"COMPARATIVE STUDY OF CYTOMORPHOLOGICAL FEATURES OF REHYDRATED AIR DRIED PAP SMEAR AND CONVENTIONAL WET FIXED PAP SMEAR IN CERVICAL CYTOLOGY."

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Dissertation submitted to the

BLDE University, Vijayapur, Karnataka

In partial fulfillment of the requirements for the award of the degree of

DOCTOR OF MEDICINE

IN

PATHOLOGY

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ACKNOWLEDGEMENT

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I sincerely adore and say thanks to Lord **Shri. Krushna** and the sacred book **'Bhagavad-Gita'**. This achievement was impossible without his support and blessings.

This achievement was conceivable due to enormous support, guidance and encouragement shown by my guru and guide Dr. **SUREKHA U. ARAKERI MD,** Professor, Department of Pathology. She is my idol and inspiration of my life. I feel very proud that she was my mentor not only for dissertation but also in publications. I feel very obliged to have her as my guide and thank her for her time, serenity and patronage.

My sincere gratitude to my co-guide **DR. MANPREET KAUR,** Professor, Department of Obstetrics and Gynecology who helped in fetching impossible looking sample size, gave me regular suggestions and guidance.

I am thankful to all compassionate and motivating staff of Department of Pathology, especially **Dr. B.R. YELIKAR, Professor and H.O.D** and also **Dr. S.B. HIPPARGI Prof.** for ardently organizing two successful cervical screening camps and **Dr. M.H. KARIGOUDAR Prof.** and **Dr. R.M. POTEKAR Prof.** for encouraging and boosting my potential.

I would also like to thank my beloved, supportive collogues cum friends **Dr. Y. Divya Prafulla**, **Dr. Jyotsna W**., **Dr. Anil Reddy K** and **Dr. Nawaz Khan** and my juniors **Dr. Shikha Sharma** and **Dr. Hamsalekha**.

I would like to thank my father **Prof. Shivaji S. Kapse**, my mother **Smita S. Kapse** and my lovely little sister **Dr. Supriya S. Kapse** who expressed their support in all circumstances in my life and their love and blessings made this endeavor achievable and easier.

Last but not least my genuine thanks to all the patients who participated in this study and co-operated whole heartedly.

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LIST OF ABBREVATIONS USED

RAD	Rehydrated Air Dried
RADPS	Rehydrated Air Dried PAP smear
C-PAP	Conventional PAP
C-PAPS	Conventional PAP smear
PAP	Papanicolaou
H&E	Hematoxylin and Eosin
US	United States
WHO	World Health Organization
CIN	Cervical Intraepithelial Neoplasia
TBS	The Bethesda System
ASC	Atypical Squamous Cells
ASC-US	Atypical Squamous Cells of-Undetermined Significance
ASC-H	Atypical Squamous Cells-cannot Exclude HSIL
LSIL	Low-Grade Squamous Intraepithelial Lesion
HSIL	High-Grade Squamous Intraepithelial Lesion
SCC	Squamous Cell Carcinoma
AGC-NOS	Atypical Glandular Cells- not Otherwise Specified
TV	Trichomonas Vaginalis
BV	Bacterial Vaginosis
HSV	Herpes Simplex Virus
CMV	Cytomegalovirus
RBC	Red Blood Cell
PHC	Primary Health Centre
FNAC	Fine Needle Aspiration Cytology
IHC	Immunohistochemistry
Fig.	Figure

ABSTRACT

BACKGROUND

Papanicolaou (PAP) smear is an effective exfoliative cytological investigation done for early detection of cervical cancer and inflammatory conditions of cervix. The routine practice is to fix PAP smear immediately in 95% ethanol. However, delay in fixation leads to air drying artifacts and poor fixation which can lead to unsatisfactory staining and difficulty in diagnosis. To circumvent this problem there was need to find out suitable alternative for conventional wet fixation methods.

OBJECTIVE

1. To compare the cytomorphological features in conventional PAP smear and rehydrated air-dried PAP smear.
2. To evaluate the efficacy of rehydrated air-dried PAP smear in cytodiagnosis of cervical lesion by comparing with cytomorphological features of conventional wet fixed PAP smear.

MATERIALS AND METHODS

The study was performed on PAP smears taken from all women coming for routine check-up or with some clinical problem in Obstetrics and Gynecology Out Patient Department and which were referred for cytological evaluation in the Cytology section of the Department of Pathology of Shri B.M. Patil Medical College, Hospital and Research Centre. The study period was from 1st October 2015 to 30th June 2017.

Paired cervical smears were prepared for 247 patients. One was fixed immediately in ethanol and was labelled as Conventional PAP smear (C-PAPS) and other was labelled as Rehydrated air-dried PAP smear (RADPS) which was air dried for 30-120 minutes

followed by rehydration in normal saline for 30secs and fixed in ethanol. Both slides were stained with PAP satin. Comparison of both smears was done for parameters like specimen adequacy, cytolysis, air drying artifact, red blood cell background, cytoplasmic staining, cell border and nuclear border and chromatin.

RESULTS

Out of 247 smears, 2.4% of RADPS were unsatisfactory whereas in C-PAPS, 7.3% were unsatisfactory. RBC background was present in 4 cases (2%) of RADPS and 104 cases (42%) of C-PAPS. Cytolysis (2% RADPS Vs 11% C-PAPS) and air-drying artifact (4% RADPS Vs 15% C-PAPS) was observed more in C-PAPS. Cytoplasmic staining (97% RADPS Vs 94% C-PAPS) was superior in RADPS. Cell border, nuclear border and chromatin of squamous and endocervical cells were better appreciated on RADPS compared to C-PAPS and also statistically significant difference was observed (P value<0.05).

CONCLUSION

Clean background, better preservation of cytomorphological features, superior cytoplasmic staining, minimal loss of cellularity, less air-drying artifacts and cytolysis makes RADS technique a satisfactory alternative for conventional wet fixation method which can be followed routinely or in conjugation with C-PAPS especially in cervical screening programs.

KEYWORDS: PAP smears, Rehydrated Air-dried smears, Wet Fixed smears

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INTRODUCTION

In developed countries like United States, incidence of cervical cancer has plunged significantly due to routine screening with PAP smear. In developing countries like India incidence of cervical cancer is more than a one quarter burden of its global burden. The incidence is as high as 7.9/100,000 population and accounts for 67,477 number of deaths per year among women aged between 30 to 69 years. In India, 5-year survival rate was reported as 46% which was much lower than other Asian countries like China, South Korea, Singapore and Thailand.[1,2]

Cervical cancer is second most leading cause of cancer mortality amongst Indian women aged 35-44 years. Cervical screening by Papanicolaou (PAP) smear study has proven as simple, non-invasive, less expensive as well as excellent screening method to curb the morbidity and mortality associated with cervical carcinoma.[1-3]

PAP smear is an effective exfoliative cytological investigation done for early recognition of cervical cancer. It also plays role in diagnosis of inflammatory lesions of cervix.[1]

PAP stain is an accurate stain for valuation of chromatin in cervical cytology and ensures optimal resemblance to corresponding cells nuclei in histopathology section.[2] Various fixatives are used in exfoliative cytology. Out of which 95% ethanol is the commonly used fixative.[3] Hence, the conventional method for fixation of PAP smear is to fix the PAP smear immediately in 95% ethyl alcohol after preparing the smear.[1] Delay in fixation can lead to air-drying artifacts and poor-fixation which can lead to unsatisfactory staining and difficulty in diagnosis. Also, faulty technique of fixation leads to loss of material. These patients need repeat smears, adding more workload for clinical and

laboratory workers. Moreover, some of the patients may be lost, for follow up as a result of nonconformity.[1,4]

To overcome these problems, few studies were carried out on rehydration of air dried cervical smear where they found that dipping of air dried PAP smears in normal saline for 30sec leads to lysis of red blood cells effectively and retains squamous and glandular cells.[5-8] In most of the studies, the quality of rehydrated air dried (RAD) smears was either equal or superior to convention PAP (C-PAPS) smears. Thus, rehydrated air-dried technique was suggested as a potential alternative to wet fixation for mass screening of cervical cytology.[1,3-8]

Hence, present study was done to assess the efficacy of RAD PAP smear technique in cytodiagnosis of cervical diseases by comparing RAD PAP smear cytomorphology with cytomorphological features of conventional wet fixed PAP smear and to find out whether rehydrated air-dried smear can be better alternative for conventional wet fixation method.

AIM

To assess whether rehydrated air-dried smear technique can be better alternative to conventional wet fixed smear technique.

OBJECTIVES

1. To study cytomorphological features in conventional wet fixed PAP smears and rehydrated air-dried PAP smears according to The Bethesda system of classification.
2. To compare the cytomorphological features in conventional wet fixed PAP smears and rehydrated air-dried PAP smears.
3. To evaluate the efficacy of rehydrated air-dried PAP smear in cytodiagnosis of cervical lesion by comparing with cytomorphological features of conventional wet fixed PAP smear.

REVIEW OF LITERATURE

ANATOMY AND HISTOLOGY OF NORMAL CERVIX

Female genital tract is composed of vulva, vagina, cervix, uterus, fallopian tubes and ovaries.

Cervix is most inferior part of uterus protruding into vagina. This cylindrical fibromuscular structure measures 2.5 to 3 cm in length. Outer aspect of the cervix is known as **ectocervix** or portio-vaginalis and inner part is **endocervix**. **Endocervical** canal connects body of uterus through internal os with vagina through the external os. Protruding inferior portion of cervix forms fornices in the superior vagina. Accumulation of exfoliated cells & pooling of secretions occurs in the fornices.[9,10]

Ectocervix is lined by stratified squamous non-keratinizing epithelium & is in continuity with vaginal epithelium distally. Lining of the endocervix is by mucin-secreting tall columnar epithelium and it is not exposed to the vaginal pH. The glandular mucosa extends into the stroma of the cervix in a racemose pattern forming branching crypts. Squamocolumar junction also called as transformation zone is the junction of endocervical mucosa with ectocervical squamous epithelium. It constitutes immature & mature metaplastic squamous epithelium along with columnar endocervical epithelium.[9,10]

The original **squamo-columnar** junction lies at the junction of native ectocervical and columnar endocervical epithelium. Whereas, functional squamo-columnar junction is situated at junction of metaplastic squamous cells with the endocervical columnar cells.

Transformation zone is the area in-between two squamocolumnar junctions and recedes into the endocervix in post-menopausal women.[10-12]

CERVICAL CYTOLOGY

Exfoliative cytology has been regarded as the most successful technique of 20th century for cervical cancer screening programs. The PAP smears created a benchmark in the screening program for pre-invasive lesions and is considered a success story for decades. The technique for PAP smear preparation is to collect exfoliated cells from the ectocervix and endocervical canal using Ayre spatula and/or endocervical brush. The sample collected is smeared on a slide & immediately fixed in 95% ethanol for minimum 30minutes.[13-16]

Adequacy Criteria:

On conventional smear for adequate smear, minimum 8000-12000 well visualized & well-preserved squamous epithelial cells are seen with exclusion of completely obscured cells.

Occurrence of transformation zone or endocervical component is not must for smear adequacy. Smears with absence of these components are interpreted as satisfactory but limited by absence of either endocervical or transformation-zone component.

It is considered that transformation or endocervical zone has been adequately sampled when atleast 10 metaplastic squamous cells or well preserved endocervical are seen singly or in clusters.

If more than 75% of squamous cells are obscured, then the smears are termed as unsatisfactory. When 50% to 75% of the cells are obscured, a statement describing the specimen as partially obscured should be followed after the satisfactory for evaluation term. If smear contains any abnormal cells, then those smears should be considered satisfactory for evaluation.[17,18]

CYTOMORPHOLOGY

Superficial cells are polygonal. large with orange-pink cytoplasm, sharply defined borders and have a pyknotic small central nucleus. Diameter superficial cell is approximately 40µm with a nuclear diameter of 3-5µm. Cells of granular cell layer shows dark blue small kerato-hyaline granules evenly distributed in the cytoplasm known as Polka-Dot sign.

Intermediate cells have folded edges and are polygonal in shape with cyanophilic cytoplasm and are approximately 30µm in diameter. Nucleus is oval to round, vesicular and diameter is 8- 10µm.

Parabasal cells are 10µm in diameter and are round to oval in shape. It has dense basophilic cytoplasm, borders are distinct and may contain vacuoles. Nuclear diameter of these cells is about 8µm and occupies about one-half of the cells and has fine nuclear chromatin with occasional nucleoli.

Metaplastic cells are usually arranged in small sheets & have dimension similar of parabasal-cells or early intermediate cells. Mild anisonucleosis may be seen with vesicular nuclei & higher N/C ratio. Cytoplasm is densely cyanophilic which may be prematurely keratinized. The cells have a spidery contour due to the presence of

cytoplasmic projections due to loosened intercellular bridges. As these cells mature, they resemble intermediate and superficial squamous cells.

Endocervical cells are columnar tall mucus-secreting cells with round to oval vesicular nuclei and 1-2 small nucleoli. Cytoplasm of endocervical cells appear clear, cyanophilic or vacuolated. They are usually seen in small sheets or groups having picket-fence pattern when seen from the sides and honey-comb appearance when viewed from above.[18,21]

Endometrial cells sometimes can be seen smears for the first 12 days of cycle following menstruation. During the menstrual phase, they are seen in well-formed 3-dimensional clusters with peripheral rim of epithelial cells and central core of stromal cells. Nucleus of these cells are small round with small nucleoli & scarce to moderate basophilic cytoplasm.[38] Presence of these endometrial cells must be stated in post-menopausal females with a note suggesting evaluation of endometrium to be performed.[20]

Normal Bacterial Flora

Lactobacillus are gram positive, aerobic rod-shaped bacilli and is normal inhabitant of lower genital-tract. On PAP stain it is identified as basophilic rod-shaped bacilli which is of varying length. It causes cytolysis of glycogen containing cell by fermenting cytoplasmic glycogen.[22]

Table 1: Cytomorphological characteristics of normal genital squamous cells. [23]

Parameters	**Superficial**	**Intermediate**	**Parabasal**	**Basal**
Size (µ)	40-60	30-60	15-25	8-10
Shape				
Polygonal (%)	85	75	5	0
Oval (%)	20	10	40	5
Round (%)	5	5	55	95
Pattern /Arrangement of cells	Single 90%	Single 80%	Single 60% Sheets 40%	Sheets 90%
Amount of cytoplasm	Abundant	Abundant	Adequate	Scanty
Cytoplasmic border curling	Rare	Common	Rare	Rare
Cytoplasmic stain	Orange	Pink or blue	Blue	Deep blue
Cytoplasmic vacuolization	None	Occasional	Occasional	None
N/C ratio	1:10	2:10	5:10	8:10
Nuclear size (µ)	5 to 7	10 to 12	8 to 13	7 to 9
Nuclear shape	Round to oval	Round-oval	Round- oval	Round
Chromatin pattern	Pyknotic	Finely granular	Granular	Coarse
Multi-nucleation	Rare	Few	Few	Rare
Nucleoli	None	Small	Occasional and prominent	None

HISTORICAL ASPECT

Dr. George N. Papanicolaou in early 1940, described that vaginal smears could be prepared to screen for cervical cancers and also introduced the PAP stain. Papanicolaou & Traut published their famous paper titled "Diagnosis of Uterine Cancer by the Vaginal Smears". Later Dr. J Ernest Ayre introduced the wooden spatula to scrape the cervix at the transformation zone in 1947 now referred to as the Ayre's spatula.[12,13]

Since Papanicolaou's introduction of PAP smear, a variety of terms have been used for convoying cytological diagnoses.[12,13]

Papanicolaou's Classes for interpretation of cytological smears

Papanicolaou in 1954 suggested the initial classification of cervico-vaginal smears & formulated series of guidelines for smear interpretation and also categorized cervical cytology interpretation into five classes:

Table 2. Papanicolaou classes for PAP smear interpretation.[19,20]

Class	Cytologic Interpretation
I	Absence of atypical or abnormal cells.
II	Atypical smear but no evidence of malignancy.
III	Suggestive of, but not conclusive for malignancy.
IV	Strongly suggestive of malignancy.
V	Conclusive for malignancy.

Later World Health Organization (WHO) graded precancerous squamous lesions in cervix into mild, moderate and severe dysplasia and carcinoma-in-situ depending on the extent of morphological changes. In 1969, Richart introduced Cervical intraepithelial neoplasia (CIN) system which emphasized on dysplasia & carcinoma in situ as a continuum. As per this system, CIN was subdivided into grade 1 to 3 according to the degree of abnormality encountered.[18,19]

Table 3: Reporting systems for cervical squamous epithelial abnormalities on cytology.[18]

Papanicolaou Class	**WHO**	**CIN**
I	-	-
II	-	-
III	Mild dysplasia	CIN-1
	Moderate dysplasia	CIN-2
	Severe dysplasia	CIN-3
IV	Carcinoma-in-situ	CIN-3
V	Carcinoma	Carcinoma

The first Bethesda workshop was conducted in 1988 at Bethesda, Maryland, presided by Robert Kurman. In this workshop discussion was concentrated on addressing the concerns related to wide inconsistency in reporting of PAP smears. During that period terminology either numeric ‘Pap Class' system or ‘dysplasia’ was used by the cytologists.

Hence, the objective of that workshop was to establish terminology that would deliver clear-cut inceptions for management and decrease inter-observer erraticism.

Subsequently, The Bethesda System (TBS) was revised in 1991 & 2001. In 2014, three fundamental principles were emerged which guided The Bethesda System (TBS) to present time was as below -

1. Terminology must converse clinically significant information from laboratory to the patient's health care provider.
2. Terminology should be reproducible & constant across different pathologists & laboratories & supple enough to be adapted in varied range of laboratory sceneries & geographic whereabouts.
3. Terminology must reflect most contemporary understanding of cervical neoplasia.[19]

As per revised TBS 2014, Squamous cell lesions were classified as atypical squamous cells of-undetermined significance (ASC-US), atypical squamous cells-cannot exclude HSIL (ASC-H), low-grade squamous intraepithelial lesions (LSIL), high-grade squamous intraepithelial lesions (HSIL) and squamous cell carcinomas. Glandular lesions have been classified as atypical glandular cells- not otherwise specified (AGC-NOS), atypical glandular cells favour neoplasia, adenocarcinoma in-situ and adenocarcinoma.[16,24]

THE 2014 BETHESDA SYSTEM[24]

Specimen type:

Conventional smear (PAP smear) vs. liquid-based preparation vs. other

Specimen adequacy:

- Satisfactory for evaluation
- Unsatisfactory for evaluation
- Specimen rejected/not processed
- Specimen processed and examined, but unsatisfactory for evaluation of epithelial abnormality

General categorization (optional):

- Negative for intraepithelial lesion or malignancy
- Other
- Epithelial cell abnormality

Interpretation/ Result:

Negative for intraepithelial lesion or malignancy:

Non-Neoplastic Findings (optional):

- Non-neoplastic cellular variations
    - Squamous metaplasia
    - Keratotic changes
    - Tubal metaplasia
    - Atrophy

        - Pregnancy-associated changes
    - Reactive cellular changes associated with:
        - Inflammation - Lymphocytic (follicular) cervicitis
        - Radiation
        - Intrauterine contraceptive device (IUD)
    - Glandular cells status post hysterectomy

Organisms:

- Trichomonas vaginalis
- Fungal organisms morphologically consistent with Candida spp.
- Shift in flora suggestive of bacterial vaginosis
- Bacteria morphologically consistent with Actinomyces spp.
- Cellular changes consistent with herpes simplex virus
- Cellular changes consistent with cytomegalovirus

Other:

Endometrial cells (in a woman aged >45 years)

Epithelial Cell Abnormalities:

Squamous Cell

Atypical squamous cells

- Of undetermined significance (ASC-US)
- Cannot exclude HSIL (ASC-H)
- Low-grade squamous intraepithelial lesion (LSIL)
- High-grade squamous intraepithelial lesion (HSIL)

- With features suspicious for invasion
- Squamous cell carcinoma

Glandular Cell

Atypical

- Endocervical cells (NOS)
- Endometrial cells (NOS)
- Glandular cells (NOS)

Atypical

- Endocervical cells, favor neoplastic
- Glandular cells, favor neoplastic
- Endocervical adenocarcinoma in situ
- Adenocarcinoma
- Endocervical
- Endometrial
- Extrauterine
- Not otherwise specified (NOS)

Other malignant neoplasms:

Adjunctive testing:

Computer-assisted interpretation of cervical cytology:

Educational notes and comments appended to cytology reports :[24]

CYTOMORPHOLOGICAL CHANGES IN VARIOUS INFECTIONS:

Trichomonas Vaginalis (TV) - It is estimated that 10-20% adult women harbor this parasite. On speculum examination, strawberry cervix is observed which occurs due to dilatation of superficial blood vessels and focal hemorrhages.

Cytomorphological changes - Marked eosinophilia in superficial, intermediated and parabasal cells. Perinuclear halo/clearing is observed in superficial squamous cells. Pale nuclei, fraying of cell borders and apoptosis of cell can also be seen. Endocervical cells may show cellular enlargement, cytoplasmic vacuolization and squamous metaplasia. Pear-shaped, gray-green organism with size 8 to 20µm. Eccentric located, pale vesicular nuclei. Cytoplasm contains eosinophilic granules.[17,22,24]

Candida- Most common pathologic fungus affecting female genital tract. Clinically patient present with intense itching, discomfort and thick milky whitish discharge.

Cytomorphological changes- Spearing of epithelial cells is more common in liquid based cytology called shish kebab effect. Budding-yeasts (3 to 7 µm); pseudo hyphae are eosinophilic to gray-brown. Fragmented leukocyte nuclei & rouleaux formation of squamous epithelial cells speared by hyphae may be seen.[17,22,24]

Bacterial Vaginosis - Commonest cause of cervicitis and vaginitis among pre-menopausal females. It occurs due to replacement of normal bacterial flora by mixed bacteria like G. vaginalis, mycoplasma, Mobiluncus and other gram-negative bacilli. Clinically patient present with profuse discharge per vagina with “fishy” odder.

Cytomorphological changes- Individual squamous cells are covered by a layer of bacteria that obscures the cell membrane, forming so called —clue cells Filmy/dirty background due to presence of small coccobacilli.[17,22,24]

Herpes virus - important and most common viral infection of female-genital tract. Speculum examination shows erosion of cervix.

Cytomorphological changes- Moderated to marked nuclear enlargement, crowding and overlapping may be seen in squamous or endocervical cells. Nuclei have "ground-glass appearance" due to intranuclear viral particles and enhancement of the nuclear envelope caused by peripheral margination of chromatin. Dense eosinophilic intranuclear inclusions surrounded by a halo or clear zone are present. Large multi-nucleated epithelial cells with molded nuclei are characteristic. [17,22,24]

Cytomegalovirus- more frequently found in AIDS patient.

