Comparison of RR100, R10 and morphologic guided list criteria in rescreening of 4000 cervical smears – an experience in a tertiary care hospital

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Abstract- **Background :** One of the most common malignancies to afflict women, cervical cancers have a high worldwide incidence. Papanicolau (Pap) test is the screening tool, ustilised widely for early detection of cervical neoplasias. However, a high load of Pap smears has resulted in errors associated with this test. Rescreening methods serve to monitor this primary cervical screening programme. This study was carried out to detect epithelial abnormalities which were otherwise missed on primary screening, using different rescreening methods and to identify the cause for such error.

Materials and methods: A retrospective study utilizing 4000 consecutive Pap smears reported in a tertiary care hospital was done. Three thousand seven hundred forty five adequate smears diagnosed as negative for intraepithelial lesion (NILM) and atypical squamous cells of undetermined significance (ASCUS) were included in the study. These smears were rescreened using 100% rapid review, 10% random review and morphologic guided list criteria (MGLC) methods. The discrepancy between the results of original cytological diagnosis and the three rescreening methods were then analysed.

Results: In the original Pap test, epithelial abnormalities accounted for only 0.85% of the total smears studied. Using RR100 and R10 methods epithelial abnormalities detected were 1.77% and 0.70% among NILM and ASCUS. No epithelial abnormalities were seen on MGLC rescreening test.

Conclusion: Rescreening methods are essential to detect screening errors, as these can have far reaching consequences in prevention of advances in cervical dysplasia. Of the various methods, 100%RR is the best available technique.

Index Terms- Rescreening, rapid review, random review, morphologic guided list criteria, Papanicolau test.

I. INTRODUCTION

Cervical carcinoma is the second most common malignancy affecting the women worldwide with an incidence of 510000/ year. (1) Various studies have detected and confirmed the association of cervical cancer with precursor lesions (50 % to 90%). (2) Ever since George N Papanicolaou introduced to the world the Papanicolaou test in 1945, it has been used extensively in cervical cancer screening programmes. An increasing load of Pap smears besides decreasing the incidence of cervical cancer, has brought to light several problems and errors associated with this test. Rescreening methods play a big role in auditing this primary cervical screening programme

besides identifying causes for error and suggesting remedial measures to be taken. One such rescreening method, 100% rapid review (RR100) involves reviewing all the negative slides in a lesser time. Rapid review is recommended as a method of monitoring laboratory and screener performance.(3) Other rescreening methods include 10% random review (R10) where random 10th slide is reviewed mainly to monitor the performance of new cytopathologist and the morphologic guided list criteria (MGLC) which involves selection of high risk cases for review, based on screening criteria. (4,5) This study utilized various rescreening methods to detect epithelial abnormalities amongst smears originally certified as Negative for intra epithelial lesion or malignancy (NILM) and atypical squamous cells of undetermined significance (ASCUS) using conventional Pap test.

II. MATERIALS AND METHODS

This retrospective study was carried out on 3645 consecutive, previously reported as negative for intraepithelial lesion (NILM) or artypical squamous cell of undetermined significance (ASCUS) on Pap smears retrieved from the archival material of Department of Pathology of a tertiary care hospital, from January to May, 2012. Conventional Pap smear reporting in the department involves thorough screening of smears by two pathologists at the rate of five minutes/smear. Relevant clinical data namely age, gynaecological history where available, were recorded for all the cases. The study was undertaken after obtaining prior institutional ethical committee clearance.

Rescreening of cases was done by the Turret technique, utilising 3 types of accepted rescreening methods namely 100 % Rapid rescreening (100% RR), 10 % Partial rescreening (10 % R), Morphological Guided List Criteria (MGLC), after proper training. Under 10X objective, the entire slide was thus scanned. Categorisation of diagnosis was done utilising the Bethesda 2001 classification for reporting of cervical smears.

- • 100% Rapid Rescreening was done in 45 seconds for each slide.
- • Every 10th available slide was screened without any time limit for 10 % R.
- • For the MGLC, smears with the following history were selected:
- o Previous cytologic abnormalities.
- o History of viral infection.
- o Postmenopausal haemorrhage.
- o Squamous intraepithelial lesion.
- o Glandular like alterations.

o   Visual inspection abnormalities.

In either of the methods, the smears found to have epithelial abnormality were then reviewed carefully for subtyping.

Criteria for inclusion: All pap smears with adequate cellularity reported as NILM or ASCUS.

