A RETROSPECTIVE STUDY ON INTEROBSERVER VARIATION OF PAP SMEAR REPORTING AT KENYATTA NATIONAL HOSPITAL

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DEDICATION

To my family for their moral support and encouragement.

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I thank God for this far that He has taken me and for the many opportunities at my disposal.

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LIST OF ABBREVATIONS

AGC	Atypical Glandular Cells.
AIDS	Acquired Immune-deficiency Syndrome.
AIS	Adenocarcinoma in Situ.
ASC-H	Atypical squamous cells high grade cannot be ruled out.
ASC –US	Atypical squamous cells of undetermined significance.
CI	Confidence Interval.
CIN	Cervical intraepithelial neoplasia.
EQC	External quality assuarance.
CIS	Carcinoma in-situ.
ERC	Ethics and Research Committee.
HIV	Human Immunodeficiency Virus.
HPV	Human Papilloma Virus.
HSIL	High grade squamous intraepithelial lesion
k	Kappa
IP/OP No	Inpatient/Outpatient number
IQC	Internal quality control
HSV	Herpes simplex virus.
KNH	Kenyatta National Hospital.
LSIL	Low grade squamous intraepithelial lesion.
PI	Principal Investigator.
TBS	The Bethesda System.
MOH	Ministry of health.
NILM	Negative for Intraepithelial lesion or Malignancy.
N/C	Nuclear Cytoplasmic ratio
NOS	Not Otherwise Specified.
SCC	Squamous cell carcinoma.
SIL	Squamous intraepithelial Lesion.
Spp	Species.
SOPs	Standard Operating Procedures.
SPSS	Statistical Packages for Social Sciences.

TBS	The Bethesda System.
UON	University Of Nairobi.
VIA	Visual Inspection with Acetic Acid
VILI	Visual inspection with Lugol's iodine
WHO	World Health Organization

ABSTRACT

Background: Interobserver variation in the cytological diagnosis of cervical lesions poses a problem for public health screening programs. Every screening program should have procedures in place to minimize this. Establishing the degree of interobserver variation would inform decisions and corrective action. This study assessed the frequency and degree of discordant diagnoses between the primary and the review results of pap smears.

Objective: To determine the interobserver variation in pap smear reporting using the Bethesda System (2001) at Kenyatta National Hospital

Study design: A laboratory based retrospective study.

Study area: The study was conducted at the KNH/UON cytology laboratory.

Study population: A total of 372 Pap smears previously reported as ASCUS or higher lesion at KNH Cytology laboratory from Jan 2011 to June 2013 (30 months) were retrieved for the study.

Materials and method: Pap smears were selected and examined to determine the cervical changes and graded using The Bethesda System of reporting cervicovaginal cytology first by the investigator then together with the supervising Pathologists.The supervisors were blinded to the primary pathologist's report. The results were then compared with the primary report. In cases with discordance between the primary and the review report, a third Pathologist acted as a tie breaker.

Results: Presence of mixed lesions (Squamous+Glandular) which had been missed in the primary report were found. More than 22% cases were downgraded to NILM. Significant 'overcalls'& 'undercalls' were also found. ASCUS had a kappa of 0.049, LSIL 0.045, HSIL 0.126, ASCH 0.231, SCC 0.376 & Glandular lesions 0.125.**Overall kappa was 0.327.**

Conclusion: Overall inter-observer agreement was **fair**, but the concordance was lower than that observed in other studies. LSIL and ASCUS lesions had very high discordance. The lesion with the best interobserver agreement was SCC while HSIL lesions had an intermediate discordance.

Recommendations: Establishment of a stringent IQC/EQA programs in the department to improve accuracy of reporting.

1.0 INTRODUCTION

Cancer of the cervix is an important public health problem in Africa with regard to its high prevalence; it is the 2nd most common female malignancy and the 2nd leading cause of cancer deaths in African women(1, 3). Cancer is the third leading cause of death in Kenya with a rate of 18,000 deaths per year (Kenya Ministry of Public Health, 2009) (4). Cervical cancer in particular is the second most prevalent cancer among women in the country, after breast cancer, and its incidence is increasing (WHO, 2010). In many developed countries, however, the incidence of cervical cancer is decreasing due to widespread implementation of cervical screening programs(3,5). If cervical precancerous lesions are discovered early through screening and are subsequently treated, the disease is almost entirely curable. The Bethesda system grades cervical intraepithelial neoplasia into two categories: low-grade squamous intraepithelial lesions (LSIL), which are manifestations of productive human papillomavirus infection and serve as markers for women who are at risk of developing *de novo* high-grade lesions such as high-grade squamous intraepithelial lesions (HSIL) and squamous cell carcinomas, which are considered the true premalignant lesions. There is evidence that most LSIL are transient infections that carry little risk for oncogenesis, whereas most HSIL are associated with viral persistence and a significant potential for progression to invasive cancer. The distinction between LSIL and HSIL is of great importance because the management of these two lesions is very different.

Interobserver variation in the cytological diagnosis of cervical lesions poses a problem for public health screening programs. Poor agreement means poor reproducibility of results and subsequently affect quality of the screening program. Every screening program should have procedures in place to minimize errors. Establishing the degree of interobserver variation would inform decisions and corrective action.

This study assessed the interobserver variation between two independent pathologists' reports on cervical dysplasia in pap smears reported at KNH cytology laboratory.

2.0 LITERATURE REVIEW

Cytology screening for prevention of cervical cancer can reduce incidence and mortality by more than 80% in settings with well organized programs and rigorous quality control given that if a woman has a significant lesion, the Pap smear can detect it in 90% to 95% of cases(6). Cytology laboratories are not completely free of diagnostic errors, especially false-negative results.

False-negative results occur at a rate of at least 5% to 10%. Even the finest laboratories miss at least 1 in 10 to 20 positive cases in routine screening. Simultaneously obtained, duplicate smear shows a false-negative rate of at least 20% due to sampling and interpretation errors (7).

In studies where patients are part of the study group and where pre-analytical, analytical & post-analytical processes are strictly observed and screened with exceptional attention, false-negative results still occur (8). The mistakes may seem obvious on retrospective review (9). False-negativity may result from limited adequacy (obscured by inflammation, transformation zone not sampled, etc) (10).

A zero screening error is an impossible standard of practice. It is both unreasonable and unachievable. Unfortunately acceptable practice standards have not been well defined in cytology. Errors of 5% to 10% may be an admirable goal and below 15% to 20% a possible standard for Pap smear accuracy (9). Errors must be judged not as individual cases but in the context of overall laboratory performance. The definition of an acceptable screening error rate is not meant to condone sloppy work or incompetence, but rather to acknowledge the reality of significant errors by competent, conscientious cytopathololologists.

Although it is a common assumption that screening errors are usually the result of professional incompetence, poor supervision, inadequate continuing education, or excessive number of smears, this is not the case in most accredited laboratories. Atypical squamous cells of undetermined significance (ASC-US) is defined as cellular abnormalities that are more marked than those attributable to reactive changes but that quantitatively or qualitatively fall short of a definitive diagnosis of squamous intraepithelial lesion.

Low-grade squamous intraepithelial lesion (LSIL) is defined as a lesion with or without human papillomavirus (HPV) changes with nuclear enlargement to at least three times that of the normal

intermediate cell and an increased N/C ratio. The superficial and intermediate cells, in sheets or isolated are generally involved. The nuclear contour may be slightly irregular with hyperchromasia and /or smudged or granular but evenly distributed chromatin.

High-grade squamous intraepithelial lesion (HSIL) is defined as small cells and more immature compared to LSIL and ASC-US. Syncytia and isolated cells are more common than sheets. Increased nuclear/cytoplasmic ratio, greater irregularity of contour, and coarsened and/or clumped chromatin are present (11).

Finally, the terminology atypical squamous cells-cannot exclude HSIL (ASC-H) reflects a mixture of true HSIL and its mimics (11).

These definitions leave some room for individual interpretation. For instance, Slater et al (4) demonstrated that LSIL cells show nuclear enlargement limited to only two times (in conventional preparations) and 1.2 times in liquid-based preparations. In spite of the improvements in terminology introduced by TBS, Stoler and Schiffman (5) showed that accuracy and reproducibility remain moderate at best among observers in cervical cytology and histology (11,12). They showed an overall kappa index of 0.46 for diagnostic reproducibility of cytology in the diagnoses of Normal, ASC-US, ASC-H, LSIL and HSIL. These results came from a study of 1996–1998 which included 4948 monolayer cytological slides. The specimens were interpreted by seven clinical centres and four pathology quality control groups. Of note, and not surprisingly, the greatest source of disagreement in monolayer cytology results involved ASCUS interpretations (11,12).

In another study, using a set of 35 slides with varied glandular abnormalities distributed to 167 laboratories, Confortini et al (6) demonstrated that the atypical glandular cell (AGC) category is also only moderately reproducible with a similar kappa value of 0.49. If the introduction of a widely accepted terminology (TBS) has not entirely solved the reproducibility issue in cervical cytology, the question arises as to the interobserver agreement on the component morphological features themselves. Very few studies have addressed this question. Schmidt et al showed poor accuracy (50.5%) and poor reproducibility (j = 0.3) in estimating nuclear area ratios: Forty five participants were asked to choose among five preset area ratios (1:1.4, ・1.5:1.9, ・2 :2.4, ・2.5:2.9, ・3:3.4) for 15 pairs of cells. The same participants repeated the assessment of the same pairs (in a different order) a week later after receiving a tutorial. Despite training before the second assessment, minimal global

improvement was observed (accuracy 53.5% and j = 0.39). The authors suggest that failure to properly assess ratios explains lack of reproducibility in the ASCUS category (13). They acknowledge that other nuclear features, such as chromaticity, chromatin texture and nuclear shape, are important diagnostic features but are more difficult to study because they are more difficult to quantify. The study investigated the reproducibility of assessment of individual cytomorphological features. They were defined as categories or ranks (either ordinal or nominal variables) (10,11).

The three Bethesda workshops (1988, 1991 and 2001) aimed to clarify the terminology for reporting results in cervical cytology. The first Bethesda system (TBS), proposed in 1988, was intended to reduce widespread confusion among laboratories and clinicians created by the use of multiple classification systems (14). Absence of common terminology had generated significant variability in the field of cervical cytology. This had been pointed out in 1972 by Leopold Koss, who referred to differences in terminology and observer variation in the detection of uterine cervical cancer hence the birth of TBS 3 standardized diagnostic terminology (11).

2.1 INTEROBSERVER VARIATION IN PAP SMEAR REPORTING

The interobserver reproducibility of cytologic interpretations is less than perfect. Even the finest laboratories with stringest QC/QA programs miss, overcall or undercall lesions. False-positive diagnoses of cervical cancer occur in 10% to 15% of cases. The following are some documented studies on interobserver variation;

2.1.1 Interobserver Variation for various Lesions

(i) ASCUS- In 2013 Schiffman M. et al had a *k* 0.47 (12). Guy La Ruche et al in 1999 had a *k* 0.000 (1). Sama D.et al in 2001 had a *k* 0.30 (45).

(ii) LSIL- In 1999 Guy La Ruche et al had a *k* 0.23 (1). Sama D.et al in 2001 had a *k* 0.39 (45). This is as shown on the Table 1 below.

Table 1 Interobserver variation for ASCUS and LSIL

ASCUS	
Study	**Kappa** *(k)*
Schiffman M. et al in 2013	*k* 0.47
Guy La Ruche et al in 1999	*k* 0.000
Sama D.et al in 2001	*k* 0.30
LSIL	
Guy La Ruche et al in 1999	*k* 0.23
Sama D.et al in 2001	*k* 0.39

The high interobserver variability can be attributed to the fact that different aspects of atypia that is used to classify ASCUS & LSIL have poor reproducibility. Also these categories do not exfoliate their cells readily.

