently healthy women were selected. Cytological materials were obtained by scraping the surface of the vagina. The obtained materials were applied with Pap Staining Method. **Results:** A total of one hundred samples of vaginal smears were included in this study, the age of the participant ranged from 20 to 70 years. Infection: Out of 100 samples 26 samples (26 %) had a bacterial infection, 3 samples (3%) had Actinomyces infection, and 71 samples (71%) were negative. Inflammations: Out of 100 samples 6 samples (6%) had acute inflammation, 5 samples (5%) had chronic inflammation and 89 samples (89%) were negative. Inflammatory changes: Out of 100 samples 22 samples (22%) had inflammatory changes and 84 samples (78%) were negative. **Conclusion:** Bacterial vaginosis appears to be the predominant cause of vaginitis. The conventional cytology for exfoliative cells from female genitalia by using PAP stain is routine, specific, and sensitive, technique easy and not expensive so it is ideal technique for screening.

#### Introduction

The vagina is muscular and tubular part of the female genital tract extends from the vulva to the cervix [1]. vagina in its nature have low ph environment (acidic ph) provided by lactobacillus bacteria which degrades glycogen into lactic acid that keep low ph to protect it from any pathogenic

organism [2]. Large and serious numbers of opportunistic diseases can attack the vagina in case of absence of lactobacillus, that cause inflammation (vaginitis) or infection (vaginosis). These diseases caused by bacteria, viruses, parasites, or fungi. The most common causes of vaginitis are: candida,

DOI: 10.21608/MID.2021.99264.1200

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chlamydia, gonorrhoea, trichomonas, human papilloma virus, herpes simplex virus, and cytomegalovirus...etc and all of these are asymptomatic diseases [3]. So, we need to apply screening program to know about these infectious agents and treat it, which sometimes convert to chronic and then to cancer. Screening in medicine is a strategy used in a certain population (females) to identify the possible presence of asymptomatic disease in individuals with pre symptomatic or unrecognized symptomatic disease [4]. As such, screening tests (PAP stain) are somewhat unusual in that they are performed on persons apparently in good health. Screening intervention is designed to identify disease in community early, thus enabling earlier intervention and management in the hope to reduce mortality and suffering from disease. Although screening may lead to an earlier diagnosis, not all screening tests have been shown to benefit the person being screened; overdiagnosis, misdiagnosis, and creating a false sense of security are some potential adverse effects of screening. For these reasons, a test used in screening program, especially for disease with low incidence, must have good sensitivity in addition to acceptable specificity [5].

### Materials and Methods

This was a descriptive cross-sectional study, it included a total of 100 married Sudanese females, volunteers were collected from different places in Sudan country, AL-Shemalia, Khartoum state, and Sinar state in AL-mujaour village. Samples were transferred to histopathology and cytology lab in AL-Yarmouk College where they were investigated.

### Sample collection

Cytological materials were obtained by scraping the surface of the vagina. The obtained materials were used for preparation of two direct smears, which were immediately fixed in 95% ethyl alcohol for 15 minutes then sent to the Histopathology laboratory at Faculty of Medical Laboratory Science, AL-Yarmouk College, Sudan for subsequent cytological processing.

### Sample processing

Ethyl alcohol fixed smears were hydrated by using descending concentrations of 70% alcohol through 50% alcohol to distilled water for two minutes in each stage. Then smears were treated with Harris' Hematoxylin for five minutes, to stain the nuclei, rinsed in distilled water and differentiated in 0.5% aqueous hydrochloric acid for a few seconds to remove the excess stain. Then they were immediately blued in tap water for 5 minutes. For cytoplasmic staining they were treated with

ascending alcoholic concentrations from 70% and 85% to 95% ethyl alcohol and then stained with EA50 and OG6 2 minutes for each. The smears were then dehydrated in absolute ethyl alcohol. The smears were then cleared in Xylene and mounted in DPX (Distrene polystyrene Xylene) mounting medium. All reagents used were from Rall Corporation, UK [6].

### Quality control of samples

All samples were collected in disposable sterile swabs to avoid contamination. Handling and transportation of collected samples were done with care, all smears were examined microscopically after fixation to detect efficiency of each smear. The quality control of staining solutions checked as John D Bancroft Theory and Practice of Histological techniques. All smears were reviewed by well professional cytologists.

