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Research Article

Spectrum of cervicovaginal Pap smears in newly established tertiary care medical institute

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ABSTRACT

Objectives: We undertook this study to find out the spectrum of the cervical smear pattern along with the clinical profiles of patients who underwent cervical Papanicolaou (Pap) smear evaluation in our newly started tertiary care center. We also tried to find the possible clinical cause for unsatisfactory smears and factors for epithelial cell abnormality.

Material and Methods: The present study was a retrospective observational study. Pap smears cases with their clinical findings mentioned on the requisition form and cytopathology observations were retrieved from the archives of the department of pathology. Fisher's exact test was used for statistical analysis.

Results: Five hundred and ninety-four cases were included in the study. The most common age group was 36–40 years. White discharge per vaginum was the most common clinical presentation. The negative for squamous intraepithelial lesions or malignancy was the most common interpretation (86.87%). Cervical erosion had statistically significant associations with unsatisfactory smears, while bacterial vaginosis had with satisfactory smears. Epithelial cell abnormality was seen in 4.62% patients. We observed a statistically significant association of cervical mucoid discharge, and inflammation with "no epithelial cell abnormality" cases, while postmenopausal bleeding was associated with "epithelial cell abnormality" cases.

Conclusion: In the presence of clinical factors like cervical erosion, which may affect the quality of Pap smear, proper sampling techniques are to be used by health-care providers. The careful evaluation of Pap smears, especially in cases of cervical mucoid discharge, postmenopausal bleeding, and inflammatory smears is required to ensure that epithelial cell abnormalities are not overlooked.

Keyword: Papanicolaou smear, Cervix, Cytology, Human papilloma virus, Vaccine

INTRODUCTION

In India and worldwide, cervical cancer is the second and fourth most commonly diagnosed cancer in females respectively and one of the leading causes of cancer death.[1,2] In India, cervical cancer is the second most common cause of cancer death.[2] Papanicolaou (Pap) is a universal stain for gynecologic and non-gynecologic cytology smears and is commonly used for oral and cervical cancer screening.[3] In developed countries, there is a decrement of 70% in the incidence of cervical cancer using Pap test.[3]

Due to effective primary (human papilloma virus [HPV] vaccine) and secondary (screening) precautionary measures, cervical cancer is considered a nearly utterly preventable disease.[1]

The World Health Organization (WHO) made a call for the global elimination of cervical cancer in 2018 using the triple-intervention approach of (a) vaccination, (b) screening of women, and (c) treating all the precancerous lesions detected during screening.[1] A cervical Pap smear examination is an easy, non-invasive, and effective screening method for cervical carcinoma.[4] The cervical Pap smears examination was started in our institute in April 2021.

Aims and objectives

We undertook this study to find out the spectrum of the cervical smear pattern along with the clinical profiles of patients who underwent cervical Pap smear evaluation. Due to a lack of liquid base cytology (LBC) and HPV testing, we also tried to find the possible clinical causes for unsatisfactory smears and factors associated with epithelial cell abnormality. The results of the present study would guide us to formulate a protocol for the cervical Pap smears evaluation in the local population's need and with available resources. Furthermore, it may suggest to start an organized cervical screening program for all eligible women.

MATERIAL AND METHODS

The present study was a retrospective observational study of all the Pap smear cases received from April 12, 2021, to October 31, 2022. The Institute's Ethical Committee approved this study vide letter number – IEC Ref No: AIIMS/BBN/IEC/July/2022/191. All the Pap smears cases with their clinical findings mentioned on the requisition form and cytopathology observations were retrieved from the archives of the department of pathology of our institute. In addition, the age and the findings of clinical presentation and examination were recorded for each case. All the Pap smears were of conventional method and stained using Pap stain. The cytological interpretation was reported according to the 2014 Bethesda System for reporting cervical cytology.

Study population

All women of age ≥21 years undergoing cervical Pap smear examination.

Data analysis

The frequency and proportion were used for data presentation. The statistical analysis, considering the nature of variables, was performed for descriptive statistics using Epi Info 7.2.5.0 and the Microsoft 365 excel sheet.

Fisher's exact test was used to find the association between the clinical parameters of satisfactory smear versus unsatisfactory smear groups. $P < 0.05$ was considered significant at 95% CI and an appropriate degree of freedom.