(i) HSIL- Guy La Ruche et al in 1999 had a *k* 0.53(1).

(ii) SCC- Sama D.et al in 2001 had a *k* 0.30 (45). Guy La Ruche et al in 1999 had a *k* 0.53 (1).

(iii) Glandular lesions- In 2001 Sama D.et al had a *k* 0.21 (45)

This is as shown on the Table 2 below.

Table 2 Interobserver variation for HSIL, SCC and Glandular Lesions

HSIL	
Study	**Kappa** *(k)*
Guy La Ruche et al in 1999	*k* 0.53
SCC	
Sama D.et al in 2001	*k* 0.30
Guy La Ruche et al in 1999	*k* 0.53
Glandular lesions	
Sama D.et al in 2001	*k* 0.21

The improved interobserver variability for HSIL & SCC can be attributed to the fact that HSIL/SCC categories exfoliate their cells more readily, there is little overlap in the criteria for their identification.

There is difficulty in recognizing glandular abnormalities and they are often overlooked. Even normal endocervical cells may show a multitude of cytomorphologic features.

2.1.2 Overall Interobserver Variation

In 1999 Guy La Ruche et al had an overall interobserver variation *k* 0.33 (l). Sama D. et al in 2001 had a *k* 0.57 (45). Schiffman M. et al in 2013 had a *k* 0.47 (12). This is as shown on the Table 3 below.

Table 3 Overall Interobserver Variation

Overall interobserver variation	
Study	**Kappa** *(k)*
Schiffman M. et al in 2013	*k* 0.47
Sama D.et al in 2001	*k* 0.57
Guy La Ruche et al in 1999	*k* 0.33

Although using TBS, interobserver variation is expected but it has to be acceptable, "*Most diagnostic errors occur because screeners are human and humans make mistakes"* [10]. A zero screening error is an impossible standard of practice. It is both unreasonable and unachievable. Unfortunately acceptable practice standards have not been well defined in cytology.

2.2 NEOPLASTIC DISEASES OF THE CERVIX

(a)Atypical Squamous cells of undetermined significance (ASC-US) These cases are daily dilemmas for cytologists (16). The decision to categorize a Pap smear as NILM , ASC-US, ASC-H, LSIL, or HSIL rests on the quantity of the altered squamous cells and the severity of the abnormalities.

In a study done at the Pathology Division of S. Orsola-Malpighi Hospital in Bologna, Italy in years 1997–2007, the high-grade lesions such as carcinoma, HSIL and also LSIL had a higher positive predictive value, as compared to ASC-US and AGC whose values were very low (18). Changes that are suspicious but not conclusive for SIL, are reported as ASC-US. Atypical "mature" squamous cells with features suspicious for a SIL are classified as ASC-US. Some cases have some features of HPV effect, such as binucleation with minimal hyperchromasia. ASC associated with atrophy are diagnosed as ASC-US when there is nuclear enlargement with hyperchromasia, when nuclei are irregular in contour and chromatin distribution, and when there is marked cellular pleomorphism with unusual shapes. In extreme cases of atrophy with inflammation, it becomes difficult to distinguish from a SIL or invasive cancer (16).

(b) Atypical squamous cells cannot exclude HSIL (ASC-H) Is a less common subtype of ASC, representing 5% to 10% of all ASC cases. This category is reserved for Pap samples that are specifically suspicious for HSIL (19).

The most common pattern is that of immature squamous cells with mild to moderate nuclear atypia (enlargement, hyperchromasia, membrane irregularity), commonly called atypical squamous metaplasia .

ASC-H has a positive predictive value for histologic CIN 2,3 that is significantly higher than that of ASC-US (50% vs 17%) (19). For this reason, women with an ASC-H Pap smear should be referred for colposcopy.

If histologic CIN 2,3 is not identified, follow-up with either repeat Pap at 6 and 12 months or HPV DNA testing at 12 months is acceptable. If there is diagnosis of ASC-US or worse on the repeat Pap or tests positive for high-risk HPV, the patient should be referred for another colposcopic examination.

2.2.1 Epithelial abnormalities - squamous

(a) Low grade squamous intraepithelial lesion (LSIL) This includes: HPV/mild dysplasia/CIN 1 is a low-risk intraepithelial lesion that is encountered in approximately 2% of all Pap samples. Many LSILs regress spontaneously, but some persist for long periods of time (21,22).

Approximately 21% progress to HSIL, but it is possible that at least some of these may have been HSILs from the beginning but were initially misclassified as LSILs. 18% of women with an LSIL Pap result prove to have HSIL (CIN 2,3) on biopsy. Less than 1% of untreated LSILs progress to invasive cancer (23,24). LSIL is a lesion of intermediate or superficial cells that shows nuclear enlargement accompanied by moderate variation in nuclear size and slight irregularities in nuclear shape and contour.

Hyperchromasia is present and can take the form of either a uniformly granular increase in chromatin or the smudgy hyperchromasia seen in some koilocytes.

Nucleoli are inconspicuous. Classic koilocytes have large, sharply defined perinuclear cytoplasmic cavities surrounded by dense rims of cytoplasm. The nuclei are sometimes enlarged and atypical. They are diagnostic of LSIL even in the absence of nuclear enlargement. Some LSILs show prominent keratinization manifested by deeply orangeophilic cytoplasm and squamous pearls.

The Kenyan MOH management guidelines for a woman with an LSIL are a repeat of the Pap at 6 or 12 months. If the repeat Pap test tests shows LSIL or greater, colposcopy is indicated. If two repeat Pap tests are negative, a return to routine Pap screening (5years in HIV-ve women) is recommended (25).

(**b) High grade squamous intraepithelial lesion (HSIL)** This includes: moderate and severe dysplasia, CIS; CIN 2 and CIN 3 With features suspicious for invasion. It is encountered in about 0.5% of all Pap samples. Almost all women (97%) with an HSIL Pap result test positive for high-risk HPV.

If untreated, it carries a significant risk of progression to cervical cancer (5,22,24) .HSIL is usually a lesion of immature squamous cells.

Pattern divides HSILs into three categories based on cell size : large cell (20%), intermediate (70%), and small cell (10%). These subtypes are helpful to keep in mind when considering what cells might mimic an HSIL (10,16).

Nuclear enlargement is generally in the same range as in LSILs, but the nuclear-to-cytoplasmic ratio is higher because the cells are smaller.

Hyperchromasia, irregular chromatin distribution, and membrane contour irregularity are all more severe than in LSIL. In HSIL, some of the nuclear changes may predominate.

Some HSILs have irregular nuclear contours but only mild-moderate hyperchromasia. The cells of HSIL are arranged in two main patterns: as distinct individual cells, or as cohesive groups of cells with indistinct cell borders (10,16).

They may have dense, squamoid cytoplasm, but HSIL cells are often completely undifferentiated in appearance and lack any defining squamous features. The cytoplasmic transparency, vacuoles and an elongated configuration can cause them to be mistaken for **cells of glandular origin**.

Although usually a lesion of small, immature squamous cells, mature keratinizing cells with marked nuclear atypia are classified as HSIL.

Distinguishing HSIL from its many mimics is an important skill of the Pathologist/cytologist.

The Kenyan MOH management guideline for a woman with an HSIL is indication for colposcopy and biopsy for confirmation and further management (25).

With the exception of adolescents and those who are pregnant, an immediate (LEEP) is recommended as the initial treatment (25).

(c). Squamous cell carcinoma (SCC) Cytology plays an important role in recognizing patients with cervical cancer. The cellular features are similar to microinvasive carcinoma, but more developed. The cells are fully malignant and show typical malignant features; A tumor diathesis is one of the key

features in the diagnosis of a fully invasive carcinoma. The absence of a diathesis reduces chances of presence of a fully invasive carcinoma (10,16).

Diathesis alone is insufficient evidence to make a firm diagnosis of cancer, presence of malignant cells is the key to differentiating a benign diathesis from a malignant diathesis.

The Kenyan MOH management guideline for a woman with SCC is referral to a specific hospital for further investigation and management (25).

2.2.2 Epithelial abnormalities - glandular

The glandular epithelium of the female genital tract includes the lining of the endocervix, endometrium, and fallopian tube. Adenocarcinomas are currently the most common invasive malignancies of the female genital tract. Unfortunately, the Pap smear is not nearly as good a screening test for glandular lesions as it is for squamous lesions. Diagnostic problems can arise because of paucity or degeneration of the glandular cells, particularly of endometrial origin. Even normal endocervical cells can show a multitude of cytomorphologic features (10). Perhaps we should pay more attention to classification of endometrial cells in cervical cytology, taking a lesson from Yanoh et al, admitting that we cannot always distinguish them simply as benign or malignant (26).

2.3 CHALLENGES IN THE DIAGNOSIS OF SQUAMOUS INTRAEPITHELIAL LESIONS

One should not infer that cytological diagnosis is unreliable because, cytological assessment does not rely on individual features but on holistic evaluation (34) .

Understanding the difficulties in interpreting nuclear features might inform strategies to improve cervical diagnoses (7). Additional investigations are necessary to confirm the cytologic findings.

2.3.1 Avoiding Overdiagnosis of Low-Grade Squamous Intraepithelial Lesions

Care must be taken not to over interpret nonspecific halos or the minimal nuclear changes of benign cells in perimenopausal women.

Without hyperchromasia or nuclear membrane irregularity, such cells are best called negative.

Cellular changes that include some hyperchromasia or nuclear membrane irregularity are suggestive of LSIL and should be categorized as ASC-US (10,16).

2.3.2 Distinguishing Low-Grade from High-Grade Squamous Intraepithelial Lesions

The distinction between cytologic LSIL and HSIL is an important one, with significantly different implications for clinical management.

HSIL is usually a lesion of immature squamous cells, and nuclear atypia (hyperchromasia, irregular chromatin distribution, and membrane contour irregularity) is more severe than in LSIL (10,14,16).

If a specimen has both LSIL and HSIL, it should be reported as an HSIL even if the HSIL cells are less numerous than the LSIL cells. In a small percentage of cases, morphologic features intermediate between typical LSIL and HSIL make grading difficult. Although there are generally fewer abnormal cells in an LSIL than in an HSIL, the quantity of cells is an unreliable discriminator (10,14,16).

Grading is difficult when the dysplastic cells are few in number, when the cytoplasm of the dysplastic cells is affected by cytolysis, or when a LSIL is accompanied by a small number of cells suggestive of but not conclusive for HSIL. Some cytologists may not be able to grade the lesion correctly when faced with the above mentioned scenario. TBS recommends the lesion to be graded using the cells with the highest degree of dysplasia (10,14,16).

Extensively keratinized SILs without definite HSIL are especially difficult to grade. In all such cases, a diagnosis of "SIL, grade cannot be determined" (or "LSIL, cannot exclude HSIL") is appropriate. This diagnosis accounts for 3% to 12% of all cytologic SILs (10,14,16).

Patients with this diagnosis have an intermediate risk (between that of cytologic LSIL and HSIL) of harboring histologic HSIL (CIN 2,3).

2.3.3 Distinguishing High-Grade Squamous Intraepithelial Lesion from Invasive Carcinoma

The criteria used to distinguish HSIL from invasive carcinoma are by no means perfect. Not infrequently, a classic case of HSIL on cytology will turn out to be invasive squamous cancer

on biopsy. Conversely, the possibility of invasive cancer is often raised in cases of HSIL in which the cells have marked nuclear abnormalities associated with abundant, heavily keratinized cytoplasm and unusual cell shapes, but the lesion turns out to be only a keratinizing HSIL on biopsy (10,14,16).