### Data analysis

Statistical Package for Social Sciences (version 16) was used for analysis and to perform Pearson Chi-square test for statistical significance ( $p$  value). The 95% confidence level and confidence intervals (95% CI), as well as the Odds Ratio (OR) were used.  $p$  value less than 0.05 was considered statistically significant.

### Ethical consent

Each participant was asked to sign a written ethical consent form during the interview, before the specimen was taken. The informed ethical consent form was designed and approved by the ethical committee of the Faculty of Medical Laboratory Research Board, College of AL-Yarmouk.

### Results

The study was conducted in Khartoum State. A total of one hundred samples of vaginal smears were included in this study, the age of the participant ranged from 20 to 70 years.

### Infections

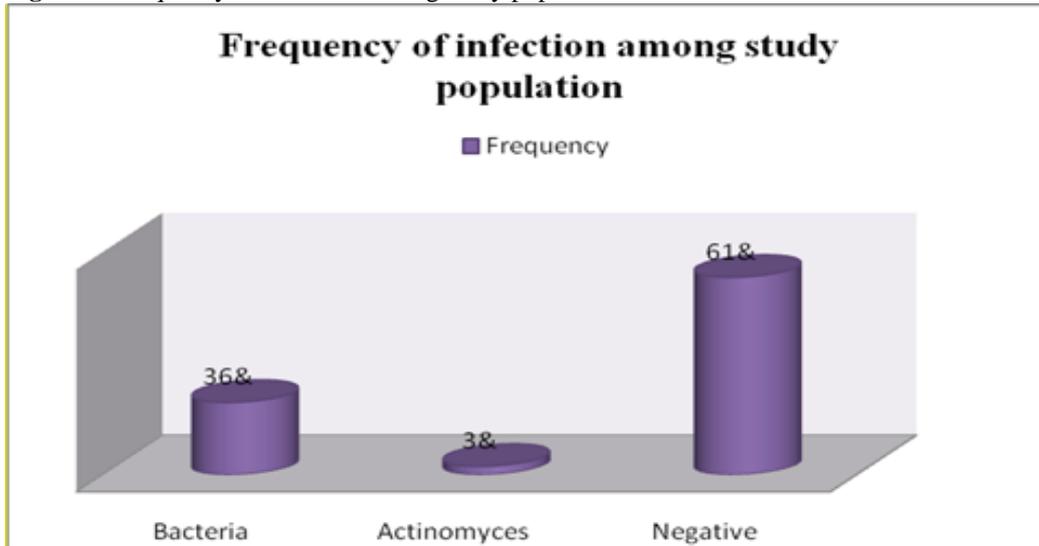
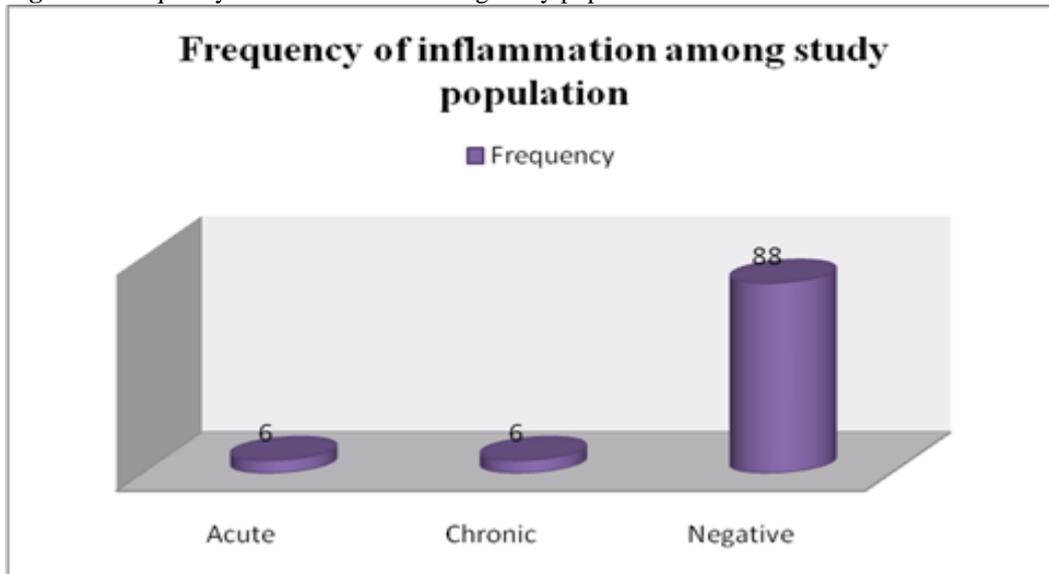
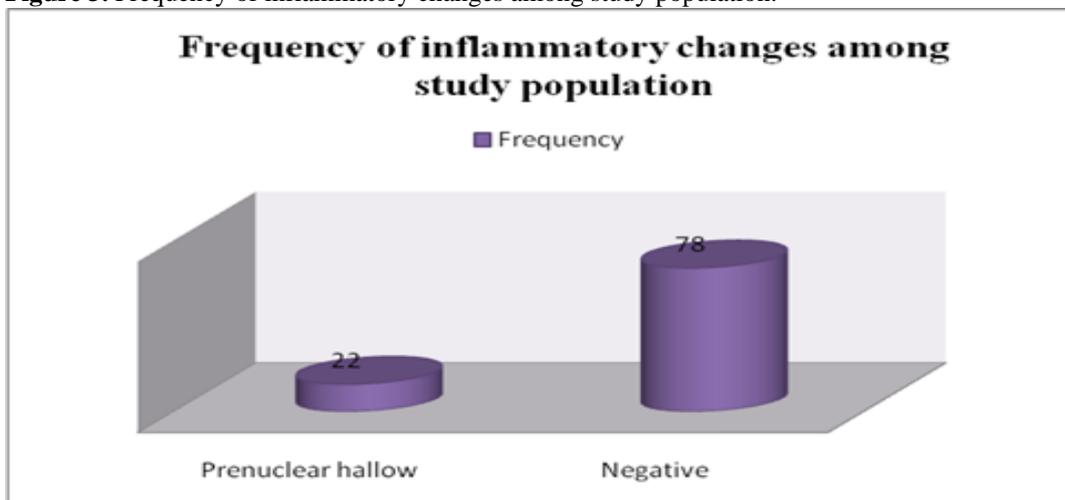
Out of 100 samples 36 samples (36 %) had a bacterial infection, 3 samples (3%) had Actinomyces infection, and 61 samples (61%) were negative as show in **figure (1)**.

