ROLE OF RAPID STAIN IN CERVICAL CYTOLOGY AND AN AUDIT OF CYTOHISTOLOGICAL CORRELATION FOR SPECTRUM OF CERVICOVAGINAL LESIONS

Objectives of the study:

(1) Evaluate the role and advantages of Rapid stain in cervical smear cytology using toluidine blue.

(2) Correlate the unsatisfactory rate with and without the use of rapid stain

(3) Correlate the findings in toluidine blue and final cytological stain.

(4) Correlate the cytological findings with histopathology diagnosis wherever available.

Justification for the conduct of study:

Carcinoma of the uterine cervix is a major health problem faced by the Indian women. India accounts for 15.2 % of the total cervical cancer deaths in the world. The situation is more alarming in rural areas due to illiteratacy and ignorancy about the hazards of cervical cancer. Screening techniques like visual inspection with acetic acid, with lugol's iodine, PAP smear and HPV-DNA testing have been suggseted. PAP smear is a simple, safe, cost effective and reliable technique used for screening of cervical lesions. Rapid stain using toluidine blue staining method has been used as an reliable way for demonstrating the adequacy of material in FNACs of different sites. However, its utility in cervical cytology has been less studied. Our attempt will be to evaluate the cases of cervical cytology using this rapid stain method and correlate the findings with the unsatisfactory rates. A check for histopathology follow up will be done and its correlation with cytology diagnosis.

Methodology:

This will be a prospective study on cervicovaginal smears received in the Dept. of Pathology, AIIMS, Mangalagiri, over a period of 3 years. All the cervical cytology smears as

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per Bethesda system of reporting will be included in the study. The conventional Pap stain and Aqueous Tol blue (1%) will be performed on the smears received. Cytomorphology of the smears will be studied and compared in reference to staining, timing and efficiency of stains. Information regarding the adequacy of sample will be immediately informed to the consultant for repeat and sent to lab.

How will you maintain the confidentiality of the subject?

The confidentiality of the patient will not be breached as only the PAP smears of all patients will be received in lab and patient identity will not be known to either the principal or the co-investigators. The patient details will not be included any time in the study.

Total Budget (Approx. in Rs.): Nil

Conflict of interest among investigators: No

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Introduction:

Carcinoma of the uterine cervix is a major health problem faced by the Indian women, and every year, approximately 1,20,000 women develop this disease.[1] India accounts for 15.2 % of the total cervical cancer deaths in the world. [1,2] Although the incidence of carcinoma cervix has declined in the urban population, in the rural areas it continues to be highly prevalent. [3] The usual 10-20 years of natural history of progression from mild dysplasia to carcinoma cervix makes this cancer as relatively early preventable disease and provides the rationale for screening. [3,4]

The international medical community has also recognized that cervical cancer is preventable and cervical cytology screening has been hailed as the most successful cancer screening method in medical history. [4,5] The quality of cervical cytology screening can be assessed by quality indices such as relative percentages of the various diagnoses, including the unsatisfactory rate and the atypical squamous cells of undetermined significance (ASCUS) squamous intraepithelial lesion (SIL) ratio. [3,5] The correlation of cytological diagnosis with available histological follow-up allows the evaluation of false positive and false negative cases. [6,7]

Rapid stain using toluidine blue a supravital stain can accentuates good cytological and nuclear details, enabling the three-dimensional view of cells in wet mount film. It is easily available, very cheap, cost effective and used for quick reporting. [8,9] There are very few studies on the utility of toluidine blue in cervical cytology.

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Aims & Objectives:

1. Evaluate the role and advantages of Rapid stain in cervical smear cytology using toluidine blue.
2. Correlate the unsatisfactory rate before and after implementation of rapid stain
3. Correlate the findings in toluidine blue and final cytological stain.
4. Correlate the cytological findings with histopathology diagnosis wherever available.

Material & Methods:

- Study period: This will be a prospective study on all cervicovaginal smears received in the Dept. of Pathology, AIIMS, Mangalagiri, over a period of 3 years. Patients ≥ 21 years of age and sexually active will be included in the study the maximum age will be ≤ 65 years. [6]
- Sample size: Sample size is inclusion of all consecutive patients' samples during the study period. Based on studies available and previous experiences the unsatisfactory rate of routine of Routine PAP is very high (approx. 11.2 % in our institute). However, the expected unsatisfactory rate with implementation of ROSE will be < 5%. Using this data, with 1% of alpha error, 20 % lost to follow up, and 95% of power the sample size was calculated to 873 cases using openepi software (3.01 version). Hence a total of 1000 cases were included for the study. Control patients in the study will the same patient where the rapid stain was not done.
- Method: Patients attending the Gynaecology OPD who meet the inclusion criteria and consent to participate in the study will undergo the routine PAP smear test. To obtain a PAP smear, a speculum is inserted into the vagina and a spatula is inserted into the opening of the cervix and twirled around to collect a sample of cells which is then smeared on glass slides. Usually, 2 smeared slides will be obtained (1 ectocervix+1 endocervix) for

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reporting. In our study, we will prepare 3 labelled slides (2 ectocervix+1 endocervix) from the collected sample. The slides with the cervical cytology requisition form will be received by the pathology department for processing. We will collect the demographic/relevant clinical information like age, sex, site of PAP smear, etc from the cervical cytology requisition form. [7-9] A record of number of slides received per patient will be kept.