Physicians understand that no diagnosis of HSIL on cytologic material excludes the possibility of invasive cancer, and that colposcopy and biopsy are necessary for confirmation. Some HSILs with

features worrisome for invasive cancer can be reported as “HSIL, with features suggestive of invasive cancer (10,14,16).

2.3.4 Distinguishing squamous cell Carcinoma from High-Grade Squamous Intraepithelial Lesion

Histologically and cytologically, SCC range from well-differentiated, keratinizing tumors to poorly differentiated, non keratinizing tumors. Some SCC cannot be distinguished cytologically from HSIL, particularly the smaller, less deeply invasive tumors(11). Others can be confidently diagnosed as invasive cancers, however. The classic pattern of SCC shows abundant necrotic debris: a granular, amorphous precipitate with nuclear debris and red blood cells called “tumor diathesis”. It is not specific for invasive cancers; a similar pattern is seen in some atrophic smears and even during heavy menstrual bleeding. When associated with hyperchromatic crowded groups of atypical cells or abundant atypical keratinized cells with unusual shapes (“tadpoles,” “fiber cells”), the pattern is diagnostic. The cells of a non keratinizing SCC look like modified HSIL cells. Like HSIL, they are hyperchromatic and have scant cytoplasm, but they have a prominent nucleolus and a highly irregular pattern of chromatin distribution. The cells of a keratinizing SCC are often bizarrely elongated . Some are long and spindle shaped, with small condensed nuclei (“fiber cells”). Others have a larger cytoplasmic body with a long tail (“tadpole cells”). Such cells are uncommon in keratinizing HSILs. Most SCC are associated with an adjacent or overlying HSIL, and therefore cytologic preparations from SCCs often contain a population of HSIL cells as well. The differential diagnosis of SCC includes HSIL. Prominent nucleoli and tumor diathesis are the principal cytologic features that help distinguish SCC from HSIL, but these features are not present in all smears from patients with SCC. A significant number of women with SCC are diagnosed as having HSIL because prominent nucleoli and tumor diathesis are absent. Conversely, a granular, tumor diathesis-like background is not specific for invasive cancers and is seen in women with atrophic vaginitis, severe cervicitis, and rare cases of HSIL (16).

2.3.5 Distinguishing squamous cell Carcinoma from marked atrophy atypia in postmenopausal women

In postmenopausal women, marked atrophy atypia is one of the most common benign mimics of a keratinizing SCC. The benign atypia of atrophy contains scattered cells with large, dark nuclei and eosinophilic or orangeophilic cytoplasm. Their large, dark nuclei are alarming, but chromatin is

usually smudgy. Such cells, if seen in a deeply atrophic squamous background, should be interpreted as ASC-US and not HSIL or invasive cancer (16) **.**

2.3.6 Distinguishing squamous cell Carcinoma from marked repair atypia

Marked repair atypia is another good mimic of nonkeratinizing SCC. Both repair and SCC contain large cells with prominent nucleoli, and mitoses are seen in both. Repair cells are recognized by their finely textured chromatin pattern, the flatness and cohesion of the sheets. If the nuclei have coarsely textured chromatin, show marked crowding, or demonstrate significant dyshesion, SCC should be considered (16).

2.3.7 Distinguishing nonkeratinizing SCC cells from endometrial cells

A minority of nonkeratinizing SCC are composed of small cells that are indistinguishable from endometrial cells . The blood that accompanies menstrual endometrial cells resembles the granular necrosis that is tumor diathesis, adding to the similarity. Mitoses, if identified, should raise the suspicion of SCC. In some cases, knowledge that the patient has a Suspicious cervical lesion or suspicious clinical symptoms (e.g., dyspareunia) may be the only clue to the correct interpretation (16).

2.3.8 Distinguishing Squamous cell Carcinoma from a mimic of Behçet disease

Behçet disease, a chronic disease of uncertain cause that is characterized by oral and genital ulcers, can mimic SCC. Smears may show numerous isolated, keratinized cells with dark, pleomorphic nuclei and large nucleoli. A history of this disorder may be critical for correct diagnosis (16).

2.3.9 Identifying Squamous cell Carcinoma in patients with Pemphigus Vulgaris (PV) disease

Smears from patients with pemphigus vulgaris, a blistering disorder that involves mucosal surfaces, may mimic a poorly differentiated SCC. A complete history may be important to avoid making an overcall, although cases of coexisting SCC and pemphigus vulgaris have been reported (16).

Cytologists/Pathologist should be aware of typical acantholytic suprabasal cells of PV to avoid overdiagnosis of neoplasia. However, underdiagnosis is possible and careful screening for malignant cells in the background of PV is necessary (18).

2.3.10 Distinguishing Squamous cell Carcinoma tumor diathesis from diathesis resulting from other causes

Although the presence of a diathesis is suspicious for infiltrating cancer, several different benign processes can be associated with a background similar in appearance to a tumor diathesis; Severe *Trichomonas* or herpes infections may causea inflammatory, dirty background.

Severely atrophic smears from postmenopausal women commonly have a granular background resembling a diathesis.

Abundant bacteria, especially cocci, may also produce a blue, granular background, mimicking a tumor diathesis (10).

2.3.11 Diagnostic problems associated with degeneration of the glandular cells

The Pap smear is not nearly as good a screening test for glandular lesions as it is for squamous lesions (35). Diagnostic problems can arise because of paucity or degeneration of the glandular cells, particularly of endometrial origin (30).

Abundance of well- preserved glandular cells, in large, cellular groups, may be obtained with an endocervical brush, which can show a spectrum of benign and malignant changes that can be difficult to interpret (30). Reactive glandular cells can possibly result in over diagnosis of both glandular and squamous abnormalities. Even normal endocervical cells can show a multitude of cytomorphologic features (10).

2.3.12 Distinguishing High-Grade Squamous Intraepithelial Lesion from atypical endocervical cells

A common error is mistaking HSILs, for atypical endocervical cells. Diagnosis of HSIL-EGI (high-grade squamous intraepithelial lesion with endocervical glandular involvement) may be possible on pap smears but will have a high sensitivity, but low specificity (18) .

Many HSILs have transparent and even vacuolated cytoplasm. Atypical cells with a rounded contour are more likely to be HSIL than AIS, and for such cases ASC-H is a more appropriate interpretation. The cells of AIS are usually recognizably columnar. For this reason, atypical endocervical cells should be reserved for cells with a recognizably columnar morphology (10).

In a study where 190 cases of patient population were surgically proven to have endometrial carcinoma (EC), 72 (41.9%) cases out of 172 with preoperative Pap test, had an abnormal Pap diagnoses, such as AGC or malignant tumours.. Tumours of higher grade/stage were more likely to be diagnosed by the Pap test ($P < 0.001$). Endometrioid carcinomas were more likely to be diagnosed as

AGC in the Pap test. In Conclusion, Only 41.9% of all EC cases in the study were diagnosed as AGC/malignant on Pap test (18).

2.3.13 Distinguishing endocervical/endometrial hyperchromatic crowded groups (HCGs) from neoplasia

Endocervical cells obtained high in the endocervical canal, which may be sampled using an endocervical brush, are normally more crowded, with higher N/C ratios, than those obtained with an ordinary spatula. The high endocervical cells can form hyperchromatic crowded groups (HCGs) mimicking neoplasia, resulting in false-positive diagnoses (36).

However, the nuclei are bland and round, and resemble other endocervical nuclei. No mitotic figures are present.

Also, in women who have undergone cone biopsy, endometrial cells from the lower uterine segment grow into the endocervix, where they may be directly sampled. These endometrial cells may show reactive/regenerative changes and exfoliate hyperchromatic crowded groups mimicking neoplasia (cone biopsy artifact) (36).

2.3.14 Identifying the real causes of Atypical Endometrial cell from its many causes /Aetiology

Atypical endometrial cell are clusters of cells with an enlarged nucleus and other features of nuclear atypia like nuclear membrane irregularity, prominent nucleoli etc. The cytoplasm is scant or moderately abundant and vacuolated.

Such cells are suspicious for endometrial adenocarcinoma (30). Presence of atypical endometrial cells carries a significant risk of cancer.

Similar changes are known to be caused by endometrial polyps, chronic endometritis, IUDs, and endometrial hyperplasia & pregnancy (16).

Cytological diagnosis of dual glandular abnormalities of the female genital tract is very difficult. Cytological features of endometrioid FTC (Fallopian tube carcinoma) are not sufficiently specific to distinguish it from endometrial carcinoma (18).

2.3.15 Identifying the real causes of reactive endocervical cell from its many causes/Aetiology

Reactive endocervical cells are also seen in microglandular hyperplasia, a benign alteration of endocervical epithelium associated with oral contraceptive use. Microglandular hyperplasia has been described in histology, where it was sometimes confused with adenocarcinoma (37). Reactive

endocervical cells are common in pregnancy, where in their extreme form they represent the Arias-Stella reaction. In their most extreme forms, reactive endocervical cells raise a differential diagnosis to LSIL, HSIL, AIS, and invasive cancer.

2.4 JUSTIFICATON

For cervical cancer screening, strategies must be developed to minimize errors (38). Management approaches for screened women differ depending on the outcome of the screening test. This demands that the reports obtained from the screening test be acceptable.

Unfortunately, there is not always a perfect, one-to-one correspondence between the cytologic diagnosis and the histologic diagnosis. In many cases, the cytology significantly undercalls or overcalls the lesion seen in the corresponding biopsy (7,39,40).

The interobserver reproducibility of cytologic interpretations is less than perfect. False-positive diagnoses of cervical cancer occur in 10% to 15% of cases.

For a screening test, false-negative diagnosis (substantially undercalling or entirely missing a lesion) is, of course, the most serious problem (8) Therefore, some diagnostic specificity may have to be sacrificed in order to enhance sensitivity (41).

Currently at KNH, pap smears are first screened by a Technologist or a Human Pathology Registrar or an Msc Clinical Cytology student who then signs it out with a Consultant Pathologist. There is no formal established system that can be reviewed to correct for deficiencies (if any).

Thus this study was done to determine the frequency and nature of discordant diagnoses between two independent reports in screening of women whose Pap smears were analyzed at KNH cytology laboratory in the years 2011 to 2013. Establishing interobserver variation in the cytological diagnosis and grading of dysplasia in cervical specimens justifies the setting up of quality control measures in public health screening programs, in order for the screening strategy to minimize substantially undercalling or overcalling a lesions (7,42). An example of a quality control measure would be regular participation in EQAs (either interlab or through a reference lab) It is also mandatory and good laboratory practice for accredited laboratories.

2.5 RESEARCH QUESTION

What is the degree of interobserver variation of pap smears using the Bethesda grading system (2001) in KNH cytology laboratory?

2.6 OBJECTIVES

2.6.1 General objective:

To determine the interobserver variation in pap smear reporting using The Bethesda System for Reporting Cervical Cytology (2001) at Kenyatta National Hospital**.**

2.6.2 Specific objectives:

1) To review all the pap smears previously reported as ASCUS and higher lesions in the period of Jan 2011 to June 2013 (30 months).
2) To determine the pattern of abnormal cervical intraepithelial lesions in the study population.
3) To determine the interobserver variation in pap smear reporting using The Bethesda System for Reporting Cervical Cytology (2001) at Kenyatta National Hospital**.**

3.0 MATERIALS AND METHODS

3.1 Study site

This study was conducted at the Kenyatta National Hospital (KNH) Cytology laboratory.

3.2 Study design

This was a laboratory based retrospective study.

3.3 study population.

Pap smears previously reported as ASCUS or higher lesions at KNH Cytology laboratory from January 2011 to June 2013 (30 months).