### Inflammations

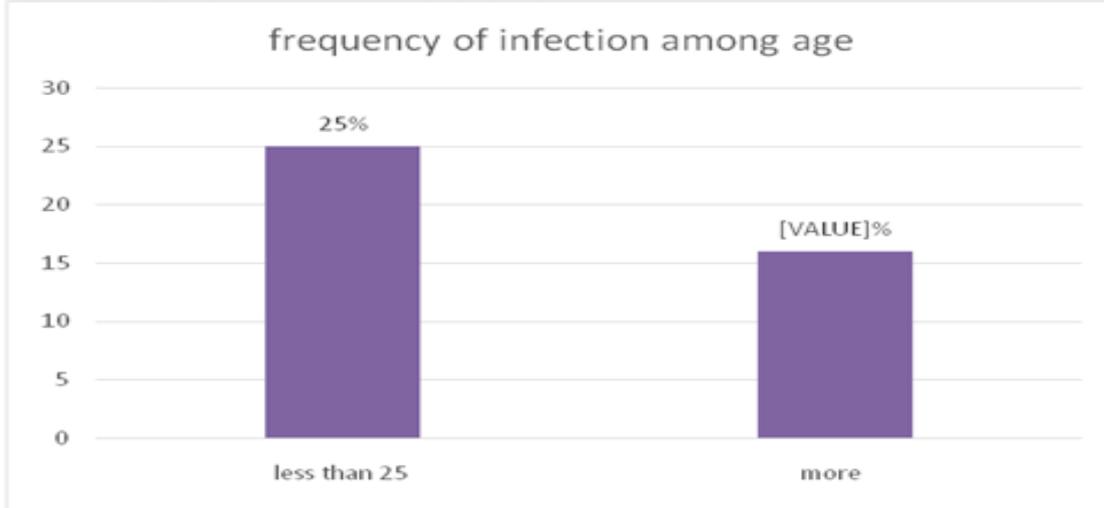
Out of 100 samples 6 samples (6%) had acute inflammation, 5 samples (5%) had chronic inflammation and 89 samples (89%) were negative that show in **figure (2)**.