Cytomorphological changes - large intra-nuclear inclusions, smaller satellite inclusions in nucleus and cytoplasm.[22]

Leptothrix - Long, curving, filamentous organisms, most commonly observed in conjunction with vaginal trichomonas infection.[17]

CYTOMORPHOLOGY OF VARIOUS EPITHELIAL CELL ABNORMALITIES:

ASC - Suggests possible presence of squamous intraepithelial lesion and rarely carcinoma. Various conditions can give rise to ASC like air drying, atrophy with degeneration, hormonal effects, inflammation and other artifacts.

ASC-US - It refers to changes that are suggestive of LSIL, but quantitatively or qualitatively insufficient for interpreting LSIL.

Cytomorphology - Nuclei are approximately 2.5 to 3 times the area of the nucleus of a normal squamous intermediate cell. Mild increase in N/C ratio and minimal nuclear hyperchromasia is seen. Irregular chromatin distribution or nuclear shape. Variation is minimal. Incomplete koilocytosis and atypical parakeratosis can also be observed. [22,24]

ASC-H - Changes evocative of HSIL, but quantitatively or qualitatively insufficient for interpreting as HSIL.

Cytomorphology - Cells are the size of metaplastic cells. Cells usually are singly scattered or in small groups of <10 cells. Nuclei are 1.5 to 2.5 times larger than normal nuclei of parabasal cells. Ratio of N/C may approximate that of HSIL.[22,24]

LSIL - Associated with Human Papilloma Virus infection.

Cytomorphology - Cells are in clusters, sheets or singly scattered. It usually affects superficial cell or intermediate squamous cell. Nuclear enlargement is 3 times or more than area of intermediate nuclei. Variations in nuclear size, number & outline is accompanied by variable degrees of nuclear hyperchromasia. Chromatin is coarse,

granular and distributed uniformly. Inconspicuous nucleoli may be seen or sometimes it may be absent. Perinuclear halo (koilocytosis) consists of sharply demarcated clear perinuclear-zone & a peripheral brim of densely stained cytoplasm is a characteristic feature. Cytoplasm may appear dense and orangophilic (keratinized). [22,24]

HSIL- Encompasses moderate and severe dysplasias (CIN I & II).

Cytomorphology- Cells are smaller and arranged in syncytial like aggregates, sheets or singly scattered.Nuclear enlargement is fickler than that seen in LSIL. Nuclei are most of the time hyperchromatic but normochromic and hypochromic nuclei can be seen. Cytoplasmic area is reduced leading to marked increase in N/C ratio. Contour of nuclear membrane is irregular and frequently shows indentations. Nucleoli are absent. Cytoplasm is delicate, immature and lacy.[16,22,24]

Squamous Cell Carcinoma (SCC) – Comprised of squamous cell of capricious degree of differentiation.

Cytomorphology - Cells are predominantly singly scattered and least commonly can be arranged in sheets. Bizarre shaped dyskeratotic- tad-pole cells which have eosinophilic cytoplasm and large irregular hyperchromatic nuclei. In non-keratinizing carcinoma, anisokaryosis is appreciated in cells arranged singly or in syncytia. Nucleus is markedly enlarged with clumped coarse chromatin and irregular nuclear membrane. Sometimes macro-nucleoli may be seen. Dirty background (tumor diathesis) comprising of necrotic debris, fibrin, blood and inflammation is seen more commonly in nonkeratinizing SCC but can be seen in keratinizing squamous-cell carcinoma.[16,17,22,24]

AGC-NOS- Generic term used if cell of origin cannot be identified i.e. endocervical or endometrial.

Cytomorphology- Cells are arranged in sheets and strips. Cell crowding with nuclear enlargement, overlapping and /or pseudo-stratification can be seen. Mild anisonucleosis with mild hyperchromatic nuclei and slight nuclear membrane irregularity is seen. Cytoplasm is abundant.

CONVENTIONAL PAP SMEARS:

The conventional PAP smear devised by Papanicolaou has been successfully used for cervical cancer screening for more than 5 decades and continues to perform well, provided the preventable causes of suboptimal smear preparation are addressed. However, the conventional PAP smears are reported to have low sensitivity which is attributed to more number of unsatisfactory smears, more air-drying artifact, increased time consumption, cellular overlapping and various obscuring factors like inflammatory cells and RBCs. This led to the advent of Rehydrated air-dried method with an objective of minimal loss of sample, increase cellularity, less air-drying artifacts, cleaner background, minimizing cellular overlap, enhancing cellular and nuclear morphology and improving smear quality.[22-25]

Rehydration technique of air dried vaginal smears was first attempted by Leoncioni *et al*[26] to overcome the difficulties of air-drying & inferior fixation, which was commonly seen in those days in the conventional wet fixation method with PAP stained smears. In spite of encouraging results of rehydration technique introduced by Leoncioni

et al, this method has not yet gained much recognition due to lack of standardization of the optimum air-drying period of re-hydration.

Studies with an attempt of development of rehydration techniques by experiments with the various rehydration-agents & with variable duration of air-drying before smear rehydration have been done by various authors. These authors found that compared to wet fixation, rehydration of air-dried smears decreased air-drying artifact.[6-8]

A retrospective study was conducted by Brad Randall *et al*[27] to compare cytomorphology of Air-Dried/Rehydrated Cervicovaginal Smears and Traditionally Fixed Smears. A total of 6,788 air-dried & 1,691 conventional smears were included in the study. They found no statically substantial differences either in degree of abnormality or percentage of abnormalities among both the technique. Conclusion was made that this new rehydrated air-dried method was a viable alternative to Conventional wet fixed method.

Gill GW *et al*[28] in their study discussed about mechanism of air drying and its cytomorphological implication. They found that diameter of nucleus in rehydrated smears was more as compared to conventional smears and nuclear chromatin is better appreciated. These authors concluded that if rehydration prior to air-drying smear is done, they may be morphologically more interpretable and had more diagnostic outcomes compared to routine wet fixation method.

In 2001,Ganesan *et al*[7] conducted a study to evaluate effect of rehydration on air-dried PAP smears & to compare it with conventional wet-fixed smears. Two sets of cervical smears were obtained from 419 women. One set was labeled as Routine and the

other was labeled as “Rehydrated”. The Rehydrated group was further subdivided into groups A–F depending on the duration of air drying. Rehydrated smears were air dried for inconstant duration, ranging from less than 30min upto 5hours. After air drying they were rehydrated with immersion in Normal-saline for 30 seconds charted by fixation and routine Papanicolaou staining. Different parameters like cellularity, cytolysis, cell border, cytoplasmic staining, nuclear border and chromatin was assessed and graded. Other parameters like hemorrhagic smears and neutrophils were also assessed and scored as 0/+1/+2/+3/+4. They concluded that staining quality was equal or superior in conventional wet fixed smears only if rehydration of air-dried PAP smears was done within two hours of preparation.

A study was conducted in Delhi, India by Gupta S *et al*[6] to assess the consequence of rehydration of air-dried cervical smears on staining quality to find out whether rehydration techniques can be adopted as a substitute method. Paramedical workers collected paired 950 conventional & rehydrated air-dried PAP smears from an urban slum. These smears were compared for staining quality by assessing different nuclear & cytoplasmic parameters. It was concluded in their study that in a resource poor setting, this technique can be conveniently adopted, as the unsatisfactory rate was low as well as staining characteristics were slightly better or same as compared to wet fixed smears.

Jaiwong K *et al*[5] in 2004 did a comparative study on cytomorphological features between rehydrated air-dried PAP smears & Conventional wet-fixed PAP smears. Total 172 Paired-cervical smears collected and prospectively evaluated. They concluded that quality of rehydrated smears was inferior to conventional smears but may be satisfactory substitute to wet-fixation in cytological cervical cancer screening.

Another study was conducted by Zare-Mirzaie A *et al*[29], in Tehran, on 117 paired wet-fixed & air-dried, rehydrated & fixed PAP smears. Staining quality of the slides was evaluated with respect to chromatin, red blood cells lysis, nuclear and cytoplasmic borders, cellularity, cytolysis and cytoplasmic staining. They found that air-drying followed by rehydration of air dried slides was a reliable method for heavily blood-stained smears and cytological features were identical to wet fixed smears.

A similar study was performed in Nigeria in 2011 by Danladi J. *et al*[8]on 100 paired cervical smear slides. One was conventional wet fixed & another air-dried, rehydrated & then fixed followed by PAP staining. They concluded that rehydration of air-dried smears was an appropriate alternative to conventional cervical smears.

Sivaram and Iyengar *et al*[7] suggested that air-dried PAP smears which were rehydrated within 30 to 120 mins & sent to laboratory could be suitably rehydrated and stained with PAP stain. In their study they found out that rehydrated air-dried PAP-stained smears were satisfactory alternative to wet-fixed ones, provided the air-drying period does not surpass >2 hrs.

A Cross-Sectional Study was conducted by Rupinder K *et al*[4] in 2013 in a tertiary care set up on paired 461 PAP smears. First was fixed in 95%ethanol & another was air-dried, subjected to rehydration followed by fixation in 95%ethanol. They concluded that rehydration of air-dried smears was modest, feasible, applicable and consistent fixation method which was analogous to the wet-fixed conventional technique used for PAP smears and can be applied for evaluation on a regular basis.

Bonime *et al*[30] tried glycerin water rehydration technique on air dried cervico-vaginal and non-gynecologic specimens. Cervical smears were smeared on slide and left for drying up to 10days. Rehydration was done by immersing slide in glycerin-water solution for 3 minutes. In their study they found that in thick smear staining of rehydrated air-dried smear with glycerin water was superior as compared to conventional wet fixed method.

In an article published by Ng WF *et al*[31] ninety fluid specimens were studied by preparing three similar smears. One slide was air-dried for Giemsa stain, one wet fixed in 95%ethyl alcohol and one withered on a hot plate at 37^0 C and then rehydrated in normal saline(NS) for thirty seconds & fixed in ethyl alcohol. The latter two were stained with PAP stain & a comparison was made retention of RBCs, retention of epithelial or mesothelial cells and cytomorphogical preservation. Giemsa-stained smear was used as a control in their study. They found that rehydrated smears showed reduction in the intensity of staining, more flattened cell clusters and slight cell enlargement. Hence, they concluded that rehydration method was advantageous for urine and blood-stained body fluids.

A cytomorphometric study was conducted by Schulte E. *et al*[32] on the effect of wet-fixed PAP and air-dried Giemsa methods on nuclear parameters in breast cancer cytology. Aspiration biopsy material from 55 benign & malignant breast lesions were smeared onto slides & stained with a routine PAP technique or with Hematoxylin and Eosin (H&E) stain. The nuclear area, nuclear perimeter & diameter were measured in each case with an image analyzer and correlated with the grade and stage of the disease.

They recommend air-drying of cytologic samples if morphometrical data has to be used to discriminate between different groups.

Chan JK *et al*[33] conducted a study in Hong Kong on 80 fine-needle aspiration cytologic cases. A direct smear was made from the aspirated material and wet-fixed immediately in 95% ethanol and another smear was quickly air dried and then rehydrated for 30 seconds in normal saline (NS) before fixation and staining was done with H&E. They found that quality of the rehydrated smears is superior or identical to that of the immediately wet-fixed smears, provided that the period of drying does not exceed 30 minutes.

Application of similar technique that is rehydrated air-dried smears on 300 cases of body cavity fluid cytology was done by Kung IT *et al*[34]. Centrifuged, concentrated cell suspensions were spread on the slides. The glass slides were dried at room-temperature and were rehydrated for 30secs in 0.9%-sodium chloride solution & fixed in 95% ethanol. They were then stained with haematoxylin and eosin. Two control smears were prepared for each case, one air-dried & rehydrated as above and another wet-fixed and both smears were stained with Papanicolaou stain. They concluded that accessibility of a crisp chromatin pattern in rehydrated air-dried smear for examination in difficult cases may help in deciding whether lesion is malignant or a reactive process.

Another study was conducted by Shidham VB *et al*[35] on 118 FNAC specimens. The cytomorphology of ADS processed H&E and PAP staining after rehydration in NS and post-fixation in 95% ethyl alcohol with 5% acetic acid were equated with respectively stained C-PAP smears. ADS were stored up to 72 hours at room temperature prior to H&E, PAP and Diff-Quick staining to assess the effects of postponing

rehydration and post-fixation. They found that RADPS showed results analogous to or better than C-PAPS.

Major drawback of conventional wet fixation method was inability to lyse red cell background. These pave a major hindrance while giving diagnosis. Many times, such smears were reported as inadequate for opinion due to presence of red cell background. Few studies were carried out in order to remove red blood cells from cervical smears like use of hypotonic solutions, tap water, acetic acid but in most of the studies normal saline was considered better alternative since it was cheap and easily available.[4-8]

Various authors mentioned that RAD smears show increase in cell size, prominent intracytoplasmic inclusions and greater cellularity as compared to CPS and thus helps in better cytomorphological assessment and interpretation of cervical smear.[4-8,28,29,31,36-38]

Air dried rehydration technique was also tried on non-gynecologic smears with FNAC, exfoliative cytology and effusion cytology as mentioned above.[27,28,29,46] Also with different staining methods like H&E[20], Giemsa[26] and IHC[39,40].

Conventional wet fixation method has been popularly followed as a part of cultured in curriculum and is being routinely used worldwide in health care settings. Few limitations of this method have been neglected such as air-drying artifacts, unsatisfactory for evaluation due to loss of cellularity while fixation, overlapping of cells, RBCs and inflammatory cells obscuring the diagnostic cells. Such limitations cannot be underestimated as it can prove costly if the precursors, pre-neoplastic and neoplastic lesion are missed.[4,6,7,41,42]

There are numerous advantages of rehydrated air-dried technique as shown by various studies conducted by different authors such as reduced number of unsatisfactory smears, lysis of RBC leading to clearer background, cellularity was maintained as there was no loss of material while fixation and reduction in Air drying artifacts. RAD method was a preferred technique by paramedics/Technicians as it was less tedious/cumbersome. There was ease in making diagnosis as there is less obscuring by RBC's and Inflammatory cells.[4,7,29-32]

RAD technique has certain disadvantages like uneven distribution of cells as two smears are prepared from same women at same time and artifactual nucleomegaly due to over-hydration. [4,6,29,31]

MATERIALS AND METHODS

SOURCE OF DATA:

A prospective study was carried out on PAP smears taken from all women coming for routine check-up or with some clinical problem in Obstetrics and Gynecology Out Patient Department and which were referred for cytological evaluation in the Cytology section of the Department of Pathology of Shri B.M. Patil Medical College, Hospital and Research Centre.

Study period: 1st October, 2015 to 30th June, 2017.

METHODS OF COLLECTION OF DATA:

- Cervical smear was prepared by using Ayre's wooden spatula.
- Two PAP smears were prepared for each case.
- One slide was immediately fixed in 95% Ethanol for 30 minutes and was labeled as C-PAPS smear.
- The other slide which was labeled as RAD was air dried for 30 minutes; rehydrated with normal saline for 30 seconds and immediately added to jar containing fixative 95% Ethanol for 30minutes.
- Both smears were stained with routine PAP stain.
- As per the study done by Sivaram and Iyengar, both RADPS and C-PAPS smears were screened, assessed and graded for cytomorphological parameters such as Cellularity, Presence or Absence of Cytolysis/Air-drying Artifact/Red cell Background etc.[7] **(Table 4)**
- All the smears were reported as per The 2014 Bethesda System.

Table 4. Comparison of cytomorphological parameters between C-PAPS and RADPS

Sr. No.	Parameter	C-PAP	RAD
1	**Cellularity**		
	Low		
	Intermediate		
	High		
2	**Cytolysis**		
	Present		
	Absent		
3	**Air-drying artifact**		
	Present		
	Absent		
4	**Red blood cell background**		
	Present		
	Absent		
5	**Cell border**		
	Distinct		
	Indistinct		
6	**Cytoplasmic staining**		
	Unsatisfactory		
	Satisfactory		
7	**Nuclear border**		
	Squamous cells		
	Distinct		
	Indistinct		
	Endocervical cells		
	Distinct		

	Indistinct		
8	**Nuclear chromatin**		
	Squamous cells		
	Crisp		
	Hazy		
	Endocervical cells		
	Crisp		
	Hazy		

INCLUSION CRITERIA:

PAP smears collected from all women coming to Obstetrics and Gynaecology Department for routine check-up or with some clinical problem were included in the study.

EXCLUSION CRITERIA:

Cases for which only wet fixed smears were collected were excluded from the study.

Fig. 1: Photograph showing Coplin Jar containing Normal Saline and Ethanol and 0.9% Normal Saline used for RAD smear.

Fig. 2: Gross appearance of RADPS and C-PAP smear in a hemorrhagic material.

STATISTICAL METHODS

Sample size:

Formula used for sample size calculation was

$$n = \frac{Z^2\, p(1-p)}{d^2}$$

Where,

n= Sample size.

Z = 1.96 at 95% confidence limit

p = Proportion of red cell background in the rehydrated air-dried PAP smear

d = Desired precision

The calculated sample size was 237.

Hence, 247 cases were included in the study.

Statistical analysis:

All characteristics were summarized descriptively. Data are presented using diagrams. For continuous variables, the summary statistics of mean and standard deviation (SD) were used. For categorical data, percentages were used. Chi-square (χ^2)/Fisher exact test was employed to determine the significance of differences between groups for categorical data. Data were analyzed using SPSS software v.17.0. Results were considered significant if the p-value was < 0.05.

RESULTS

The present study was undertaken to evaluate the efficacy of rehydrated air-dried PAP smear in cytodiagnosis of cervical lesion by comparing with cytomorphological features of conventional wet fixed PAP smear.

Comparative study of 247 cases of conventional PAP smears and rehydrated air-dried PAP smears were made during the study period.

AGE

Age group of patients in the study group ranged from 20 to 80 years with the youngest patient aged 20 years and the oldest 80 years with a mean age of 36.8 years. Majority of the patients were in the age group of 31-40 years. **(Table 5)**

Table 5: Age wise distribution of cases.

Age group (Years)	Number of cases (N)	Percent (%)
20-30	82	32
31-40	96	39
41-50	51	21
51-60	14	6
61-70	2	1
71-80	2	1
Total	**247**	**100**

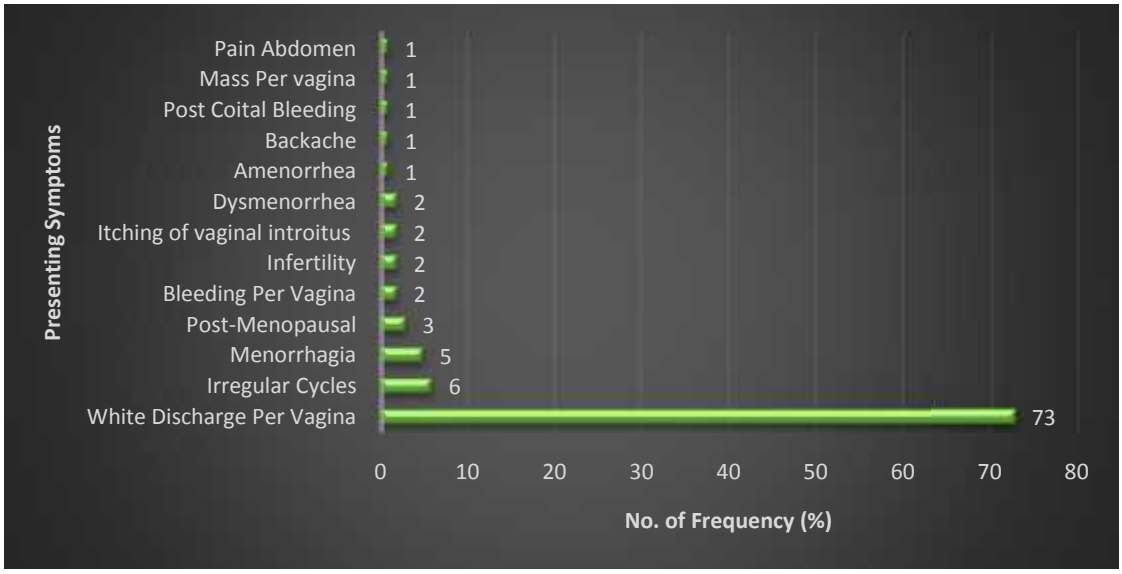
CLINICAL PRESENTATION

The most common clinical presentation was white discharge per vagina. **(Table 6 and 7)**

Table 6: Distribution of Presenting Symptoms (n = 247)

Presenting Symptoms	Number of cases (N)	Percent (%)
White Discharge Per Vagina	180	73
Irregular Cycles	13	6
Menorrhagia	11	5
Post-Menopausal	8	3
Bleeding Per Vagina	6	2
Infertility	6	2
Itching of vaginal introitus	6	2
Dysmenorrhea	5	2
Amenorrhea	3	1
Backache	3	1
Post Coital Bleeding	3	1
Mass Per vagina	2	1
Pain Abdomen	1	1

Table 7: Bar diagram showing distribution of Presenting Symptoms (percentage)



SAMPLE ADEQUACY

Out of 247 cases adequate samples were obtained in 229 (92.7%) cases of conventional PAP smear and 241 (97.6%) cases of Rehydrated air-dried PAP smear. **(Table 8, Table 9)**

Table 8: Adequacy of samples in Conventional and Rehydrated air-dried PAP smear.