3.4 Selection criteria

3.4.1 Inclusion criteria

Pap smears which were reported as ASCUS or higher lesions at KNH Cytology laboratory from January 2011 to June 2013 (30 months).

3.4.2 Exclusion criteria

1) Cases where the slide preparations missed from among the Pap smears which had been reported as ASCUS or higher lesions at KNH Cytology laboratory from January 2011 to June 2013 (30 months).
2) Unsatisfactory on review slide preparations from among the Pap smears which had been reported as ASCUS or higher lesions at KNH Cytology laboratory from January 2011 to June 2013 (30 months).

3.5 Sample size determination

This study sought to ascertain the degree of agreement between two observers on the same samples of pap smears. Therefore, Fisher's formula was applied to find the minimum sample size required to estimate the degree of concordance between the two readers within a certain degree of confidence (in this case 95% confidence interval).

Sample size calculation was done using the following formula:

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

n – Sample size

Z – 1.96 (95% confidence interval)

P – Estimated degree of disagreement – 41% (1)

d – Margin of error (precision error) = ±5%

Substituting into the formula,

$$n = \frac{1.96^2 \times 0.41\,(1- 0.41)}{0.05^2}$$

n = 371.7

n = 372

Thus 372 Pap smears which were reported as ASCUS or higher lesions at KNH Cytology laboratory from January 2011 to June 2013 (30 months),were selected for the study.

3.6. Sampling method

Sampling was done starting from January 2011 upto June 2013 (30 months) by the principal investigator with assistance from qualified technical staff in KNH Cytology laboratory.All pap smears that had been reported as ASCUS or worse including those that had been reported by the supervising pathologists were included in the study.

Consecutive sampling technique was used to select Pap smear preparations. The cases were selected, totalled, and their ratios determined in order to arrive at the above calculated sample size.

3.7 Cytological assessment and Reporting

All the Pap smears were first screened by the PI and then reported with the supervisors (Consultant Pathologists). The 372 Pap smear prepartions were divided equally amongst the three supervisors.

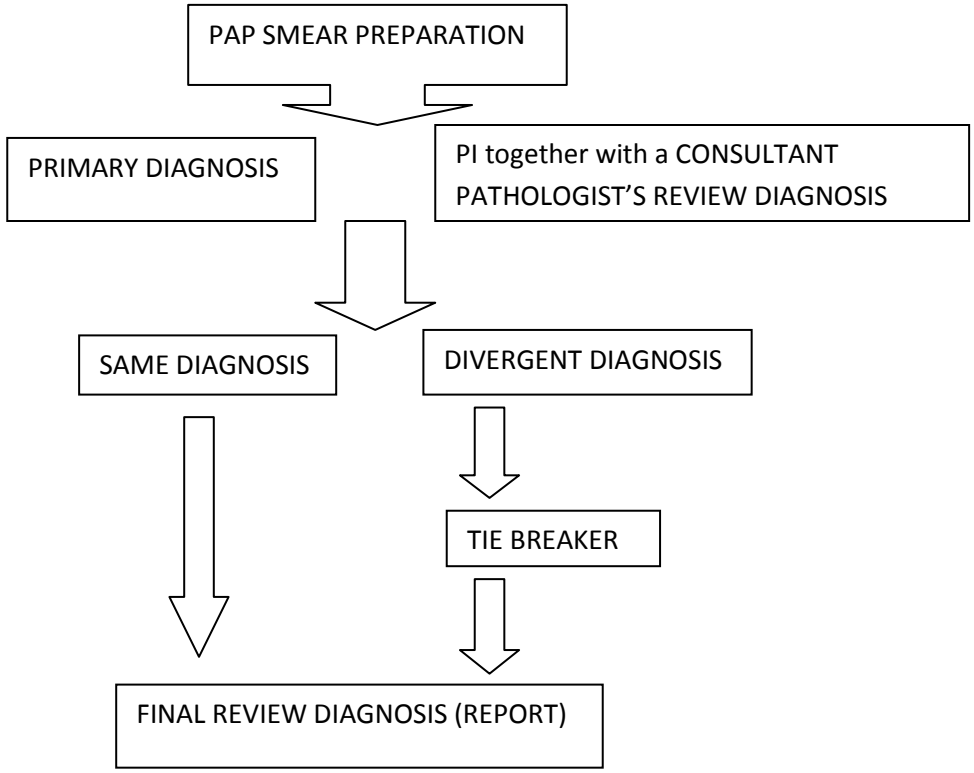
Smears with discordant results from the primary reports were reviewed by a different Consultant Pathologist (i.e from among the three supervisors) who was the tie breaker.

All the three Consultant Pathologists have a vast experience in Pap smear reporting and hence each one of them did tie breaking for his/her peer.Criteria for selection of the tie breaker was that each Pathologist did tie breaking on a different set of Pap smears from the one he/she had reported.

Presence of neoplastic or non-neoplastic cytologic findings was reported using The Bethesda Reporting System (TBS) 2001[Appendix 1]. The information on which pathologist initially reported and signed out the pap smear was not extracted from the laboratory records so review was done blindly.

Report generated by the PI together with supervising Pathologists formed the "Review report" and was compared with the Primary results for determination of interobserver variation.

Figure 1 Schematic chart on Cytological Review Diagnosis



3.8 Biosafety measures

Gloves were worn during selection, storage, screening and reporting of the pap smear samples. Selected smears were put in safe slide boxes and transported to a holding area of KNH/UON

laboratories. The selection of the specimens was done at KNH/UON laboratories under the supervision of qualified technologists.

3.9 Variables

Dependent variables in this study were the types of intraepithelial lesion or malignancy i.e ASCUS, LSIL, HSIL, AGC, ASCH, SCC or AIS.

Independent variables in this study were age, type of contraception used, parity, state of menstrual cycle (pre or post menopause) and HIV status.

The correlation between Dependent and Independent variables was not possible to analyse. This is because some clinical information including the type of contraception used, parity, state of menstrual cycle (pre or post menopause) and HIV status were not provided in the laboratory reports.

In addition distribution of of the intraepithelial lesions or malignancy over the three years was analysed for both primary and review reports.

3.10 Data Management & Statistical Analysis.

Data was collected by the PI. It was then entered into Microsoft Excel (Ms 2007), and analyzed using SPSS version 17.0 for Windows (SPSS Inc). Data was analyzed and presented in form of frequencies, proportions/percentages, graphs, charts and photomicrographs.

Kappa (k) statistic was used to calculate the degree of agreement between the primary and the review reports.

3.11 Interpretation of Kappa Values

Cohen's kappa is a statistical method used to compare the degree of consensus between raters (inspectors), for qualitative (categorical) items for example, Measurement of Systems Analysis. It uses a contingency table approach. This is the statistical method that was used to analyze the data for this study. Kappa values have a range of -1 to +1 and are interpreted as shown in Table 4 below.

Table 4 Interpretation of Kappa Values

Kappa Value	Kappa Agreement
< 0	Less than chance agreement
0.01–0.20	Slight agreement
0.21– 0.40	Fair agreement
0.41–0.60	Moderate agreement
0.61–0.80	Substantial agreement
0.81–0.99	Almost perfect agreement

3.12 Quality assurance.

The sample preparations were reviewed by a Consultant pathologist. Smears with discordant results were reviewed by a different pathologist who was the tie breaker. All the three Consultant Pathologists have a vast experience in Pap smear reporting and they consulted each other on some cases while tie breaking.

3.13 Ethical Considerations.

Permission to carry out the research was sought and obtained from the UON/KNH ethics and research committee before commencement of the study.

Data was and will not be published in any way that can be directly connected to an individual patient or pathologist to observe confidentiality (43).

The obtained review reports were categorized accordingly for proper management of patients as follows:

(A). -Correct *(correct diagnosis and agreement)*

(B).-Incorrect (*misdiagnosis and disagreement):* -If so;- (i).-Category 1

(ii).-Category 2

(iii).-Category 3

Category 1: A diagnostic error, which would have a definitive influence on clinical management and possible outcome.

Category 2: A diagnostic misinterpretation or oversight, which had the potential to affect clinical management or outcome.

Category 3: A minor discrepancy of lesion categorisation likely to be of little clinical significance.

Category 1 and 2 Necessitated sending addendum reports to the clinician for re-evaluation of management of the patient.

IP/OP number of patients in category 1 and 2 above was used to obtain clinical records from which clinician and patient contact details were retrieved. Addendum report were written and communicated to the clinician. The patient was then contacted for re-evaluation of clinical management.

3.14 Results Dissemination.

Scientific paper will be published in a peer review journal.

4.0 RESULTS

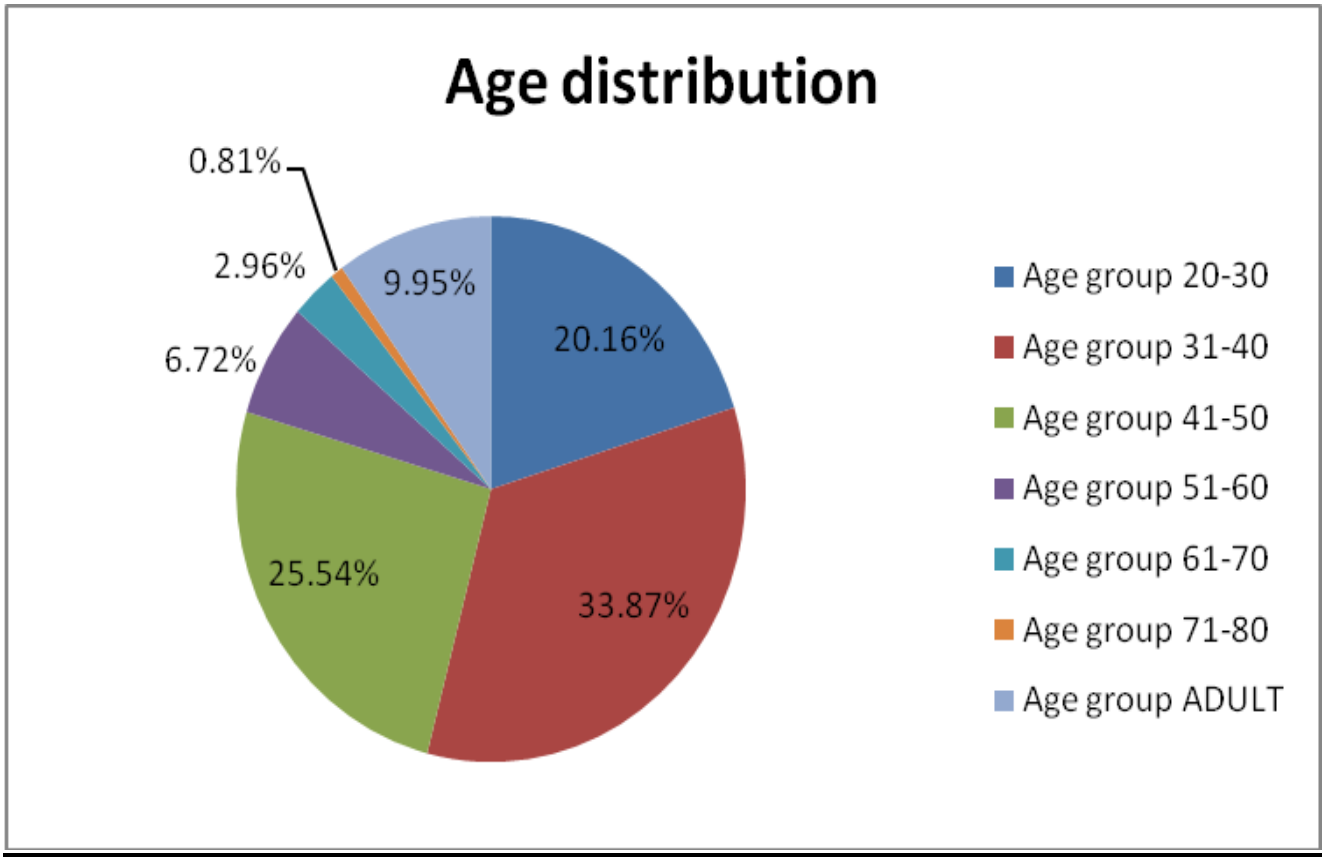
4.1 Demographic characteristics

Age distribution

In the study period, i.e between January 2011 upto June 2013 (30 months), 8256 Pap smears were reported in KNH Cytology laboratory. Of these 1019 (12.3% of total smears reported) Preparations were reported as ASCUS and above, of which 221 cases (21.7% of abnormal cases & 2.7% of total smears reported) had missing slides.