### Inflammatory changes

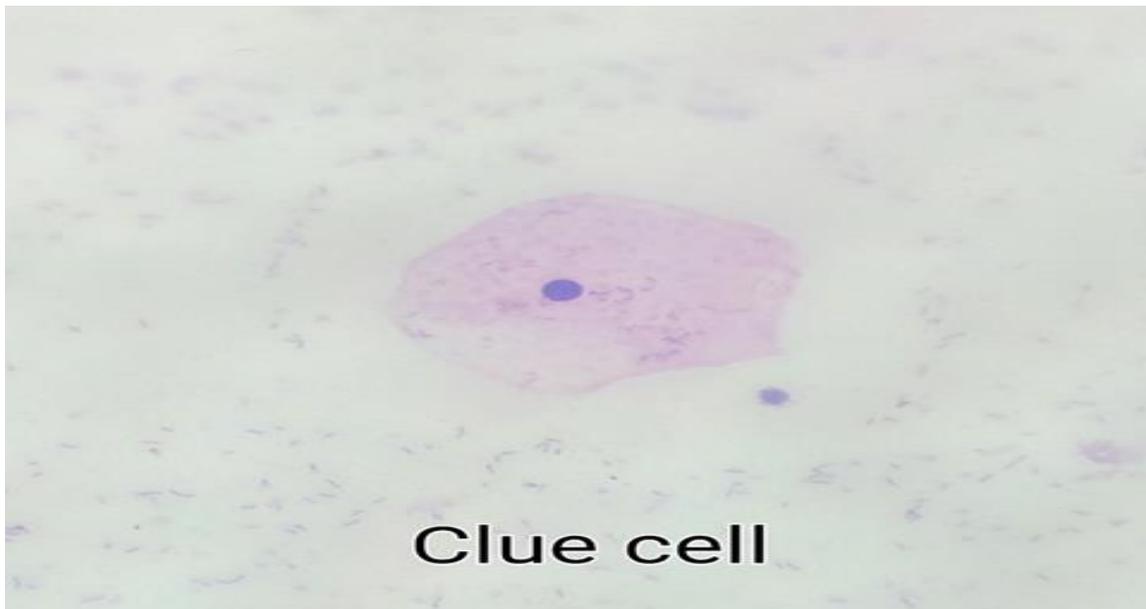
Out of 100 samples 22 samples (22%) had inflammatory changes and 84 samples (78%) were negative that show in **figure (3)**.

**Figure 1.** Frequency of infection among study population.**Figure 2.** Frequency of inflammation among study population.**Figure 3.** Frequency of inflammatory changes among study population.

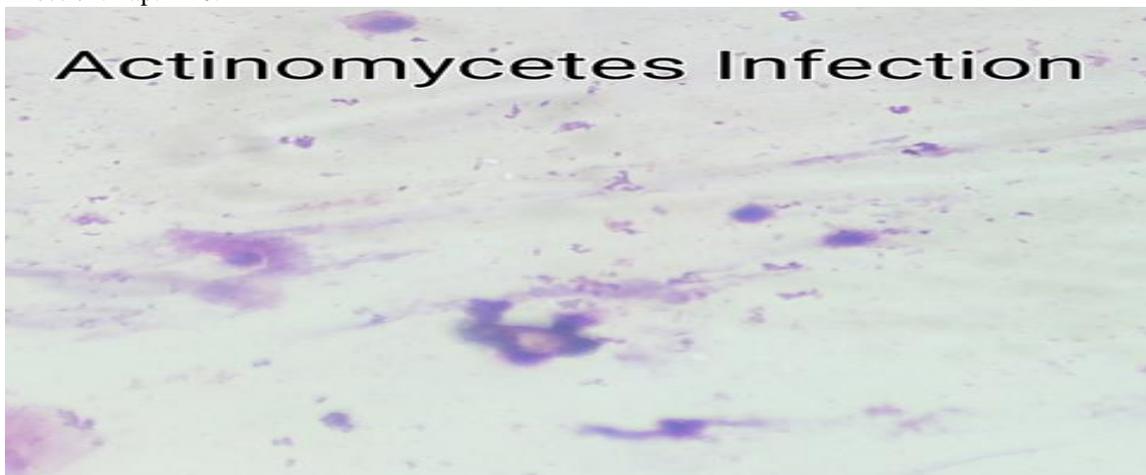
**Figure 4.** Frequency of infection among age group.