Criteria for exclusion:

1. Non available smears.
2. Smears with epithelial abnormalities and inadequate cellularity (removed after rescreening).

Results obtained on RR100%, R10 and MGLC were then compared with the original diagnosis to detect lesions originally reported as NILM /ASCUS. The discrepancy between the results obtained by different rescreening methods, were analysed.

III. RESULTS

Four thousand consecutive Pap smears reported in the Pathology department of a tertiary care hospital were retrieved. The age group of these patients ranged from 17-86 years with majority (33%) being between 31-40 years. Of these, 255 cases were excluded from the study after rescreening, as 195 smears were inadequate and 60 cases had epithelial abnormalities. The summary and comparison of the results of RR100, R10 and MGLC rescreening methods and original diagnosis is illustrated in table 1.

Table 1: Comparison of results of original diagnosis, RR 100, R10 and MGLC

Diagnosis		Number of cases (%)			
		Original diagnosis (n=3745)	RR100 (n=3745)	R10 (n=382)	MGLC (n=363)
1.	NILM	3713 (99.15)	3679 (98.23)	379 (99.30)	353 (76.20)
a)	NILM without other pathology	3246 (87.42)	3114 (84.64)	310 (81.79)	40 (8.60)
b)	Candidiasis	150 (4.04)	157 (4.27)	11 (2.90)	58 (12.50)
c)	Bacterial Vaginosis	131 (3.53)	135 (3.67)	13 (3.17)	9 (1.90)
d)	Trichomonas Vaginalis	30 (0.81)	34 (0.92)	-	-
e)	Atrophy	146 (3.93)	225 (6.11)	40 (10.56)	-
f)	Inflammatory atypia	4 (0.11)	7 (0.19)	3 (0.79)	3 (0.60)
g)	Combined Infections	2 (0.05)	4 (0.05)	-	-
h)	Others	4 (0.11)	3 (0.15)	3 (0.79)	-
2.	ASCUS	32 (0.85)	43 (1.15)	0.50%	-
3.	LSIL	-	14 (0.37)	0.20%	-
4.	HSIL	-	1 (0.03)	-	-
5.	ASC-H	-	2 (0.05)	-	-
6.	AGUS	-	6 (0.17)	-	-

Abbreviations: LSIL – Low grade squamous intraepithelial lesion. HSIL- High grade squamous intraepithelial lesion, ASC-H - atypical squamous cells – HSIL cannot be ruled out, AGUS - atypical glandular cells of undetermined significance.

Total of 3745 cases were included in the study of which 3713 (99.15%) and 32 (0.85%) were reported as NILM and ASCUS, respectivitely by conventional Pap smear testing were included in the study. Four hundred sixty seven cases of NILM also had additional pathology such as infections, atrophy or inflammatory atypia. Bacterial vaginosis (BV) was the most commonly detected infection followed by Trichomonas vaginalis infection (TV). Inflammatory atypia, atrophy associated changes and combined infections were also noted. Other epithelial changes included Herpes simplex virus infection, radiotherapy induced changes and granulomatous inflammation.

On 100% Rapid Review, sixty six cases (1.77%) were detected to have epithelial abnormalities. Amongst these epithelial abnormalities, the most commonly detected condition was ASCUS, followed by LSIL. Other abnormalities detected were AGUS, ASC-H and HSIL in decreasing frequency. Amongst the infections, predominance of Candidiasis, followed by Bacterial vaginosis was seen and were picked up in slightly increased numbers compared to the original diagnosis. Higher numbers of atrophic smears were also detected on rescreening.

For R10 rescreening method, every 10th slide of 4000 consecutive slide was analysed. However, 18 slides were not available for rescreening. R10 recreening of 382 slides was done, the results of which are tabulated in table 2. One case was recategorised as LSIL and two cases as ASCUS.

Table 2: Comparison of original diagnosis and R10 rescreening

Diagnosis		Original diagnosis Number of cases (%) (n=382)	R10 Number of cases (%) (n=382)
1.	NILM	380(99.48)	379(99.30)
a)	NILM without other pathology	330 (86.84)	310 (81.79)
b)	Candisiasis	12 (3.16)	11 (2.90)
c)	Bacterial Vaginosis	13 (3.43)	12 (3.17)
d)	Trichomonas Vaginalis	-	-
e)	Atrophy	17 (4.48)	40 (10.56)
f)	Inflammatory atypia	6 (1.57)	3 (0.79)
g)	Combined Infections	-	-
h)	Others	2 (0.52)	(0.79)
2.	ASCUS	2 (0.52)	2 (0.50)
3.	LSIL	-	1 (0.20)

On MGLC screening, 463 cases reported originally as NILM were studied. The diagnosis of candidiasis, bacterial

vaginosis and trichomoniasis were comparatively higher than the original diagnosis. Epithelial abnormalities, atrophy and inflammatory atypia were not detected by this technique.

