



## SPECTRUM OF INFECTIVE ORGANISMS ON LIQUID BASED CYTOLOGY IN A TERTIARY CARE HOSPITAL

### Pathology

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### ABSTRACT

**Background :** Pap cervicovaginal infection is common presentation of females in gynecology department. Most of the females are of reproductive age group. Cervical pap smear is a simple test done to find out any infective organism in cervical region. The present study aims to identify the prevalence of infective organisms in liquid based cytology in symptomatic as well as non symptomatic females.

**Material and methods:** 2000 liquid based pap smear were included in the study. They were stained and examined for any pathogenic organism.

**Results:** The results showed that majority of them were negative for intraepithelial lesion with infective organism in 72 pap smears.

**Conclusion:** The study highlights the importance of a simple pap test which is highly effective in screening of organisms along with epithelial abnormalities.

### KEYWORDS

infective organisms, cervical pap smear, liquid based cytology.

### BACKGROUND

Pap smear is a simple, cheap, quick and painless procedure performed in outdoor patient department to screen patients for cervical cancer (Michalas, 2000). Inflammatory pap smears are a significant component of smears reported as negative for intraepithelial lesions. The majority of them are non-specific inflammation and thus inflammatory pap smears are mostly underinvestigated. Infections are commonly encountered in reproductive age group and most of these females are asymptomatic with inflammatory pap smears. Majority of those who are symptomatic present with history of discharge per vaginum. Vaginal discharge is caused by overgrowth of pathogens due altered normal flora. Poor genital hygiene is the common cause of these infections in Indian females (Mishra, 1997). The most common pathogens encountered are bacterial vaginosis (BV), candida and trichomonas (Mishra, 1997).

The most common cause of discharge is BV. It has been commonly associated with complications after gynaecological surgeries and in pregnancy whereas trichomonas during pregnancy is related with preterm deliveries and low birth weight (Heller et. al, 2006; Azargoon et. al, 2006; Cotch et. al, 1997).

The sensitivity, specificity and positive predictive value of pap smear for BV and trichomonas are 88.2%, 98.6%, 96.8 % and 98%, 96%, 88% respectively (Platz-Christensen et. al, 1995; Loo et. al, 2009). There is lack of any guidelines for work up and follow up of the patients with inflammatory pap smears. Intensive search for infectious organisms should be done in inflammatory pap smears. This study illustrates the importance of liquid based cytology (LBC) pap smear in diagnosis of cervical infection.

### MATERIAL AND METHODS

This retrospective study was carried out in a tertiary care hospital in north India and included 2000 LBC pap smears over a period of 15 months. All the females attending to the gynaecology OPD and undergoing LBC pap test were included in the study. An informed consent was taken from all the patients included in the study in a language they understand. The presenting complaints of patients were discharge per vaginum, pain abdomen and abnormal bleeding. LBC smears were prepared by Surepath™ method and smears were examined by experienced cytopathologist.

Diagnostic criteria for various infections were as follow: BV - presence of clue cells or coccobacilli, vaginal candidiasis - presence of pseudohyphae or budding yeast forms, trichomoniasis - presence of trophozoite forms, actinomyces - presence of filamentous hyphae as cotton wool appearance, herpes simplex virus - multinucleation, margination of chromatin and moulding of nuclei in squamous epithelial cells and microfilariasis - presence of larval form of microfilaria. The frequency of various infections was estimated.

### RESULTS

A total of 2000 LBC pap smears were examined. 1609 of them were negative for intraepithelial lesions (NILM) and 51 were unsatisfactory either due to low cellularity, hemorrhage or dense inflammation. Inflammatory smear were divided as minimal, mild, moderate and dense (Figure.1)

Among these 1609 NILM, 72 were having inflammation with infective etiology (Table.1 and 2). The most common infection encountered was bacterial vaginosis found in 39 cases followed by candidiasis in 26 cases (Figure 2 and 3). Nine cases were of trichomonas vaginalis (Figure. 4). Two of each actinomyces (Figure.5) and microfilaria and one case of herpes simple virus infection (Figure.6)

A correlation between severity of inflammation and infection was calculated and it was found that moderate to dense inflammation was present in smears with bacterial vaginosis ( $p < 0.001$ ). However, this association was not significant with other infections. Also there was no significant correlation between presence /absence of inflammation and infection.

Coinfection with BV and TV was found in 3 cases, whereas BV and candida coinfection was found in one case. All three coinfections were found in females of age group 31-40 years.

### DISCUSSION

Cervical infections are very common in developing countries like India. In our study the prevalence of infection in females living in north India was 3.6 %. Another study conducted in south India showed a prevalence of 19% which is very high in comparison of the present study (Madhivanan et. al, 2008). The reason for such a large difference can be attributed to low literacy rate leading to less awareness of cervicovaginal infections in northern Indian females in comparison to south Indian females, which leads to less OPD visits for mild gynaecological problems in these females. Other studies conducted in Iran and Africa had a prevalence of 28.5% and 38 % respectively which is very high in comparison to the present study (Shobeiri et. al, 2006; Romoren et.al, 2007). The present study shows that bacterial vaginosis (BV) is the most common infection with a prevalence of 44.4% whereas a study by G. vandana found BV in 54%<sup>11</sup>. Namazi et al studied that the prevalence of candida is 25.5% which is lower than that found in the present study (36.12%). Molana & Ghazi-Saeidi concluded that the prevalence of trichomonas infection is 1.37% which is far less than that found in our study (12.50) (Namazi et. al, 2005; Sarbour et. al 2018).