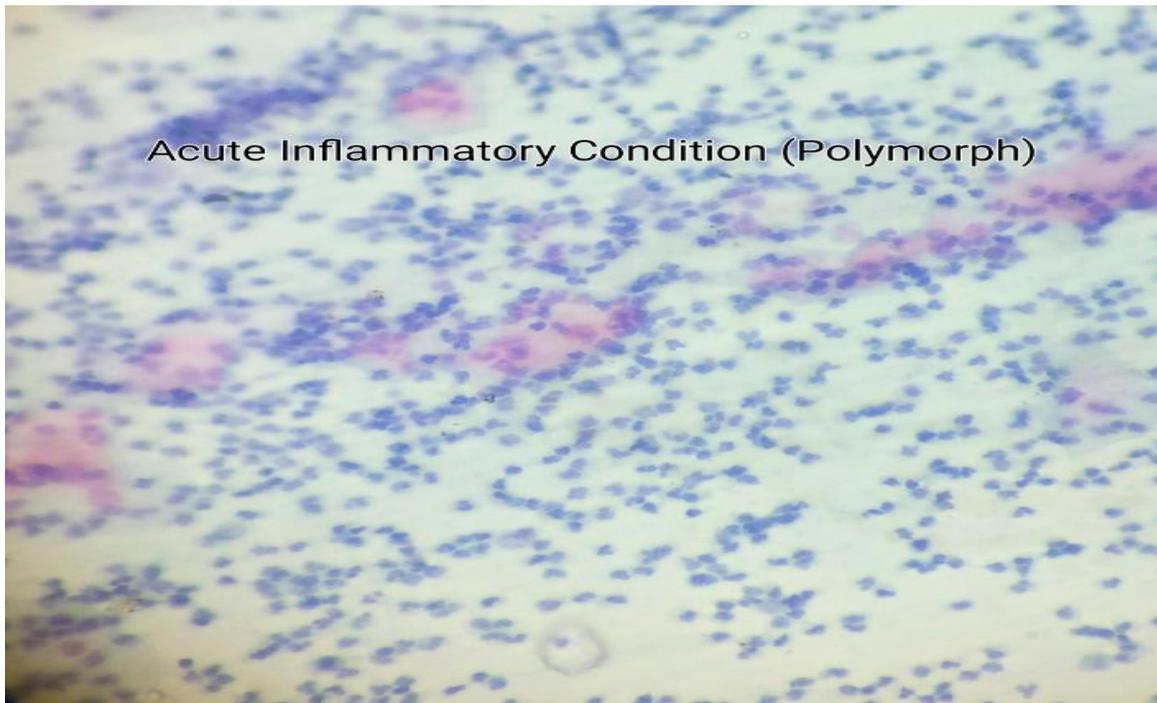
**Photograph No (1).** Vaginal smear taken from apparently healthy women. The smear showing epithelium cells. Pap. X40.