RESULTS

Five hundred and ninety-four cervicovaginal Pap smear cases were identified during the study period. The patient's ages ranged from 21 to 80 years, and the mean, median, and mode were 38.89, 38, and 40 years, respectively. The most common age group was 36–40 (148/594, 24.92%), followed by 31–35 (104/594, 17.51%) and 41–45 (96/594, 16.16%) [Table 1]. From clinical presentation and examination, white discharge per vaginum (36.87%, 219/594) was the most common clinical presentation, followed by cervical erosion (30.81%, 183/594) [Table 2].

On cytological evaluation, the unsatisfactory smears accounted for 8.92% (53/594), with a higher percentage in age >45 (9.92%, 12/121) than 8.67% (41/473) when age was <45 years. The common causes of unsatisfactory smears were low squamous cell cellularity and the cell morphology obscured by inflammation or blood.

The negative for squamous intraepithelial lesions or malignancy (NILM) was the most common interpretation category accounting for 86.87% (516/594) of all Pap smears [Figure 1]. On the other hand, the epithelial cell abnormality (both squamous epithelial cells and glandular epithelial cells) was observed in 4.62% (25/541) of satisfactory smears.

Epithelial cell abnormality was seen in 7.34% (8/109) of cases with age >45, and when age <45 years, the epithelial cell abnormality was observed in 3.94% (17/432) patients. No epithelial cell abnormality was observed in ages ≤25 years and between 61 and 65 years [Table 1]. The white discharge per vaginum (8/25) was the most common clinical presentation, followed by cervical erosion (7/25) and hypertrophied cervix (5/25) in cases with epithelial cell abnormality. In addition, atypical squamous cells (ASCs) of undetermined significance (ASC-US) (36%, 9/25), ASC cannot exclude high-grade squamous intraepithelial

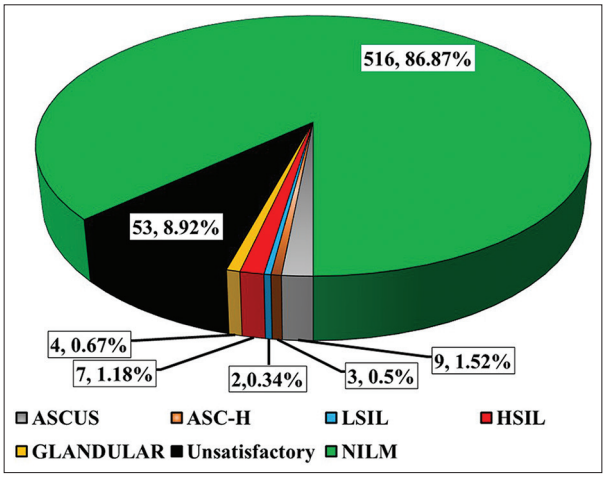

Figure 1: Distribution of cytological category.

Table 1: Age-wise distribution of cases.

	Satisfactory	Unsatisfactory	Total	Epithelial cell abnormality	No epithelial cell abnormality	Total
21–25	35	3	38	0	35	35
26–30	79	8	87	2	77	79
31–35	99	5	104	3	96	99
36–40	130	18	148	8	122	130
41–45	89	7	96	4	85	89
46–50	61	6	67	4	57	61
51–55	18	3	21	1	17	18
56–60	11	1	12	2	9	11
61–65	12	2	14	0	12	12
>65	7	0	7	1	6	7
Total	541	53	594	25	516	541

lesion (HSIL) (ASC-H) (12%, 3/25), low-grade squamous intraepithelial lesion (LSIL) (8%, 2/25), HSIL (28%, 7/25), and atypical glandular cell (not otherwise specified, NOS) (16%, 4/25) were observed [Figure 2].

The statistical analysis was performed for the association between epithelial abnormality and age group and provided clinical parameters. Since unsatisfactory smears were not considered as NILM or other epithelial abnormality, hence not included for statistical analysis here. We observed a statistically significant association of cervical mucoid discharge and inflammation with "no epithelial cell abnormality" cases when compared with "epithelial cell abnormality" cases, while postmenopausal bleeding was associated with epithelial cell abnormality [Table 2].

