

ORIGINAL ARTICLE

MORPHOLOGICAL FEATURES AND CYTOPATHIC EFFECTS OF ORGANISMS IN THE CERVICAL SMEAR OF INFERTILE VERSUS FERTILE WOMEN: A COMPARATIVE ANALYSIS**Sumaira Javed, Zilli Huma*, Humaira Imtiaz, Sara Jadoon, Fayyaz Ahmed**, Alruba Taimur*****

Department of Anatomy, Ayub Medical College, Abbottabad, *Department of Anatomy, Khyber Medical University, Peshawar, Department of **Pathology, ***Physiology, Ayub Medical College, Abbottabad, Pakistan

Background: Infertility is a challenging health problem around the world affecting 22% women in Pakistan. Approximately 75% of women are infected with sexually transmitted diseases (STDs) during their lives at least once, and present with cervical epithelial cell lesions. Sexually transmitted infections (STIs) are responsible for pelvic inflammatory disease which is known to be the commonest cause of infertility. This study aimed to explore whether infertile women have a higher incidence of infectious organisms in their cervical smears than fertile controls. **Methods:** A total of 150 individuals were recruited for the study. Cervical smears of 100 infertile women were compared with 50 fertile women in the age range of 20–40 years. Fifty fertile patients were taken as controls (Group-A), 50 primary infertile patients (Group-B) and 50 secondary infertile patients (Group-C) in the outpatient departments of Gynaecology and Obstetrics, Ayub Teaching Hospital, Abbottabad. **Results:** A significant difference was observed regarding the prevalence of infectious organisms in their cervical smears ($p < 0.004$), with higher rate in infertile women. The secondary infertile women group showed significant rise ($p < 0.05$) in the rate of isolation of infectious organisms as compared to primary infertile women. **Conclusion:** Infectious organisms are present at a remarkably higher rate in infertile women in comparison to fertile women. Women with secondary infertility have a greater possibility of colonization with infectious organisms than women with primary infertility. Screening through Pap smear should be done in infertile population to detect these organisms at an early stage.

Keywords: Infertility, Pap smear, Cervical epithelium, Sexually transmitted diseases

Pak J Physiol 2024;20(3):23–6, DOI: <https://doi.org/10.69656/pjp.v20i3.1645>

INTRODUCTION

Infertility is a significantly alarming health issue for couples of reproductive age all over the world with an increasing incidence due to changes in lifestyle and environmental stress factors.¹ Pelvic inflammatory disease (PID) caused by sexually transmitted infections (STIs) is considered the most common cause of infertility.² PID, is a crucial health issue commonly observed in sexually active women of reproductive age. The commonest cause of PID is sexually transmitted infections like *Chlamydia trachomatis* and the Human Papilloma Virus (HPV).³

Four main agents, i.e., *Neisseria gonorrhoea*, *Chlamydia trachomatis*, *Trichomonas vaginalis* and Syphilis, mainly responsible for sexually transmitted infections, were identified in a study conducted by the World Health Organization (WHO), who were known to cause almost 500 million new cases each year throughout the world.⁴ Sexually transmitted infections are significantly involved in the causation of cervical epithelial cell abnormalities, hence cervical carcinoma.

Three-fourths of women in their lifetime are infected with STDs at least once, which represents as abnormal cervical cytology.⁵ Cervical cancer is regarded as a sexually transmitted disease because of increased association of cervical cancer with sexually

transmitted infections.⁶ Multiple studies have suggested that infertile women are more susceptible to acquire cervical carcinoma preceded by cervical epithelial cell abnormalities because of their increased risk of exposure to either STDs or to exogenous hormones.⁷

Certain non-cancerous conditions such as infections and inflammation can also be detected by using Pap smear cytology.⁸ Although it is not the primary test for diagnosing infectious organisms in cervical smears but it can be used effectively to diagnose various genital infections. It is very useful in investigating the cellular changes caused by various viruses and also helps in detection of different bacterial organisms as well as sexually acquired organisms.

Cervical smears can also identify fungal infections present in the specimen.^{1,9} Pap smear is an affordable, comparatively simple and easily tolerable tool utilized for early investigation of cervical and vaginal infections in under developed countries. Hence, it is used in infertility management protocols in some centres worldwide.^{10–12}

The aim of this study was to identify the various infectious organisms in infertile women using Pap smear which ultimately leads to cervical epithelial abnormalities.

