

Determination of Prevalence and Clinical Significance of Detection of Micro-organisms in Pap Smear with Seasonal Variation

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ABSTRACT

Introduction: The most common problem in reproductive age group women is vaginitis. In the vaginal flora an extensive and diverse spectrum of pathogenic agents may be observed. The important infectious agents in vaginal flora are Bacterial Vaginosis (BV), Candida (Can) and *Trichomonas vaginalis* (TV). The pap smear is a screening test to diagnose cervical cancer and has also been used as a diagnostic test in cervical infection caused by different pathogenic organisms.

Aim: To determine the prevalence and clinical significance of micro-organisms in pap smears.

Materials and Methods: A two year retrospective Pap smear study was done from January 2014 to December 2015, in a tertiary care unit, Saveetha Medical College and Hospital, Thandalam, Chennai, India. This study was undertaken to determine the prevalence, clinical significance and seasonal

variation in cervicovaginal infections. The Chi-square test was used for statistical analysis. A p-value of <0.05 was considered significant for statistical evaluation.

Results: The total number of pap smears examined was 6424, out of which the total pap positive smears for infectious organisms were 896(13.94%). The prevalence of organisms among total infectious organisms is 51.4% (BV), 30% (Can), 12.4% (TV) and mixed infections 3.5% and 2.7% (BV+TV and BV+Can). Comparison of seasonal variation in prevalence of vaginal infectious agents was also analyzed.

Conclusion: It is concluded that cervical cytology is important for diagnosis of cervical infections. The most important cause of cervico-vaginitis are bacterial vaginosis, next important cause is candidiasis. It is important to mobilize all reproductive age women to undergo pap smear examination and to prevent complications caused by infectious agents.

Keywords: Clue cells, Cytobrush, Pruritis, Vaginal discharge, Yeast forms

INTRODUCTION

Cervico-vaginal infections are common in the reproductive age group of females. The pap smear is a screening test to know the premalignant and malignant lesions and also useful for diagnosis of cervico-vaginal infections and also mixed infections. It is a simple, quick, and painless procedure. Pap smear was first introduced by George Papanicolaou in 1943. It is a screening test to detect the presence of abnormal cells in the cervix that could become cancerous if not diagnosed early [1,2]. About two thirds of patient present with vaginal discharge, abdominal pain, postcoital bleeding, dyspariunia and other symptoms. Bacterial vaginosis, candidiasis and trichomoniasis are responsible for 90% of the cases of diseases of infectious origin, and less than 1% of these cases show mixed infection. Actinomycosis is common in women who used IUD [3].

MATERIALS AND METHODS

A retrospective analysis of cervical pap smear study was

done over a period of two years from January 2014 to December 2015 in a Tertiary Care Unit, Department of Pathology, Saveetha Medical college and Hospital, Thandalam, Chennai, India.

Patients were included in the study if a pap smear was performed for routine screening and medical necessity. The study was approved by scientific review board and ethical clearance was obtained from the Ethical Committee of Saveetha University. Prior to data collection, the objectives of the study were explained in community meetings. A sample of 6424 pap smears were analyzed.

The pap smear material was obtained by using cytobrush and placed immediately into a vial of pap spin collection fluid (EZI prep). Liquid Based Cytology (LBC) preparations were made with the cytospin 4 cytocentrifuge (Nanocyt). The smears were stained with Papanicolaou method and analyzed by pathologist based on Bethesda system 2001 [4].

STATISTICAL ANALYSIS

A SPSS window, version 16 software was used for statistical calculations. The Chi-square test was used for statistical analysis. A p-value of <0.05 was considered significant for statistical evaluation.