The fungal organism morphologically consistent with *Candida* spp. was observed in 7.24% (43/594), shift of flora suggestive of bacterial vaginosis in 13.13% (78/594), inflammation in 22.56% (134/594), and trichomonas in 0.67% (4/594) [Table 2 and Figures 3].

We also performed statistical analysis for the association between satisfactory and unsatisfactory smears with age, clinical presentation, and clinical examination. The cervical erosion showed a statistically significant association with unsatisfactory smears, while bacterial vaginosis was associated with satisfactory smears [Table 2].

DISCUSSION

A Pap smear test is the foremost screening technique for identifying precancerous cervical intraepithelial neoplasia and the early stage of carcinoma.[5] Although the Pap smear test prevents cervical cancer, the community's knowledge of the test is relatively low.[1] Because there is a protracted preinvasive stage, screening for cervical cytology is valuable and is one reason to consider invasive cervical carcinoma a preventable carcinoma.[6] To eliminate cervical cancer (4/100,000 women worldwide), the WHO recommends screening 70% of women twice between the ages of 35 and 45.[1] Forty-one percentages of cases belonged to the generation of 36–45 years in our study, the recommended age group by the WHO to achieve the target of cervical cancer's elimination. Similarly, Tummidi *et al.* observed most common age group of screening patients was 31–40 years, followed by 41–50 years.[7] Sharif YH and Baamer *et al.* observed that mean age of the study population was 31.55 ± 6.66 years (ranging from 21 to 60 years) and 45.12 ± 6.66 years (ranging from 21 to 75 years), respectively.[6,8] The age range in our study was 21–80 years, with a mean age of 38.89 years.

After excluding asymptomatic and healthy cervix cases, the most commonly reported clinical symptoms and examination findings are white discharge per vaginum, abdominal pain, abnormal bleeding, postcoital bleeding, irregular cycle, postmenopausal bleeding, intermenstrual bleeding, hypertrophied cervix, cervical erosion, bleed on touch cervix, and uterovaginal prolapse.[5,6,8,9] We observed white discharge per vaginum, abdominal pain, cervical mucoid discharge, and irregular cycle as common clinical symptoms, and cervical erosion, cervical hypertrophy, and bleed on touch cervix as common examination findings. In 2014 Bethesda System, the "Epithelial cell abnormality: Squamous" includes squamous intraepithelial lesion (SIL) category, which comprises a spectrum of squamous cell lesions starting from the precancerous lesions of LSIL to HSIL and invasive squamous cell carcinoma (SCC).[10] Depending on the qualitative and quantitative constraints of the material, certain ambiguous morphological characteristics that are indicative of squamous cell

Table 2: Comparison of clinicocytomorphological features.

	Satisfactory	Unsatisfactory	Fisher's exact test *P* value	Epithelial cell abnormality	No epithelial cell abnoramality	Fisher's exact test *P* value
White discharge per vaginum						
Yes	204	15	0.23	8	196	0.67
No	337	38		17	320	
Cervical erosion						
Yes	157	26	*0.005*	7	150	1.00
No	384	27		18	366	
Abdominal pain						
Yes	102	7	0.36	2	100	0.20
No	439	46		23	416	
Hypertrophied cervix						
Yes	83	13	0.11	5	78	0.57
No	458	40		20	438	
Cervical mucoid discharge						
Yes	88	4	0.11	0	88	*0.02*
No	453	49		25	428	
Irregular cycle						
Yes	67	5	0.66	2	65	0.76
No	474	48		23	451	
Nabothian cyst						
Yes	41	5	0.59	3	38	0.43
No	500	48		22	478	
Heavy menstrual bleeding						
Yes	40	4	1.00	3	37	0.42
No	501	49		22	479	
Bleed on touch cervix						
Yes	30	4	0.53	1	29	1.00
No	511	49		24	487	
Polymenorrhagia						
Yes	22	4	0.28	1	21	1.00
No	519	49		24	495	
Amenorrhea						
Yes	23	2	1.00	2	21	0.29
No	518	51		23	495	
Itching in Vulva						
Yes	22	2	1.00	1	21	1.00
No	519	51		24	495	
Postmenopausal bleeding						
Yes	18	0	0.25	3	15	*0.045*
No	523	53		22	501	
Foul smell from vagina						
Yes	14	2	0.65	0	14	1.00
No	527	51		25	502	

(*Contd...*)

Table 2: (*Continued*).