METHODOLOGY

This cross-sectional comparative study was carried out in the outpatient departments of Ayub Teaching Hospital, Abbottabad, recruiting 150 patients after getting ethical approval from Khyber Medical University, Peshawar. The sample size was calculated using sample size calculator. A comparison of cervical smears for the presence of infectious organisms was done among each of the three groups by using quota sampling. The three groups were Group A (fertile, controls), Group B (primary infertile) and Group C (secondary infertile), with 50 patients each. Lab work was done at Histopathology Departments of Ayub Medical College, Abbottabad and KMU Institute of Basic Medical Sciences, Peshawar.

Patients aged 20–40 years were included who showed compliance to vaginal examination and were willing to give cervical smear. Patients having acute pelvic infection or presenting with any other systemic disease were excluded. Women with minor ailments, other than the infertility problem presenting to Obs/Gyn OPDs who had delivered a child within the last year were selected for the control (fertile) group.

Infertile women were labelled as primary infertile who had failed to conceive after trying for 2 years at least. Women who conceived earlier but later were not able to conceive after trying for a minimum of 2 years at least were labelled as secondary infertile. Cervical smears were taken from these patients and stained with Haematoxylin and Eosin (H&E) stain and Papanicolaou (Pap) stain. The slides were fixed and examined for infectious organisms. (Table-1)

Table-1: Characteristic morphological features and cytopathic effects of organisms in cervical smear

Organism	Morphology	Cytopathic effect
Lactobacilli	Rod shaped bacteria	'Cytolysis'- absence of intact squamous cells, bare nuclei
<i>Trichomonas vaginalis</i>	Protozoa, 15 µm, tear drop shaped,	'Cannon ball cells'
<i>Gardenerella vaginalis</i>	Anaerobe	'Clue cells' (large no of bacteria obscuring cell borders of squamous cells)
<i>Candida albicans</i>	Hyphae and spores	nonspecific inflammatory changes (perinuclear vacuoles, parakeratosis- orangeophillia of superficial & intermediate cells)
Human Papilloma Virus (HPV)	Not visible on light microscope	A halo around nucleus, slightly enlarged pinched nucleus pushed towards the periphery, a thick cytoplasmic rim around the periphery, loss of round nuclear contours
Herpes simplex Virus (HSV)	Not visible on light microscope	3 M's (Margination of chromatin, Molding of nuclei, Multinucleation)
Actinomyces like organisms	Clusters of filamentous bacteria in many shapes and sizes	Dusty appearance, dust bunny

The descriptive statistical analysis was conducted on SPSS-22. Variables were represented as Mean±SD. Inferential statistical analysis was conducted by applying Kruskal Wallis test and Mann Whitney U tests for qualitative variables.

RESULTS

Various infectious organisms were observed and identified in cervical smears of patients (Figure-1) according to the criteria tabulated as Table-1. The overall group comparison showed a highly significant difference ($p<0.004$) between groups. The multiple group comparisons showed significant differences between Fertile (group A) and Secondary infertile (group C) and primary infertile (group B) and secondary infertile (group C) ($p=0.003$ and 0.047 , respectively). The comparison of Group A and Group B did not show any significant differences. Cervical smears from Secondary infertile women (Group C) showed more infectious organisms compared to the other 2 groups. (Table-2).

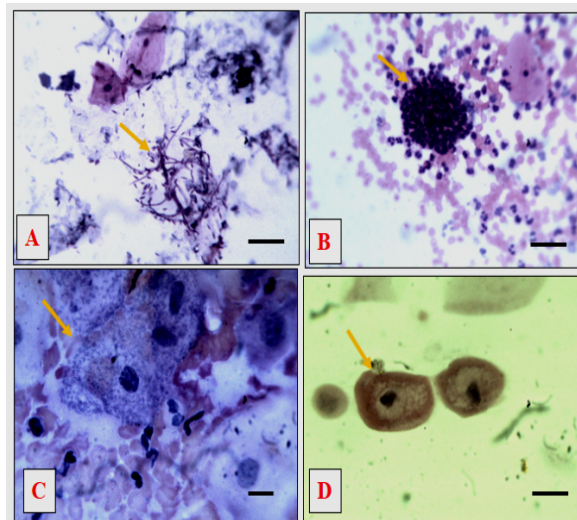


Figure-1: Photomicrographs of infectious organisms

A: *Candida albicans* organism: arrow showing pseudohyphae (Scale 50 µm, 40×). B: *Trichomonas vaginalis*: arrow at cannonball cells or Poly balls (Scale 50 µm, 40×). C: *Gardnerella vaginalis*: arrow at Clue cells with *Coccobacilli* (Scale 10 µm, 100×). D: Human Papilloma Virus (HPV) cytopathic effect, pinched nucleus pushed to the periphery and a clear halo around it (Scale 50 µm, 40×)