Comparison of the three methods of rescreening and original diagnosis showed candidiasis and bacterial vaginosis being diagnosed better on MGLC. Atrophic smears and inflammatory atypia were highest in R10. No epithelial abnormality was noted in MGLC. ASCUS was the most common diagnosis, followed by LSIL. As expected, because of the higher number of slides rescreened using RR100, epithelial abnormalities were detected more often to the rate of 1.77% as compared to R10 (0.7%). MGLC method of rescreening was the least compatible with no epithelial abnormality being detected at all.

Recategorization of cases on R100 rescreening has been illustrated in table 3. Of the 32 ASCUS identified during the original screening, rapid review (100% RR) confirmed 23 as ASCUS the distribution of which is demonstrated in table 2. Out of cases which were downgraded, 5 were called as inflammatory atypia , 2 as atrophic smears with related atypia ; two cases were upgraded as LSIL on RR100. Among the cases identified as NILM, 20 and 11 cases were recategorised as ASCUS (Figure 1) and LSIL (Figure 2) respectively. Six cases were recognized as AGUS (Figure 3) and one as HSIL (Figure 4).
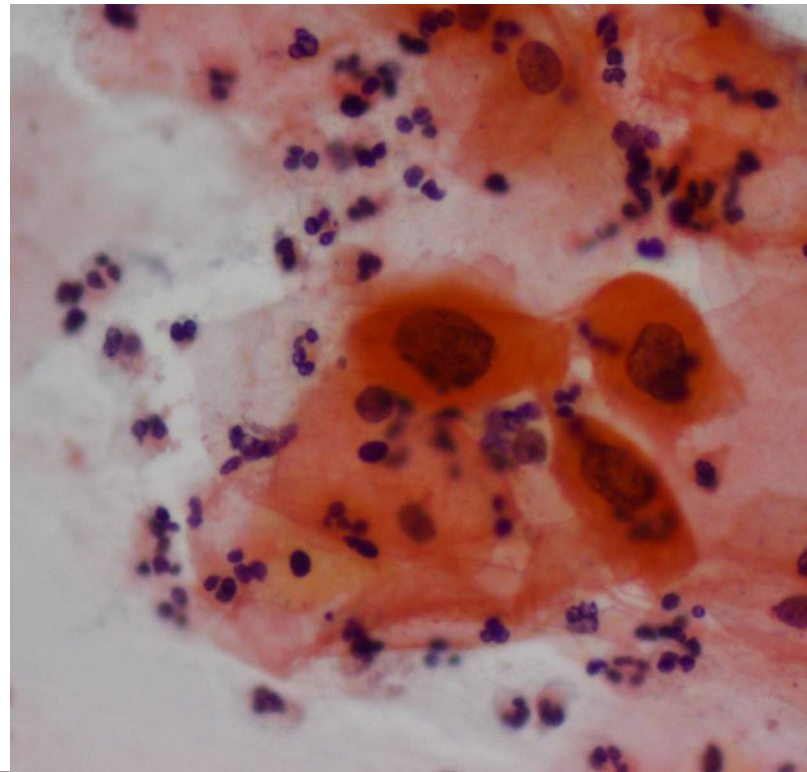