	Satisfactory	Unsatisfactory	Fisher's exact test *P* value	Epithelial cell abnormality	No epithelial cell abnoramality	Fisher's exact test *P* value
Obesity						
Yes	16	0	0.38	2	14	0.17
No	525	53		23	502	
Other discharge						
Yes	34	4	0.77	1	33	1.00
No	507	49		24	483	
Increase vascularity of cervix						
Yes	11	2	0.33	1	10	0.45
No	530	51		24	506	
Spotting						
Yes	9	1	0.61	1	8	0.35
No	532	52		24	508	
Postcoital bleeding						
Yes	6	1	0.48	1	5	0.25
No	535	52		24	511	
Hypomenorrhea						
Yes	5	1	0.43	1	4	0.21
No	536	52		24	512	
Burning micturation						
Yes	21	0	0.24	0	21	0.62
No	520	53		25	495	
Dyspareunia						
Yes	8	1	0.57	1	7	0.32
No	533	52		24	509	
Age groups						
21–≤45	432	41	0.72	17	415	0.13
>45	109	12		8	101	
Infection/inflammation						
Candida						
Yes	42	1	0.16	2	40	1.00
No	499	52		23	476	
Shift in flora suggestive of bacterial vaginosis						
Yes	76	2	*0.03*	1	75	0.23
No	465	51		24	441	
Trichomonas vaginalis						
Yes	04	0	1.00	0	04	1.00
No	537	53		25	512	
Inflammation						
Yes	122	12	1.00	1	121	*0.03*
No	419	41		24	395	

p Value <0.05 is significant

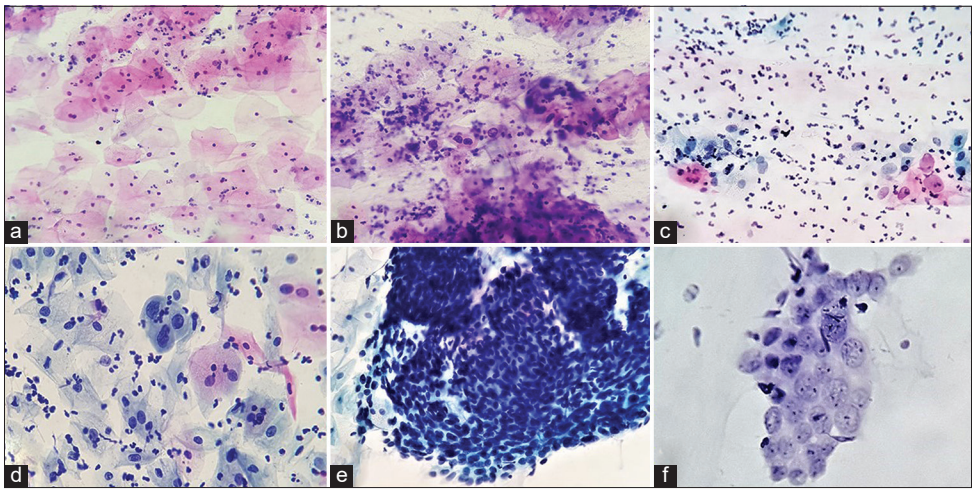

Figure 2: (a) Negative for squamous intraepithelial lesions or malignancy: Superficial squamous cells without any cytological atypia (Papanicolaou Stain ×400). (b) Atypical squamous cells of undetermined significance: Occasional atypical cell in the inflammatory background (Papanicolaou Stain ×400). (c) Low-grade squamous intraepithelial lesion: Variable sized atypical cell, irregular nuclear membrane with inconspicuous (Papanicolaou Stain ×400). (d) Atypical squamous cells cannot exclude high-grade squamous intraepithelial lesion (HSIL): Cells with coarse chromatin, irregular nuclear membrane, high nucelo: cytoplasmic ratio (Papanicolaou Stain ×400). (e) HSIL: Syncytial sheet of parabasal type cells, variation in the size and the shape of the nuclei, hyperchromatic nuclei, irregular nuclear membrane, and high nucelo:cytoplasmic ratio (Papanicolaou Stain ×400). (f) Atypical glandular cell – Endocervical cell: Round to oval atypical cell with high nucelo: Cytoplasmic ratio. (Papanicolaou Stain ×400).