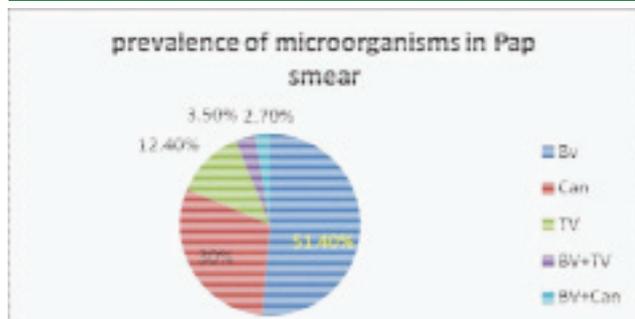
RESULTS

The total number of pap smears examined was 6424, the samples were obtained from the women who attended the Gynaecology OPD in the age group of 15 to > 50 years and the mean age group commonly affected is 20 to 40 years [Table/Fig-1]. Out of 6424 cases, the pap positive smears for infectious organisms were 869 (13.94%). The prevalence of organisms among total infectious organisms is 51.4 % (BV), 30% (Can), 12.4% (TV), and mixed infections 3.5% and 2.7% (BV + TV and BV + Can) [Table/Fig-2]. The prevalence of Bacterial Vaginosis (7.12%), *Candida* species (4.61%), *Trichomona vaginalis* (1.73%) and mixed infections [BV+Can (0.37%), BV+TV (0.48%)] respectively from total cases. Comparison of seasonal variation in prevalence of cervico-vaginal infectious agents was also analyzed [Table/Fig-3]. It is important to mobilize all reproductive age women to undergo pap smear examination and to prevent complications caused by infectious agents.

Organism	15 - 30 Years	31 - 40 Years	41 - 50 Years	> 50 Years	Total	p-value
BV	147	188	110	16	461	0.252
	16.4%	21.0%	12.3%	1.8%	51.4%	
Can	106	109	45	9	269	0.023
	11.8%	12.2%	5.0%	1.0%	30.0%	
TV	25	52	24	10	111	0.006
	2.8%	5.8%	2.7%	1.1%	12.4%	
BV+TV *	9	17	5	0	31	0.356
	1.0%	1.9%	.6%	.0%	3.5%	
BV+Can	9	7	7	1	24	0.622
	1.0%	.8%	.8%	.1%	2.7%	

[Table/Fig-1]: Frequency of of BV,Candida,TV and mixed infections in different age groups.

*p-value of Can age in years, TV age in years were <0.05. Hence, the results are statistically significant.



[Table/Fig-2]: Frequency of Bacterial Vaginosis, *Candida*, *Trichomona vaginalis* and mixed infections.

Organisms/Season	Winter	Summer	Monsoon	Total	p-value
BV	132	106	223	461	0.001
	14.7%	11.8%	24.9%	51.4%	
Can	98	43	128	269	0.076
	10.9%	4.8%	14.3%	30.0%	
TV	40	11	60	111	0.044
	4.5%	1.2%	6.7%	12.4%	
BV+TV *	5	4	22	31	0.062
	0.6%	0.4%	2.5%	3.5%	
BV+Can	5	0	19	24	0.009
	0.6%	0.0%	2.1%	2.7%	

[Table/Fig-3]: Seasonal variations in prevalence of the infectious agents.

Test of significance: p-value here taken as 0.05

BV months, TV months, BV+Can months were <0.05. Hence, the results are statistically significant. There is a difference between the groups.

DISCUSSION

Cervicovaginal infection is commonest problem in most of the women who are in reproductive age group [2]. The Papanicolaou test has become an important screening test for cervical cancers and also useful for diagnosis of cervicovaginal infections [5].