A total of 372 (36.5% of abnormal cases & 4.5% of total smears reported) Pap smear Preparations were selected for the study. The ages of the patients were retrieved from the laboratory register forms, of which 37 reports did not have patients' ages recorded.The ages ranged from 20 up to 78 years with a mean age of 35 years and a median of 37years. Other socio demographic and clinical information including the type of contraception used, parity, state of menstrual cycle (pre or postmenopausal) and HIV status was not provided in the laboratory reports and were therefore excluded from analysis.

Figure 2 Distribution of Age



4.2 Distribution of lesions in the study population.

4.2.1 Distribution of cervical lesions in primary report

Of the selected 372 Pap smear preparations, 87 were from the year 2011. Others were 149 and 136 preparations from years 2012 and 2013 respectively. The prevalence of the different grades of lesions was obtained from the selected population totalled, and their ratios determined in order to arrive at a sample size of 372 pap smear preparations.This is as shown on the table 5 below.

Table 5 Distribution of lesions in the primary report

CERVICAL LESION	**YEAR**			**TOTAL** n (%)
	2011 n (%)	**2012** n (%)	**2013** n (%)	
ASCUS	17 (5)	31 (8)	55 (15)	**103** (28)
LSIL	37 (10)	52 (14)	46 (12)	**135** (36)
HSIL	20 (5)	50 (13)	24 (6)	**94** (25)
ASCH	1 (0.3)	4 (1)	3 (0.8)	**8** (2)
SCC	2 (0.5)	3 (0.8)	6 (1.5)	**11** (3)
GLANDULAR	10 (3)	9 (2)	2 (0.5)	**21** (6)
TOTAL	**87** (23)	**149** (40)	**136** (37)	**372** (100)

4.2.2 Distribution of lesions in the study population on Review

The distribution of lesion over the years varied between the primary and the review reports. Upon review, some lesion were downgraded and others upgraded. Presence of a mixed pathology which had been missed in the primary report over the years 2011 to 2013 was found as shown on the Table 6 below.

Table 6 Distribution of lesions on review

LESION	YEAR			TOTAL n (%)
	2011 n (%)	**2012** n (%)	**2013** n (%)	
ASCUS	6 (1.6)	31 (8)	23 (6)	**60** (16)
NILM	22 (6)	24 (6)	36 (9.6)	**82** (22)
LSIL	18 (5)	34 (9)	30 (8)	**82** (22)
HSIL	28 (7.5)	40 (11)	26 (7)	**94** (25)
ASCH	2 (0.5)	6 (1.6)	5 (1.3)	**13** (3.5)
SCC	7 (1.8)	10 (3)	15 (4)	**32** (9)
GLANDULAR	4 (1)	4 (1)	1 (0.3)	**9** (2)
TOTAL	**87** (23)	**149** (40)	**136** (37)	**372** (100)

4.2.3 Comparison of distribution of lesions in primary and the review reports

A total of 15 (4.03%) cases with mixed lesion which were missed in the primary report were picked in the review report. Eighty two cases (22.04%) were downgraded to negative (NILM-Negative for intraepithelial lesion or malignancy). Majority of these cases, 57 (15.32%) had been signed out as ASCUS of which the interobserver variation has insignificant changes on patients'clinical management. However there were the following significant 'overcalls' ; 17 (4.57%) LSILs cases, 3 (0.81%) HSILs cases, and 4 (1.08%) AGC cases were downgraded to NILM. For this study, reactive, reparative and inflammatory changes were grouped together as NILM.

Other significant 'overcalls' were as follows; 2 (0.54%) cases which had been signed out as HSIL in the primary report were downgraded to ASCUS, 3 (0.81%) HSIL cases were downgraded to LSIL.

Significant 'undercalls' were as follows; For ASCUS category, 2 (0.54%) cases were upgraded to HSIL microivasion cannot be excluded, 1 (0.27%) case to HSIL and 2 (0.54%) cases to SCC. Two (0.54%) cases were upgraded to mixed lesions of LSIL/AGC and HSIL/AGC respectively.

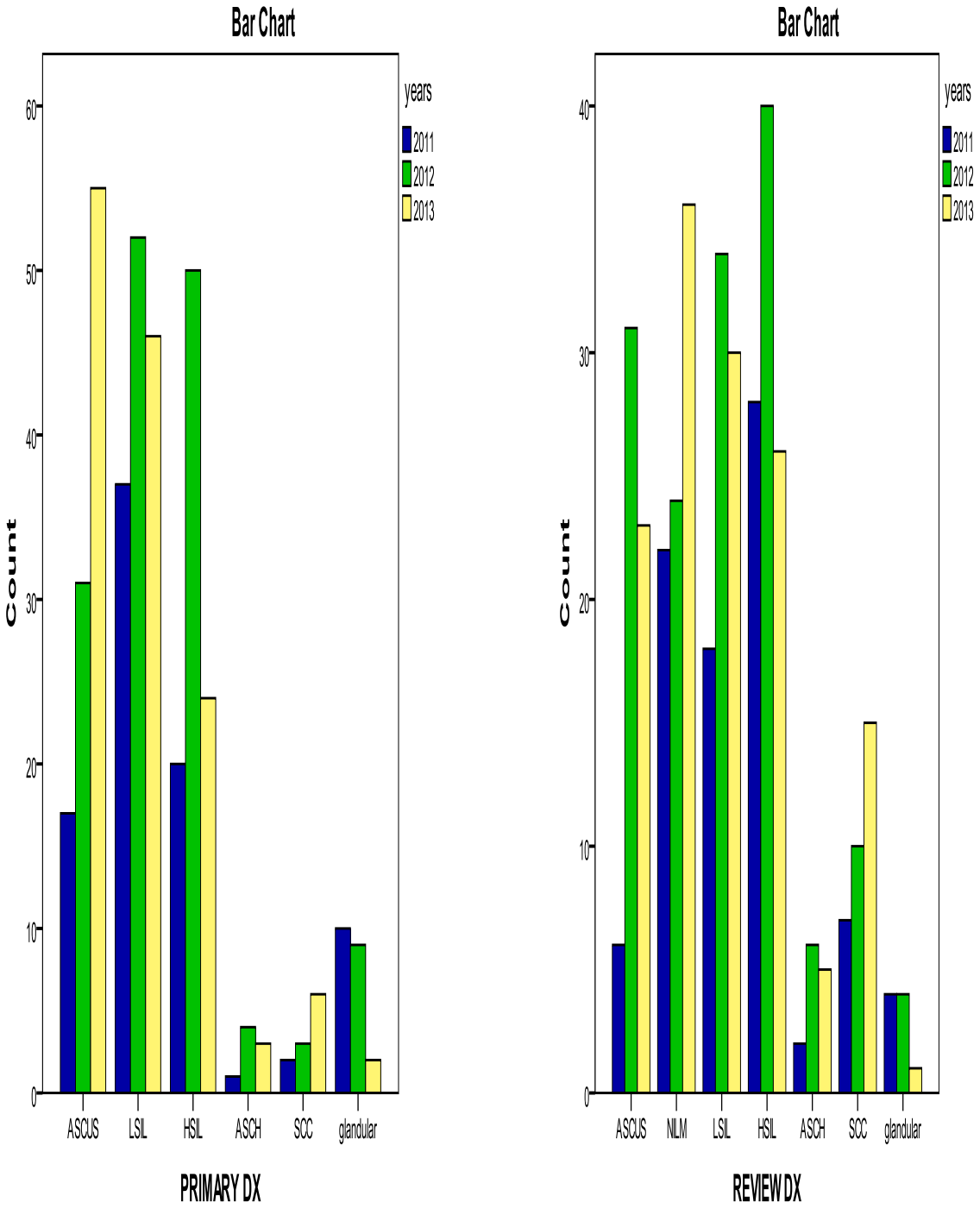
For LSIL category 8 (2.15%) cases were upgraded to HSIL, 2 (0.54%) cases to ASCH, 3 (0.81%) cases to SCC and 2 (0.54%) cases to HSIL microivasion cannot be excluded. Two (0.54%) cases of LSIL were upgraded to mixed lesions of SCC/AGC and 2 (0.54%) cases to ASCH/AIS and HSIL/AGC respectively. This is as shown on the Table 7 below.

Table 7 Distribution of lesions - Primary reports versus review reports

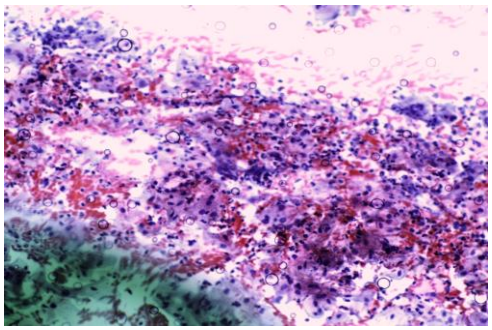
LESION	YEAR									
	2011		2012		2013		TOTAL			
	1st	2nd	1st	2nd	1st	2nd	1st	1st %	2nd	2nd %
NILM	0	22	0	24	0	36	**0**	**0%**	**82**	**22.04%**
ASCUS	17	6	31	31	55	22	**103**	**27.69%**	**59**	**15.86%**
LSIL	37	17	52	34	46	30	**135**	**36.29%**	**81**	**21.77%**
HSIL	16	23	44	30	15	14	**75**	**20.16%**	**67**	**18.01%**
HSIL Microinvasion cannot be excluded	3	1	2	4	4	6	**9**	**2.42%**	**11**	**2.96%**
ASCH	1	1	4	5	3	5	**8**	**2.15%**	**11**	**2.96%**
AGC	8	1	9	3	2	1	**19**	**5.11%**	**5**	**1.34%**
HSIL/AGC	1	4	4	5	5	5	**10**	**2.69%**	**14**	**3.76%**
SCC	2	6	3	10	6	12	**11**	**2.96%**	**28**	**7.53%**
AIS	2	1	0	0	0	0	**2**	**0.54%**	**1**	**0.27%**
ADENOSQUAMOUS	0	2	0	0	0	0	**0**	**0%**	**2**	**0.54%**
LSIL/AGC	0	1	0	0	0	0	**0**	**0%**	**1**	**0.27%**
ASCH/AGC	0	1	0	0	0	0	**0**	**0%**	**1**	**0.27%**
SCC/AGC	0	1	0	1	0	3	**0**	**0%**	**5**	**1.34%**
ASCUS/AGC	0	0	0	0	0	1	**0**	**0%**	**1**	**0.27%**
HSIL Microinvasion cannot be excluded /AGC	0	0	0	1	0	1	**0**	**0%**	**2**	**0.54%**
ASCH/AIS	0	0	0	1	0	0	**0**	**0%**	**1**	**0.27%**
TOTAL	**87**	**87**	**149**	**149**	**136**	**136**	**372**	**100%**	**372**	**100%**