Smear	C-PAPS		RADPS		p value
	No. of cases	Percent	No. of cases	Percent	
Satisfactory	229	92.7	241	97.6	
Unsatisfactory	18	7.3	6	2.4	**P=0.0213***
Total	**247**	**100**	**247**	**100**	

Table 9: Bar diagram showing adequacy of samples in Conventional and Rehydrated Air-dried PAP smear. (percentage)

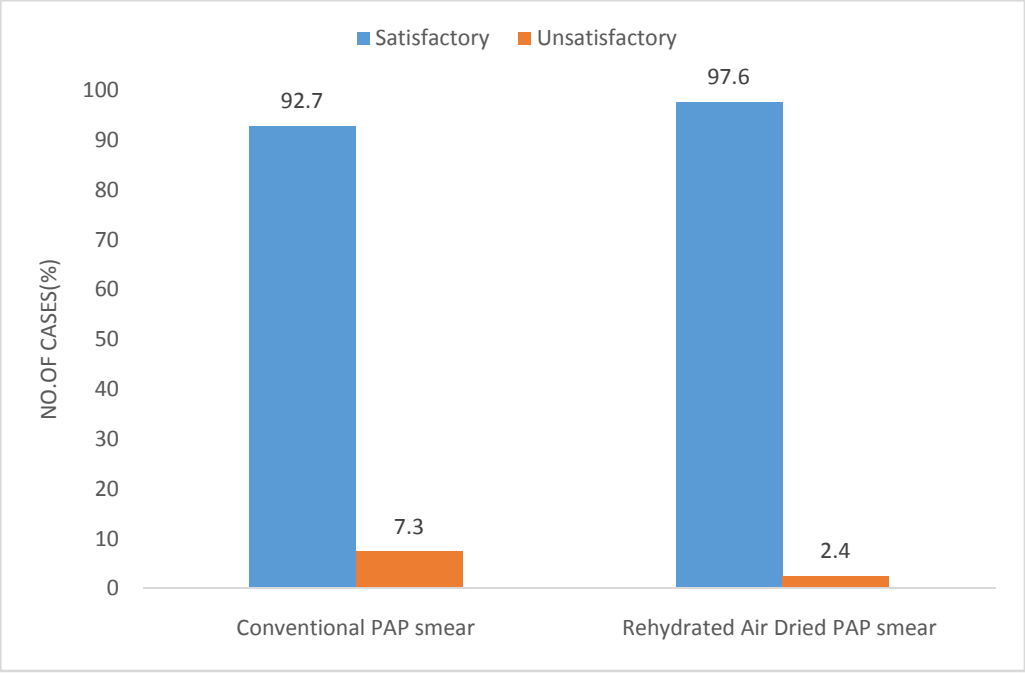


Table 10: Cytomorphological diagnosis in C-PAP smear and RAD PAP smear.(n = 247)

Morphological Distribution	C-PAPS		RADPS		p value
	No. of cases	Percent	No. of cases	Percent	
Inflammatory Smear	162	65.6	167	67.6	0.7039
Normal study	37	15	40	16.1	0.7127
Unsatisfactory	18	7.3	6	2	0.021*
Bacterial vaginosis	8	3.2	10	4.7	0.6311
Atrophic smear	4	1.6	6	2.4	0.5229
Trichomonas Vaginalis	2	0.8	2	0.8	1
Candida	2	0.8	2	0.8	1
Oestrogenic effect	1	0.4	1	0.4	1
ASCUS-US	3	1.2	3	1.2	1
AGC-NOS	2	0.8	2	0.8	1
LSIL	1	0.4	1	0.4	1
HSIL	5	2.1	4	1.6	0.7034
ASCUS-H	2	0.8	2	0.8	1
Squamous Cell carcinoma	0	0	1	0.4	0.3168

Table 11: Bar diagram showing comparison of pre-neoplastic and neoplastic squamous and glandular epithelial abnormalities diagnosed on C-PAP smear and RAD PAP smear.

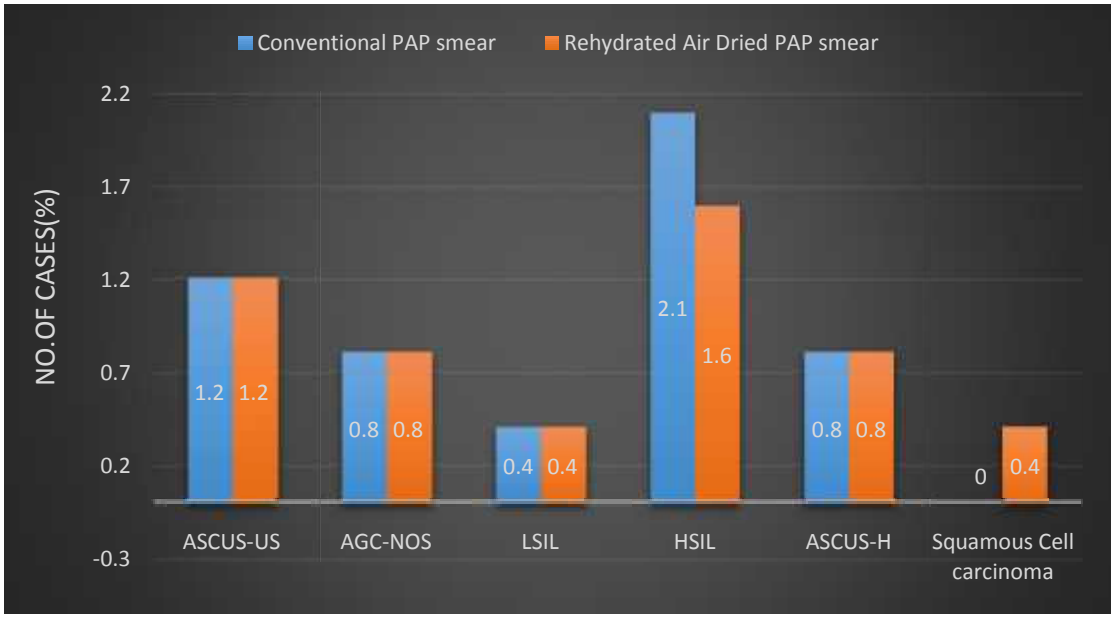
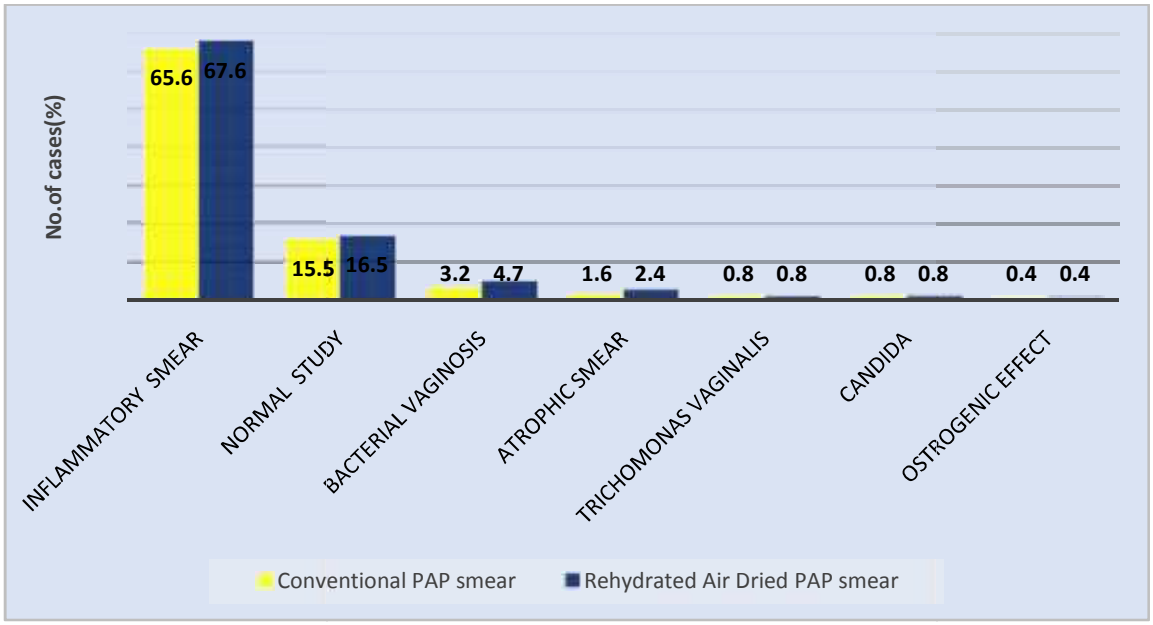


Table 12: Bar diagram showing comparison of non- neoplastic cases diagnosed on C-PAP smear and RAD PAP smear.



Out of 247 rehydrated air dried cervical smears studied, 234 (95%) were reported as non-neoplastic lesions and 13(5%) cases were reported as preinvasive/preneoplastic/neoplastic lesions.

In C-PAPS cytological diagnosis was not possible in 7.3% cases however in RAD PAP smear in only 2.4% cases diagnosis was unsatisfactory. By both technique common lesion diagnosed on cervical cytology was inflammatory smear (162 cases) followed by normal study (38 cases), bacterial vaginosis (8 cases), atrophic smear (4 cases), candidal infestation (2 cases), trichomonas vaginalis (2 cases) and Oestrogenic effect (1 case).

Two cases of atrophic smear and bacterial vaginosis were diagnosed as unsatisfactory on C-PAP smear.

By the rehydrated air-dried technique, the most common non-neoplastic lesion was inflammatory smear (167 cases) followed by normal study (41 cases), bacterial vaginosis (10 cases), atrophic smear (6 cases), candida infestation (2 cases), trichomonas vaginalis (2 cases).

Diagnosis of HSIL, ASCUS, AGC-NOS, ASC-H and LSIL was rendered in 4, 3,2,2 and 1case respectively by both C-PAP smear and RAD PAP smear.

However, 1 case of SCC were diagnosed on RAD PAP smear was reported as HSIL on C-PAP smear. **(Table 10, 11 and 12)**

In present study, histopathological correlation was available in three cases. Out of three cases, one was diagnosed as LSIL and another was diagnosed as ASC-H on C-PAP smear and on RAD PAP smear. On cervical biopsy, both cases of LSIL and ASC-H were diagnosed as chronic non-specific inflammation. In one case discordance was

observed between C-PAPS and RADPS. On C-PAPS it was diagnosed as HSIL and on RADPS it was diagnosed as SCC. On histopathological study of this case, it was diagnosed as large cell non-keratinizing squamous cell carcinoma.

Table 13: Comparison of general cytomorphological features in C-PAP Smear and RAD PAP smear (n = 247)

Sr. No.	Cytomorphological features	Conventional PAP smear		Air Dried PAP smear		P value
		N	Percent (%)	N	Percent (%)	
1	**Cellularity**					
	Low	46	19	24	10	
	Intermediate	82	33	68	28	**P=0.0015***
	High	119	48	155	62	
2	**Cytolysis**					
	Present	27	11	6	2	
	Absent	220	89	241	98	**P=0.0002***
3	**Air-drying artifact**					
	Present	36	15	11	4	
	Absent	211	85	236	96	**P=0.0001***
4	**Red blood cell background**					
	Present	104	42	4	2	
	Absent	143	58	243	98	**P=0.015***
5	**Cell border**					
	Distinct	212	86	239	97	**P<0.0001***
	Indistinct	35	14	8	3	
6	**Cytoplasmic staining**					
	Unsatisfactory	14	6	7	3	**p=0.1797**
	Satisfactory	233	94	240	97	

Table 14: Bar diagram showing comparison of general cytological features in C-PAP smear and Air-dried PAP smear

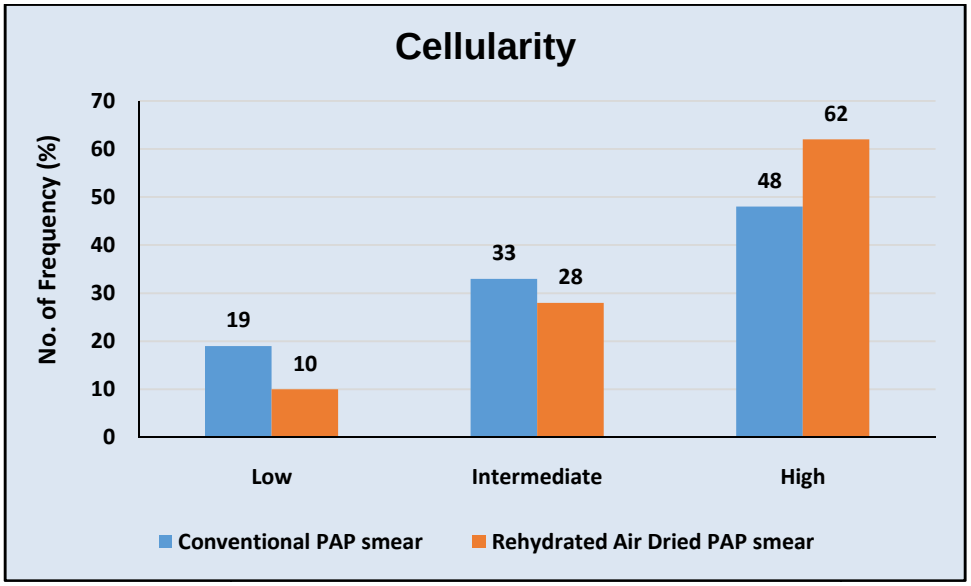


Table 15: Bar diagram showing comparison of cytomorphological features – Cytolysis, Air-drying Artifact and Red blood cell Background.

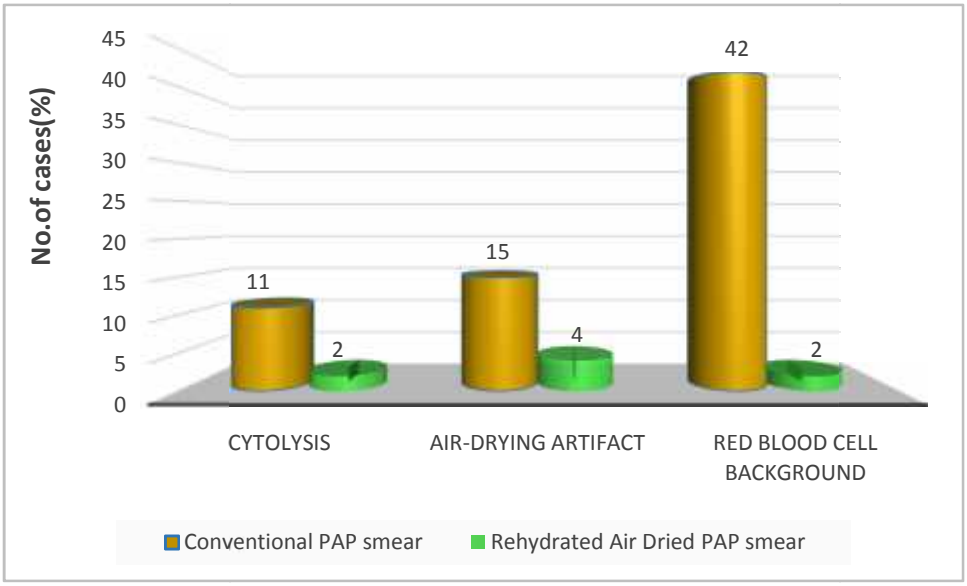


Table 16: Bar diagram showing comparison of Cytomorphological features – Cell border

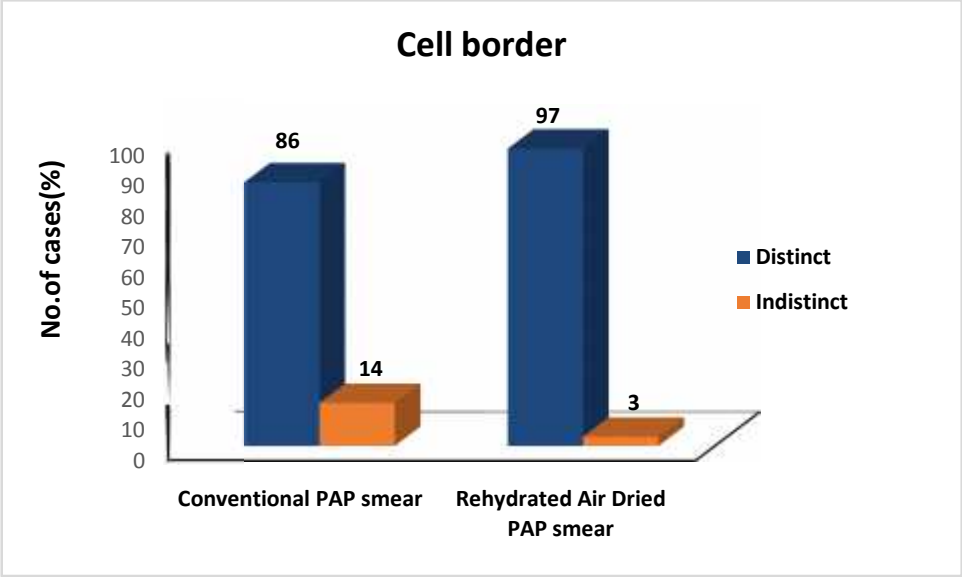
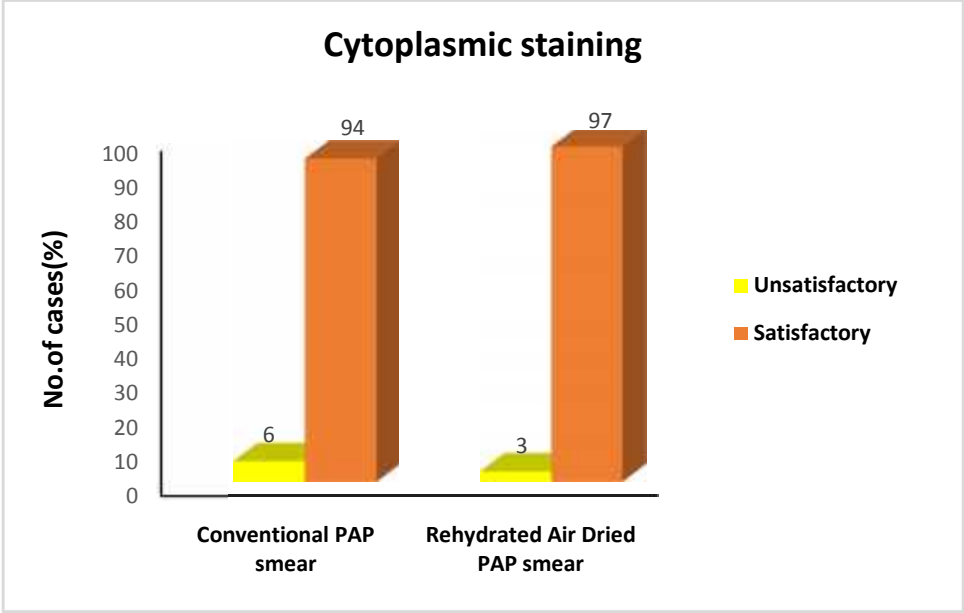


Table 17: Bar diagram showing comparison of cytomorphological features – Cytoplasmic staining



Cellularity was high in most of the RAD PAP smears as compared to C-PAP smear. Cytolysis was more in C-PAPS compared to RADPS. Air drying artifacts were more in C-PAPS compared to RADPS. Red blood cell background was absent in most of the RADPS.

Cell borders were more distinctly seen in RAD PAP smear. Cytoplasmic staining was satisfactory in more number of cases of RAD PAP smears. **(Table 13-17)**

Table 18: Comparison of nuclear features in squamous and endocervical cells on C-PAP smear and Air-dried PAP smear. (n = 247)

Sr. No.	Morphological features	C-PAPS		RADPS		P value
		N	Percent (%)	N	Percent (%)	
1	**Nuclear border**					
	Squamous cells					
	Distinct	220	89	238	96	
	Indistinct	27	11	9	4	**P=0.0019***
	Endocervical cells					
	Distinct	67	91	70	97	**P=0.0933**
	Indistinct	7	9	2	2	
2	**Nuclear chromatin**					
	Squamous cells					
	Crisp	216	87	241	98	
	Hazy	31	13	6	2	**P=0.0001***
	Endocervical cells					
	Crisp	60	81	68	94	**P=0.0141***
	Hazy	14	19	4	6	

Table 19: Bar diagram showing comparison of Nuclear border of squamous cells.

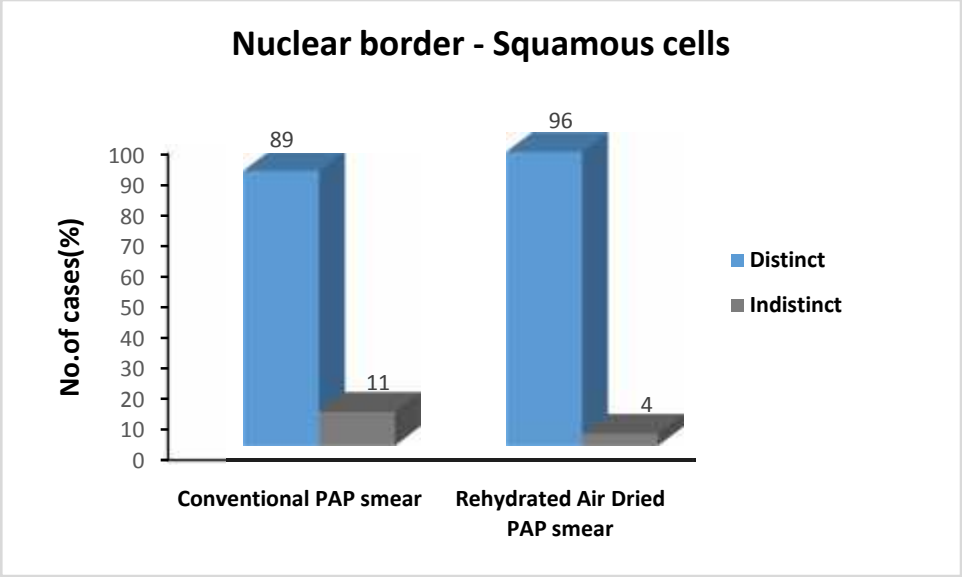


Table 20: Bar diagram showing comparison of Nuclear border of endocervical cells.

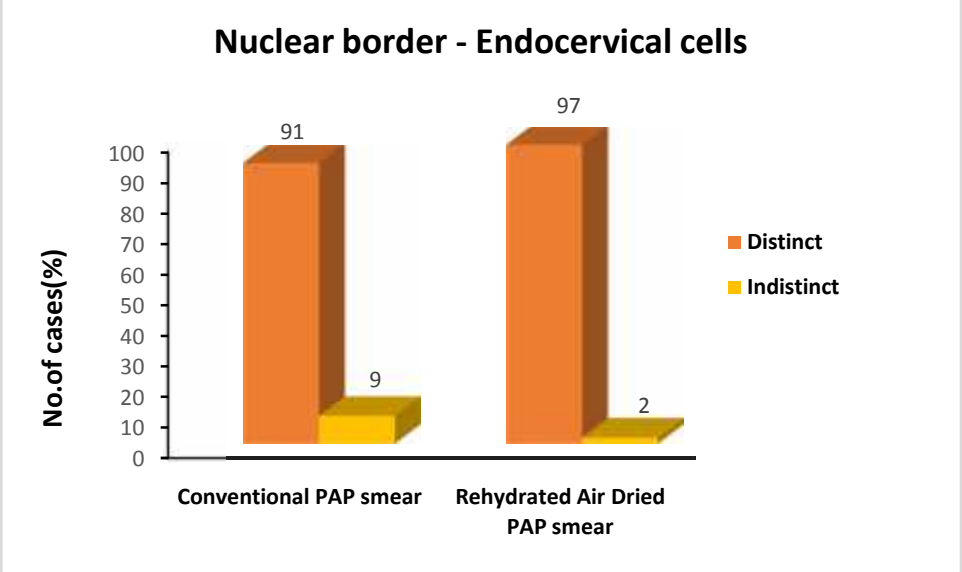


Table 21: Bar diagram showing comparison of Nuclear chromatin of squamous cells.