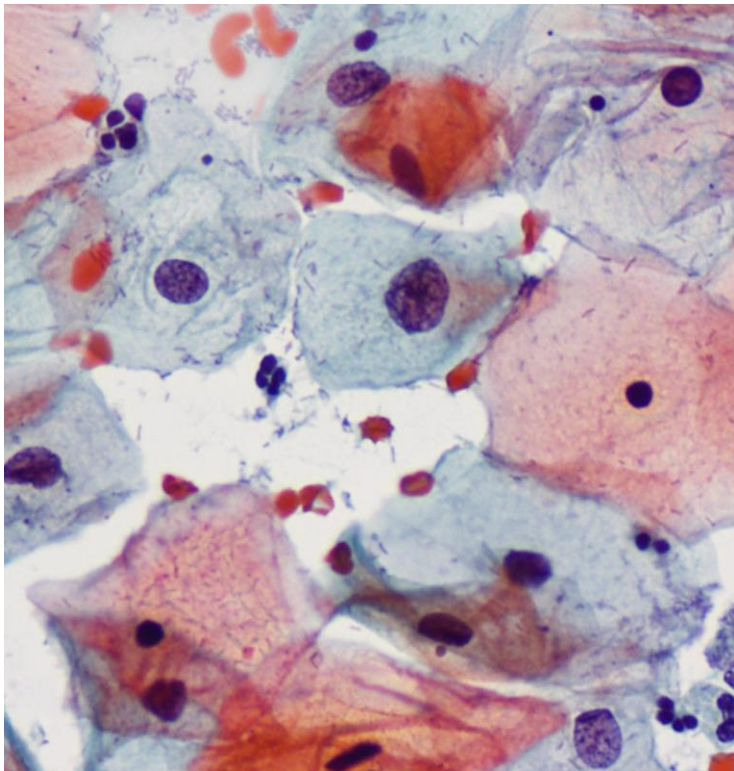**Figure 2. Original diagnosis – NILM, RR100 – LSIL (PAP,400X)**

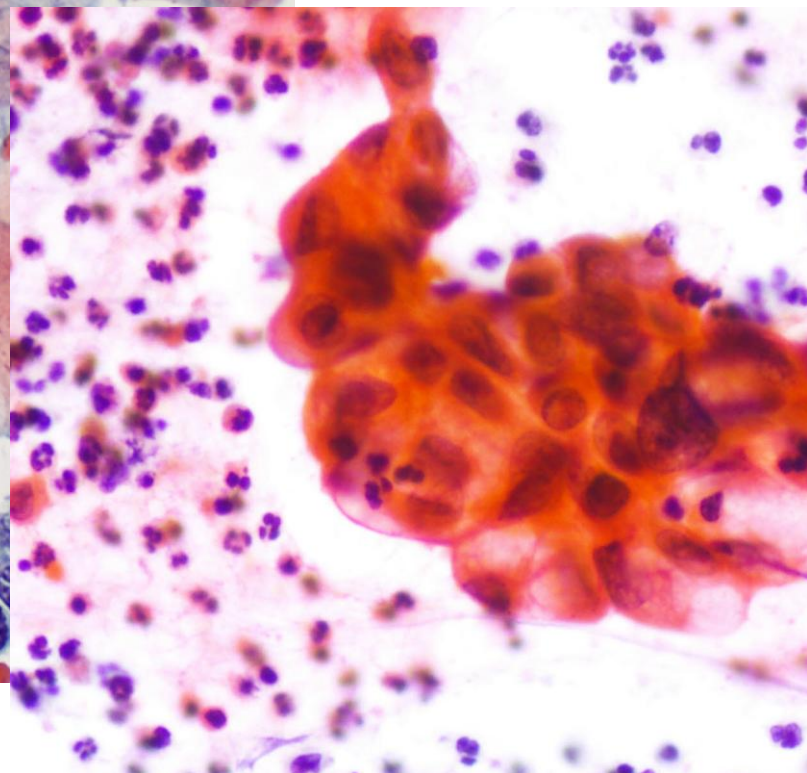**Figure 1. Original diagnosis – NILM, RR100 – ASCUS (PAP,400X)**