1st-Primary report **2nd-Review report** **%-Percentage**

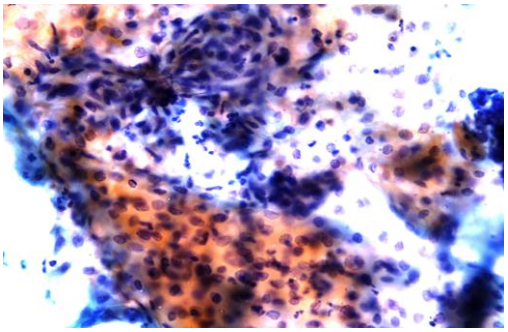
Figure 3 Comparison of distribution of lesions between the Primary & the Review report



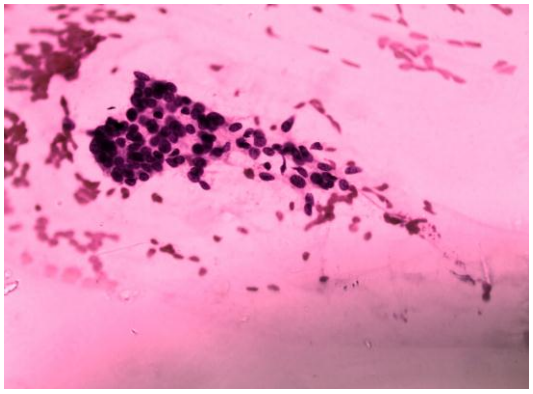
4.2.4 Example of Photomicrographs (A-R) showing various lesions



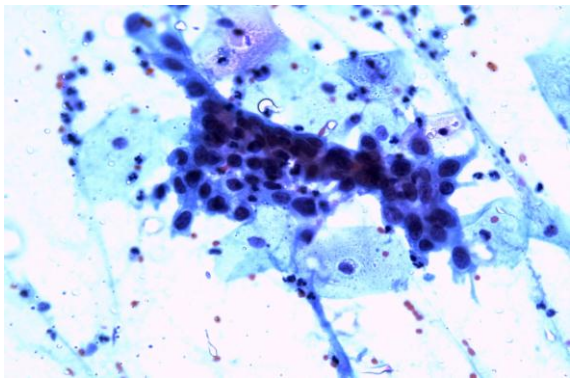
(A) ×40



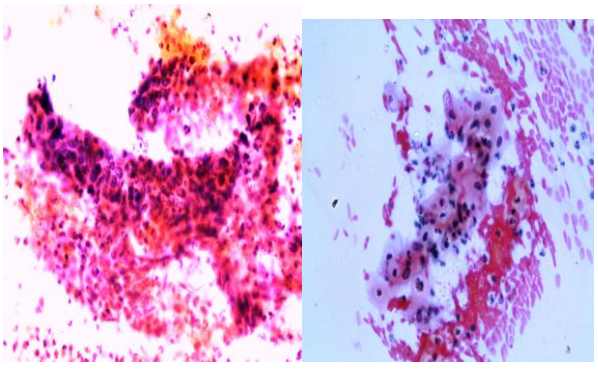
(B) ×40



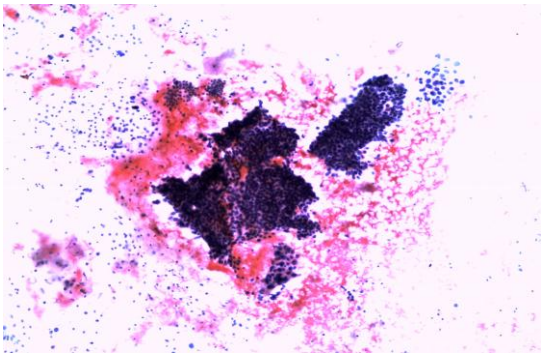
(C) ×40



(D) ×40

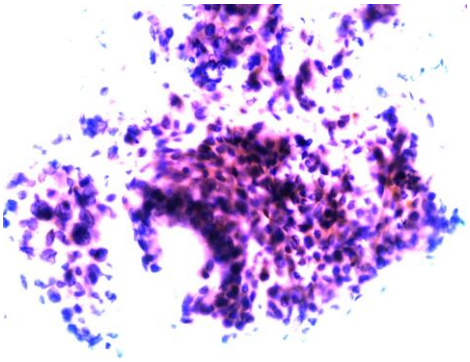


(E) ×40

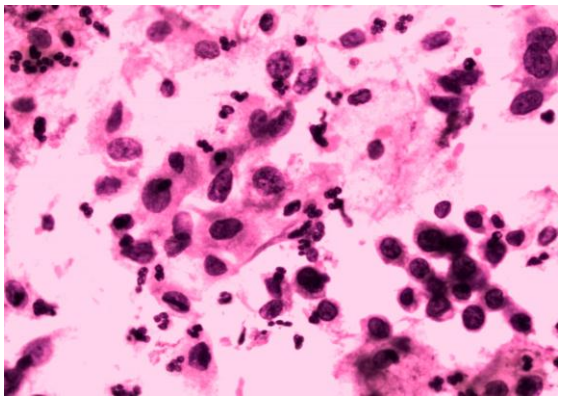


(F) ×10

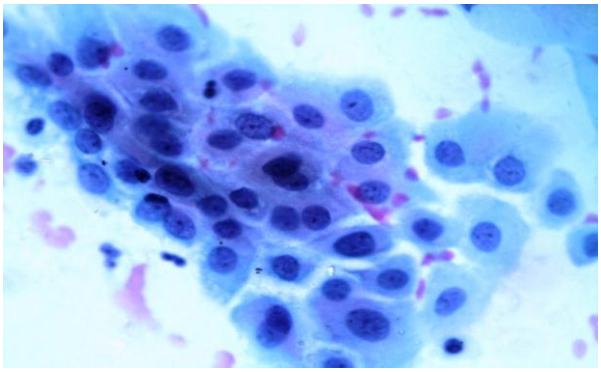
(A) SCC Previously undercalled as HSIL**. (B)** Adenosquamous carcinoma, Previously undercalled as AGC, Mixed lesion was missed. **(C)** AIS**,** Previously undercalled as AGC, Note the feathering of cells. **(D)** HSIL Prevously signed out as AGC. **(E**) ASCUS/AGC signed out as AGC. **(F)** SCC/AGC signed out as AGC, Squamous lesion was missed.



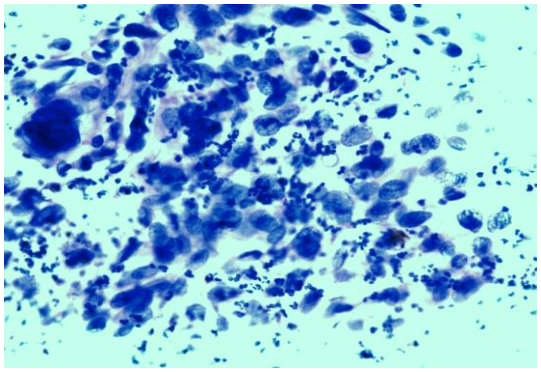
(G) ×40



(H) ×63



(I) ×63



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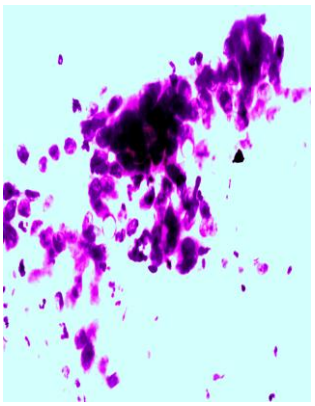
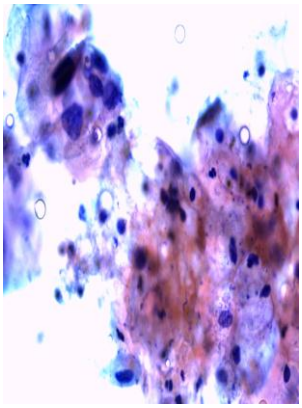
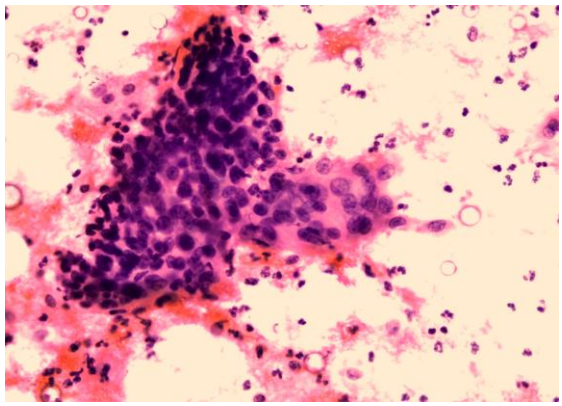


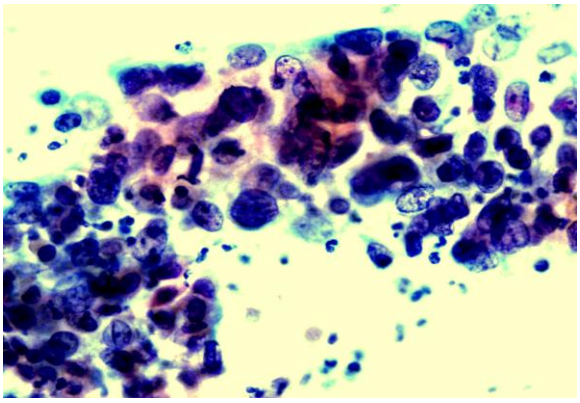


(K) ×63

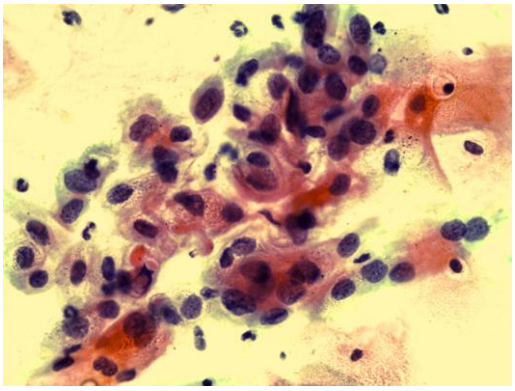


(L) ×63

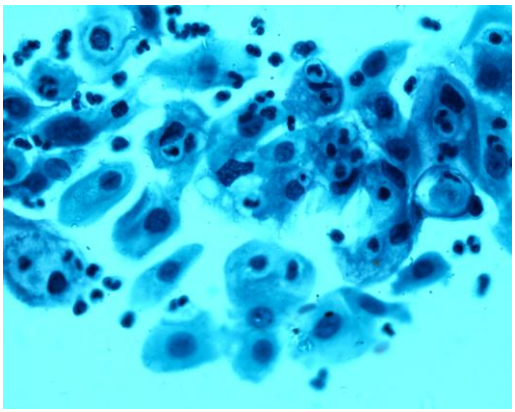
(G) HSIL with glandular extension signed out as HSIL/AGC, Note the crypt in the lumen with a squamous involvement. (**H)** HSIL signed out as ASCH. **(I)** HSIL microinvasion cannot be excluded, signed out as ASCH. However G,H & I above have insignificant change towards patient management. **(J)** HSIL microinvasion cannot be excluded. Previously undercalled as ASCUS. **(K)** HSIL/AGC. Previously undercalled as ASCUS. Mixed lesion was missed**. (L)** HSIL with inflammation, Previously undercalled as ASCUS, atrophic smear.