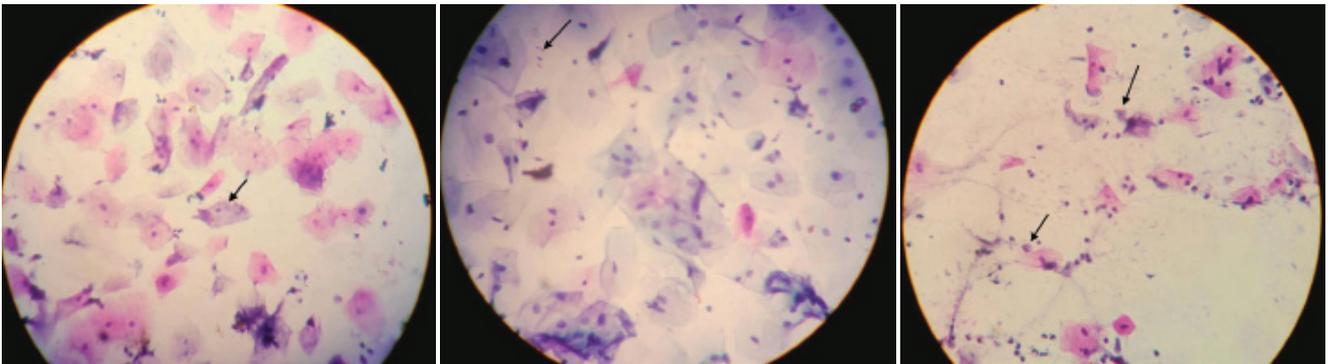
Several studies have identified BV as the leading vaginal infection [6-8]. Simoes Barbosa A et al., studying 142158 women in Brasilia, reported BV prevalence as 17.2% [9]. In Turkey, Krabulut A et al., reported frequency of BV to be 8.3% [10]. In this study, BV was found as the main infectious agent in 51.4 % among total infectious agents, which is very high and 7.12% among total cases of the patients, which is similar to Krabulut's result.

The diagnosis of BV was made by the presence of clue cells i.e., mature squamous cells are covered by cocobacilli, typically extending beyond the cell margin and relative absence of lactobacilli on mixed flora [Table/Fig-4]. Half of the patients with BV are asymptomatic; the Pap smear study may be only means of diagnosis [11].

For the diagnosing BV most of the studies proved that the finding of clue cells in the Pap smear examination had 100% sensitivity and a 96% specificity [12]. The frequency of clue cells are decreased due to the less variable pH in older women and in younger women BV is more frequent with related pH alterations [13]. In the present study, younger and middle aged women had significantly higher prevalence of BV.

The diagnosis of BV is important as it causes endometritis, urinary tract infections, preterm delivery, chorioamnionitis and pelvic inflammatory disease [14]. BV also increases the risk of HIV acquisition [15]. Other mixed infections are also observed are BV with TV and BV with Candidiasis.

The most common fungal disease in women is vaginal candidiasis and affects 75% of women at some stage in their



[Table/Fig-4]: Shift in flora suggestive of Bacterial vaginosis. Numerous small bacteria adherent to the squamous cells (clue cells). **[Table/Fig-5]:** *Candida* (arrow), budding yeast forms of fungus (papx400). **[Table/Fig-6]:** *Trichomonas vaginalis* (arrows). This organism has a pale oval nucleus and faint red granules (papx400).

lifetime. The patients present with curdy white discharge and pruritis. *Candida* species was diagnosed when yeast forms or pseudohyphae are present [Table/Fig-5] and mixed infections candida with BV are seen. Adad et al., reported an increase of *Candida* infection over the last decade (8.1% in 1978 to 22.5% in 1998) [7]. Present study showed 30% of higher prevalence rate among total infectious agents and 4.61% of *Candida* infection from total cases similar to Adad et al., and Bukhari et al., 6.5% [7,2]. In this study, younger and middle aged women had significantly higher prevalence of candidiasis.

The most common sexually transmitted disease in sexually active women in all age groups is *Trichomonas vaginalis* (TV) [16]. Approximately 57-10 million people are affected by TV globally, majority living in developing countries [17,18]. In Nigeria an overall prevalence of TV is 2.6% [19] and in Asian studies were as follows- 2.9% women in China, 18.2%, 25% and 28.1% women from Palestine, Turkey and Saudi Arabia are affected. Occurrence of TV was 12.4% in the present study, which had higher prevalence rate among total infectious agents and constituted 1.73% of total cases. Current study showed middle aged women had significantly higher prevalence of TV.