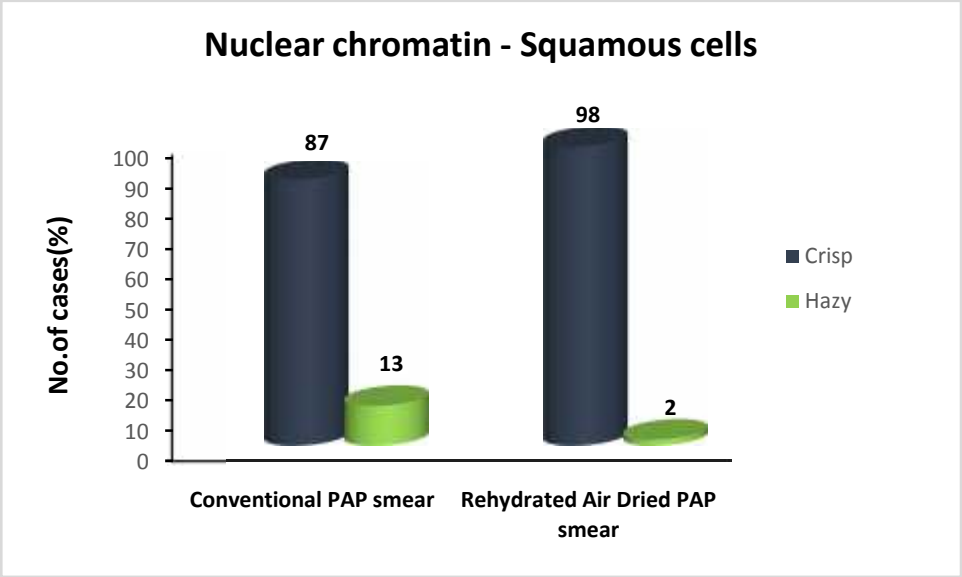
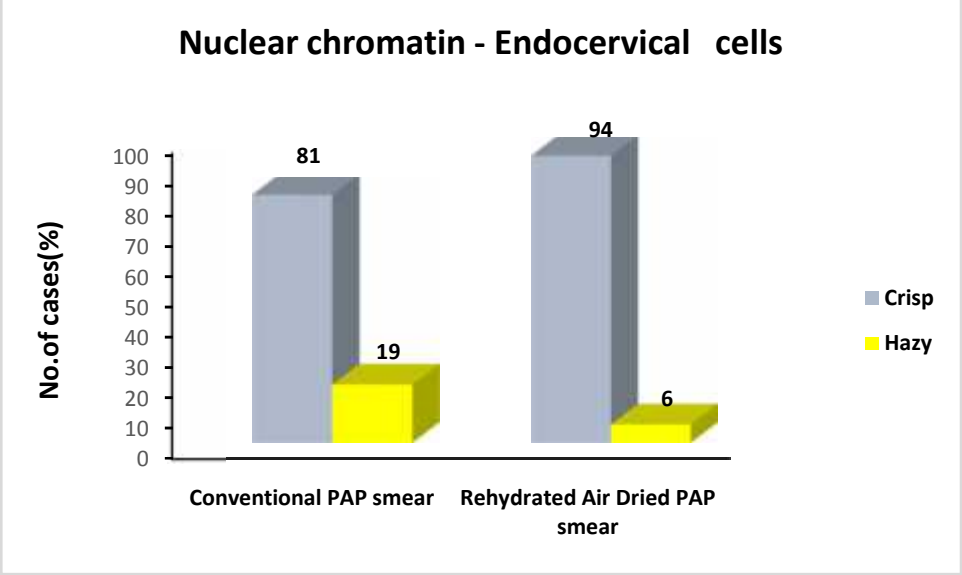


Table 22: Bar diagram showing comparison of Nuclear chromatin of endocervical cells.



Nuclear borders of squamous and endocervical cells was more distinct in RAD PAP smear and also crisp nuclear chromatin was found more in RAD PAP smears as compared to C-PAP smears.**(Table 18-22)**

PHOTOMICROGRAPH'S

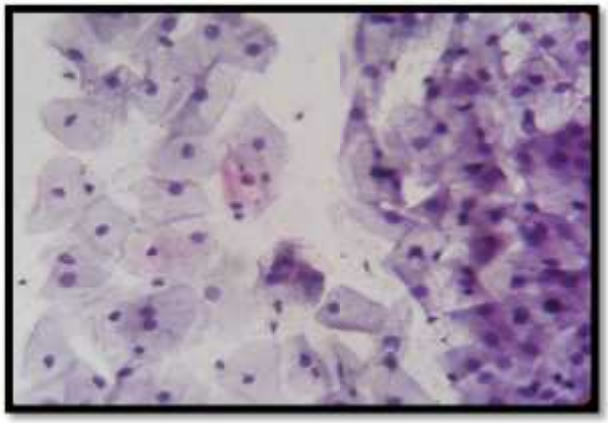


Fig. 3 -Photomicrograph showing Normal study in C-PAPS. (PAP Stain, 400x)

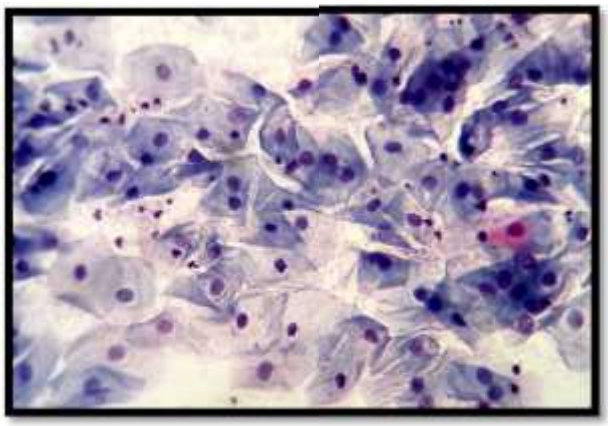


Fig. 4 -Photomicrograph showing Normal study in RADPS. (PAP Stain, 400x)

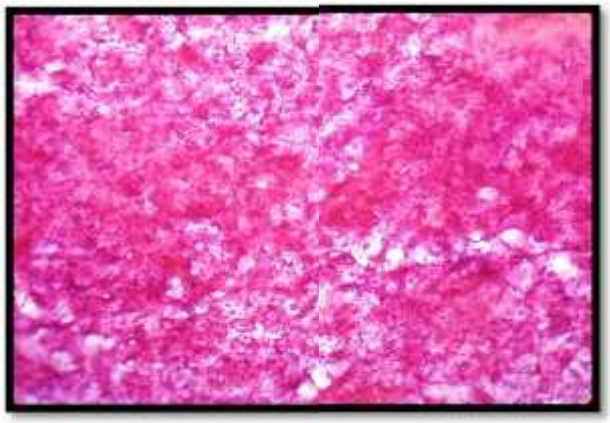


Fig. 5 -Photomicrograph of inflammatory smear showing cellular obscuring by inflammatory cells in C-PAPS. (PAP Stain,

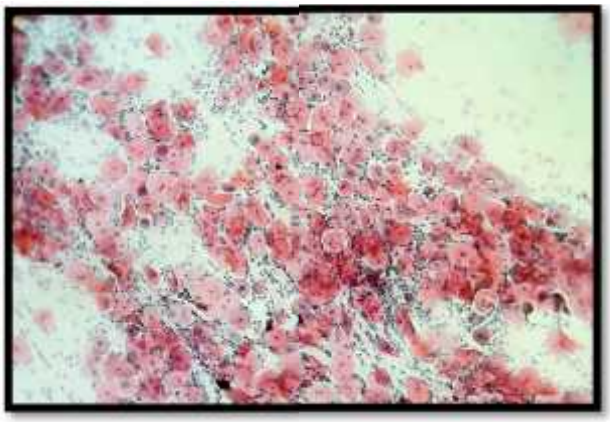


Fig. 6 -Photomicrograph showing inflammatory smear in RADPS. (PAP Stain, 100x)

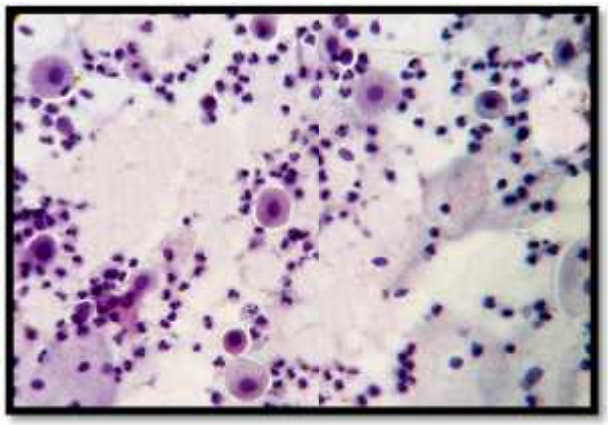


Fig. 7 -Photomicrograph showing Atrophic Smear in C-PAP (PAP Stain,

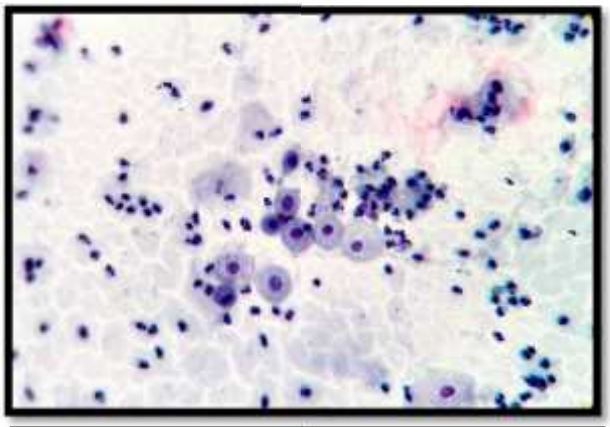


Fig. 8 -Photomicrograph showing Atrophic Smear in RADPS. (PAP Stain,

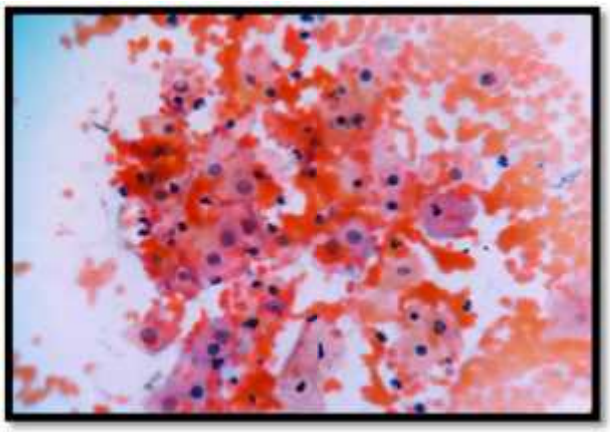


Fig. 9 -Photomicrograph showing hemorrhagic background obscuring the sperm morphology in C-PAPS. (PAP

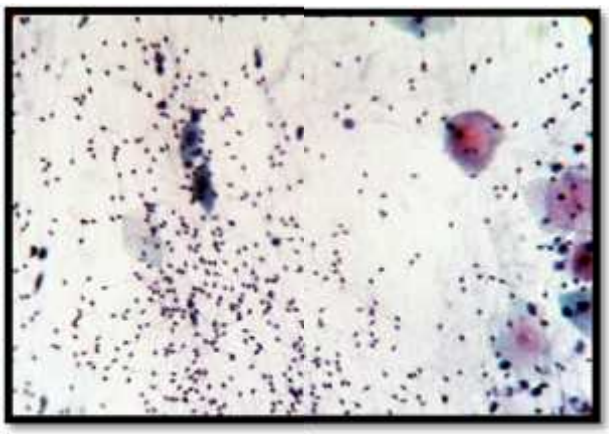


Fig. 10 -Photomicrograph showing sperms in RADPS which were obscured by RBCs in RADPS. (PAP Stain, 400x)

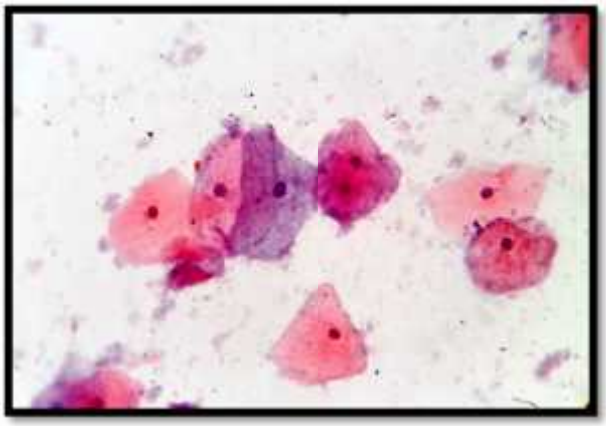


Fig. 11 -Photomicrograph showing Bacterial Vaginosis in C-PAPS. (PAP Stain, 400x)

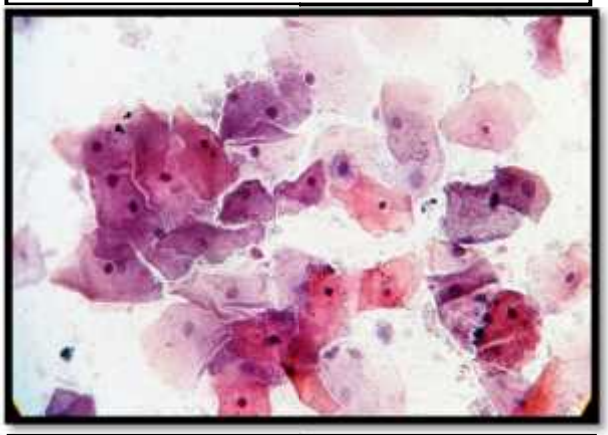


Fig. 12 -Photomicrograph showing Bacterial Vaginosis in RADPS. (PAP Stain, 400x)

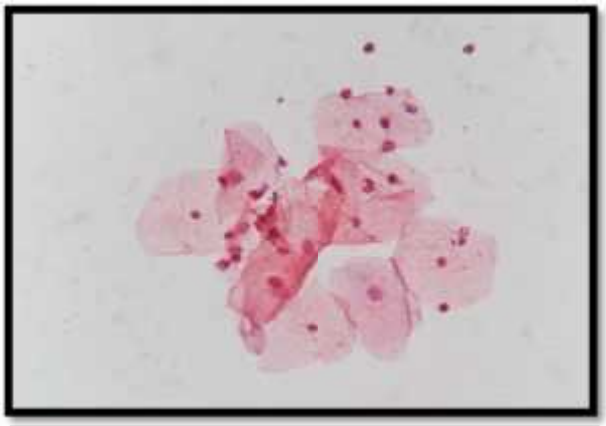


Fig. 13 -Photomicrograph showing low cellularity and air-drying artifact in C-PAPS. (PAP Stain, 400x)

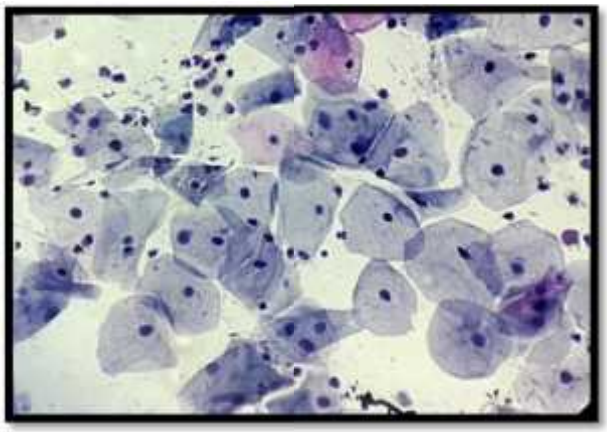


Fig. 14 -Photomicrograph showing high cellularity and absence of air drying artifact in RADPS.(PAP Stain, 400x)

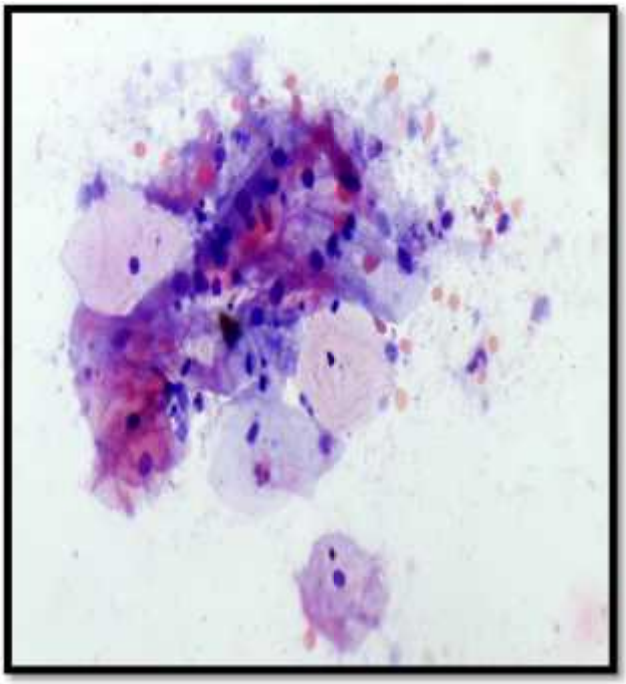


Fig. 15 -Photomicrograph showing candidal infection in C-PAPS. (PAP Stain, 400x)

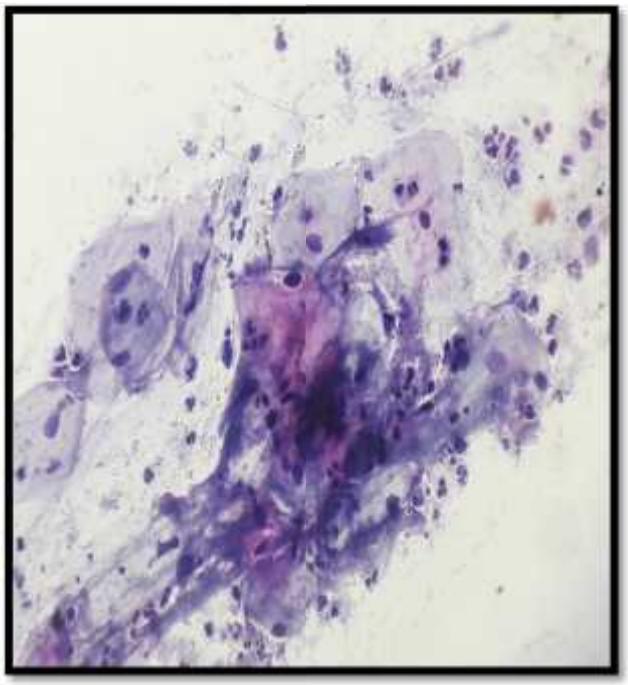


Fig. 16 -Photomicrograph showing candidal infection in RADPS.(PAP Stain, 400x)

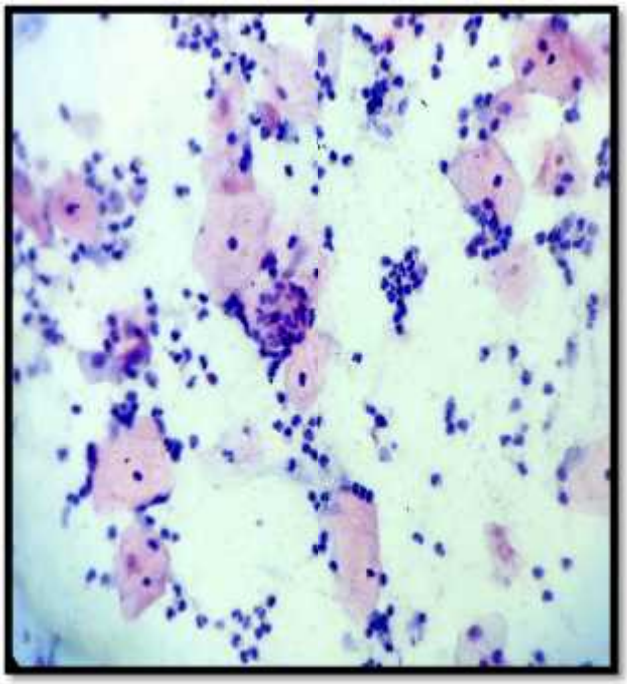


Fig. 17 -Photomicrograph showing Trichomonas Vaginalis in C-PAPS. (PAP Stain, 400x)

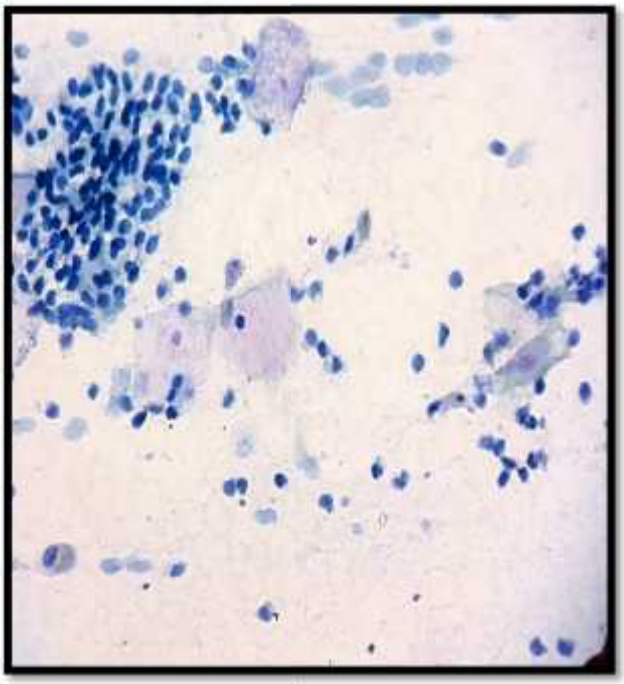


Fig. 18 -Photomicrograph showing Trichomonas Vaginalis in RADPS. (PAP Stain, 400x)

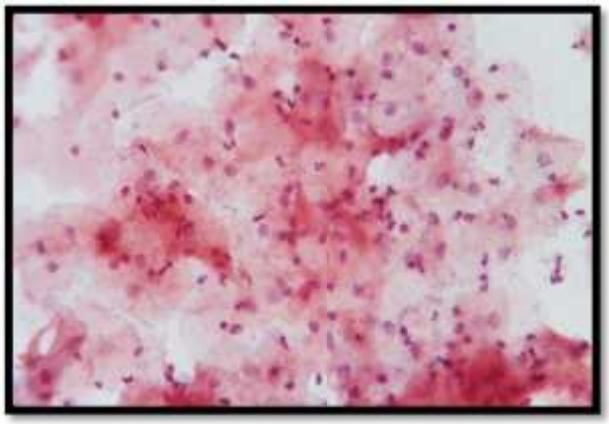


Fig. 19 -Photomicrograph showing indistinct cell border, unsatisfactory cytoplasmic staining and cytolysis in C-PAPS.(PAP Stain, 400x)