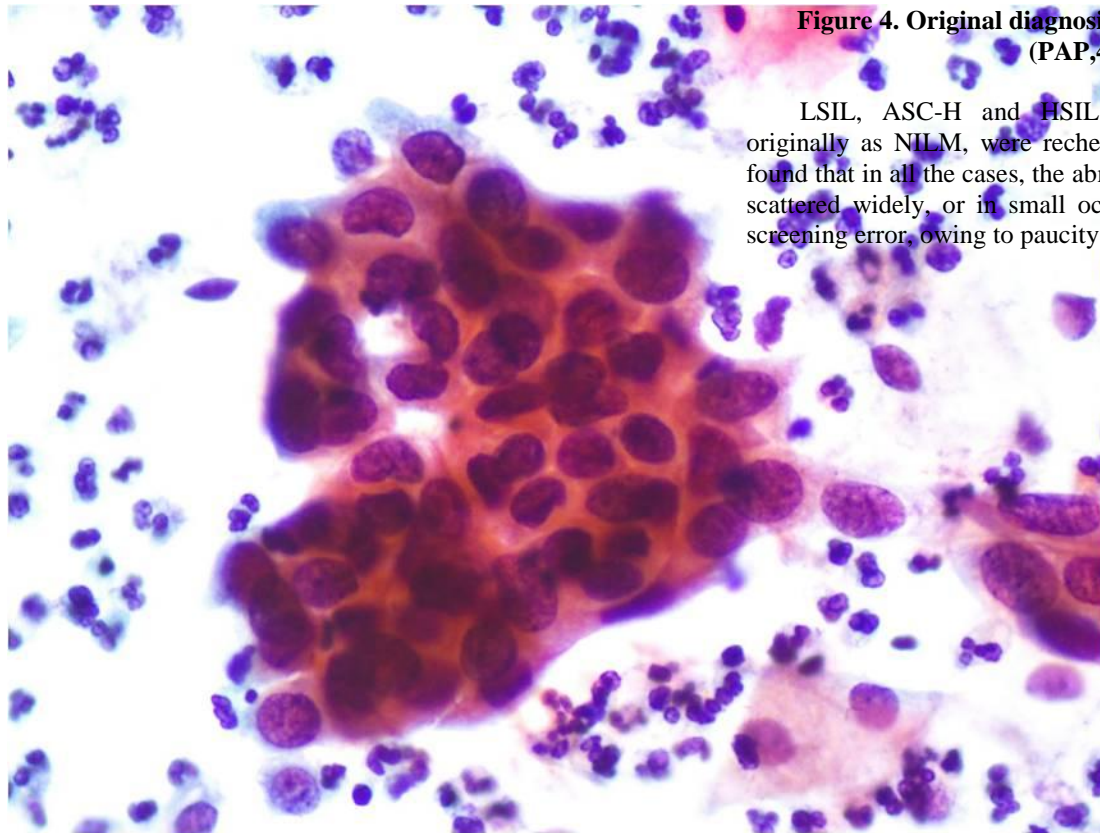**Figure 3. Original diagnosis – NILM, RR100 – AGUS (PAP,400X)**

Figure 4. Original diagnosis – NILM, RR100 – HSIL (PAP,400X)

LSIL, ASC-H and HSIL cases that had been called originally as NILM, were rechecked for cause of error. It was found that in all the cases, the abnormal cells were either less and scattered widely, or in small occasional clusters, making for a screening error, owing to paucity and focal nature of these cells.

Table 3: Recategorization of cases on R100 rescreening

Original diagnosis	RR100 category									
	NILM	LSIL	HSIL	SCC	ASC-H	Adenocarcinoma	Inflammatory atypia	Atrophy	AGUS	ASCUS
ASCUS (n = 32)	-	2	-	-	-	-	5	2		23
NILM (n=3713)	3679	12	01	-	2	-	-	-	6	20

IV. DISCUSSION

This study was retrospectively carried out to detect the efficacy of the Pap Screening programme in our institute and to identify the rescreening method with the best veracity. RR100%, R 10 and MGLC methods of screening done on 4000 consecutive smears, were considered for our study. Mattosinho and co researchers (6) utilised 2954 smears for 100% RR, Amaral et al 5215 smears for 100% RR and 10% R (7). Utagava et al , Farrel et al , Shield et al , Farekar and co researchers have also carried out studies on similar number of cases (8,9,10,11). The highest number of smears studied was 1,42,208 by Baker, Melcher and Smith in their research (12).

Inadequacy in our series was detected to be 5.12 % (195 cases). This is concurrent with Dr. Farekar`s study, which showed inadequacy of 5.3% (11). Farekar and Boxer found 0.6% to be inadequate on rapid rescreening (13). Lee and researchers got 1.6% inadequate smears (4). The specimen inadequacy rate is important and widely used quality assurance index in cervico-vaginal cytology. Data from Diehl et al showed 16% of unsatisfactory samples with subsequent follow-up showed SIL or malignancy. (14)

RR100% detected 1.77% cases with epithelial abnormality in this study on rescreening of NILM and ASCUS smears, with ASCUS accounting for 1.15%, followed by LSIL (0.37%), HSIL (0.03%), AGUS (0.17); ASC-H was the least. ASCUS cases that are optimally characterized, represent a more significant underlying lesion. In a study by Farell et al, one minute screening of 2925 smears detected additional 21 cytological abnormalities (9 ASCUS, 10 LSIL, 2 HSIL and 10 infections). (9) Atypical glandular cells of undermined significance is a difficult diagnosis to make and constitutes less than 1% of a cytology laboratory workload. (7) There was no statistically significant difference in additional pickup rates at different screening times. (9) In our study, a 45 seconds screening of 3745 smears detected 66 epithelial abnormalities with an additional

load of 41 epithelial abnormalities detected from smears earlier called NILM.