TV was diagnosed as a unicellular organism of ovoid or pear shaped with single nucleus present [Table/Fig-6]. The pelvic inflammatory disease, infertility, genitourinary tract infection premature labor, ectopic pregnancy, and there was an increased chance in the risk of both the transmission and acquisition of Human Immunodeficiency Virus (HIV) in TV patients [15]. Vikki M et al., studies showed that epidemiological association between TV infection and subsequent cervical neoplasia and carcinoma [20]. The cause for the development of cancer was chronic inflammation either specific or nonspecific has been associated with malignancy [21].

In the previous literature mixed infections (BV+ Can, BV+TV) were not determined but were slowly started to find the mixed infections in pap smear. This study showed mixed infections of 3.5% and 2.7 % (BV+TV and BV+ Can) of prevalence rate

among total infectious agents and BV+ can infection was 0.37% and TV with BV was 0.48% of total cases.

Regarding seasonal variation in the detection of micro-organisms in the cervical canal was published by a few research studies and the results varied from study to study. No seasonal variation was reported by some authors. The occurrence of candidiasis was variable in different seasons and an increased incidence rate was seen in rainy season as reported by Sodhani [22]. Present study also correlated with Sodhani P et al. A study by Rietveld et al., and Sodhani et al., reported that trichomoniasis exhibited a higher incidence in winter and lower incidence in summer [23]. The rainy season (July-October) gave intermediate values. In this case, the early and exogenous rhythmicity of the monsoon might interact with the endogenous rhythm of the immune system. Shrader S et al., reported no seasonal variation in the detection of TV [24]. *Trichomonas* can thrive in moist environment, like in bathing water, on toilet seats, on wet clothing and can survive up to 45 minutes [25]. The countries with a more moderate climate and with more gradual changes of the various seasons over the year, no data are yet available on seasonal patterns detected through cervical screening

The study was undertaken to document a seasonal variation in genital infections as detected in cervical smears. A seasonal variation of BV, *Candida*, TV and mixed infections (BV+Can, BV+TV) were reported with higher detection rate in rainy season followed by summers.

LIMITATION

Microbiological study was not included, as it was not of much use in this study.

CONCLUSION

The study concludes that cervical cytology is important for diagnosis of cervical infections. The most important cause of cervicovaginitis are BV, next important cause is candidiasis. Higher incidence of BV and *Candida* infection are seen in younger to middle aged women and TV was in middle aged group. Seasonal variation was observed for all

infectious organisms and mixed infections showed higher incidence rate in rainy season. It is important to mobilize all reproductive age women to undergo pap smear examination and to prevent complications caused by infectious agents and also gynecological disease prevention, community and school based STD prevention programs are needed.