- Rapid stain- aqueous TBS (1%) will be used for assessing the adequacy of sample and making a provisional diagnosis, following which the slides will be sent for routine PAP stain.[10] The results from both the techniques will be compared to evaluate the efficacy of the rapid stain technique using PAP smear as the gold standard.
- Information regarding the adequacy of sample will be immediately informed to the consultant in case of inadequacy repeat procedure if required will be done and sent to lab. The rapid stain will be compared for cytomorphology of the smears with reference to staining, timing and efficiency of stains. [11-14]
- Where ever possible if biopsy has been obtained by the Department of Obstetrics & Gynaecology simultaneously. We will correlate the findings of histopathology with the cytology findings.

Inclusion criteria:

- All patients giving consent for the procedure and study (patients ≥ 21 years of age and sexually active.
- ≤ 65 years of age.
- Vulnerable person (pregnant women, differently abled, employees/students/staff, elderly, economically and socially disadvantaged, refugees/migrants/homeless)

Exclusion criteria:

- Non-co-operative patients or patients who did not give consent for the procedure.
- Patients who denied being included in the study.
- Age ≤ 20 years or > 65 years
- Sample if not submitted in fixative, Broken slides.

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Result Analysis:

All cases who underwent PAP smears will be evaluated for correlation of rapid stain findings and final cytological diagnosis based on PAP stained slides. Correlation of unsatisfactory cases with and without the use of rapid stain will be evaluated. Turn-around time along with sensitivity, specificity, PPV, NPV, diagnostic accuracy will be analysed. All smears and corresponding histopathology correlation will be reviewed separately and categorized based on the Bethesda system of cervical cytology reporting system. Correlation of sensitivity of smears and histopathology will be done. Correlation with various other studies will also be done. SPPS v23.0 will be used for analysis of the results. All statistical results will be considered significant at a p value < 0.05. However, since sole dependence on p values can introduce fallacies in interpretation, we will be drawing our inferences based on a combination of effect sizes, confidence intervals, p values and area under the curve.

Ethical Issues:

Institutional Ethics Committee permission will be sought and obtained prior to commencement of study.

Implications:

The study will be an attempt to look for the following points

1. Turn-around time of sample.
2. Non diagnostic rate with and without rapid stain.
3. Study the spectrum of infectious and premalignant cases with evaluation of index in cervical cytopathology.
4. Reduced visit of patients to repeat procedure and improved patient compliance.

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References:

1. Srivastava AN, Misra JS, Srivastava S, Das BC, Gupta S. Cervical cancer screening in rural India: Status & current concepts. Indian J Med Res 2018; 148:687-96.
2. Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. Int J Cancer. 2010;127(12):2893-2917. doi:10.1002/ijc.25516
3. Bangalore: National Cancer Registry Programme; 2016. National Cancer Registry Programme. Three-year report of population-based cancer registries: 2012-2016.
4. Crasta J, Chaitra V, Simi CM, Correa M. An audit of cervicovaginal cytology in a teaching hospital: Are atypical glandular cells under recognised on cytological screening? J Cytol. 2009;26(2): 69–73.
5. Vallikad E. Cervical cancer: The Indian Perspective. Int J Obstet Gynecol. 2006;95: S215-33.
6. Nagose VB, Halder NR, Deshpande SA, Rathod SS, Jadhav VA. Spectrum of cervical cytological lesions in premenopausal and postmenopausal women. Annals of pathology and laboratory medicine 2017; 4(6): A795-800.
7. Birdsong GG, Davey DD. Specimen adequacy. In: Nayar R, Wilbur DC. The Bethesda system for reporting cervical cytology. Defination, Criteria and explanatory notes, 3rd ed. Switzerland: Springer International Publishing; 2015. p. 1-28Deepthi KN, Aravinda Macharla. Lesions of uterine cervix by cytology and histopathology- A prospective study for a period of two years. Indian Journal of Pathology and Oncology. 2017; 4(2): 193-198.
8. Kanchan K, Santosh T, Agnihotri M, Sathe P, Naik L. This 'Rose' Has No Thorns - Diagnostic Utility of 'Rapid On-Site Evaluation' (Rose) In Fine Needle Aspiration Cytology. Indian journal of surgical oncology 2019;10(4):688-98.
9. Ammanagi AS, Dombale VD, Patil SS. On –site toluidine blue staining and screening improves efficiency of fine needle aspiration cytology reporting. Acta Cytol. 2012;56(4):347-51.
10. Patil PV, Nikumbh DB. Efficacy of toulidine blue staining in cervicovaginal cytology over conventional Papanicolaou stain. IP Archives of Cytology and Histopathology Research 2018;3(2):47-51.
11. Alves PM, Ferreira FJN. Experimental validation of a rapid staining method and digital cytology for cervical and anal cancer. J Bras Patol med Lab 2018;54(4):253-254.
12. Izhar S, Kaur R, Masih K. Efficacy of rapid, economical, acetic acid, Papanicolaou stain in cervical smears as an alternative to conventional Papanicolaou stain. J Cytol. 2014;31(3):154-157.
13. Thakur M, Guttikonda VR. Modified ultrafast papanicolaou staining technique:a comparative study. J Cytol. 2017;34(3):149-153.

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