

# Significance of Unusual Microorganisms in Cervico-Vaginal Smears – A Tripod of Cases

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## ABSTRACT

The commonly employed screening test for detection of cervical carcinoma is Pap smear examination. However, it can also be used for screening cervico-vaginal infections. Several microorganisms are encountered in Pap smears such as bacteria, fungi, parasites etc.

The occurrence of few such organisms may be rare and when encountered should be reported with caution for several reasons such as to avoid unnecessary toxic anti-fungal therapy. *Chlamydia* is a sexually transmitted disease. The infection with *Chlamydia* and *trichomonas* may show changes in cells which mimic malignancy. So in our case report, we present three such cases and discuss the importance of reporting them.

**Keywords:** *Aspergillus*, *Chlamydia*, *Leptothrix*, Pap smear, *Trichomonas vaginalis*

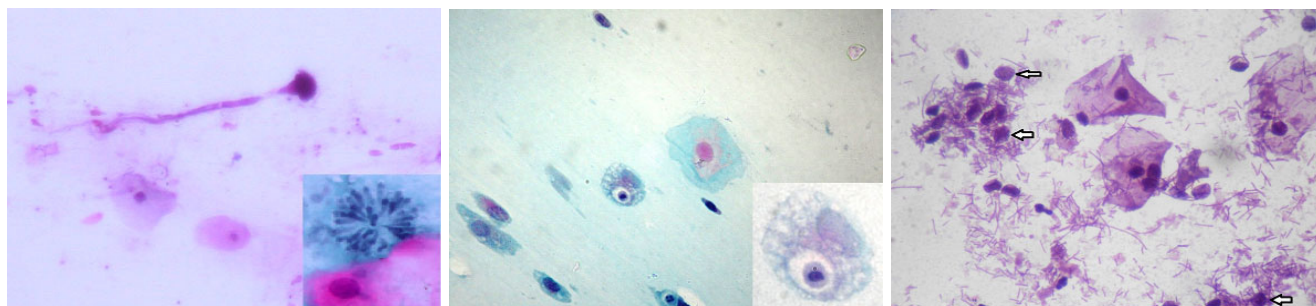
## CASE REPORT

**Case 1-** A 45-year-old woman was referred to the OBG department with complaints of white discharge per vaginum. There was no associated history of bleeding per vaginum, dyspareunia, weight loss. Gynaecological examination revealed an unhealthy cervix. Results of routine clinical and laboratory examinations were within normal limits. She underwent routine Papanicolaou smear examination. Smear examination showed acute inflammatory cells along with conidiophores and conidia of *Aspergillus* species [Table/Fig. 1]. Screening for Human Immunodeficiency virus (HIV) serology yielded negative results. Negative culture indicated that the presence of *Aspergillus* species was due to contamination.

**Case 2-** A 40-year old lady was referred to the OBG department with a history of dysfunctional uterine bleeding.

There was no associated history of white discharge per vaginum, dyspareunia or weight loss. Routine laboratory examinations were within normal limits. She underwent routine Pap smear examination. Smear showed inflammatory cells along with cells showing coccoid bodies with a clear halo and vacuolated cytoplasm, consistent with features of *chlamydial* infection [Table/Fig.2].

**Case 3 -** A 65-year old lady came with a history of white discharge per vaginum. Routine Pap smear examination was negative for dysplasia or malignancy but showed both *Leptothrix* and *Trichomonas vaginalis* (co-infection). *Trichomonas* was identified as 15-30 µm, pear shaped structures with a centrally located nucleus whereas *Leptothrix* as long, curving and filamentous bacteria [Table/Fig.3].



**[Table/Fig-1]:** Microphotograph of *Aspergillus* - showing conidiophores with conidia (Pap,X400). Inset-“fruiting body” or conidia (Pap,1000X)

**[Table/Fig-2]:** Microphotograph of *Chlamydial* infection showing tiny elementary coccoid bodies surrounded by narrow clear zones seen in the cytoplasm of squamous cells (Pap,400X) Inset (Pap,1000X) **[Table/Fig-3]:** Microphotograph showing pear shaped structures with a centrally located nucleus (*Trichomonas vaginalis*) [white arrows] and long, curving, filamentous bacteria (*Leptothrix*) (Pap,400X) (images from left to right)

The final diagnosis offered for all the above three cases were inflammatory smear with no evidence of dysplasia or malignancy.

## DISCUSSION

Pap smear is a simple, quick screening test for diagnosing cervical cancers. However, Pap smear can also be employed to detect cervico-vaginal infections especially in women who present to the Gynae OPD with White Discharge Per Vaginum (WDPV). Cervico-vaginal infections can be caused by bacteria, fungi or parasites. Some are commonly encountered whereas others are rare and occur under certain circumstances. Hence it is essential to identify such rare organisms and to signify its importance to the clinician to ensure safe treatment strategies for the patients. We herein report three such cases in women with varied clinical presentations. We discuss the cytomorphological features and the differential diagnosis of these cases.

Cervico-vaginal infections are commonly encountered problems in women. Sullam et al reported a prevalence of 52.8% with a spectrum consisting of *Candida albicans* (28%), *Trichomonas vaginalis* (8.7%), *Aspergillus* species (7.4%), *Streptococci* (4.6%) and *Chlamydia trachomatis* (4.2%) [1].