REFERENCES

- [1] Narasimha A, Nirup NC, Chandana B, Nishanth N, Harendra Kumar ML. Spectrum of infections in cervico-vaginal Pap smears. *J Clin Biomed Sci.* 2014;4(1):222-25.
- [2] 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. *Diag Cytopathol.* 2012;40:35-41.
- [3] Fitzhugh V, Heller D. Significance of micro-organisms on Pap smear. *J Low Genit Tract Dis.* 2008;12:40-51.
- [4] Solomon D, Davey D, Kurman R. The 2001 Bethesda system terminology for reporting results of cervical cytology. *JAMA.* 2002;287:2114-19.
- [5] Levi AW, Harigopel M, Hui P, Schofoeld K, Chhieng DC. Comparison of affirm VP III and Papanicolaou tests in the detection of Infectious vaginitis. *Am J Clin Pathol.* 2011;135:442-47.
- [6] Takei H, Ruiz B, Hicks J. Cervicovaginal flora. Comparison of conventional papsmears and a liquid based thin layer preparation. *Am J Clin Pathol.* 2006;125:855-59.
- [7] Adad SJ, de Lima RV, Sawan ZT, Silva ML, de Souza MA, Saldanha JC, et al. Frequency of *Trichomonas vaginalis*, *Candida* sp. and *Gardinerella vaginalis* in cervical vaginal smears in four different decades. *Sao Paulo Med J.* 2001;119:200-05.
- [8] Allosworh J, Pepert J. Prevalence of bacterial vaginosis. 2001-2004. National Health and Nutrition Examination Survey data. *Obstetric and Gynaecol.* 2007;109:114-20.
- [9] Simoes Barbosa A, Coutinho Feijo G, de Siva J. A six year follow up survey of sexually transmitted diseases in Brasilia, the capital of Brazil. *Braz J Infect Dis.* 2002;6:110.
- [10] Karabulut A, Alan T, Ekiz M. Evaluation of cervical screening results in a population at normal risk. *Int J Gynaecol Obstet.* 2010;110:40-42.
- [11] Pray M. Routine Pap smears for the diagnosis of bacterial vaginosis. *Diag Cytopathol.* 1999; 21:10-13.
- [12] Enrique OE, Andrés PL, Francisco MA, Pamela GH. Bacterial vaginosis: diagnosis and prevalence. *Rev Chil Gynecol.* 1996;61(1):28-33.
- [13] Murta EF, Silva AO, Silva EA, Adad SJ. Frequency of infectious agents for vaginitis in non and hysterectomized women. *Arch Gynecol Obstet.* 2005;273(3):152-56.
- [14] Davis JD, Connor EE, Clark P, Wilkinson EJ, Duff P. Correlation between cervical cytology results and gram stain as diagnostic tests for bacterial vaginosis. *Am J Obstetgynecol.* 1997;177:532-35.
- [15] McMillan A. The detection of genital tract infection by Papanicolaou-stained tests. *Cytopathology.* 2006;17(6):317-22.
- [16] Schwebke JR, Burgess D. Trichomoniasis. *Clin Microb Rev.* 2004;17:794-803.
- [17] Garland SM. *Trichomonas vaginalis*: why we should be screening. *Venereology.* 2001;14:116-20.
- [18] Adu-Sarkodi Y. *Trichomonas vaginalis* screening goes global. *Sex Transm Infect.* 2004;80:201-03.
- [19] Adulazeez A, Alo E, Livingstone R. Epidemiology of urino-genital trichomoniasis in a North-eastern State, Nigeria. *The Internet J Parasitic Dis.* 2007;2(2).
- [20] Vikki M, Pukkala E, Nieminen P, Hakama M. Gynecological infections as risk determinants of subsequent cervical neoplasia. *Acta Oncol.* 2000;39:71-75.
- [21] Dasari P, Rajathi S, Kumar SV. Colposcopic evaluation of cervix with persistent inflammatory Pap smear: A prospective analytical study. *Cyto Journal.* 2010;7:16.
- [22] Sodhani P, Murthy NS, Sartdana S. Seasonal variation in genital tract infections as detected on Papnicolaous smear examination. *Diagn Cytopathol.* 1994;10:98-99.
- [23] Rietveld WJ, Boon ME, Meulman JJ. Seasonal fluctuations in the cervical smear detection rates for (pre) malignant changes and for infections. *Diagn Cytopathol.* 1997;17:452-55.
- [24] Shrader S, Hernandez E, Gaughan J. Is there a seasonal difference in the detection of *Trichomonas vaginalis* by cytology. *Sci World J.* 2003;17:45-50.
- [25] Nanda N, MG RG, Kurdgelashvili G, Wendel KA. Trichomoniasis and its treatment. *Expert Rev Anti Infect Ther.* 2006;4:125-35.

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