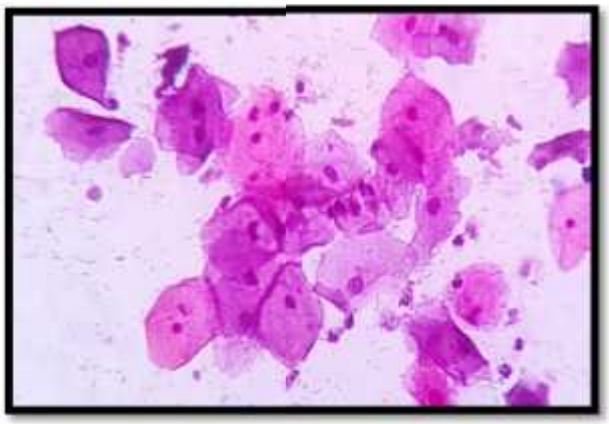


Fig. 20 -Photomicrograph showing distinct cell border, satisfactory cytoplasmic staining and absence of cytolysis in RADPS. (PAP Stain, 400x)

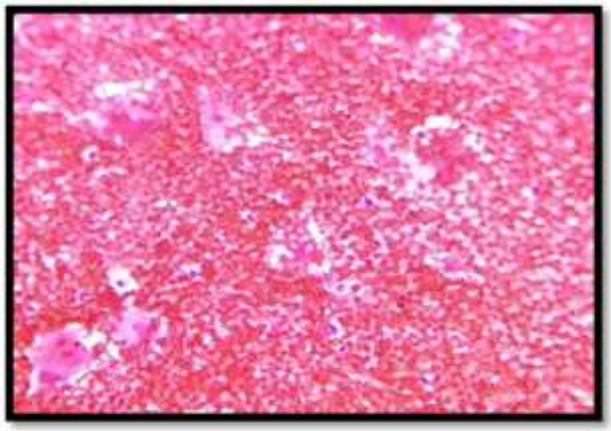


Fig. 21 -Photomicrograph showing RBCs obscuring visualization of cells in C-PAPS. (PAP Stain, 400x)

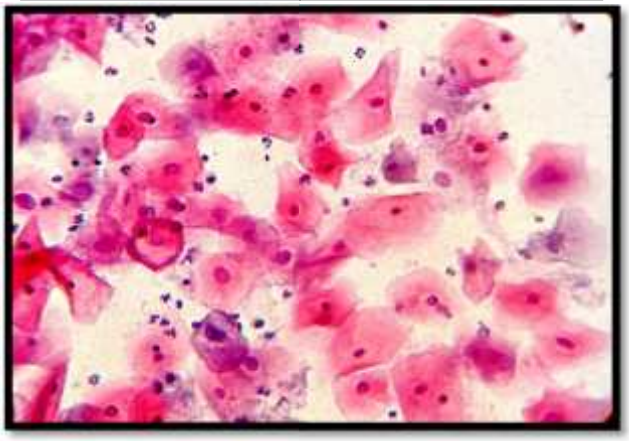


Fig. 22 -Photomicrograph showing clean background in RADPS. (PAP Stain, 400x)

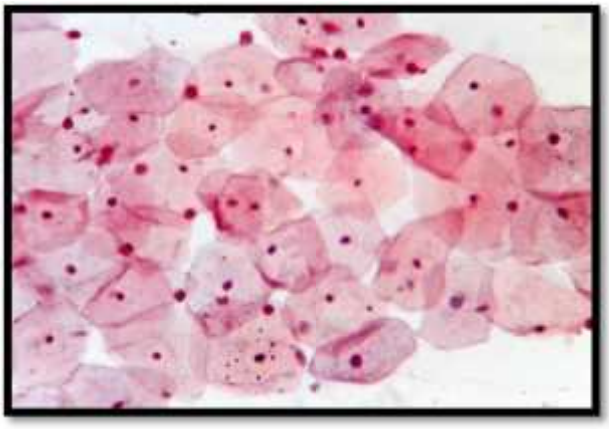


Fig. 23 -Photomicrograph showing indistinct nuclear border and hazy nuclear chromatin of squamous cell C-PAPS.(PAP Stain, 400x)

Fig. 24 -Photomicrograph showing distinct nuclear border and crisp chromatin of squamous cell RADPS. (PAP Stain, 400x)

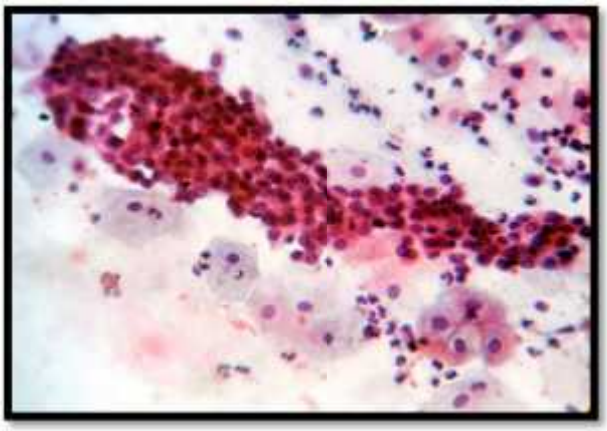


Fig. 25 -Photomicrograph showing indistinct nuclear border and hazy nuclear chromatin of endocervical cells in C-PAPS. (PAP Stain, 400x)

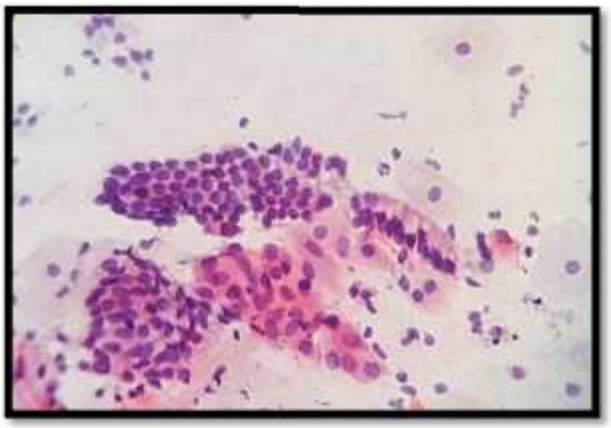


Fig. 26 -Photomicrograph showing distinct nuclear border and crisp chromatin of endocervical cells in RADPS. (PAP Stain, 400x)

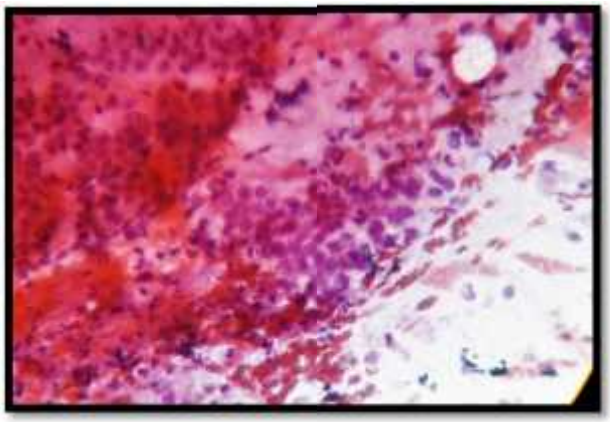


Fig. 27 -Photomicrograph showing AGCNOS in C-PAPS. (PAP Stain, 400x)

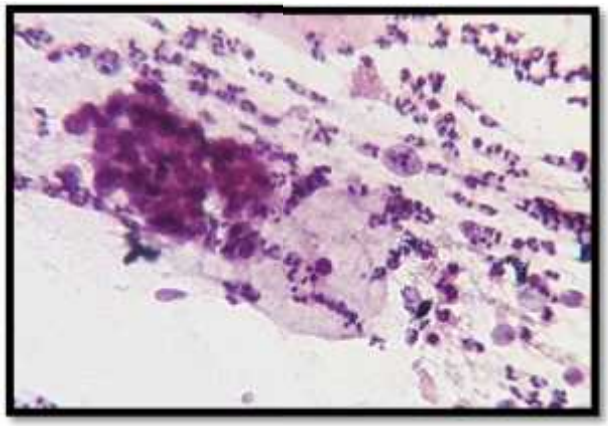


Fig. 28 -Photomicrograph showing AGCNOS in RADPS. (PAP Stain, 400x)

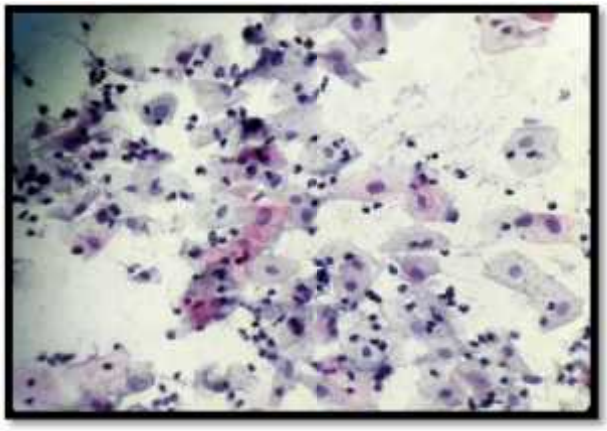


Fig. 29 -Photomicrograph showing ASCUS in C-PAPS. (PAP Stain, 400x)

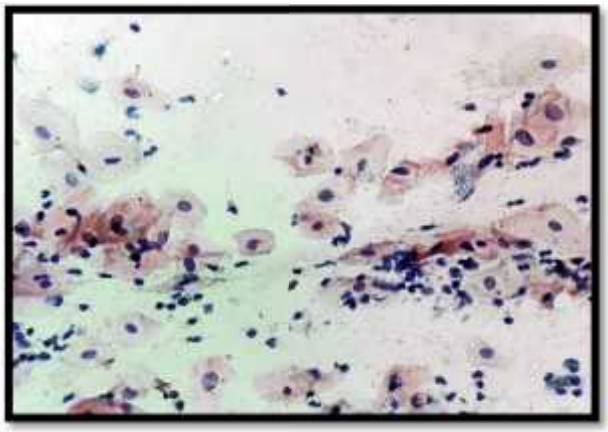


Fig. 30 -Photomicrograph showing ASCUS in RADPS. (PAP Stain, 400x)

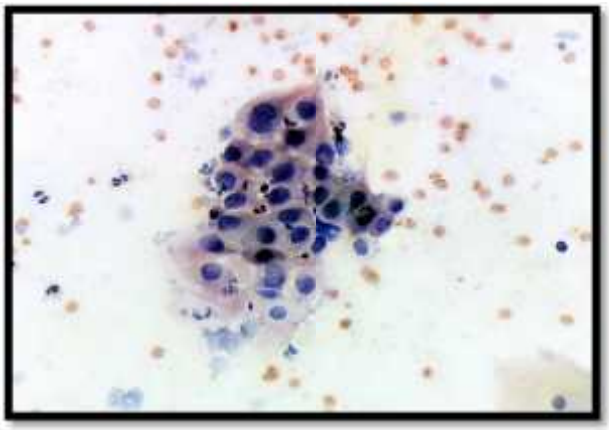


Fig. 31 -Photomicrograph showing ASC-H in C-PAPS. (PAP Stain, 400x)

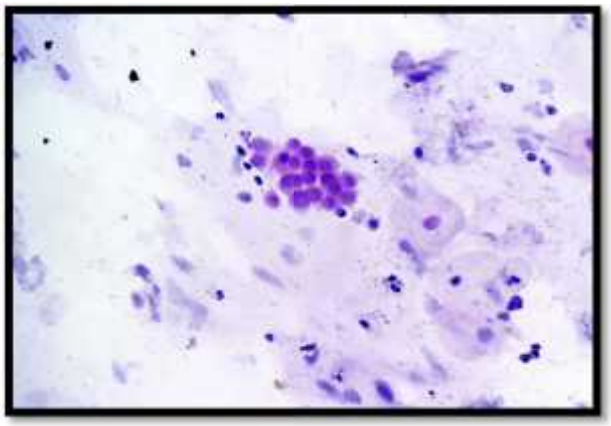


Fig. 32 -Photomicrograph showing ASC-H in RADPS. (PAP Stain, 400x)

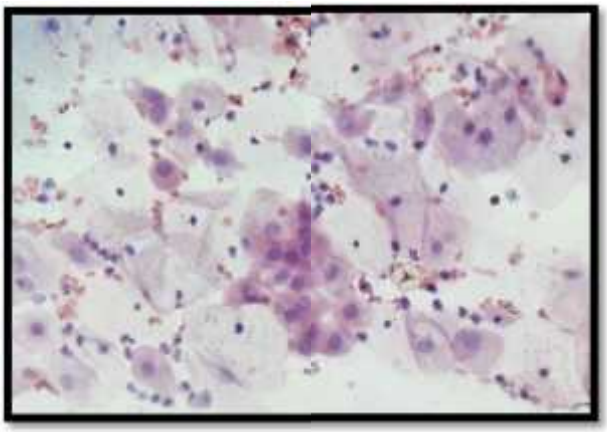


Fig. 33 -Photomicrograph showing LSIL in C-PAPS. (PAP Stain, 400x)

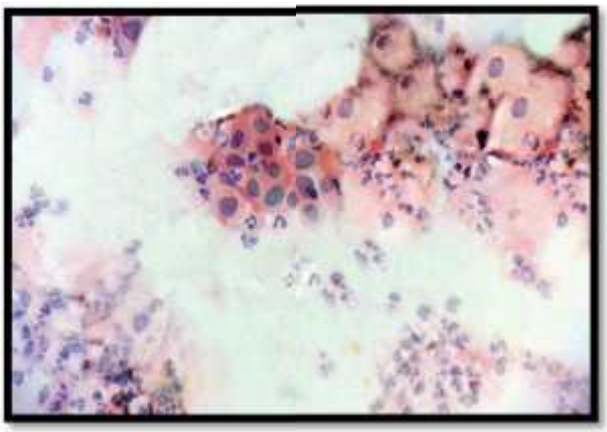


Fig. 34 -Photomicrograph showing LSIL in RADPS. (PAP Stain, 400x)

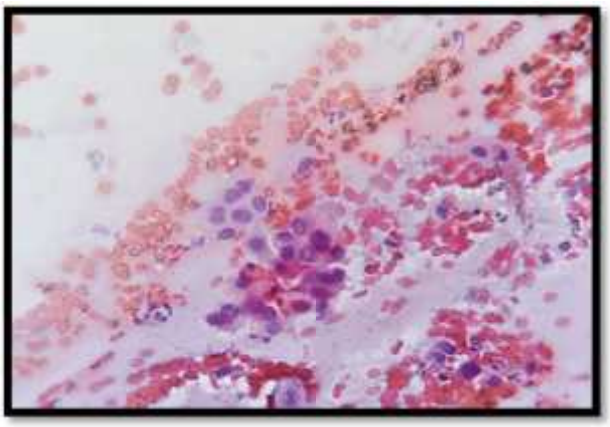


Fig. 35 -Photomicrograph showing HSIL in C-PAPS. (PAP Stain, 400x)

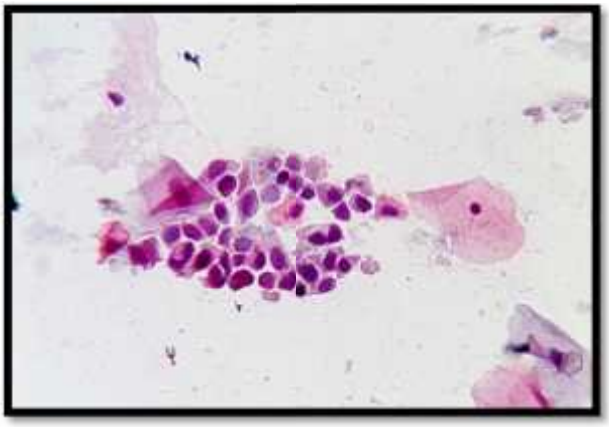


Fig. 36 Photomicrograph showing HSIL in RADPS. (PAP Stain, 400x)

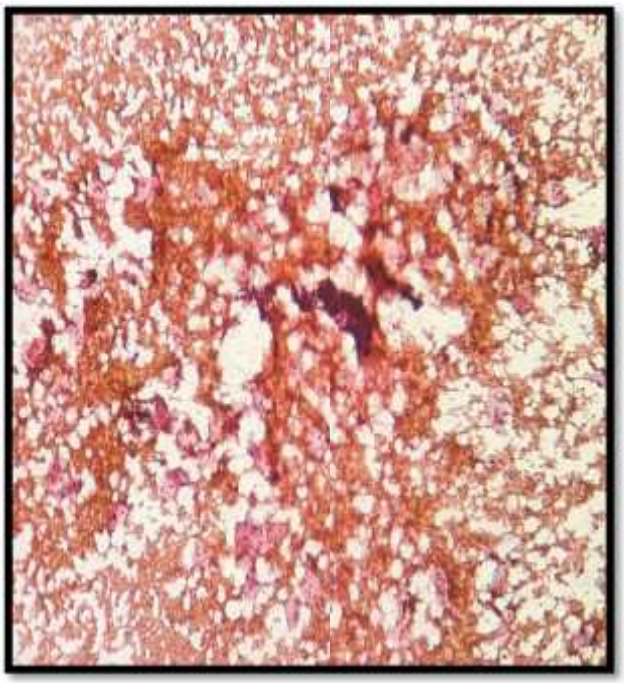


Fig. 37 -Photomicrograph of C-PAP smear diagnosed as HSIL showing RBCs in the background. (PAP Stain, 100x)

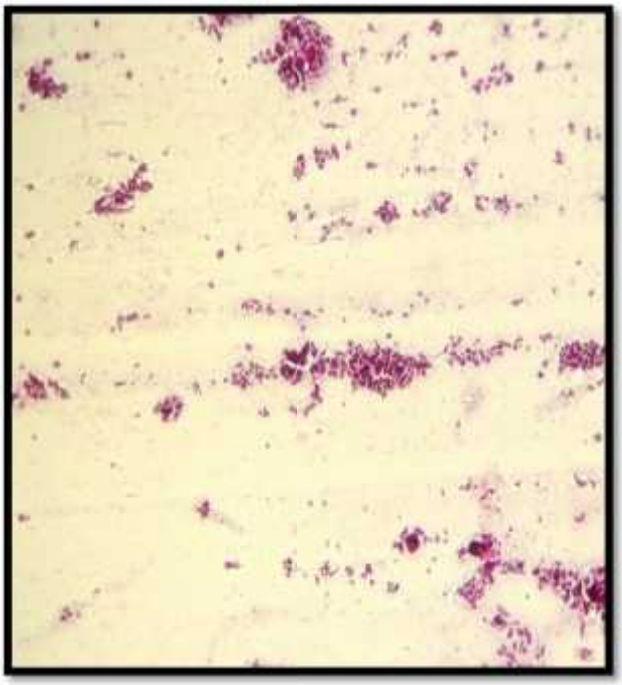


Fig. 38 -Photomicrograph of RADPS diagnosed asSCC.(PAP Stain, 100x)

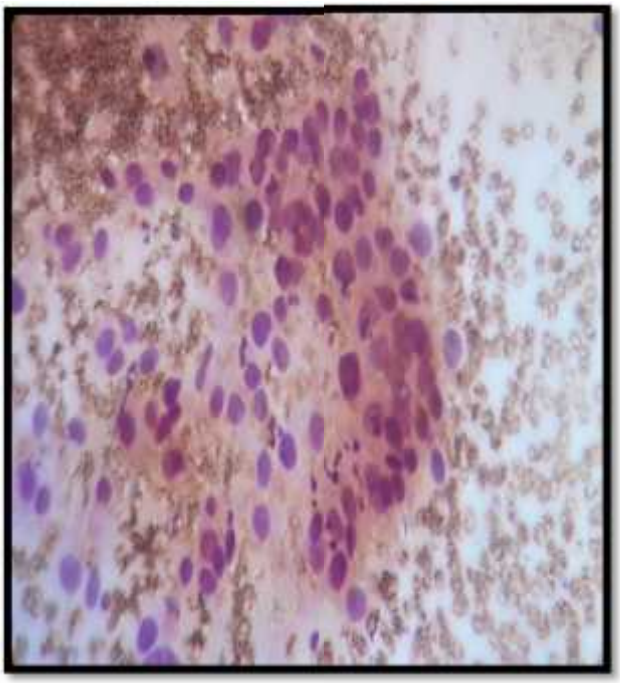


Fig. 39 -Photomicrograph of C-PAP smear diagnosed as HSIL showing RBCs in the background. (PAP Stain, 400x)

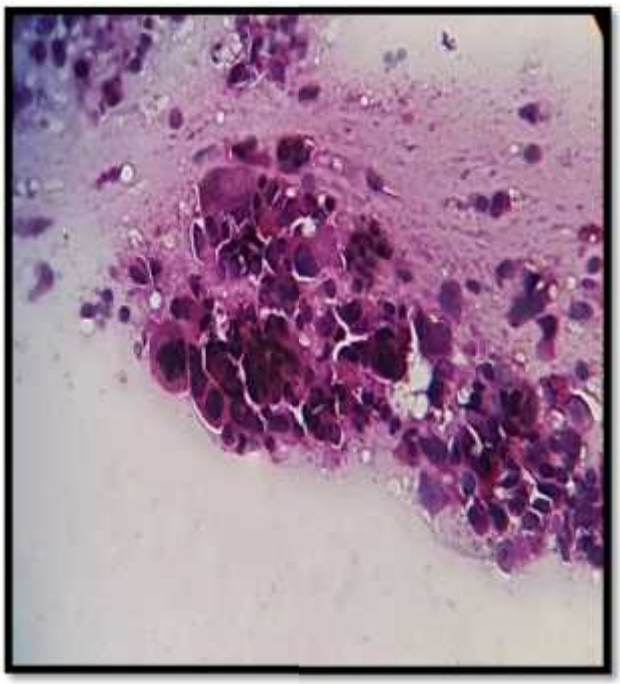


Fig. 40 -Photomicrograph of RADPS diagnosed asSCC.(PAP Stain, 400x)

DISCUSSION

PAP smear is simple, non-invasive and effective method for screening as well as diagnosing cervical abnormalities. It is proved by the decrease in mortality rate of cervical cancer in developed countries. In developing countries like in Asian and African continent, the morbidity and mortality caused by cervical cancer is more, especially in the rural settings. At the time of presentation, most cases (85%) present in advanced and late stages.[1,2] Screening programs in the resource poor settings as well as increasing the accuracy of PAP smear reporting can help to curb down the incidence of cervical cancer.[4]

In most of the rural health settings like primary health center, where paramedical staff play a pivotal role in PAP smear preparation should have proper knowledge and should also know the importance of proper fixation methods. Most of the times PAP smears are not collected due to lack of proper facility for preservation.[4,6]

Various studies were done, to find out whether RAD technique method can replace conventional wet fixation technique in PAP smears. These authors used several rehydrating agents like hypotonic solutions, normal saline, tap water and aqueous glycerin. Normal saline was considered as best rehydrating fluid as it was simplest, cheapest and easily available in the laboratories.[4,20,21,23] In our study we used normal saline as a rehydrating agent.