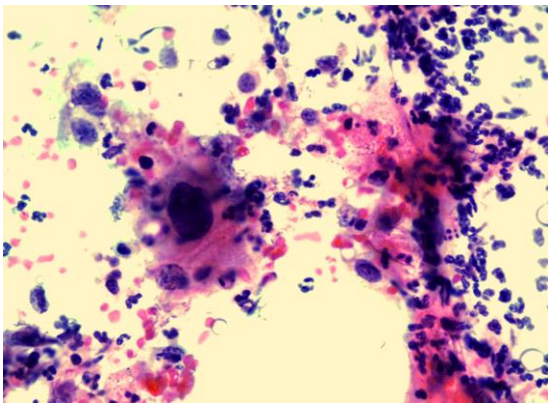
(M) ×63



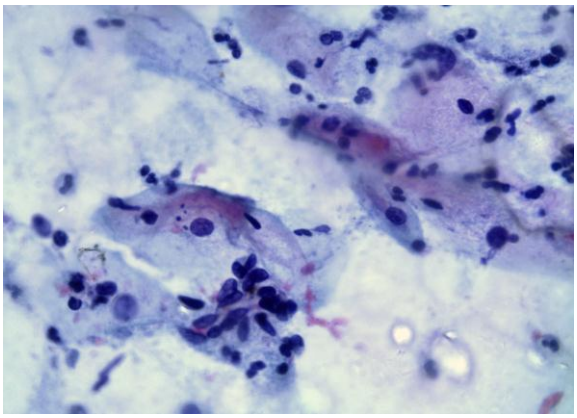
(N) ×63



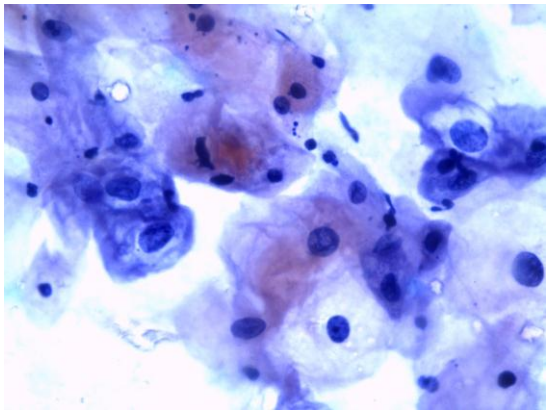
(O) ×63



(P) ×63



. **(Q) ×63**



(R) ×63

(M) HSIL,Microinvasion cannot be excluded. Previously undercalled as ASCUS. **(N)** HSIL, Previously undercalled as LSIL. **(O)** HSIL, Previously undercalled as LSIL. **(P)** SCC, Previously undercalled as LSIL. **(Q)** HSIL, Previously undercalled as LSIL. **(R)** LSIL, Previously overcalled as HSIL. Note the Koilocytic changes on the intermediate sized cells.

4.3 Interobserver variation between the Primary and the Review report

4.3.1 Interobserver variation between the Primary and the Review report for Different Lesions

ASCUS had a kappa of 0.049, LSIL had a kappa of 0.045, HSIL had a kappa of 0.126, ASCH had a kappa of 0.231, SCC had a kappa of 0.376 & Glandular lesions had a kappa of 0.125 as shown on the table 8 below.

Table 8 Interobserver variation for Different Lesions

LESION	**YEAR**			
	2011 *k* **(n)**	**2012** *k* **(n)**	**2013** *k* **(n)**	**OVERALL** *k* **(n)**
ASCUS	0.017 **(17)**	0.131 **(31)**	0.028 **(55)**	0.049 **(103)**
LSIL	0.041 **(37)**	0.045 **(52)**	0.047 **(46)**	0.045 **(135)**
HSIL	0.348 **(20)**	0.076 **(50)**	0.076 **(24)**	0.126 **(94)**
ASCH	0.00 **(1)**	0.50 **(4)**	0.410 **(3)**	0.231 **(8)**
SCC	1.00 **(2)**	0.80 **(4)**	0.097 **(5)**	0.376 **(11)**
GLANDULAR	0.138 **(10)**	0.031 **(9)**	1.000 **(2)**	0.125 **(21)**

4.3.2 Overall Interobserver variation between the Primary and the Review report

The **overall agreement** between the primary report and the review report was fair with a kappa of **0.327.** The contingency table that was used for the calculation is as shown below Table 9.

Table 9 Interobserver variation for Overall kappa (review report vs primary report)

		REVIEW Diagnosis							
		ASCUS	NILM	LSIL	HSIL	ASCH	SCC	GLANDULAR	TOTAL
PRIMARY Diagnosis	ASCUS	26	58	10	4	2	2	1	**103**
	LSIL	29	17	68	13	3	5	0	**135**
	HSIL	2	3	3	63	2	18	3	**94**
	ASCH	1	0	0	4	3	0	0	**8**
	SCC	0	0	0	5	1	5	0	**11**
	GLANDULAR	2	4	1	5	2	2	5	**21**
	TOTAL	**60**	**82**	**82**	**94**	**13**	**32**	**9**	**372**

5.0 DISCUSSION

This retrospective study was done to determine the frequency and nature of discordant diagnoses between two independent reports in screening of women whose Pap smears were analyzed at KNH cytology laboratory in the years 2011 to 2013.

The patient ages were retrieved from the laboratory register files, they ranged from 20 upto 78 years with a mean age of 35years and a median of 37 years. The mean age compares well with that of a previous study by Guy La Ruche et al in 1999 (1).

The age group that had the highest frequency of lesions was 31-40 years. This can be attributed to the known fact that the majority of lesions manifest in the fourth decade of life.

Other socio-demographic and clinical information including the type of contraception used, parity, date of menstrual cycle (pre or post menopause) and HIV status was not provided in the laboratory reports. Consequently, it was not possible to do a correlation of the above mentioned variables with the type of lesions and as a result, analysis on the pattern of abnormal cervical intraepithelial lesions in the study population was not possible.

Interobserver variation in the cytological diagnosis of cervical lesions poses a problem for public health screening programs. Although using TBS, **interobserver variation** is expected, but it has to be acceptable (10). This study assessed the degree of discordant diagnoses between the primary and the review results.

About 4% cases with mixed lesion which had been missed in the primary report were diagnosed in the review report. A total of 22% cases were downgraded to negative (NILM-Negative for intraepithelial lesion or malignancy). Majority of these cases had been signed out as ASCUS of which the interobserver variation had insignificant changes on patients'clinical management.

Sources of interpretative variability was the fraction of HSIL that were reviewd as either LSIL or ASCUS. Transition from HSIL to LSIL reflects the difficulty referenced in the literature of trying to separate mild (CIN I) from moderate dysplasia (CIN II). On the other hand, transition from HSIL to ASCUS and vice versa represents the controversy surrounding small atypical cells of immature metaplastic type. Another source of variability was the fraction of LSIL that were reviewed as either

NILM or ASCUS. This transition reflects problems in implementation of criteria for recognizing HPV cytopathic effects and other cytomorphological features necessary for the classification of an LSIL category.

Squamous Lesions

ASCUS had very low agreement ***k* 0.049**. Schiffman M. et al in 2013 had a *k* 0.47 (12) .Guy La Ruche et al in 1999 had a *k* 0.000(1). Sama D.et al in 2001 had a *k* 0.30 (45). **LSIL** had a very low agreement with ***k* 0.045**. Guy La Ruche et al in 1999 had a *k* 0.23 (1). Sama D.et al in 2001 had a *k* 0.39 (45).

The high interobserver variability can be attributed to the fact that different aspects of atypia that is used to classify ASCUS & LSIL have poor reproducibility e.g N/C ratio, nuclear size etc. ASCUS can be mistaken for reactive, inflammatory & reparative changes. Also these categories do not exfoliate their cells readily. Presence of 100 abnormal cells represent a threshold below which detection is possible, but not always reliable, by routine screening.

The kappa value for ASCUS in this study compares with Guy La Ruche et al in 1999 (1) but does not compare well with other studies. This can be attributed to the fact that most of the ASCUS category were overcalled in the primary report.

HSIL had a poor agreement with a kappa of **0.126**. This is low compared to Guy La Ruche et al in 1999 who had a *k* 0.53(1). **SCC** had a fair agreement with a kappa of **0.376**. Sama D.et al in 2001 had a *k* 0.30 (45). Guy La Ruche et al in 1999 had a *k* 0.53 (1) .

The improved interobserver variability can be attributed to the fact that HSIL/SCC categories exfoliate their cells more readily, there is little overlap in the criteria for their identification, therefore the reason for low *k* for HSIL in this study is uncertain. Many HSIL category were upgraded to SCC. This could be due to differences in application of criteria for classification of lesions by different pathologists.

Glandular Lesions

Glandular lesions had a poor agreement with a kappa of **0.125**. Sama D.et al in 2001 had a *k* 0.21 (45) The low kappa can be attributed to the high degree of overlap with squamous neoplasia and

reactive/non-neoplastic glandular changes .In this study 4.03% cases with mixed lesion (Squamous + glandular) which were missed in the primary report were diagnosed on review.

There is difficulty in recognizing glandular abnormalities and often overlooked. Even normal endocervical cells may show a multitude of cytomorphologic features.

Overall Interobserver Variation

The **overall interobserver** variation between the primary and review report had a fair agreement with kappa of **0.327**. This compares well with Guy La Ruche et al in 1999 who had a *k* 0.33 (l). Sama D. et al in 2001 had a *k* 0.57 (45). Schiffman M. et al in 2013 had a *k* 0.47 (12).

This could be due to differences in application of criteria for classification of lesions by different pathologists.

5.1 Study limitations

Socio-demographic data capture was minimal for this study since it was done using archived data,due to lack of patient information on laboratory request forms e.g HIV status, parity & state of menstrual cycle (pre or post menopause). Consequently, it was not possible to do any correlation of abnormal lesions with socio-demographic variables.

Due to missing slides from the filling cabinets, it was not possible to include all the abnormal Pap smear slide preparations for the entire period of study time i.e. 30 Months. Missing slides may all have had significant lesions and therefore this could potentially have biased the results.