Manrique and co researchers, in their study, reported prevalence of lesions in initial evaluation as 5.5% and 100% rescreening detected additional 1.3% of lesions. The 10% random rescreening and screening based on clinical criteria detected 0.03% and 0.25% respectively. (15) Our study showed detection of 1.7% epithelial abnormalities in RR100 and 0.7% in 10% rapid rescreening. Baker and colleagues performed rapid 30 seconds rescreening and reported a five times increase in pick up of missed positive cases compared to 10% random rescreening. (12) Five cases of AGUS were diagnosed in Tan's study. In our study six cases of AGUS are detected (0.17%). (16)

On reviewing every 10thsmear in our study, 18 were found to be inadequate and very few epithelial abnormalities were detected (0.7%) with two cases of ASCUS and one case of LSIL. Utagava detected similarly, 0.3% ASC-H, 0.1% ASCUS, and HSIL each. (8) Lee and collegues detected 1.2% of abnormal cases with 4 ASCUS and 1 LSIL. (4)

MGLC has been suggested by Utagava as one of the quality control strategies to improve internal cytological diagnosis. 21% of their cases demonstrated cellular changes. (4) Comparatively in our series, no epithelial abnormality was detected using the aforementioned criteria. Morphologic guided criteria seems to be an efficient option to avoid errors in lesion categorization. (4) Study by Mattosinho and co researchers have involved a set of criteria with some clinical information and cytomorphologic findings for the past 10 years. (6)

Comparison of three methods of quality control of gynaecological diagnosis, 100% RR, 10% R and morphologic guided list criteria was extensively studied by Utagava and researchers. They then upon came to the conclusion that more lesions were detected on 100% RR as compared to the other two methods. (8) This correlates very well with our study. In a study by Amaral et al, 100% rapid rescreening showed sensitivity twice that of the 10% random rescreening. (7) The better performance of rapid rescreening is due to its objective as not to provide precise diagnosis but to separate the smears of negative from epithelial lesion. Well trained cytotechnologists are able to identify abnormal smears in 1 minute rapid rescreening. (6)

Although RR can miss abnormalities such as high grade dyskaryotic cells, it is a good alternative. The value of RR is well established and it is superior in performance when compared to 10% reveiw. (6)

Study by Farrell et al and others showed rapid rescreening for 2 minutes is as effective as conventional screening in detecting HSIL. (9,17) In our study using a time limit of 45 seconds, 1.77% of the epithelial abnormalities were detected, with LSIL and HSIL being 14(0.37%), 1(0.03%) respectively. In a study done by Gupta et al 3.7% of additional lesions were picked up, majority being ASCUS. (5)

It is observed that rapid review is best done by experienced staff as they have the ability to detect background clues. (11) Since the RR techniques are dependent on training, a slightly higher sensitivity is reached by the trained cytologists. (3)

Manrique and co researchers, in their study, reported prevalence of lesions in initial evaluation as 5.5% and 100% rescreening detected additional 1.3% of lesions. The 10% random rescreening and screening based on clinical criteria

detected 0.03% and 0.25% respectively. (15) Our study showed detection of 1.77% epithelial abnormalities in RR100 and 0.7% in 10% rapid rescreening. Baker and colleagues performed rapid 30 seconds rescreening and reported a five times increase in pick up of missed positive cases compared to 10% random rescreening. (12,18) Five cases of AGUS were diagnosed in Tan's study. (17) In our study six cases of AGUS are detected (0.1%).

Pap smear being a widespread screening programme currently in use worldwide; requires well trained cytopathologists to make it more effective in the goal of prevention of cervical cancer and detection of precancerous cervical lesions. Auditing utilising 100% RR and continuous training of personnel involved in various aspects of the programme will go a long in the successful implementation of this programme.

V.   CONCLUSION

Our study reaffirms the view of other researchers that considerable training and experience are required for rescreening to be effective, as subtle abnormalities and background clues can be missed. This study proves that 100% rapid rescreening is the most favourable method, as compared to R10 and MGLC. It further affirms that it can be as effective in detection of higher grades of epithelial abnormalities, and showed a better performance in picking up lower graded lesions. An analysis of cause of error and retraining of concerned personnel can go a long way in improving the Pap smear utilised Cervical Cancer detection programme.

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