The mean age of the patient in the present study was 36.8yrs. Age of the youngest patient was 20yrs and oldest was of 80yrs. Similar observations were noted in study done by Jaiwong K *et al*[5] and Rupinder *et al*[4].

Out of 247 paired PAP smears, 97% of RAD PAP smears and 92% of C-PAP smears where found to be satisfactory for evaluation. Only 6 cases (2.4%) of RAD PAP smears were found to be unsatisfactory, however in C-PAP smears 18 cases (7.3%) were unsatisfactory. These findings were similar to observation found in studies conducted by Rupinder K. *et al*[4] and Ganesan *et al*[7]. **(Table 23)**

The possible explanation for more satisfactory material and more cellularity in RAD PAP smear was that air-drying leads to better adhesion of cells to the slide. Also, there was loss of material in wet fixation from thick smear while immersing in fixative. In air drying this loss of material in thick smear is not seen.[27]

Table 23: Comparison of Adequacy of samples in Conventional and Rehydrated air-dried PAP smear with other studies.

Authors	**RADPS (%)**	**C-PAP (%)**
Present study	97	92
Rupinder K. *et al*[4]	90.5	90.5
Ganesan *et al*[7]	96	94

Overall cellularity was high in 62% cases in RADPS however in only 48% cases high cellularity was noted in C-PAPS. Low cellularity was more in C-PAPS that is in 46%. In RADPS in only 24% cases low cellularity was observed. Gupta *et al*[6]in their study had similar findings. RADPS had more cellularity owing to less loss of specimen while fixation and less fixation artifact per se, less obscuring of cells by RBCs and inflammatory cells. As the Air-dried smears were made at leisure, a thin and uniform

preparation of RADPS was possible compared to C-PAPS wherein smears are hurriedly prepared as they are supposed to fix immediately.[26-28]

In this study the optimum time for air drying for RAD PAP smears was 30-120mins to avoid air drying artifact. Maximum duration mentioned in various studies was up to 4 days. However, these authors also mentioned that air drying artifact, cytolysis and contamination by organisms was more if smears were kept for longer duration.[7,23]

Air drying artifact was seen in 11% and 15% of RADPS and C-PAPS respectively. As the RADPS were subjected to rehydration, lesser air-drying artifacts were seen in RADPS. While in study conducted by Jaiwong K *et al*[5] air drying artifact was 44.76% in RADPS and 33.72% in C-PAPS smears.

In the present study cytolysis was more amounting to 11% in C-PAPS whereas it was 2% in RADPS. A study conducted by Zare-Mirzaie *et al*[29]showed more cytolysis in C-PAPS amounting to 27.4% and in RADPS it was 19.7%. These findings are correlating with our study findings with high percentage of cases showing cytolysis in C-PAPS. However, contrasting result that is more cytolysis in RADPS was seen in study conducted by Gupta S *et al*[6] and Jaiwong K *et al*[5] wherein cytolysis was observed in 17.8% and 47.67% in RADPS and 15.7% and 34.88% in C-PAPS respectively, however the difference was statistically insignificant.

In the present study smears were rehydrated for 30 secs to lyse the RBCs and avoid air drying artifacts. Hemolysis was evident grossly as pinkish appearance of normal saline after immersing heavily blood-stained smears in normal saline due to lysis RBCs. In the present study Red cell background was seen in only 2% of RADPS as

compared to 42% of C-PAPS. Similar findings were observed in study conducted by various authors.[5-7,29] RBC Background in study done by Gupta S*et al*[6],Ganesan *et al*[7],Jaiwong K *et al*[5] and Zare-Mirzaie *et al*[29]was seen in 12%, 31%, 13.94%, 29.1% C-PAPS compared to 3%, 8%, 3.49% and 6% in RADPS. These authors in their study noticed lysis of majority of the background RBCs with only few intact RBCs.[6,7,23.28] Mechanism of RBC lysis was explained in a study conducted by Gill GW *et al*[28]. As per these authors clean background due to RBC lysis accounted for more number of satisfactory specimens in rehydrated air-dried technique. The advantage of cleaner background was that the infectious agents were distinctly identifiable and also easy to pick up on the RADPS. Also, it was easy to diagnose precursors, pre-neoplastic and neoplastic conditions.[4,5,6,7,21,23]

Distinct cell border was seen in 97% RADPS and 86% of C-PAPS. This might be due to more number of air drying artifacts and cytolysis in C-PAPS.

Moreover, in our study we found that size of squamous cells was increased which has been documented in various studies. [4,5,6,7,21,23] Therefore, there was ease in diagnosing epithelial as well as glandular cell abnormalities on RADPS.

Cytoplasmic staining was found to be superior in RADPS as compared to conventional C-PAPS. Unsatisfactory staining of smears was 6 % in C-PAPS which was more as compared to RADPS where only in 3% cases unsatisfactory staining was noted. In study conducted by Ganesan *et al*[7], Gupta S*et al*[6],Jaiwong K *et al*[5] and Zare-Mirzaie *et al*[29] satisfactory cytoplasmic staining was in 59.5%, 79%, 100% and 62.4% C-PAPS smear and 60.6%, 87.8%, 100% and 65.8% in RADPS. Zare-Mirzaie *et al*[29]in their study observed excellent cytoplasmic staining in 37.6%cases of RADPS compared to 34.2%

cases ofC-PAPS smears. Factors favoring better cytoplasmic staining in RADPS were better penetration as well as fixation of smears due to lysis of RBCs and less obscuring by inflammatory cells leading to thin and uniform smears.[6,7,8,22]**(Table 24)**

Table 24: Comparison of general cytomorphological features in C-PAPS Smear and RADPS with other studies.

Parameter	Present study (n=247)		Ganesan *et al*[7] (n=397)		Gupta S *et al* [6] (n=950)		Jaiwong K *et al*[5] (n=172)		Zare-Mirzaie *et al*[29] (n=117)	
	C-PAP	RADS	C-PAP	RADS	C-PAP	RADS	C-PAP	RADS	C-PAP	RADS
Cellularity										
Low	19%	10%	-	-	4.9%	5.9%	11.04%	8.13%	-	-
Intermediate	33%	28%	-	-	14.9%	15.9%	81.39%	84.30%	-	-
High	48%	62%			80.2%	78.2%	7.55%	7.55%	56.4%	58.9%
Cytolysis										
Present	11%	2%	-	-	15.7%	17.8%	34.88%	47.67%	27.4%	19.7
Red blood cell background										
Present	42%	2%	31%	8%	12%	3%	13.94%	3.49%	29.1%	6%
Cell border										
Distinct	86%	97%	-	-	83.1%	81.8%	80.23%	75.58%	NSD	-
Indistinct	14%	03%	-	-	16.9%	18.2%	19.76%	24.41%	-	-
Cytoplasmic staining										
Unsatisfactory	06%	03%	34.5%	25.4%	21.0%	12.2%	0	0	-	-
Satisfactory	94%	97%	59.5%	60.6%	79.0%	87.8%	100%	100%	62.4%	65.8%
Excellent	-	-	-	-	-	-	-	-	34.2%	37.6%

Nuclear border of squamous and endocervical cells were more distinctly visible in RADPS. Out of 247 smears, indistinct nuclear border in squamous cell was seen in 11% of C-PAPS, 4% of RADPS and in endocervical cells in 9% & 2% of C-PAPS and RADPS respectively. Similar results were seen in study conducted by Jaiwong K *et al*[5]. Study conducted by Zare-Mirzaie *et al*[29]showed no statically significant difference in distinctness of nuclear border of squamous cell. While, Gupta S *et al*[6] in their study showed C-PAPS had more distinct squamous cell nuclear border as compared to RADPS, but difference was not significant statistically.

Crispness of nuclear chromatin of squamous and endocervical cells was more evident in RADPS compared to C-PAPS. Nuclear chromatin of squamous cell and endocervical cell was crisp in 87% and 80% of C-PAPS, which was less compared to 98% and 99% of RADPS. Jaiwong K *et al*[5] in their study observed that in C-PAPS crisp nuclear chromatin of squamous and endocervical cell was seen in 96.5% and 84% respectively and in RADPS it was 87.8% and 76.5%. In their study crispness of nuclear features were better in C-PAPS than RADPS. Hazy nuclear chromatin of squamous as well as endocervical cell was more evident in C-PAPS and less in RADPS. Possible explanation for this is more cytolysis and air-drying artifact on C-PAPS. The air drying had added advantage as there was increase in nuclear size, flatter and depth of focus on nuclei is shallower, which give better cytomorphology and advantage in taking photograph.[22,28,30] Hence, it was easy to diagnose precursors, pre-neoplastic and neoplastic conditions better in RADPS as compared to C-PAPS. **(Table 25)**

Table 25: Comparison of nuclear features in squamous and endocervical cells on C-PAP smear and Rehydrated air-dried PAP smear with other studies.

Morphological features	Present study (n=247)		Ganesan *et al*[7] (n=397)		Gupta S *et al*[6] (n=950)		Jaiwong K *et al*[5] (n=172)	
	C-PAP	RADS	C-PAP	RADS	C-PAP	RADS	C-PAP	RADS
Nuclear border								
Squamous cells								
Distinct	89%	96%	66.8%	66%	78.6 %	76.1%	97.09 %	88.9%
Indistinct	11%	4%	33.2%	34%	21.4 %	23.9%	2.9%	11%
Endocervical cells								
Distinct	91%	97%	-	-	-	-	86.5%	79.8%
Indistinct	9%	2%	-	-	-	-	13.5%	20.2%
Nuclear chromatin								
Squamous cells								
Crisp	87%	98%	58.9%	56.1 %	73.6 %	71.7%	96.5%	87.8%
Hazy	13%	2%	41%	43.8 %	26.4 %	28.3%	3.5%	12.2%
Endocervical cells								
Crisp	80%	99%	-	-	-	-	84%	76.5%
Hazy	20%	1%	-	-	-	-	16%	23.5%

Various authors mentioned that there are only few disadvantages of RADS such as air-drying artifacts, cytolysis and contamination by organisms if smears are kept for longer period for air drying. Further studies should be conducted for standardizing the maximum time for which air drying can be done as well as effect of environmental factors on PAP smear. Another disadvantage is due to over hydration (>30 seconds) which can cause artifactual pseudo-nucleomegaly. This can be prevented by restricting

duration of rehydration for 30seconds.[4,7,23] In our study we found that Rehydrated air-dried technique is better substitute for traditional C-PAP method if air drying and rehydration timings are maintained. Rehydrated Air-dried smear technique is easy, inexpensive/cost effective, applicable, technician friendly as well as efficient method which can act as adjuvant to conventional C-PAP method.

CONCLUSION

In developed countries, PAP smear has proved to be an effective non-invasive modality in curbing down the morbidity and mortality associated with cervical cancer. Conventional wet fixation method is most commonly followed method and is considered as best method for cervical smears study. However, it has certain limitations. The problems faced with this method are availability of ethanol, more number of air drying artifact, loss of cellularity, hemorrhagic background and cytolysis which lead to more number of unsatisfactory smears. Due to the sensitivity of the conventional PAP technique is low. In order to overcome this problem, rehydrated air-dried technique can be a satisfactory alternative technique as the unsatisfactory rates are low and the air-drying artifacts and cytolysis can also be prevented. Added advantage of this technique is clean background, superior cytoplasmic staining and better preservation of cytomorphological features which helps in easy diagnosis of infections and neoplastic lesions. In resource poor settings and in remote areas, this technique can be successfully applied as it is simple and less cumbersome. Also, RAD can be practiced routinely or in conjugation with C-PAP in tertiary care setup. This might help in early detection of cervical cancer and thus helps to bring down the burden of morbidity and mortality associated with cervical cancer.

Limitations of the study –

Since split smears were prepared from the same sample of same women at same time which may lead to uneven distribution of cells. This may be one of the cause for disparity in the cellularity among two smears.

The efficacy as well as diagnostic utility in ASCUS, ASC-H, AGC-NOS, LSIL, HSIL and SCC was not possible due to less number of samples of these lesions. For establishing efficacy of RAD technique in these lesions, study with more number of samples should be carried out.

SUMMARY

A prospective study to compare cytomorphological features of Rehydrate Air dried PAP smear with conventional Wet Fixed PAP smears was undertaken to evaluate its diagnostic utility, during 1st October 2015 to 30th June 2017 in the Department of Pathology, _____

_____

The salient features observed in this study are –

A comparative study for 247 cases of Rehydrated air-dried PAP smears and Conventional Wet Fixed PAP smears were prepared. Age group of patients ranged from 20 to 80 years with a mean age of 36.8 years. The most frequent presenting complaint was white discharge per vagina seen in 73% of the women.

Satisfactory cell samples were obtained in 92.7% cases of conventional PAP smear and 97.6% cases of Rehydrated air-dried PAP smear. Out of 247 cases, 234(95%) cases were non-neoplastic lesion. By conventional technique, the most common non-neoplastic lesion was inflammatory smear in 162 cases (65.58%) followed by normal study 38 cases in (15.38%), bacterial vaginosis in 8 cases (3.2%), atrophic smear in 4 cases (1.61%) and 2 cases each of candida infestation and trichomonas vaginalis amounting to 0.80% each. The most common neoplastic lesion in C-PAPS was found to be HSIL accounting for 5 cases (2.1%) followed by ASCUS in 3 cases (1.21%), ASCUS-H in 2 cases (0.80%), AGC-NOS in 2 cases (0.80%) and LSIL in 1 case (0.40%). In few cases of C-PAPS sperms were not visualized due to obscuring by hemorrhagic

background while they were easily picked up on RADPS due to lysis of RBCs, which may be helpful in Medico legal cases.

Comparison of cytomorphological features between RADPS and C-PAPS was carried out for parameters such as adequacy, cellularity, cytolysis, air drying artifact and red blood cell background, cell border, cytoplasmic staining, nuclear border and chromatin of squamous and endocervical cells and the difference was statistically significant at 5% level of significance.

Air drying technique can be potential alternative for conventional wet fixation method. As this technique is a simple, convenient and less cumbersome for technicians hence, it can be successfully used routinely as well as in resource poor settings and cervical cancer screening camps.

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ANNEXURE – I

INSTITUTIONAL ETHICAL COMMITTEE CLEARANCE CERTIFICATE

ANNEXURE II

RESEARCH INFORMED CONSENT FORM

I, the undersigned, _____, S/O D/O W/O _____, aged ____years, ordinarily resident of _____ do hereby state/declare that Dr. _____ of _____ Hospital has examined me thoroughly on _____ at _____ (place) and it has been explained to me in my own language that I am suffering from _____ disease (condition) and this disease/condition mimic following diseases. Further Doctor informed me that he/she is conducting dissertation/research titled _____under the guidance of Dr. _____requesting my participation in the study. Apart from routine treatment procedure, the pre-operative, operative, post-operative and follow-up observations will be utilized for the study as reference data.

Doctor has also informed me that during conduct of this procedure like adverse results may be encountered. Among the above complications most of them are treatable but are not anticipated hence there is chance of aggravation of my condition and in rare circumstances it may prove fatal in spite of anticipated diagnosis and best treatment made available. Further Doctor has informed me that my participation in this study help in evaluation of the results of the study which is useful reference to treatment of other

similar cases in near future, and also, I may be benefited in getting relieved of suffering or cure of the disease I am suffering.

The Doctor has also informed me that information given by me, observations made/ photographs/ video graphs taken upon me by the investigator will be kept secret and not assessed by the person other than me or my legal hirer except for academic purposes.

The Doctor did inform me that though my participation is purely voluntary, based on information given by me, I can ask any clarification during the course of treatment / study related to diagnosis, procedure of treatment, result of treatment or prognosis. At the same time, I have been informed that I can withdraw from my participation in this study at any time if I want or the investigator can terminate me from the study at any time from the study but not the procedure of treatment and follow-up unless I request to be discharged.

After understanding the nature of dissertation or research, diagnosis made, mode of treatment, I the undersigned Shri/Smt _____ under my full conscious state of mind agree to participate in the said research/dissertation.

Signature of patient:

Signature of doctor:

Witness: 1.

2.

Date:

Place:

ANNEXURE-III

PROFORMA

NAME	:	OP/IP No.	:
AGE	:	D.O. A	:
RELIGION	:	D.O. D	:
OCCUPATION	:		
RESIDENCE	:		
Presenting Complaints	:		
Past history	:		
Personal history	:		
Family history	:		
Treatment history	:		

General physical examination:

Pallor	present/absent
Icterus	present/absent
Clubbing	present/absent
Lymphadenopathy	present/absent
Edema	present/absent
Built	poor/average/well

VITALS: PR: RR:

BP: TEMPERATURE:

WEIGHT:

SYSTEMIC EXAMINATION:

Per vaginal/ Per speculum finding:

Clinical Diagnosis:

INVESTIGATIONS:

- PAP smear reporting as per the BETHESDA SYSTEM:

1. Specimen
2. Specimen Adequacy
3. General Categorization
4. Interpretation
5. Impression

- Comparison of cytomorphological features between Air Dried and Wet Fixed PAP smear

Table 2. Comparison of cytomorphological parameters between two smears

Sr. No.	Parameter	RAD	C-PAP
1.	**Cellularity**		
	Low		
	Intermediate		
	High		
2.	**Cytolysis**		
	Present		
	Absent		
3.	**Air-drying artifact**		
	Present		
	Absent		
4.	**Red blood cell background**		
	Present		
	Absent		

5.	**Cell border**		
	Distinct		
	Indistinct		
6.	**Cytoplasmic staining**		
	Unsatisfactory		
	Satisfactory		
7.	**Nuclear border**		
	Squamous cells		
	Distinct		
	Indistinct		
	Endocervical cells		
	Distinct		
	Indistinct		
8.	**Nuclear chromatin**		
	Squamous cells		
	Crisp		
	Hazy		
	Endocervical cells		
	Crisp		
	Hazy		

KEY TO MASTER CHART

Sr. No.	Parameter	Point Score	Sr. No.	Parameter	Point Score
1	**Cellularity**		**7**	**Nuclear border**	
	Low	0		**Squamous cells**	
	Intermediate	1		Distinct	0
	High	2		Indistinct	1
2	**Cytolysis**			**Endocervical cells**	
	Present	0		Distinct	0
	Absent	1		Indistinct	1
3	**Air-drying artifact**		**8**	**Nuclear chromatin**	
	Present	0		**Squamous cells**	
	Absent	1		Crisp	0
4	**Red blood cell background**			Hazy	1
	Present	0		**Endocervical cells**	
	Absent	1		Crisp	0
5	**Cell border**			Hazy	1
	Distinct	0			
	Indistinct	1			
6	**Cytoplasmic staining**				
	Unsatisfactory	0			
	Satisfactory	1			

KEY TO MASTER CHART

Abbreviation	Full form
A	Amenorrhea
B	Backache
BPV	Bleeding Per Vagina
D	Dysmenorrhea
GW	Generalized Weakness
I	Infertility
IC	Irregular Cycles
IT	Itching
MPV	Mass Per vagina
M	Menorrhagia
PA	Pain Abdomen
PCB	Post Coital Bleeding
PM	Post-Menopausal
WDPV	White Discharge Per Vagina
Sr. no.	Serial Number
ns	not seen
C/F	Clinical features
CI	Cytological Impression
CL	Cellularity
CY	Cytolysis
ADF	Air Drying Artifact
RBCB	Red blood cell background
CB	Cell Border
CS	Cytoplasmic Staining
NBSC	Nuclear Border squamous of cell
NBEC	Nuclear Border of endocervical cell
NCSC	Nuclear chromatin of squamous cell

NCEC	Nuclear chromatin of endocervical cell
ASCUS	Atypical Squamous Cell of Undermined Significance
ASC-H	Atypical Squamous Cell Cannot exclude HSIL
AGC-NOS	Atypical glandular Cell Not Otherwise Specified
LSIL	Low Grade Intraepithelial Lesion
HSIL	High Grade Intraepithelial Lesion
IS	Inflammatory Smear
TV	Trichomonas Vaginalis
CD	Candida
BV	Bacterial vaginosis
AS	Atrophic Smear
OE	Oestrogenic effect
NS	Normal study
UFE	Unsatisfactory for Evaluation
SCC	Squamous Cell Carcinoma