Presence of microfilaria in two cases was a very rare finding as till now a very few cases are reported in literature (Sood et. al, 2014). One of the patient was an antenatal female. Actinomyces was present in two cases, both of them were using intrauterine devices (IUD) for a long

time and now they had complain of pain abdomen and vaginal discharge. These are gram positive filamentous non spore forming bacteria generally found in IUD users. Kim YJ et. al found the prevalence of actinomyces was 0.26 % of which 80.8 % females were IUD users. This is lower than that found in our study (Kim Et. al, 2014). A single case of herpes simple virus was found in 2000 pap smears, showing the low prevalence of the entity.

The differences in various studies may be due to studies with variable sample size in different geographical areas, different socioeconomic and cultural population, variability in sample collection and interpretation by cytopathologist.

There was coinfection of BV and TV in the present study in two cases, although it was not significant but in a study conducted by Demirezen et al in Turkey on 600 pap smears, they found a significant association of BV and TV in 44% of cases and explained that presence of TV alters the vaginal pH by phagocytosis which results in overgrowth of anaerobic bacterias (Barouti et. al, 2013). The association of infection and presence of inflammation are observed by many studies, most of them state that there is no direct cause and effect relationship between the two. At times infections are not associated with inflammation and vice versa (Ayres et. al, 1997). Burke et al studied that patients with inflammation in pap smear had high probability of infection than those who don't have inflammation (Burke et al, 2004). The present study also had significant association between inflammation and infective etiologies.

The present study highlights the importance of cervical pap smear in diagnosis of entities other than epithelial cell abnormalities. The smears which are negative for intraepithelial lesion/malignancy should be examined thoroughly to rule out infective causes. Dense inflammatory smears should be examined carefully as most of the time the infective organisms are obscured by inflammation, however this problem has been minimized with liquid based cytology which removes the background debris, hemorrhage and decreases the inflammatory cells to a large extend. Hospital based studies are required for estimation of the prevalence and spectrum of cervicovaginal infection which are further helpful in planning and implementation of various health schemes.

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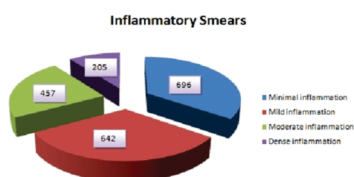
**The authors declare that there is no conflicts of interest**

**Table 1. Spectrum of infective organism in LBC PAP smears**

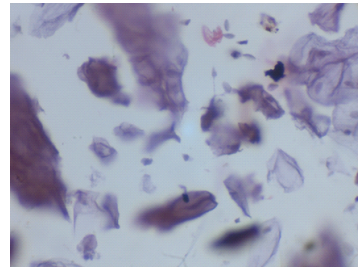
| Infective organism    | Number | percentage |
|-----------------------|--------|------------|
| Gardenella vaginalis  | 32     | 44.44%     |
| Candida               | 26     | 36.12%     |
| Trichomonas Vaginalis | 09     | 12.50%     |
| Actinomyces           | 02     | 2.78%      |
| Microfilaria          | 02     | 2.78%      |
| Herpes simple virus   | 01     | 1.38%      |
| Total                 | 72     | 100%       |

| Age group   | BV | Candida | TV | Actinomyces | Microfilaria | HSV |
|-------------|----|---------|----|-------------|--------------|-----|
| 20-30 years | 20 | 15      | 01 | 01          | 01           | 00  |
| 31-40 years | 10 | 09      | 07 | 01          | 01           | 01  |
| 41-50 years | 07 | 02      | 01 | 00          | 00           | 00  |
| >50 years   | 02 | 00      | 00 | 00          | 00           | 00  |

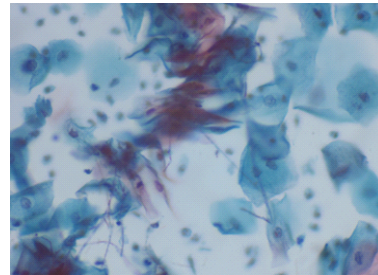
Table 2. Age wise distribution of various infections.( BV-Bacterial vaginosis, TV- Trichomonas vaginalis)



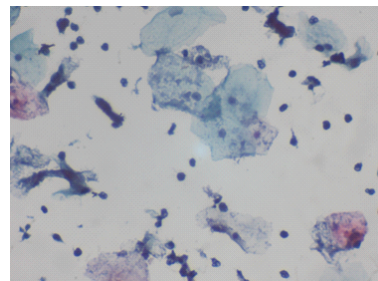
**Figure 1. Pie diagram showing number of cases with severity of inflammation.**



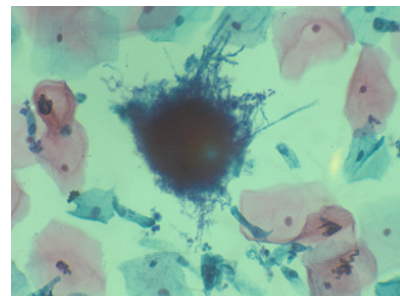
**Figure 2. Giemsa stain 200X Liquid based pap smear showing clue cell (Bacterial vaginosis)**



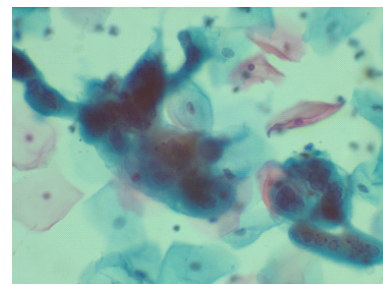
**Figure 3. Giemsa stain 200X Liquid based pap smear showing pseudohyphae of candida.**



**Figure 4. Giemsa stain 400X Liquid based pap smear showing trichozoite forms of trichomonas.**



**Figure 5. Giemsa stain 200X Liquid based pap smear showing filaments of actinomyces.**



**Figure 6. Giemsa stain 400X Liquid based pap smear showing multinucleation, nuclear molding**

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