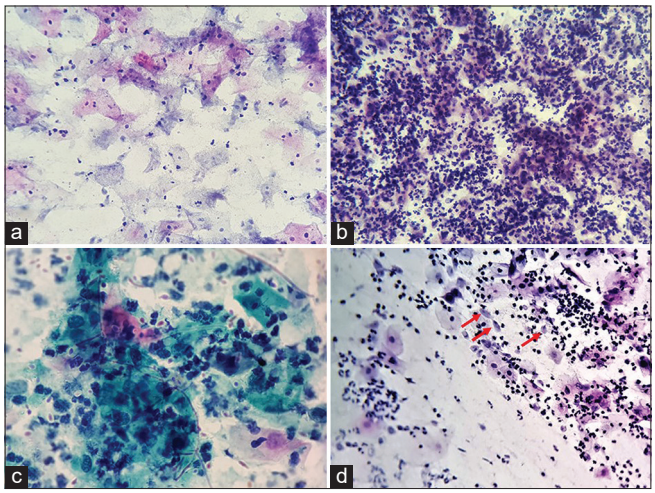

Figure 3: Bacterial vaginosis: Change in normal vaginal bacterial flora from lactobacilli to cocco-bacilli (Papanicolaou Stain ×400). Inflammatory Smear: Predominantly neutrophils obscuring cell morphology – unsatisfactory smear (Papanicolaou Stain ×400). Candida Infection: Eosinophilic to gray-brown-Yeasts and pseudohyphae of organisms (Papanicolaou Stain × 400). Trichomonas: Gray-blue, "pear-shaped," small, round-to-oval organisms with an eccentric nucleus (Papanicolaou Stain × 400).

abnormalities may fall under an equivocal category: "Atypical Squamous Cells" (ASCs), which are categorized into two categories; ASCs undetermined significance (ASC-US) or ASCs cannot exclude HSIL (ASC-H).[10] The NILM is the most common category reported in cervicovaginal Pap smears ranging from 68% to 96.31%.[5-7,9] In accordance with the results of earlier studies, we also found NILM in 86.87% of the cases (the most common interpretation category). However, it conflicts with the findings of Baamer *et al.*, who found that ASC-US (26.1%) was the most frequent interpretation category, and NILM was seen in only 8.9% of their cases.[8] NILM is a general category that may or may not be associated with organisms or other non-neoplastic cellular findings (such as squamous or tubal metaplasia, atrophy, and reactive cellular changes) but have no cellular evidence of neoplasia.[10]

The unsatisfactory smears were 8.92% in the present study, with a higher percentage in age >45 years (9.92%, 12/121) than 8.67% (41/473) when the age was <45 years. However, this difference was not statistically significant. The unsatisfactory smears ranged from 1% to 12.1% in the previous studies.[5,7,11] Tummidi *et al.* observed the most unsatisfactory smears in cases with only one Pap smear slide.[9] Patients with unsatisfactory smears should undergo a careful evaluation since they may have benign or pre-neoplastic or neoplastic diseases.[12] Sharma *et al.* conclude that older age groups and cervical erosion are predictors for unsatisfactory conventional Pap smears.[12] They also

observed that white discharge and lower abdominal pain are associated with unsatisfactory Pap smears.[12] We also observed a significant difference in satisfactory and unsatisfactory Pap smears in cases with cervical erosion and bacterial vaginosis. The unsatisfactory smears could be due to cell obscuration by mucus, inflammatory cells, or blood and low squamous cell cellularity.[12] Similar findings were also observed in the present study. To decrease the rate of unsatisfactory smears in conventional Pap smears, gynecologists must take extra precautions with proper sampling techniques. They should also keep in mind the predictors for unsatisfactory conventional Pap smears. However, the rate of unsatisfactory Pap smears significantly reduces in LBC compared to conventional Pap smears.[13] However, in resource-constrained settings such as in developing countries, the issue of unsatisfactory Pap smears exists as conventional Pap smears test is used at most screening centers. Therefore, it is crucial to determine the clinical factors linked to unsatisfactory Pap smear results.