MASTER CHART

Sr. no.	PAP No.	OP/IP No.	Name	Age (in years)	C/F	CI OF RAD	CI OF CPAP	CL of RAD	CL of CPAP	CY of RAD	CY of CPAP	ADF of RAD	ADF of CPAP	RBCB of RAD	RBCB of CPAP	CB of RAD	CB of CPAP	CS of RAD	CS of CPAP	NBSC of RAD	NBSC of CPAP	NBEC of RAD	NBEC of CPAP	NCSC of RAD	NCSC of CPAP	NCEC of RAD	NCEC of CPAP
1	1735 /15	OP/2015/345792	Sharnama G	40	WDPV	IS	IS	2	2	1	1	1	1	1	1	0	0	1	1	0	0	0	0	0	0	0	1
2	1736 /15	IP/2015/30268	BasammaLayappa	58	WDPV	UFE	UFE	0	0	1	0	1	0	1	0	1	0	1	1	0	1	0	0	0	1	0	0
3	1737 /15	OP/2015/346252	Gourabai	40	IC	NS	NS	1	1	1	1	1	1	1	0	0	1	1	1	0	0	0	0	0	0	0	0
4	1738 /15	OP/2015/346928	Neelamma	40	WDPV	IS	IS	1	1	1	1	0	0	1	1	1	1	1	1	0	0	0	0	1	0	0	0
5	1746 /15	OP/2015/344798	Nagaratna	29	A	IS	IS	2	2	1	0	1	0	1	1	0	1	1	0	0	1	0	0	0	1	0	0
6	1755 /15	OP/2015/351394	BasammaManaguli	47	WDPV	IS	IS	2	1	1	1	1	1	1	1	0	0	1	1	0	0	0	1	0	1	0	1
7	1776 /15	OP/2015/352836	Sneha Patil	50	M	IS	IS	2	2	1	1	1	0	1	0	0	1	1	0	0	0	0	0	0	1	0	1
8	1777 /15	OP/2015/352679	DevammaSomanal	40	WDPV	LSIL	LSIL	2	1	1	1	1	1	1	0	0	0	1	1	0	1	0	0	0	1	0	0
9	1778 /15	OP/2015/352573	YallawwaChalawadi	50	WDPV	IS	IS	2	2	1	1	1	1	1	1	0	0	1	1	0	0	0	0	0	0	0	0
10	1779 /15	OP/2016/354583	Girija	45	IC	NS	NS	0	0	1	1	1	1	1	1	0	1	1	1	0	0	0	0	0	0	0	0
11	1780 /15	OP/2015/352901	TellawwaGolar	35	WDPV	IS	IS	1	0	1	1	1	1	1	0	1	1	1	1	1	0	1	0	0	0	0	1
12	1781 /15	OP/2015/353923	Neelamma G	30	WDPV	IS	IS	2	2	1	1	1	1	1	0	0	0	1	1	0	0	0	0	0	0	0	0
13	1782 /15	OP/2015/355049	Sumitra Patani	42	Ic	BV	BV	2	2	1	0	1	0	1	0	0	1	1	1	0	1	ns	ns	0	1	ns	ns
14	1809 /15	OP/2015/355050	Indrabai	45	D	IS	IS	2	2	1	0	1	0	1	0	0	1	1	1	0	1	ns	ns	0	1	ns	ns
15	1818 /15	OP/2015/358806	Gangabai	40	WDPV	IS	IS	2	1	1	1	1	1	0	0	0	1	1	1	0	0	ns	0	0	0	ns	0
16	1819 /15	OP/2015/360298	GanguPati	33	WDPV	IS	IS	1	1	1	0	1	0	1	0	0	1	1	0	0	1	0	0	0	0	0	0
17	1820 /15	OP/2015/358350	SiddammaShelagi	50	WDPV	IS	IS	2	2	1	1	1	1	1	0	0	1	1	0	0	0	0	0	0	0	0	0
18	1837 /15	OP/2015/362969	Bouramma	37	IC	NS	NS	2	2	1	0	1	0	1	1	0	0	1	1	0	0	ns	ns	0	0	ns	ns
19	1848 /15	OP/2015/365267	Lalita Rathod	55	WDPV	IS	IS	1	1	1	1	1	1	1	1	0	0	1	1	0	0	ns	ns	0	0	ns	ns
20	1849 /15	OP/2015/365722	ShantammaI	20	Ic	IS	IS	2	2	1	1	1	0	1	0	0	1	1	1	0	0	ns	ns	0	0	ns	ns
21	1850 /15	IP/2015/31402	Tippawwa Y	65	PM	IS	IS	0	0	0	0	0	0	1	0	1	1	1	1	1	1	ns	ns	1	1	ns	ns
22	1859 /15	OP/2015/367522	PutalabaiPawar	47	WDPV	IS	IS	2	1	0	0	0	0	1	1	0	0	1	1	0	0	ns	ns	0	0	ns	ns
23	1915 /15	OP/2015/377816	Yogita Poddar	30	WDPV	IS	IS	0	0	1	1	1	1	1	0	0	0	1	1	0	1	0	1	1	1	0	1
24	1916 /15	OP/2015/377823	Ningamma	59	WDPV	IS	IS	2	2	1	1	1	1	1	1	0	0	1	1	0	0	0	0	0	0	0	0
25	1923 /15	OP/2015/377818	Mahadevi	35	WDPV	NS	NS	0	0	1	1	1	0	1	1	0	0	1	1	0	0	ns	0	0	0	ns	0
26	1932 /15	OP/2015/381670	Gangubai Rathod	45	IC	NS	NS	0	0	1	0	1	1	1	1	0	1	1	1	0	1	ns	ns	0	1	ns	ns
27	2025 /15	IP/2015/35397	Rangamma Y	43	WDPV	IS	IS	2	2	1	1	1	1	1	1	0	0	1	1	0	0	ns	ns	0	0	ns	ns
28	2242 /15	OP/2015/439329	Basamma S	40	WDPV	NS	NS	1	0	1	1	1	1	1	1	0	0	1	1	0	0	ns	ns	0	0	ns	ns
29	2259 /15	IP/2015/39118	Nagawwa B	28	A	IS	IS	2	2	1	1	1	1	1	1	0	0	1	1	0	0	ns	ns	0	0	ns	ns
30	2260 /15	OP/2015/442799	Girija Desai	38	WDPV	IS	IS	1	0	1	1	1	1	1	1	0	0	1	1	0	0	ns	ns	0	0	ns	ns

31	2261 /15	OP/2015/442630	JakkavvaKabade	38	WDPV	IS	IS	2	0	1	1	1	1	1	1	0	1	0	1	0	0	ns	ns	0	1	ns	ns
32	2336 /15	OP/2015/458105	Renuka	25	M	IS	IS	2	0	1	0	1	0	1	1	0	1	1	1	0	1	ns	ns	0	1	ns	ns
33	2337 /15	OP/2015/455400	Nandini Meti	23	WDPV	IS	IS	2	2	1	1	1	1	1	1	0	0	1	1	0	0	ns	ns	0	0	0	0
34	2338 /15	IP/2015/39970	Neelakka Ramesh	38	WDPV	IS	IS	2	2	1	1	1	0	1	1	0	0	1	1	0	0	0	0	0	0	0	0
35	2239 /15	IP/2015/39989	sharadabiradar	38	WDPV	IS	IS	1	1	1	1	1	1	1	0	0	0	1	1	0	0	ns	ns	0	0	ns	ns
36	2347 /15	OP/2015/458905	Reshma Shaikh	32	WDPV	IS	IS	1	0	1	0	1	0	1	1	0	1	1	0	0	1	ns	ns	0	1	ns	ns
37	2353 /15	OP/2015/459528	Surekha Rajure	30	D	IS	IS	2	1	1	1	1	1	1	0	0	0	1	1	0	0	ns	ns	0	0	ns	ns
38	2354 /15	OP/2015/458929	Neelawwa S.	42	WDPV	IS	IS	0	0	0	1	0	1	1	0	1	0	0	1	1	0	ns	ns	1	0	ns	ns
39	2434 /15	OP/2015/474009	Bharati	60	PM	IS	IS	1	1	1	0	1	0	1	0	0	1	1	1	0	1	ns	ns	0	1	ns	ns
40	008 /16	IP/2016/65	Renuka Rakesh	20	WDPV	IS	IS	2	2	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0
41	20 /16	IP/2016/223	laxmi	25	WDPV	IS	IS	2	2	1	1	1	1	1	0	0	0	1	1	0	0	ns	ns	0	0	ns	ns
42	24 /16	OP/2016/5625	renuka	43	M	IS	IS	2	2	1	1	1	0	1	1	0	1	1	0	0	1	ns	ns	0	1	ns	ns
43	35 /16	OP/2016/7080	Sharubai Rathod	38	WDPV	NS	NS	2	1	1	1	1	1	1	1	0	0	1	1	0	0	ns	ns	0	0	ns	ns
44	37 /16	OP/2016/7081	sujatha	40	WDPV	IS	IS	2	2	1	1	1	1	1	1	0	0	1	1	0	0	ns	ns	0	0	ns	ns
45	38 /16	OP/2016/9566	shivamma	48	WDPV	NS	NS	2	2	1	1	1	1	1	0	0	0	1	1	0	0	ns	0	0	0	ns	0
46	39 /16	OP/2016/13525	prerna	37	WDPV	IS	IS	2	2	1	1	1	1	1	1	0	0	1	1	0	0	ns	ns	0	0	ns	ns
47	68 /16	OP/2016/13666	DoddawwaMadar	80	PM	NS	NS	2	1	1	1	1	1	1	1	0	0	0	1	0	0	ns	ns	0	0	ns	ns
48	73 /16	OP/2016/13667	basalingamma	27	WDPV	IS	IS	2	2	1	1	1	1	1	0	0	0	1	1	0	0	0	0	0	0	0	0
49	87 /16	OP/2016/202569	sangeeta	36	Ic	IS	IS	2	2	1	1	1	1	1	0	0	0	1	1	0	0	ns	ns	0	0	ns	ns
50	88 /16	OP/2016/20278	Umashree	45	WDPV	NS	NS	2	2	1	1	1	1	1	1	0	0	1	1	0	0	ns	ns	0	0	ns	ns
51	89 /16	OP/2016/20279	Vallamma	47	WDPV	IS	IS	2	2	1	1	1	1	1	1	0	0	1	1	0	0	ns	ns	0	0	ns	ns
52	102 /16	OP/2016/20277	TasaleemaChabanur	40	MPV	IS	IS	2	1	1	1	1	1	1	0	0	0	1	1	0	0	ns	ns	0	0	ns	ns
53	108 /16	OP/2016/20289	Reena	29	WDPV	IS	IS	2	1	1	1	1	1	1	0	0	0	1	1	0	0	ns	ns	0	0	ns	ns
54	119 /16	OP/2016/23698	LakshmibaiGodekar	42	WDPV	IS	IS	2	2	1	1	1	1	0	1	0	0	1	1	0	1	0	1	0	0	0	1
55	120 /16	OP/2016/22551	Kamala Biradar	55	WDPV	NS	NS	0	1	1	1	1	0	1	0	1	0	1	1	0	0	ns	ns	0	0	ns	ns
56	127 /16	OP/2016/25023	Neelakka P Dhage	22	IC	IS	IS	1	1	1	0	0	0	1	0	0	1	1	0	0	0	ns	ns	0	1	ns	ns
57	151 /16	OP/2016/29923	Mahadevi Chikeari	38	WDPV	IS	IS	2	2	1	1	1	1	1	1	0	0	1	1	0	0	ns	ns	0	0	ns	ns
58	158 /16	OP/2016/30924	Asha	30	WDPV	IS	IS	2	1	1	1	1	1	1	1	0	0	1	1	0	0	ns	ns	0	0	ns	ns
59	166 /16	OP/2016/29925	Vasanti Patil	25	If	IS	IS	2	2	1	1	1	1	1	1	0	0	1	1	0	0	ns	ns	0	0	ns	ns
60	178 /16	OP/2016/35679	Mangal Bagali	25	WDPV	IS	IS	1	1	1	1	1	1	1	0	0	0	1	1	0	1	0	1	0	1	0	1
61	179 /16	OP/2016/36703	Manjula Biradar	28	WDPV	IS	IS	2	2	1	1	1	1	1	0	0	0	1	1	0	0	ns	ns	0	0	ns	ns
62	192 /16	OP/2016/38021	Muttawwa	35	WDPV	IS	IS	2	1	1	1	1	1	1	1	0	0	1	1	0	0	0	0	0	0	0	0
63	196 /16	OP/2016/40818	Reshma G	20	WDPV	IS	IS	1	1	1	1	1	1	1	0	0	0	1	1	0	0	0	1	0	0	0	1
64	221 /16	OP/2016/40819	Kashibai	30	WDPV	NS	NS	1	1	0	1	0	1	1	0	1	0	1	1	1	0	ns	ns	1	0	ns	ns
65	246 /16	OP/2016/153652	Renuka	25	WDPV	IS	IS	1	1	1	0	1	0	1	0	0	1	1	1	0	1	ns	ns	0	1	ns	ns
66	254 /16	OP/2016/52723	Parveen Mokashi	28	WDPV	UFE	UFE	0	0	1	1	1	1	1	1	0	0	1	1	0	0	ns	ns	0	0	ns	ns
67	255 /16	OP/2016/54431	Sangitha More	50	IC	UFE	UFE	0	0	1	1	1	1	1	1	0	0	1	1	0	0	0	0	0	0	0	0
68	289 /16	OP/2016/60519	KalavatiKumabar	47	WDPV	NS	NS	2	2	1	1	1	1	1	1	0	0	1	1	0	0	ns	ns	0	0	ns	ns
69	298 /16	OP/2016/60589	Lalita Ravi	28	WDPV	IS	IS	0	0	1	1	1	1	1	1	0	0	1	1	0	0	ns	ns	0	0	ns	ns
70	301	OP/2016/60590	Parvati	30	If	NS	NS	2	1	1	1	1	1	1	0	0	0	1	1	0	0	0	0	0	0	0	0
71	303 /16	OP/2016/60341	Sangamma	47	M	IS	IS	2	1	1	1	1	1	1	1	0	0	1	1	0	0	ns	ns	0	0	ns	ns
72	304 /16	OP/2016/60592	Godavari	50	WDPV	IS	IS	1	0	0	1	0	1	1	1	0	0	1	1	1	0	ns	ns	1	0	ns	ns

73	306 /16	IP/2016/5198	Danamma G	37	WDPV	IS	IS	2	2	1	1	1	1	1	0	0	0	1	1	0	0	0	0	0	0	0	0
74	307 /16	IP/2016/5369	IrammaIrappa	36	Ic	IS	IS	2	2	1	1	1	1	1	0	0	0	1	1	0	0	0	0	0	0	0	0
75	308 /16	IP/2016/5371	Shivamma G	50	WDPV	IS	IS	2	2	1	1	1	1	1	0	0	0	1	1	0	0	ns	ns	0	0	ns	ns
76	310 /16	OP/2016/63296	Jayashree Chalawadi	25	WDPV	IS	IS	0	0	1	1	1	1	1	1	0	0	1	1	0	0	ns	ns	0	0	ns	ns
77	318 /16	OP/2016/64142	Mahanandakumbar	22	WDPV	IS	UFE	1	0	0	0	1	0	1	0	0	1	1	1	0	0	0	0	0	0	0	0
78	319 /16	OP/2016/64147	Indrabai	35	D	NS	UFE	1	0	1	0	1	1	1	0	0	1	1	1	0	1	0	0	0	0	0	1
79	320 /16	OP/2016/64260	Suramma	30	WDPV	IS	IS	1	1	1	1	1	1	1	0	0	0	1	1	0	0	0	0	0	0	0	0
80	323 /16	OP/2016/64261	Arati	35	WDPV	IS	IS	0	0	1	1	0	0	1	1	0	0	1	1	0	0	ns	ns	0	1	ns	ns
81	324 /16	OP/2016/64293	Chandbe	40	WDPV	NS	NS	1	1	1	0	0	0	1	1	0	0	1	1	0	1	ns	ns	0	0	ns	ns
82	325 /16	OP/2016/64299	Basamma S	35	IC	NS	NS	1	1	1	0	1	1	1	0	0	1	1	1	0	0	ns	ns	0	0	ns	ns
83	326 /16	OP/2016/64305	Basamma T	35	WDPV	IS	IS	1	1	1	0	1	1	1	1	0	0	1	1	0	0	ns	ns	0	1	ns	ns
84	327 /16	OP/2016/64308	Jagadevi J	28	WDPV	NS	NS	2	2	1	1	1	1	1	1	0	0	1	1	0	0	ns	ns	0	0	ns	ns
85	328 /16	OP/2016/64689	Holiyamma s	30	WDPV	BV	BV	1	0	1	0	1	0	0	0	0	1	1	0	0	1	0	1	0	1	0	1
86	330 /16	OP/2016/64704	Laxmi M	50	WDPV	IS	IS	0	1	1	0	1	0	1	1	0	1	1	1	0	0	ns	ns	0	1	ns	ns
87	331 /16	OP/2016/64798	Neelamma M	40	WDPV	IS	IS	1	1	1	1	1	1	0	0	0	0	1	1	0	0	0	0	0	1	0	1
88	332 /16	OP/2016/64809	Mallamma G	25	If	IS	IS	1	1	1	1	1	1	1	0	0	0	1	1	0	0	ns	ns	0	0	ns	ns
89	343 /16	OP/2016/64864	Boramma S	50	WDPV	IS	IS	1	1	1	1	1	0	1	0	0	0	1	1	0	0	0	0	0	0	0	0
90	344 /16	OP/2016/64858	Siddamma K	28	M	NS	NS	1	1	1	1	0	0	1	0	0	1	1	1	0	0	ns	ns	0	1	ns	ns
91	345 /16	OP/2016/64857	Bharati M	30	WDPV	TV	TV	1	1	1	0	1	1	1	1	0	0	1	1	0	0	ns	ns	0	1	ns	ns
92	346 /16	OP/2016/64856	Kalamma A	40	PM	NS	NS	1	1	1	1	1	0	1	1	0	0	1	1	0	1	ns	ns	0	1	ns	ns
93	347 /16	OP/2016/64854	Prabhavati K	36	WDPV	NS	NS	1	1	1	1	1	0	1	0	0	1	1	1	0	0	0	0	0	1	0	0
94	348 /16	OP/2016/64851	Siairabanu A	41	WDPV	IS	IS	1	1	1	1	1	0	1	0	0	0	1	1	0	0	0	0	0	0	0	1
95	349 /16	OP/2016/64849	Gouramma V	35	WDPV	IS	IS	2	2	1	1	1	1	1	0	0	0	1	1	0	0	ns	ns	0	0	ns	ns
96	350 /16	OP/2016/64847	Laxmibai A	60	BPV	AS	AS	2	1	1	1	1	1	1	0	0	0	1	1	0	0	0	0	0	0	0	0
97	351 /16	OP/2016/64840	Malamma d	45	B	AS	AS	1	1	1	1	1	1	1	0	0	0	1	1	0	0	ns	ns	0	0	ns	ns
98	352 /16	OP/2016/64837	Sujata Biradar	35	WDPV	CAD	CAD	1	0	1	1	1	1	1	0	0	0	1	1	0	0	ns	ns	0	0	ns	ns
99	353 /16	OP/2016/64838	Bharati	40	GW	IS	IS	2	2	1	1	1	0	1	0	0	0	1	1	0	0	ns	ns	0	0	ns	ns
100	354 /16	OP/2016/64833	Akkamahadevi s	50	D	IS	IS	2	2	1	1	1	1	1	0	0	0	1	1	0	0	0	0	0	0	0	0
101	355 /16	OP/2016/64829	Ambramma s	35	Ic	CAD	CAD	2	2	1	1	1	1	1	0	0	0	1	1	0	0	ns	ns	0	0	ns	ns
102	356 /16	OP/2016/64866	Parammavva m	60	WDPV	AS	AS	1	1	1	1	1	1	1	0	0	0	1	1	1	1	ns	ns	0	0	ns	ns
103	357 /16	OP/2016/64821	Shivaleela r math	34	WDPV	IS	IS	2	2	1	1	1	1	1	0	0	0	1	1	0	0	0	0	0	0	0	0
104	358 /16	OP/2016/64813	Parvati t biradar	33	WDPV	NS	NS	2	2	1	1	1	1	1	0	0	0	1	1	0	0	0	0	0	0	0	0
105	367 /16	OP/2016/64871	Chandramma b	40	WDPV	UFE	UFE	2	0	1	1	1	1	1	0	0	0	1	1	0	0	0	0	0	0	0	0
106	368 /16	OP/2016/64873	Sharanamma s	26	If	NS	NS	2	2	1	1	1	1	1	0	0	0	1	1	0	0	ns	ns	0	0	ns	ns
107	369 /16	OP/2016/64875	Vijayalaxmi y	21	WDPV	NS	NS	2	2	1	1	1	1	1	0	0	0	1	1	0	0	0	0	0	0	0	0
108	370 /16	OP/2016/64891	Sangamma v	40	WDPV	ASCUS	ASCUS	2	2	1	1	1	1	1	0	0	0	1	1	0	0	ns	ns	0	0	ns	ns
109	371 /16	OP/2016/64892	Parwati s hiregoudar	35	B	NS	NS	2	2	1	1	1	1	1	1	0	0	1	1	0	0	ns	ns	0	0	ns	ns
110	372 /16	OP/2016/64893	Vijayalaxmi M	35	WDPV	BV	BV	2	2	1	1	1	1	1	0	0	0	1	1	0	0	0	0	0	0	0	0
111	373 /16	OP/2016/64876	Gangabaimelinamagi	60	MPV	AS	AS	2	2	1	1	1	1	1	1	0	0	1	1	0	0	ns	ns	0	0	ns	ns
112	374 /16	OP/2016/64877	Sharada gundalageri	25	WDPV	IS	IS	2	2	1	1	1	1	1	0	0	0	1	1	0	0	0	0	0	0	0	0
113	375 /16	OP/2016/64878	Premarathod	30	WDPV	NS	NS	2	1	1	1	1	1	1	0	0	0	1	1	0	0	ns	ns	0	0	ns	ns
114	376 /16	OP/2016/64880	Akkammabellur	25	WDPV	IS	IS	2	2	1	1	1	1	1	0	0	0	1	1	0	0	ns	ns	0	0	ns	ns