The most common fungal infection encountered is *Candida* species [2]. One uncommon fungal infection which we encountered in our routine practice was *Aspergillus* showing conidia and conidiophores. *Aspergillus* is a common environmental fungus transmitted by airborne conidia and causes severe respiratory infections and fungemia in immunosuppressed individuals [2]. The fungus has septate hyphae, 3-6 mm in width, with acute angle branching. They form conidial head or "fruiting bodies" that produce spores in cavities [2]. The characteristic features that allow species identification include the length and width of the conidiophores, size and contour of the vesicle, arrangement of phyllides, colour, size and length of chains of conidia [2]. An important clue to diagnose a particular *Aspergillus* species and to differentiate it from other similar fungi is identification of fruiting bodies and hyphae [3]. Fruiting bodies help in differentiating *Aspergillus* species from its mimics such as Zygomycetes and *Candida* species [2]. Fruiting bodies and hyphae of *Aspergillus* are rarely encountered in routine Pap smears. There are several case reports on occurrence of *Aspergillus* in Pap smears [3,4]. But all these authors have emphasised the fact that when one encounters such rare organism, it warrants a thorough clinical examination and other necessary investigations to confirm the diagnosis as infection or contamination. In our case, *Aspergillus* was a contaminant, as cultures were negative and the patient was not immunosuppressed. The other contaminants that may be encountered in routine cervico-vaginal smears include *Alternaria* and *Cladosporium* which are airborne fungal spores [5].

Coccioid bacteria and erythrocytes can also be confused for fungal spores. Fungal spores are bigger than coccioid bacteria

and smaller than erythrocytes [5].

Hence, it is essential to rule out contamination to prevent unnecessary toxic anti-fungal therapy, and true infection may require a thorough clinical examination and more vigilant investigations to study the immune status of the individual [3].

*Chlamydia trachomatis* is one among the most prevalent sexually transmitted pathogens which cause non gonococcal urethritis, epididymitis, proctitis in men, mucopurulent cervicitis, urethritis, salpingitis in women and various infections in newborn infants. It is also linked to infertility in women and associated with certain complications of pregnancy such as premature rupture of foetal membranes, premature delivery and post-partum endometritis [6]. Hare et al., have shown its association with follicular cervicitis and carcinoma in situ [7].

The cells infected with *Chlamydia* are large "atypical" metaplastic cells and endocervical columnar cells with vacuolated cytoplasm containing coccioid or inclusion bodies with three phases of development [8]. Gupta et al., [8] have reported various stages of chlamydial infection. In the first stage, *Chlamydia* particles occur as fine acidophilic coccioid bodies and may represent the infective stage of the disease. This stage is followed by localisation, condensation and transformation of these particles into fine perinuclear vacuoles containing fine eosinophilic or basophilic granular bodies, may represent the non-infective replication of the organism. In the final stage relatively large intra cytoplasmic vacuoles form that contain either a central homogenous target like condensation surrounded by a halo or numerous densely packed coccioid structures diffusely distributed throughout the vacuole [8]. Our case showed features suggestive of final stage of chlamydial infection.

As radiation, chemotherapy, cryosurgery and folate deficiency can cause cytoplasmic vacuolations one should be careful in diagnosing Chlamydial infections [9]. Other findings which can lead to misdiagnosis include condensed mucus secretions in cytoplasmic vacuoles or coccioid bacteria covering degenerative cytoplasmic vacuoles [8,9]. Because the infection with *Chlamydia trachomatis* is of major clinical significance, the consensus is that the identification of this agent should always be confirmed by culture using McCoy cells which is the standard diagnostic test [10]. Other sensitive diagnostic techniques include immuno-fluorescence using a monoclonal antibody, an enzyme-linked immunosorbent assay procedure, or by Polymerase Chain Reaction (PCR). Our case was confirmed by culture [10].

*Leptothrix vaginalis* is a filamentous bacteria found in the vagina of young females commonly during pregnancy [11]. A study by Carvalho et al., [11] showed that 52% of their patients presented with thick, yellowish vaginal discharge associated with vulvo-vaginal pruritus and irritation of the vaginal mucosa. *Leptothrix* usually takes the shape of S or U and is long, curved, and filamentous. The close differential diagnoses are a) *Doderlein bacilli* when its filaments are short,

but *L. vaginalis* is a little wider and dark in colour. b) *Monilia* but *Leptothrix* does not show any segments, branches or spores unlike *Monilia*. *Leptothrix* per se may not have any clinical significance. But many at times, this usually occurs as a co-infection along with trichomonads. So finding *leptothrix* in Pap smears may give us the clue of the co-existing *trichomoniasis* [11]. In our case also we had both *leptothrix* and *trichomonas vaginalis* together.

So what is the significance of reporting *trichomonas* infection?

A study by Bertini et al., [12] showed that in trichomoniasis, the superficial and intermediate cells may show inflammatory atypia, nuclear hypertrophy, slight hyperchromasia, cytoplasmic vacuolization and a rare binucleation. As these features could be confused for cervical carcinoma, one should be aware in reporting such lesions [12]. Recent studies have proposed that *T. vaginalis* infection may increase the risk of HIV-I acquisition [13] and may also be associated with adverse pregnancy outcomes such as premature rupture of membranes, preterm delivery and low birth weight. Hence, one should emphasise care while reporting smears with trichomoniasis [14].

## CONCLUSION

Pap smear examination is commonly employed for cervical cancer screening. However, one may encounter a variety of infections which is of public health importance. Accurate diagnoses of these infections are essential to provide accurate treatment strategies as well as to prevent any complications. Hence, recognition of cytomorphological features are critical in proper interpretation and diagnosis.

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