Table-2: Percentage distribution of infectious organisms in study groups

Organisms	Group-A Fertile N=50	Group-B Primary infertile n=50	Group-C Secondary infertile n=50
Cannonball cells (<i>Trichomonas vaginalis</i>)	–	2.0	10
Clue cells (<i>Gardenerella vaginalis</i>)	–	0	4.0
Pseudohyphae (<i>Candida albicans</i>)	–	0	2.0
Cytopathic cells (<i>Human Papilloma Virus</i>)	–	2.0	0

DISCUSSION

Several studies have been done on the presence of microorganisms in cervical smears. Although cervical cytology is not a standard or primary method for detection of infectious organisms, but as a component of Bethesda classification system it can be extremely useful in diagnosis of various pathogenic organisms by either finding the infectious organisms themselves or by discerning the peculiar cytological cellular changes. Various studies have shown the frequent presence of *Trichomonas vaginalis* and *Gardenerella vaginalis* in cervical smears.^{13,14} In our study, the most common infectious organisms observed was *Trichomonas vaginalis* represented by ‘Cannonball cells’ in cervical smears of infertile patients, followed by *Gardenerella vaginalis* represented by ‘Clue cells’. Both organisms were observed in patients between the ages of 20–30 years. This difference in prevalence of infectious organisms according to age range is proved by other studies as well.^{15,16} The main reason may be because of high sexual activity in this age group or poor resistance against vaginosis among young patients. Another possibility is that bacterial vaginosis represented predominantly by *Gardenerella vaginalis* is influenced by vaginal pH, showing an increased risk of bacterial vaginosis in the luteal phase when vaginal pH is more alkaline and there is a decrease in normal healthy vaginal flora.

Candida albicans representing fungal infection was observed in 2% smears from infertile women which is quite low as compared to other studies on prevalence of fungal infection.^{17,18} A reason may be that the Pap test is not a reliable method for diagnosis of fungal infection. *Candida* is much more easily diagnosed on vaginal smears or wet mounts as compared to cervical smears. Other factors affecting the prevalence may be sexual habits, cultural conditions, and socioeconomic status.

Our study shows the presence of bacterial vaginosis, candidiasis and trichomoniasis in the infertile population as does a Nigerian and Indian study by Okonofua FE *et al*¹⁹ and Bose A *et al*²⁰ respectively, who reported that pathogenic organisms are found much frequently in infertile women residing in their lower genital tract, although no comparison of secondary infertile with primary infertile women regarding the presence of infectious organisms was done. Our findings strongly suggest presence of genital tract infections in primary infertile women and secondary infertilities having a relatively higher incidence and are suggestive of a probable cause of infertility.

CONCLUSION

Women with secondary infertility have a higher level of pathogenic organisms in their cervical smears in addition to the more threatening incidence of cervical epithelial

cell abnormalities than women with primary infertility. These findings strongly favour the positive connection between genital tract infections and infertility.