Epithelial cell abnormality ranged from 1.2% to 32%.[5-7,9,11] However, Baamer *et al*. observed 91% of cases with epithelial cell abnormality.[8] The different epithelial cell abnormalities in the previous studies ranged as ASC-US 0.5–26.1%, ASC-H 0.1–16.7%, LSIL 0.1–19.4%, HSIL 0.2–9.2%, SCC 0.3–0.9%, and glandular cell abnormality including adenocarcinoma 0.2–18.6%.[5-11] We observed ASC-US, ASC-H, LSIL, HSIL, and atypical glandular cells (NOS) in 1.52%, 0.5%, 0.34%, 1.18%, and 0.67%, respectively, within the range reported in the previous studies. We have not categorized any Pap smear as SCC or adenocarcinoma during the duration of the study. We have also compared clinical and a few cytological parameters for epithelial cell abnormality. We observed a statistically significant association between cervical mucoid discharge, postmenopausal bleeding, and inflammatory Pap smears with epithelial cell abnormality versus no epithelial cell abnormality. This suggests that the cervicovaginal Pap smear should be carefully examined in these cases to ensure that epithelial cell abnormalities are not overlooked.

We could not find any study in PubMed search that established a similar association of epithelial cell abnormality with clinical and cytological parameters.

The virus-associated cytopathic changes were also not observed in the present study. However, the American Cancer Society's new guideline recommends the human papillomavirus test as a preferred type of cervical cancer screening test.[14] In addition, they recommend all women between the age of 25 and 65 get screened every 5 years with an HPV test alone.[14] Women can be tested with an HPV/Pap cotest every 5 years or a Pap test every 3 years if HPV testing alone is not available.[14] However, p16 and ProExC are immunomarkers related to aberrant cell cycle due to the oncogenic effects of HPV along with Ki-67, cellular proliferation immunomarker may be used on cell block of LBC to improve the accuracy of cervical cancer screening and reduce unnecessary invasive examinations.[15] Ours is a newly established center; therefore, HPV or immunocytochemistry testing is not currently available in our institution.

Due to the non-availability of LBC and HPV tests, we tried to find factors affecting the quality of conventional Pap smears to reduce the rate of unsatisfactory smears. Furthermore, finding the clinical and cytological parameters suggests the possibility of epithelial cell abnormality.

Limitation

The limitations of the present study were that histology of all cases was not available, and cytological findings were not compared with histology. In addition, the LBC can help to reduce the rate of unsatisfactory smears, and HPV tests were also not available in our institute.

CONCLUSION

The unsatisfactory Pap smear was the second most common observed category after NILM in the present study. In the presence of clinical factors like cervical erosion, which may affect the quality of Pap smear, proper sampling techniques are to be used by health-care providers to reduce the unsatisfactory smear rate. We also suggest careful evaluation of cervicovaginal Pap smears in cases of cervical mucoid discharge, postmenopausal bleeding, and inflammatory Pap smears to ensure that epithelial cell abnormalities are not overlooked.

COMPETING INTEREST STATEMENT BY ALL AUTHORS

Authors declare "No Competing Interest".

AUTHORSHIP STATEMENT BY ALL AUTHORS

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Study conception/design: Manas Ranjan Sethy, Jitendra Singh Nigam, Ashutosh Rath, Abhimanyu Sharma,

Data acquisition: Manas Ranjan Sethy, Jitendra Singh Nigam, Ashutosh Rath, Abhimanyu Sharma, Immanuel Pradeep.

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ETHICS STATEMENT BY ALL AUTHORS

The institute's ethical committee approved this study vide letter no- IEC Ref No: AIIMS/BBN/IEC/July/2022/191.

The cases were submitted without identifiers.

LIST OF ABBREVIATIONS (In alphabetic order)

ASC-H – Atypical squamous cells cannot exclude HSIL
ASC-US – Atypical squamous cells of undetermined significance
HPV – Human papilloma virus
HSIL – High-grade squamous intraepithelial lesion
LBC – Liquid base cytology
LSIL – Low-grade
NILM – Negative for squamous intraepithelial lesions or malignancy
NOS – Not otherwise specified
WHO – The World Health Organization

EDITORIAL/PEERREVIEW STATEMENT

To ensure the integrity and highest quality of CytoJournal publications, the review process of this manuscript was conducted under a **double-blind model** (authors are blinded for reviewers and *vice versa*) through automatic online system.

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