115	382 /16	OP/2016/64881	Holigamma	30	WDPV	IS	IS	2	1	1	1	1	1	1	1	0	0	1	1	0	0	ns	ns	0	0	ns	ns
116	400 /16	OP/2016/71567	Savitri C Naik	25	Ic	IS	IS	2	2	1	1	1	1	1	1	0	0	1	1	0	0	0	0	0	0	0	0
117	401 /16	OP/2016/72343	BashiraVijapur	36	WDPV	IS	IS	2	2	1	0	1	0	1	0	0	1	1	0	0	1	ns	ns	0	1	ns	ns
118	481 /16	OP/2016/72344	Lalitha Hali	35	WDPV	IS	IS	1	1	1	1	1	1	1	0	0	0	1	1	0	0	ns	ns	0	0	ns	ns
119	641 /16	IP/2016/10755	Shoba Babu	31	WDPV	IS	IS	2	2	1	1	1	1	1	0	0	0	1	1	0	0	0	0	0	0	0	0
120	642 /16	OP/2016/118924	BismillaDarga	23	WDPV	IS	IS	2	2	1	1	1	1	1	0	0	0	1	1	0	0	0	0	0	0	0	0
121	675 /16	OP/2016/123767	ShantabaiKadimani	40	IC	IS	IS	2	2	1	1	1	1	1	1	0	0	1	1	0	0	ns	ns	0	0	ns	ns
122	682 /16	OP/2016/125395	Laxmi Biradar	28	WDPV	UFE	UFE	2	0	1	1	1	1	1	1	0	1	1	0	0	1	ns	ns	0	1	ns	ns
123	683 /16	OP/2016/125395	Laxmi Biradar	28	WDPV	IS	IS	1	0	1	0	1	0	1	1	0	1	1	1	0	1	ns	ns	0	1	ns	ns
124	755 /16	OP/2016/125395	Laxmi Biradar	28	M	UFE	UFE	1	1	1	1	1	1	1	0	0	0	1	1	0	0	ns	ns	0	0	ns	ns
125	840 /16	OP/2016/154455	KreshaJavali	25	WDPV	IS	IS	2	2	1	1	1	1	1	0	0	0	1	1	0	0	ns	ns	0	0	ns	ns
126	841 /16	OP/2016/153757	MayammaBudhyal	25	WDPV	IS	IS	2	0	1	1	1	1	1	0	0	0	1	1	0	0	0	0	0	0	0	0
127	895 /16	OP/2016/167033	Bharati B	26	PM	IS	IS	2	2	1	1	1	1	1	1	0	0	1	1	0	0	ns	ns	0	0	ns	ns
128	896 /16	OP/2016/166789	Rekha D Kadam	26	WDPV	IS	IS	2	2	1	1	1	1	1	1	0	0	1	1	0	0	ns	ns	0	0	ns	ns
129	952 /16	OP/2016/177797	SushilaKollali	35	IC	NS	NS	2	2	1	1	1	1	1	0	0	0	1	1	0	0	ns	ns	0	0	ns	ns
130	961 /16	OP/2016/177915	Yalamma H	45	WDPV	ASC-H	ASC-H	2	1	1	1	1	1	1	0	0	0	1	1	0	0	ns	ns	0	0	ns	ns
131	1084 /16	OP/2016/201061	NeelabaiPawar	35	WDPV	IS	IS	2	1	1	1	1	1	1	0	0	0	1	1	0	0	ns	ns	0	0	ns	ns
132	1158 /16	OP/2016/213696	ManglaPujeri	22	WDPV	IS	IS	2	2	1	1	1	1	1	0	0	0	1	1	0	0	ns	ns	0	0	ns	ns
133	1159 /16	OP/2016/213510	NagammaBilagaker	18	WDPV	IS	IS	2	2	1	1	1	1	1	1	0	0	1	1	0	0	ns	ns	0	0	ns	ns
134	1216 /16	OP/2016/222602	ParvatibaiMalagar	50	WDPV	IS	UFE	1	1	1	1	1	1	1	0	0	0	1	1	0	0	ns	ns	0	0	ns	ns
135	1226 /16	OP/2016/224495	Kamalakshi	38	WDPV	IS	IS	1	2	1	1	1	1	1	1	0	0	0	1	1	0	ns	ns	0	0	0	ns
136	1231 /16	OP/2016/224496	Prathibha	36	WDPV	IS	IS	2	2	1	1	1	1	1	1	0	0	1	1	0	0	ns	ns	0	0	ns	ns
137	1237 /16	OP/2016/227062	RanubaiGayakwad	35	WDPV	IS	IS	2	2	1	1	1	1	1	1	0	0	1	1	0	0	ns	ns	0	0	ns	ns
138	1298 /16	OP/2016/237690	SukanyaAwati	42	GW	IS	IS	1	1	1	1	1	1	1	0	0	0	1	1	0	0	ns	ns	0	0	ns	ns
139	1300 /16	OP/2016/235807	Sumitra Gundakanal	50	WDPV	NS	NS	2	2	1	1	1	1	1	1	0	0	1	1	0	0	ns	ns	0	0	ns	ns
140	1309 /16	IP/2016/22270	Annapurna C	43	WDPV	NS	NS	2	2	1	1	1	1	1	1	0	0	1	1	0	0	0	0	0	0	0	0
141	1321 /16	IP/2016/22679	Prema	35	M	IS	IS	2	1	1	1	1	1	1	1	0	0	1	1	0	0	ns	ns	0	0	ns	ns
142	1333 /16	OP/2016/243781	MayawwaBalabatti	31	WDPV	IS	IS	1	1	1	1	1	1	1	1	0	0	1	1	0	0	ns	ns	0	0	1	1
143	1361 /16	OP/2016/246548	UmalaLamani	60	WDPV	OE	OE	2	2	1	1	1	1	1	1	0	0	1	1	0	0	ns	ns	0	0	ns	ns
144	1370 /16	OP/2016/247694	DanammaHiremath	30	WDPV	IS	IS	2	2	1	1	1	1	1	0	0	0	1	1	0	0	ns	ns	0	0	ns	ns
145	2264 /16	OP/2016/417989	Lakshmibai	40	WDPV	IS	UFE	1	0	1	1	1	1	1	1	0	0	1	1	0	0	ns	ns	0	0	ns	ns
146	2272 /16	OP/2016/421501	Shamala	45	WDPV	IS	IS	2	2	1	1	1	1	1	1	0	0	1	1	0	0	ns	ns	0	0	ns	ns
147	2274 /16	IP/2016/40203	RakamabaiJettappa	45	WDPV	IS	IS	1	1	1	1	1	1	1	1	0	0	1	1	0	0	0	0	0	0	0	0
148	2428 /16	OP/2016/448047	Drakshayani Patil	46	WDPV	IS	IS	2	2	1	1	1	1	1	1	0	0	1	1	0	0	ns	ns	0	0	ns	ns
149	009 /17	OP/2017/1895	Noorjahan	46	WDPV	IS	IS	1	1	1	1	1	1	1	1	0	0	1	1	0	0	ns	ns	0	0	ns	ns
150	010 /17	OP/2017/1260	Savitri Gangade	30	WDPV	BV	BV	2	2	1	1	1	1	1	0	0	0	1	1	0	0	ns	ns	0	0	ns	ns
151	011 /17	OP/2017/1534	BasammaKashinkunti	50	WDPV	IS	IS	2	1	1	1	1	1	1	1	0	0	1	1	1	0	0	ns	0	0	ns	ns
152	012 /17	OP/2017/1204	Indra Chopra	41	WDPV	IS	IS	2	2	1	1	1	1	1	1	0	0	1	1	0	0	0	ns	0	0	0	0
153	19 /17	OP/2017/2803	NeelammaNatikar	52	WDPV	IS	IS	2	2	1	1	1	1	1	1	0	0	1	1	0	0	ns	ns	0	0	ns	ns
154	20 /17	OP/2017/3183	Savitri Shinde	20	M	IS	IS	2	2	1	1	1	1	1	0	0	0	1	1	0	0	ns	ns	0	0	ns	ns
155	21 /17	OP/2017/2901	Bharati V Ajanal	32	If	IS	IS	2	2	1	1	1	1	1	1	0	0	1	1	0	0	ns	ns	0	0	ns	ns
156	29 /17	OP/2017/4201	KashibaiHipparagi	46	WDPV	NS	UFE	2	2	1	1	1	1	1	1	0	0	1	1	0	0	ns	ns	0	0	ns	ns

157	45 /17	OP/2017/6556	Mallawwa	22	WDPV	IS	IS	2	2	1	1	1	1	1	1	0	0	1	1	0	0	0	0	0	0	0	0
158	54 /17	OP/2017/7867	Mahadevi Basappa	40	WDPV	IS	IS	2	2	1	1	1	1	1	0	0	0	1	1	0	0	ns	ns	0	0	ns	ns
159	188 /17	OP/2017/35810	Rajashree	35	WDPV	NS	NS	1	1	1	1	1	1	1	0	0	0	1	1	0	0	0	0	0	0	0	0
160	189 /17	OP/2017/35811	Mahadevi	38	WDPV	IS	IS	2	1	1	1	1	1	1	1	0	1	1	1	0	0	ns	ns	0	0	ns	ns
161	190 /17	OP/2017/35812	Shanta	40	WDPV	NS	NS	2	2	1	1	1	1	1	0	0	0	1	1	0	0	0	0	0	0	0	1
162	191 /17	OP/2017/35813	Nyamatbi M	35	M	IS	IS	1	1	1	1	1	1	1	1	0	0	1	1	0	0	ns	ns	0	0	ns	ns
163	192 /17	OP/2017/35814	Katavva	45	WDPV	IS	IS	1	1	1	1	1	1	1	0	0	0	1	1	0	0	ns	0	0	0	ns	0
164	194 /17	OP/2017/35817	Geeta Madar	35	WDPV	NS	NS	1	1	1	1	1	1	1	1	0	0	1	1	0	0	ns	ns	0	0	ns	ns
165	195 /17	OP/2017/35818	Sushila B	35	WDPV	NS	NS	1	0	1	1	1	0	1	1	0	0	1	1	0	0	ns	ns	0	0	ns	ns
166	196 /17	OP/2017/35820	Chandrawwa P	35	WDPV	IS	IS	0	0	1	1	1	1	1	1	0	0	1	1	0	0	ns	ns	0	0	ns	ns
167	197 /17	OP/2017/35821	Renuka R	40	WDPV	NS	NS	0	0	1	1	0	1	1	1	0	0	1	1	0	0	ns	ns	0	0	ns	ns
168	198 /17	OP/2017/35822	RatnaBagale	35	IC	NS	NS	0	1	1	1	1	1	1	1	0	0	1	1	0	0	ns	ns	0	0	ns	ns
169	199 /17	OP/2017/35824	Shantawwa	40	WDPV	NS	NS	2	2	1	1	1	1	1	1	0	0	1	1	0	0	ns	ns	0	0	ns	ns
170	200 /17	OP/2017/35825	Sharada	36	WDPV	NS	NS	1	0	1	1	1	1	1	1	0	0	1	1	0	0	ns	ns	0	0	ns	ns
171	201 /17	OP/2017/35827	Geeta K	27	A	IS	IS	2	1	1	1	1	0	1	1	0	0	1	1	0	0	ns	ns	0	0	ns	ns
172	202 /17	OP/2017/35830	Sharada S	45	WDPV	IS	IS	2	1	1	1	1	1	1	0	0	0	1	1	0	0	0	ns	0	0	0	ns
173	203 /17	OP/2017/35831	MayakkaDoni	25	WDPV	IS	IS	1	1	1	1	1	1	1	1	0	0	1	1	0	0	1	1	0	0	ns	ns
174	211 /17	OP/2017/37474	Mabubee	30	WDPV	IS	IS	0	1	1	1	1	1	1	1	0	0	1	1	0	0	ns	ns	0	0	ns	ns
175	213 /17	OP/2017/37337	Bhimavva	60	WDPV	IS	IS	1	0	1	1	1	1	1	0	0	0	1	1	0	0	ns	ns	0	0	ns	ns
176	223 /17	OP/2017/40631	Meere	36	M	IS	IS	2	2	1	1	1	1	1	0	0	0	1	1	0	0	ns	ns	0	0	ns	ns
177	224 /17	OP/2017/40607	Bhagyashri	31	WDPV	NS	UFE	2	1	1	1	1	1	1	1	0	0	1	1	0	0	ns	ns	0	0	ns	ns
178	229 /17	OP/2017/40619	Sumitra	55	WDPV	IS	IS	2	1	1	1	1	1	1	1	0	0	1	1	0	0	ns	ns	0	0	ns	ns
179	236 /17	OP/2017/43697	Nirmala kolar	56	PA	IS	IS	1	1	1	1	1	1	1	1	0	0	1	1	0	0	ns	ns	0	0	ns	ns
180	268 /17	OP/2017/48807	Veena	42	WDPV	BV	UFE	2	0	1	1	1	1	1	1	0	0	1	1	0	0	ns	ns	0	0	ns	ns
181	384 /17	OP/2017/74146	Gangamma	40	WDPV	AS	UFE	2	0	1	1	1	1	1	0	0	0	1	1	0	0	ns	ns	0	0	ns	ns
182	388 /17	OP/2017/74174	Danamma	20	WDPV	IS	UFE	2	2	1	1	1	1	1	1	0	0	1	1	0	0	ns	ns	0	0	ns	ns
183	390 /17	OP/2017/74182	Renuka	25	WDPV	IS	IS	2	0	1	1	1	1	1	1	0	0	1	1	0	0	0	0	0	0	0	0
184	391 /17	OP/2017/73163	Geeta Lavagi	26	If	IS	IS	2	2	1	1	1	1	1	1	0	0	1	1	0	0	ns	ns	0	0	ns	ns
185	406 /17	OP/2017/75648	Revamma	40	WDPV	IS	IS	2	2	1	1	1	1	1	1	0	0	1	1	0	0	ns	ns	0	0	ns	ns
186	462 /17	OP/2017/84332	Amenbee S	40	WDPV	IS	UFE	1	0	1	1	1	1	1	1	0	0	1	1	0	0	ns	ns	0	0	ns	ns
187	464 /17	OP/2017/84337	Satyawwa S	34	WDPV	ASCUS	ASCUS	2	2	1	1	1	1	1	1	0	0	1	1	0	0	0	0	0	0	0	0
188	463 /17	OP/2017/84334	Parvathi B	40	BPV	HSIL	HSIL	2	2	1	1	1	1	1	1	0	0	1	1	0	0	ns	ns	0	0	ns	ns
189	465 /17	OP/2017/84341	Renuka K	27	WDPV	IS	IS	2	2	1	1	1	1	1	0	0	0	1	1	0	0	0	0	0	0	0	0
190	466 /17	OP/2017/84393	Haseena G	29	WDPV	NS	NS	2	2	1	1	1	1	1	1	0	0	1	1	0	0	ns	ns	0	0	ns	ns
191	467 /17	OP/2017/84344	Bouramma Kumar	35	WDPV	BV	BV	2	2	1	1	1	1	1	1	0	0	1	1	0	0	ns	ns	0	0	ns	ns
192	468 /17	OP/2017/84349	Sonam A	25	WDPV	BV	BV	2	2	1	1	1	1	1	0	0	0	1	1	0	0	ns	ns	0	0	ns	ns
193	469 /17	OP/2017/84353	Kesharibai A	45	WDPV	BV	UFE	1	1	1	1	1	1	1	1	0	0	1	1	0	0	ns	ns	0	0	ns	ns
194	470 /17	OP/2017/84352	Yellawa P	45	BPV	AGC-NOS	AGC-NOS	2	2	1	1	1	1	1	0	0	0	1	1	0	0	ns	ns	0	0	ns	ns
195	471 /17	OP/2017/84356	Rukamabai	65	PM	AS	UFE	2	0	1	0	1	1	1	1	0	1	1	0	0	1	0	0	0	1	0	ns
196	472 /17	OP/2017/84360	Hafuza S	32	PCB	IS	IS	2	2	1	1	1	1	1	1	0	0	1	1	0	0	0	0	0	0	0	0
197	473 /17	OP/2017/84361	Kousar I	26	WDPV	IS	IS	0	0	1	1	1	1	1	0	1	0	0	0	1	1	0	0	0	0	0	ns
198	474 /17	OP/2017/84362	Hameeda A	40	WDPV	ASCUS	ASCUS	2	2	1	1	1	1	1	0	0	0	1	1	0	0	ns	ns	0	0	ns	ns

199	475 /17	OP/2017/84364	Jyothi H Kalal	30	WDPV	ASC-H	ASC-H	2	2	1	1	1	1	1	0	0	0	1	1	0	0	ns	ns	0	0	ns	ns
200	476 /17	OP/2017/84365	Haseena G	36	WDPV	IS	IS	2	2	1	1	1	1	1	1	0	0	1	1	0	0	ns	ns	0	0	ns	ns
201	477 /17	OP/2017/85314	Yasmeen Irfan	32	M	IS	IS	2	2	1	1	1	1	1	1	0	0	1	1	0	0	0	0	0	0	0	0
202	478 /17	OP/2017/84375	Radha R	32	PCB	IS	IS	2	2	1	1	1	1	1	0	0	0	1	1	0	0	ns	ns	0	0	ns	ns
203	479 /17	OP/2017/84384	RameezaSindagi	36	WDPV	BV	BV	2	2	1	1	1	1	1	1	0	0	1	1	0	0	ns	ns	0	0	ns	ns
204	480 /17	OP/2017/84387	SatavvaHarijan	40	WDPV	TV	TV	2	2	1	1	1	1	1	0	0	0	1	1	0	0	ns	ns	0	0	ns	ns
205	481 /17	OP/2017/84393	Nasareen K	38	WDPV	AGC-NOS	AGC-NOS	1	2	1	1	1	1	1	1	0	0	1	1	0	0	ns	ns	0	0	ns	ns
206	482 /17	OP/2017/84411	Nazama A	36	WDPV	IS	IS	2	2	1	1	1	1	1	1	0	0	1	1	0	0	ns	ns	0	0	ns	ns
207	490 /17	OP/2017/87943	Basamma	25	WDPV	IS	IS	1	2	1	1	1	1	1	0	0	0	1	1	0	0	0	0	0	0	0	0
208	505 /17	OP/2017/90406	Poornima tuppad	38	WDPV	IS	IS	2	1	1	1	1	1	1	1	0	0	1	1	0	0	ns	ns	0	0	ns	ns
209	506 /17	OP/2017/90400	Sunanda	40	WDPV	IS	IS	2	2	1	1	1	1	1	1	0	0	1	1	0	0	ns	ns	0	0	ns	ns
210	507 /17	OP/2017/90414	Anjum nadaf	28	WDPV	IS	IS	0	2	1	0	1	1	1	1	0	1	0	1	0	1	ns	ns	0	0	ns	ns
211	508 /17	OP/2017/90427	Shivakka	80	PM	IS	IS	2	0	1	1	1	1	1	0	0	0	1	1	0	0	ns	ns	0	0	ns	ns
212	510 /17	OP/2017/90427	Surekha	30	WDPV	IS	IS	2	0	1	1	1	1	1	0	0	0	1	1	0	0	ns	ns	0	0	ns	ns
213	523 /17	OP/2017/91777	Savitri	40	WDPV	IS	IS	2	2	1	1	1	1	1	1	0	0	1	1	0	0	ns	ns	0	0	ns	ns
214	525 /17	OP/2017/93025	Shreedevi	29	WDPV	IS	IS	1	1	1	1	1	1	1	1	0	0	1	1	0	0	ns	ns	0	0	ns	ns
215	539 /17	OP/2017/94808	Siddamma	46	WDPV	BV	BV	2	2	1	1	1	1	1	1	0	0	1	1	0	0	0	0	0	0	0	0
216	541 /17	OP/2017/97969	Mahadevi kotyal	42	WDPV	IS	IS	0	1	1	1	1	1	1	1	0	0	1	1	0	0	ns	ns	0	0	ns	ns
217	542 /17	OP/2017/97966	Surekha doni	24	WDPV	IS	IS	2	2	1	1	1	1	1	0	0	0	1	1	0	0	ns	ns	0	0	ns	ns
218	543 /17	OP/2017/97963	Sangeeta	35	WDPV	IS	IS	2	1	1	1	1	1	1	1	0	0	1	1	0	0	ns	ns	0	0	ns	ns
219	545 /17	OP/2017/97950	Shridevi	29	D	IS	IS	0	2	1	1	1	1	1	1	0	0	1	1	0	0	ns	ns	0	0	ns	ns
220	559 /17	OP/2017/99036	Shailanavi	35	WDPV	IS	IS	1	2	1	1	1	1	1	1	0	0	1	1	0	0	ns	ns	0	0	ns	ns
221	560 /17	OP/2017/99041	Madiwalawwa	45	WDPV	HSIL	HSIL	1	1	1	1	1	1	1	1	0	0	1	1	0	0	ns	ns	0	0	ns	ns
222	561 /17	OP/2017/99050	Zannatbi	24	WDPV	IS	IS	2	2	1	1	1	1	1	1	0	0	1	1	0	0	ns	ns	0	0	ns	ns
223	562 /17	OP/2017/99058	Ameenbee	35	IC	NS	NS	2	2	1	1	1	1	1	1	0	0	1	1	0	0	ns	ns	0	0	ns	ns
224	563 /17	OP/2017/100353	Kalavati	22	WDPV	IS	IS	1	1	1	1	1	1	1	1	0	0	1	1	0	0	ns	ns	0	0	ns	ns
225	565 /17	OP/2017/100367	Kasturi	40	BPV	HSIL	HSIL	2	1	1	1	1	1	1	1	0	0	1	1	0	0	ns	ns	0	0	ns	ns
226	571 /17	OP/2017/100349	Keerti	28	WDPV	IS	IS	2	2	1	1	1	1	1	0	0	0	1	1	0	0	ns	ns	0	0	ns	ns
227	577 /17	OP/2017/103657	Kashibai	27	B	IS	IS	2	2	1	1	1	1	1	1	0	0	1	1	0	0	ns	ns	0	0	ns	ns
228	578 /17	OP/2017/100413	Niraja	47	WDPV	IS	IS	0	0	1	0	1	0	1	0	0	1	1	0	0	0	ns	ns	0	0	ns	ns
229	591 /17	OP/2017/106973	Anuradha pawar	24	WDPV	IS	IS	1	2	1	1	1	1	1	1	0	0	1	1	0	0	0	0	0	0	0	0
230	593 /17	OP/2017/106988	Bhagyashree	20	WDPV	IS	IS	2	0	1	1	1	1	1	1	0	0	1	1	0	0	0	0	0	0	0	0
231	594 /17	OP/2017/105320	Annapurna Hiremath	45	PCB	IS	IS	1	2	1	1	1	1	1	1	0	0	1	1	0	0	ns	ns	0	0	ns	ns
232	604 /17	OP/2017/106969	IndumathiBilur	39	WDPV	IS	IS	2	2	1	1	1	1	1	0	0	0	1	1	0	0	ns	ns	0	0	ns	ns
233	608 /17	OP/2017/108104	kannabai	25	IC	IS	IS	1	1	1	1	1	1	1	1	0	0	1	1	0	0	ns	ns	0	0	ns	ns
234	629 /17	OP/2017/108104	kasturi	25	WDPV	IS	IS	1	1	1	1	1	1	1	1	0	0	1	1	0	0	ns	ns	0	0	ns	ns
235	630 /17	OP/2017/108104	sakkubai	55	PM	IS	IS	0	0	1	1	1	1	1	1	0	0	1	1	0	0	0	0	0	0	ns	ns
236	631 /17	OP/2017/108104	Mahadevi	25	WDPV	IS	IS	2	1	1	1	1	1	1	1	0	0	1	1	0	0	ns	ns	0	0	ns	ns
237	633 /17	OP/2017/111465	Sunanda	30	WDPV	IS	IS	2	2	1	1	1	1	1	1	0	0	1	1	0	0	0	0	0	0	0	0
238	643 /17	OP/2017/112490	Reshma Pal	37	WDPV	IS	IS	2	2	1	1	1	1	1	1	0	0	1	1	0	0	0	0	0	0	0	0
239	647 /17	OP/2017/114634	Sumalata	35	WDPV	IS	IS	2	2	1	1	1	1	1	1	0	0	1	1	0	0	ns	ns	0	0	ns	ns
240	649 /17	OP/2017/114638	Gourabai	45	WDPV	IS	IS	1	1	1	1	1	1	1	1	0	0	1	1	0	0	ns	ns	0	0	ns	ns

241	651 /17	OP/2017/113742	Samim Banu	41	BPV	IS	IS	2	0	1	1	1	1	1	1	0	0	1	1	0	0	ns	ns	0	0	ns	ns
242	657 /17	OP/2017/115302	Rajashreepatil	43	WDPV	IS	IS	1	1	1	1	1	1	1	1	0	0	1	1	0	0	ns	ns	0	0	ns	ns
243	681 /17	OP/2017/119360	Sumangala	35	WDPV	IS	IS	1	1	1	1	1	1	1	1	0	0	1	1	0	0	ns	ns	0	0	ns	ns
244	683 /17	OP/2017/119350	Siddamma	30	IC	IS	IS	2	2	1	1	1	1	1	1	0	0	1	1	0	0	ns	ns	0	0	ns	ns
245	684 /17	OP/2017/119338	Ningamma	26	WDPV	IS	IS	2	0	1	1	1	1	1	1	0	0	1	1	0	0	0	0	0	0	0	0
246	686 /17	OP/2017/121505	Sudha dodmani	25	WDPV	IS	IS	2	1	1	1	1	1	1	1	0	0	1	1	0	0	ns	ns	0	0	ns	ns
247	753 /17	OP/2017/134837	Bharati	40	BPV	SCC	HSIL	2	2	1	1	1	1	1	0	0	0	1	1	0	0	ns	ns	0	0	ns	ns