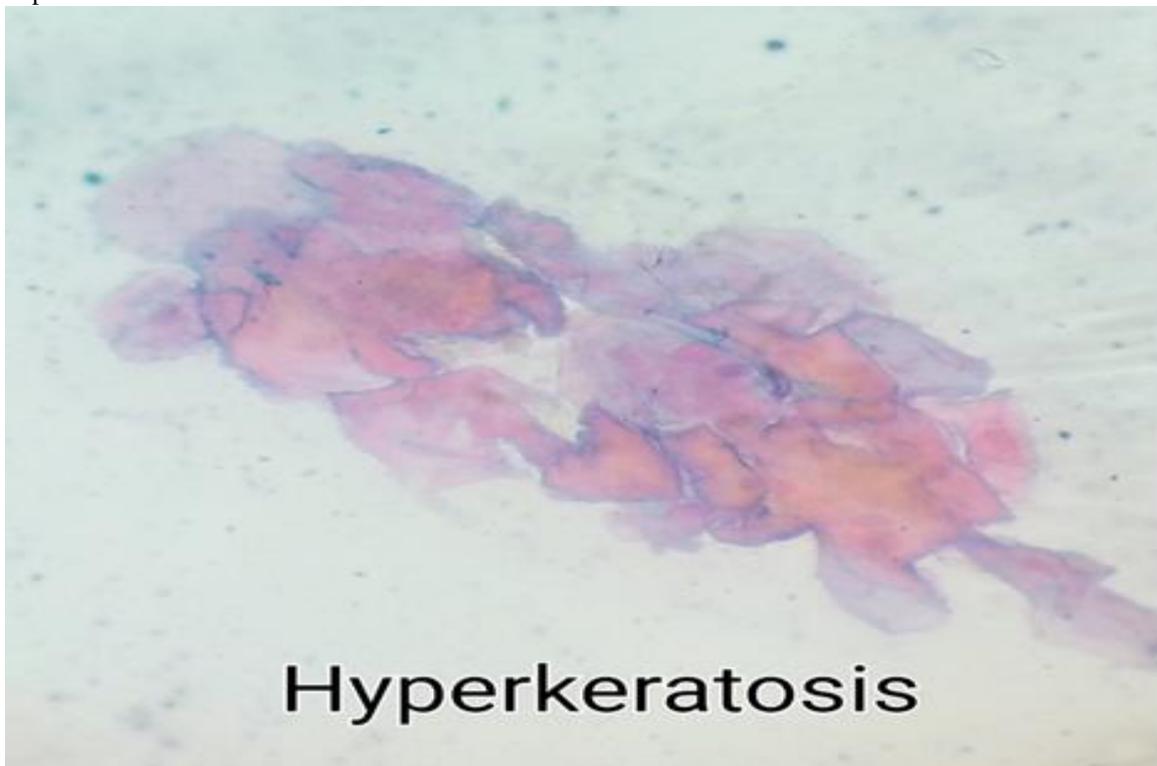
**Photograph No (2).** Vaginal smear taken from apparently healthy women. The smear showing Actinomycetes infection. Pap. X40.



**Photograph No (3).** Vaginal smear taken from apparently healthy women. The smear showing acute inflammatory condition (polymorph). Pap. X40.



**Photograph No (4).** Vaginal smear taken from apparently healthy women. The smear showing hyperkeratosis. Pap. X40.



#### Discussion

Vaginal cytology is used for screening sexually active women to enable early detection of

precancerous lesions and prevent mortality due to cancer. Although not mandatory in the current Bethesda terminology, it is a common practice to give a diagnosis of infectious microorganisms seen

in the vaginal cytology. Regarding bacterial vaginitis, several studies have identified it as the leading vaginal infection [7]. Bacterial vaginosis (BV) is the most common vaginal dysbiosis to affect women globally [8]. Bacterial vaginosis is the most prevalent vaginal condition, affecting 30% of women globally [9]. Bacterial vaginosis is associated with an increased risk of a broad range of gynaecological and obstetric sequelae including preterm delivery, spontaneous abortion, and early pregnancy loss [10]. **Hart** studying 5365 patients in Australia, found BV prevalence to be 13.7% [11]. **Simoes Barborasa et al.**, studying 142158 women in Brasilia, reported BV prevalence as 17.2% [12]. In Turkey, **Karabulut et al.**, reported frequencies of BV to be 8.3% [2]. In our study, we consider BV as the main infectious agent in 26% of the patients, which is semi similar to Karabulut's result. If left untreated, BV can cause serious complications. Bacterial vaginosis has been implicated in preterm delivery of low birth-weight infants due to chorioamnionitis, puerperal endometritis, postoperative vaginal cuff cellulitis, and pelvic inflammatory disease. Since up to half of the women with BV are asymptomatic, the cervical cytology may be only means of diagnosis. Some authors have suggested that the descriptive diagnosis for BV as "predominance of coccobacilli consistent with a shift in vaginal flora" is vague and probably not well-understood by most pathologists who collect routine vaginal cytology. Successive studies proved that the finding of "clue cell" in the cervical cytology examination had 100% sensitivity and a 96% specificity for the diagnosis BV. Clue cells have a decreased frequency in older women because in this age range, the pH is less variable and BV is more frequent in young women that are related pH alteration. Also, in our study, younger women had a significantly higher prevalence of BV. Cervicovaginal candidiasis is the most common fungal disease in the world. Approximately 75% of women are affected from vulva vaginal candidiasis at some point in their life time. Hart reported that candidiasis is a main infectious agent with 17.6% prevalence rate [11]. **Toloi et al.** reported *Candida* in 26% [13].