REFERENCES

1. Cong J, Li P, Zheng L, Tan J. Prevalence and risk factors of infertility at a rural site of northern China. *PLoS One* 2016;11(5):e0155563.
2. van Hamont D, Nissen LH, Siebers AG, Hendriks JC, Melchers WJ, Kremer JA, *et al*. Abnormal cervical cytology in women eligible for IVF. *Hum Reprod* 2006;21(9):2359–63.
3. Yagur Y, Weitzner O, Barchilon Tiosano L, Paitan Y, Katzir M, Schonman R, *et al*. Characteristics of pelvic inflammatory disease caused by sexually transmitted disease —An epidemiologic study. *J Gynecol Obstet Hum Reprod* 2021;50(9):102176.
4. Mitchell C, Prabhu M. Pelvic inflammatory disease: current concepts in pathogenesis, diagnosis and treatment. *Infect Dis Clin North Am* 2013;27(4):793–809.
5. Nnadi D, Nwobodo E, Ekele B, Sahabi S. Screening for cervical cancer: A review of outcome among infertile women in a tertiary hospital in North-West Nigeria. *Ann Med Health Sci Res* 2014;4(3):383–7.
6. Abeyewickreme I. Cervical cytology screening in a sexually transmitted diseases clinic for the first time in Sri Lanka. *Genitourin Med* 1989;65(2):98–102.
7. Almobarak AO, Elhoweris MH, Nour HM, Ahmed MA, Omer AF, Ahmed MH. Frequency and patterns of abnormal Pap smears in Sudanese women with infertility: What are the perspectives? *J Cytol* 2013;30(2):100–3.
8. Jayapalan S, Bindu RS. Papanicolaou smear: A diagnostic aid in sexually transmitted infections. *Indian J Sex Transm Dis AIDS* 2020;41(2):143–8.
9. Sami N, Saeed Ali T. Perceptions and experiences of women in Karachi, Pakistan regarding secondary infertility: results from a community-based qualitative study. *Obstet Gynecol Int* 2012;2012:108756.
10. Mishra R, Bisht D, Gupta M. Primary screening of cervical cancer by Pap smear in women of reproductive age group. *J Family Med Prim Care* 2022;11(9):5327–31.
11. Whitman-Elia GF, Baxley EG. A primary care approach to the infertile couple. *J Am Board Fam Pract* 2001;14(1):33–45.
12. Morrison J, Carroll L, Twaddle JO, Obisesan KA, Grimshaw J, Leyland A, *et al*. Pragmatic randomised controlled trial to evaluate guidelines for the management of infertility across the primary care–secondary care interface. *BMJ* 2001;322(7297):1282–4.
13. Konje JC, Otolorin EO, Ogunniyi JO, Obisesan KA, Ladipo OA. The prevalence of *Gardnerella vaginalis*, *Trichomonas vaginalis* and *Candida albicans* in the cytology clinic at Ibadan, Nigeria. *Afr J Med Med Sci* 1991;20(1):29–34.
14. Faur AC, Ghenciu LA, Gurban CV, Lazăr DC. Unusual fungal species in routine cervicovaginal Papanicolaou smears. *Clin Exp Obstet Gynecol* 2023;50(8):157.
15. Fernández Limia O, Lantero MI, Betancourt A, de Armas E, Villoch A. Prevalence of *Candida albicans* and *Trichomonas vaginalis* in pregnant women in Havana City by an immunologic latex agglutination test. *MedGenMed* 2004;6(4):50.
16. Nai GA, Mello AL, Ferreira AD, Barbosa RL. [Frequency of *Gardnerella vaginalis* in vaginal smears of hysterectomized women]. *Rev Assoc Med Bras* (1992). 2007;53(2):162–5. [Article in Portuguese]
17. Bukhari MH, Majeed M, Qamar S, Niazi S, Syed SZ, Yusuf AW, *et al*. Clinicopathological study of Papanicolaou (Pap) smears for diagnosing of cervical infections. *Diagn Cytopathol* 2012;40(1):35–41.
18. Okoro CL, Okoro VM, Uwaezuoke JC, Ogbulie JN. Antimicrobial assessment of bacterial and fungal isolates from women with infertility challenges in Imo State, Nigeria. *Gha Alt Med J* 2022;3(3):10–7.



19. Okonofua FE, Ako-Nai KA, Dighitoghi MD. Lower genital tract infections in infertile Nigerian women compared with controls. *Genitourin Med* 1995;71(3):163–8.
20. Bose A, Acharya M, Panigrahi K, Pradhan P, Dash K. Pap smear

changes and cervical inflammation in women with infertility in correlation to clinical and microbiological findings. *Int J Acad Med* 2023;9(3):145–50.

Address for Correspondence:

Dr Sumaira Javed, Assistant Professor Anatomy, Ayub Medical College, Abbottabad, Pakistan. **Cell:** +92-335-5511279
Email: sumairazia@gmail.com

Received: 28 Feb 2024

Reviewed: 28 Aug 2024

Accepted: 29 Aug 2024

Contribution of Authors:

SJ: Study design, acquisition of data, manuscript writing

ZH: Drafting and critical review

HI: Conception of study and critical review

SJ: Drafting of manuscript

FA: Analysis and interpretation of data

AT: Critical review and analysis

Conflict of interest: None to declare

Funding: None