*Candida* spp. vulvovaginitis, or vulvovaginal candidiasis (VVC), considered the second cause of genital infection among women in menacme, occurs by excessive multiplication, favored by predisposing factors of yeast, found in the vaginal microbiota of women in reproductive phase [14, 15]. **Adad et al.** reported that infection by *Candida* sp, has presented a large increase over the last decade (8.1% in 1988 to 22.5% in 1998) while **Kent** reported a fall in the frequency of

*Candida albicans* in USA and a stabilization of infection by this agent in Skandinavia [1,7]. In our study *Candida* sp. was detected in 2.78% of all vaginal smears. The frequencies of all infectious agents in our study were much lower than those published in the literature. It is probable that lower indices are related to socioeconomic power of the patients. The conservative lifestyle in our country may contribute to lower rates in sexually transmitted disease.

Actinomycosis is a chronic infectious disease, caused by a Gram-positive anaerobic bacteria of the genus *Actinomyces*, with *Actinomyces israelii* being the most prevalent species. *Actinomyces*, a group of filamentous micro colonies, do not form spores, measure up to 1  $\mu$ m diameter, and are slow-growing. The bacterium is endogenous: it exists within the oral flora and is also a common inhabitant of the female genital tract. Therefore, the infection is considered opportunistic and requires a breach in the mucosal lining, since this constitutes the first line of defense against the germ. After gaining access to deeper tissues with impaired blood supply, the germs proliferate in the form of branching filamentous rods, producing suppurative abscesses and/or granulomas. The infection then spreads by direct extension using sinus tracts and can involve other organs in proximity with the primary entry site [16,17]. *Actinomyces* species are obligate anaerobic bacteria that demonstrate branching and filamentous growth. First report of *Actinomyces* in vaginal cytology was detected by **Gupta et al.** in 1976 [18]. They claimed that *Actinomyces* sp. could be easily identified on cytological smear. In literature many of published studies confirmed authors' findings via use of culture or immunofluorescent antibodies. For microbiologists, cervicovaginal cultures are the most difficult cultures to be evaluated in a clinical microbiology practice because it's expensive, difficult, and have very complicated processes in diagnosis, especially of *Actinomyces* sp. There have been a few studies published regarding seasonal variation in the detection of microorganisms in the vagina among Sudanese women.

### Conclusion

We conclude that cervical cytology is well suited for diagnosis of cervical infections. Bacterial vaginosis appears to be the predominant cause of vaginitis. The conventional cytology for exfoliative cells from female genitalia by using PAP stain is routine, specific, and sensitive, technique easy and not expensive so it is ideal technique for screening. So, we recommend by create a fixe screening

program legacy by it from ministry of health and government in all Arab counties.

#### Author contributions

**MAE and AAI:** Study design, conduct of the study, collection, and interpretation of data, manuscript writing, and revision.

**EMA and MIO:** Study design and manuscript writing and revision.

**SS:** Collection and interpretation of data, manuscript writing, and revision

**IBYE and AEAA:** Statistical analysis, interpretation of data, manuscript writing, and revision

All authors have read and approved the final manuscript.

#### Competing interests

The authors declare that they have no conflict of interest.

**Funding:** None.

#### Acknowledgements

The authors would like to acknowledge the support of all colleagues during design and finalization of the present study.

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