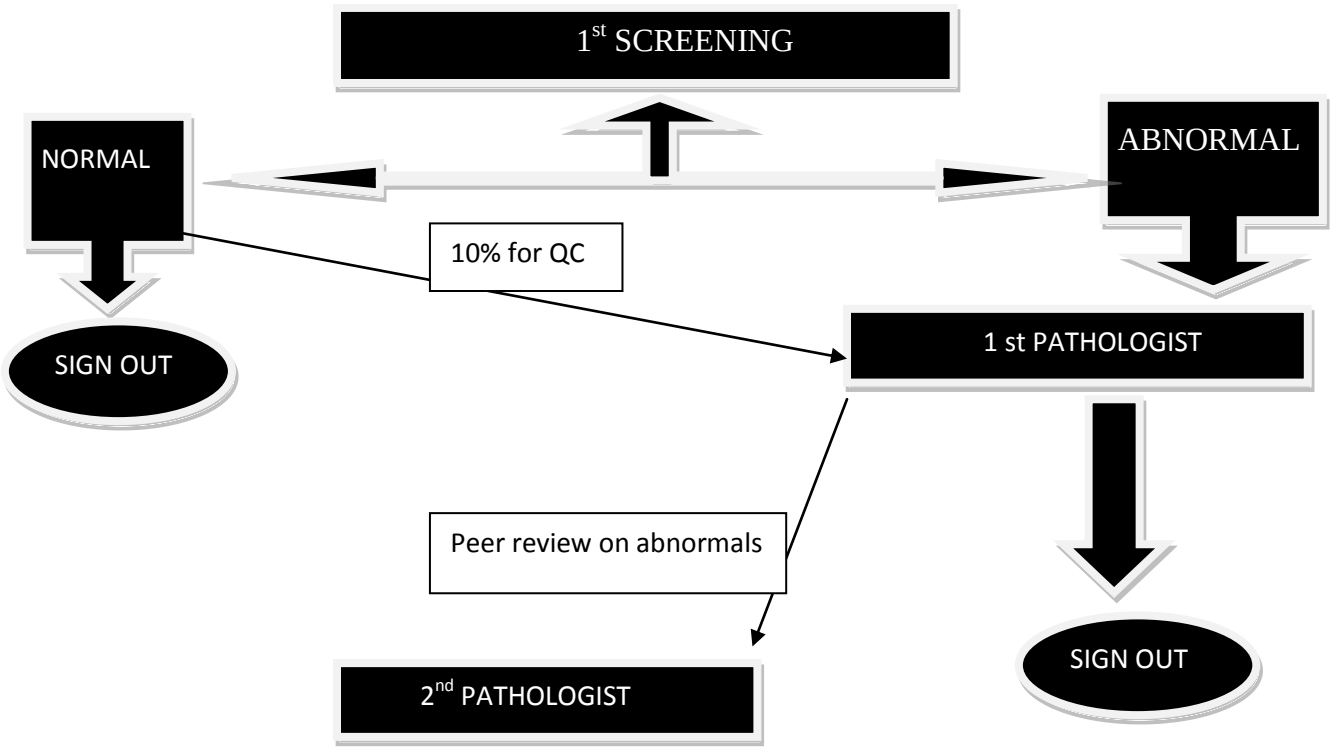
5.2 CONCLUSIONS

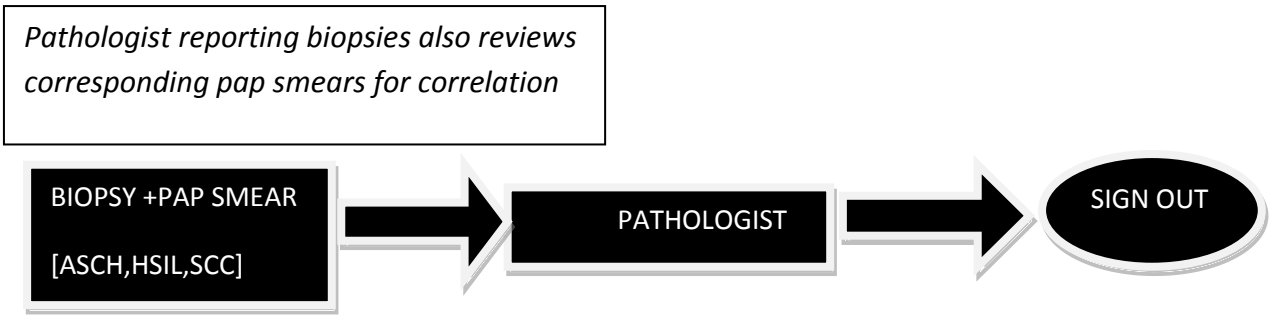
- Overall inter-observer agreement was **fair**, but the concordance was lower compared to other studies.
- LSIL and ASCUS lesions had highest discordance. The lesion with the best interobserver agreement was SCC while HSIL lesions had an intermediate discordance.
- There was significant undercall & overcall of lesions with an impact on clinical management of patients.

5.3 RECOMMEDATIONS

- To improve accuracy of reporting by improving/establishing IQC program.
- Establish a screening algorithm with an IQC program as suggested below to improve performance.
- Periodic participation in EQA/PT programs to improve accuracy of reporting.
- To improve accuracy of reporting through mandatory CME programs for pathologists & cytotechnologists.
- Re-training clinicians on importance of completeness of laboratory requisition forms and utilization of special pap smear request proformas to improve patient data capture.

Figure 4 SUGGESTED ALGORITHM FOR CERVICAL PAP SMEAR SCREENING





REFERENCES

1. Ruche G La, Mensah-ado I, Bergeron C, Welffens-ekra C. Cervical Screening in Africa: Discordant Diagnosis in a Double Independent Reading. J Clin Epidemiology. 1999;52(10):953–8.

2. Pengsaa P, Sriamporn S, Kritpetcharat O et al. A Comparison of Cytology with Pap Smears Taken by a Gynecologist and with a Self-sampling Device. Asian Pacific J Cancer Prev. 2003;4(66):99–102.

3. Bergeron,c lgo zgu¨ lj alg-adbkhwsre. Cervical screening in the Mediterrenean countries. J Clin Cytopathology, 2011;22:27–38.

4. Kenya Ministry of Public Health and Sanitation, Kenya Ministry of Medical Services. Draft National Cancer Control Strategy 2010-2015.Nairobi. 2009.

5. Congress E, European S, Farnsworth A, Syrjanen Y. The impact of cytological cervical screening and its changing role in the future. J Clin Cytopathology 2010, 21;355–8.

6. Ullal A, Roberts M, Bulmer JN, Mathers ME, Wadehra V. The role of cervical cytology and colposcopy in detecting cervical glandular neoplasia. J Clin Cytopathology 2009, 20;359–66.

7. Castanon A, Ferryman S, Patnick J, Sasieni P. Review of cytology and histopathology as part of the NHS Cervical Screening Programme audit of invasive cervical cancers. J Clin Cytopathology 2012, 23;13–22.

8. Graaf Y Vander, Vooijst GP. False negative rate in cervical cytology. J Clin Pathol 1987;40;438–42.

9. Health N, Cervical S, Marshall A. The challenge of cervical screening : to find and treat high-grade cervical intraepithelial neoplasia at risk of progression in women of childbearing age. J Clin Cytopathology 2012, 23;3–5.

10. Richard M. DeMay. The Art & Science of Cytopathology. Pathologists' American society of Cancer, editor. Chicago: Jeffrey Carlson,Andrea Meenahan,Michael Methe,Beena Rao,Phillip Rogers,Jennifer Schima,Joshua Weikersheimer; 1st ed. p. 62–185.

11. Bigras G, Wilson J, Russell L, et al. Interobserver concordance in the assessment of features used for the diagnosis of cervical atypical squamous cells and squamous intraepithelial lesions (ASC-US, ASC-H, LSIL and HSIL). Cytopathology: official journal of the British Society for Clinical Cytology [Internet]. 2013 Feb,24(1):44–51.

12. Schiffman M. Interobserver Reproducibility of Cervical Cytologic and Histologic Interpretations Realistic Estimates From the ASCUS-LSIL Triage Study. 2013;285(11):1500–5.

13. Denton K. The proposed BSCC terminology for abnormal cervical cytology. J Clin Cytopathology 2008, 19;398–9.

14. Nayar R. Support from authors of the Bethesda system. J Clin Cytopathology 2008, 19, p. 399–400.

15. Sriamporn S, Kritpetcharat O, Nieminen P. Consistency of Cytology Diagnosis for Cervical Cancer between Two Laboratories. Asian Pacific Journal of Cancer Prevention, Vol 6. 2005;6(0):208–12.

16. Edmund S. Cibas BSD. Cytology Diagnostic principles and Clinical Correlates. 3rd ed. Philadelphia: Saunders Elsievier; 2009. p. 1–63.

17. Lee KR, Granter SR. Use of Statistical Analysis of Cytologic Interpretation to Determine the Causes of Interobserver Disagreement and in Quality Improvement. Journal of Cancer (Cancer Cytopathology) 1997 / Volume 81 / Number 4. 1997;212–9.

18. N. Zagorianakou, P. Zagorianakou, E. Nastou and M. Gouva. Acceptability of female students in Greece. J Clin Cytopathology, 22 (Suppl. 1). 2011;22:55–183.

19. An L. The boundary between HSIL and LSIL. J Clin Cytopathology 2009,44:1–2.

20. Wright PK, Marshall J, Desai M. Comparison of SurePath â and ThinPrep â liquid-based cervical cytology using positive predictive value , atypical predictive value and total predictive value as performance indicators. J Clin Cytopathology 2010, 21;374–8.

21. Hunter C, Duggan MA, Duan Q, Power P, et al. Cytology and outcome of LSIL: cannot exclude HSIL compared to ASC-H. J Clin Cytopathology 2009, 20;(Table 1):17–26.

22. Herbert A. EU guidelines for Cervical Screening: Issues that outcome is not always achieved. Journal of Cytopathology 2011;22:6–26.

23. Blanks RG, Kelly RS. Comparison of cytology and histology results in English cervical screening laboratories before and after liquid-based cytology conversion: do the data provide evidence for a single category of high-grade dyskaryosis. J Clin Cytopathology 2010, 21;368–73.

24. Congress E, Kingdom U, Syrjanen K, Screening C, Unit E, Blanks R. CIN treatment and follow-up. J Clin Cytopathology 2009,9;353–4.

25. Ministry of Public Health & Sanitation, Ministry of Medical Services. National Guidelines for Prevention and Management of Cervical, Breast and Prostate Cancers. 2012. p. 12–101.

26. Adhya C. How accurate is cytology for endometrial carcinoma diagnosis? J Clin Cytopathology 2009, 14;p. 345–6.

27. Agc A, Nos AGC, Nomenklatur M, et al. Atypical glandular cells in cervical cytology: what are we talking about? Terminology and the impact of molecular techniques. J Clin Cytopathology 2009, 20;347–50.

28. Chummun K, Fitzpatrick M, Lenehan P, Boylan P, Mooney E, Flannelly G. Diagnostic and therapeutic dilemma associated with atypical glandular cells on liquid-based cervical cytology. J Clin Cytopathology 2012, 23;378–82.

29. Kumar N, Bongiovanni M, Molliet M, Pelte M, Egger J, Pache J. Diverse glandular pathologies coexist with high-grade squamous intraepithelial lesion in cyto-histological review of atypical glandular cells on ThinPrep specimens. J Clin Cytopathology 2009, 20;351–8.

30. Gupta N, Desai M, Hermansen P, Davies J. Commonly seen in Cervical Cytology Thin preparations. J Clin Cytopathology 2013;138–40.

31. Adhya AK, Mahesha V, Srinivasan R, et al. Atypical glandular cells in cervical smears: histological correlation and a suggested plan of management based on age of the patient in a low-resource setting. J Clin Cytopathology 2009, 20;375–9.

32. Finall AI, Olafsdottir R. Outcomes of cervical liquid-based cytology suggesting a glandular abnormality J Clin Cytopathology 2009, 20;367–74.

33. Austin RM, Zhao C. Type 1 and type 2 cervical carcinomas: some cervical cancers are more difficult to prevent with screening. J Clin Cytopathology 2012, 23;6–12.

34. Koss LG. How accurate are observations of diagnostic features of SIL and ASC?J Clinical Cytopathology 2012. p. 1–2.

35. Yanoh K, Norimatsu Y, Hirai Y, et al. New diagnostic reporting format for endometrial cytology based on cytoarchitectural criteria. J Clin Cytopathology 2009, 20;388–94.

36. Gupta N, John D, Dudding N, Crossley J, Smith JHF. Factors contributing to false-negative and potential false-negative cytology reports in SurePathÔ liquid-based cervical cytology. J Clin Cytopathology 2013, 24;39–43.

37. Risse EKJ, Holierhoek JP, Boon ME. Increased diagnostic accuracy of atypical glandular cells in cervical liquid-based cytology using cell blocks. J Clin Cytopathology 2011, 22;253–60.

38. Sarmadi S, Sanii S. Quality control in cervicovaginal cytology by cytohistological correlation. J Clin Cytopathology 2013, 24;(i):33–8.

39. Sherman SM, Moss E, Redman CWE. The invasive cervical cancer review: psychological issues surrounding disclosure. J Clin Cytopathology 2013, 24;77–80.

40. Moss EL, Moran A, Douce G, et al. Cervical cytology / histology discrepancy: a 4-year review of patient outcome. J Clin Cytopathology 2010, 21;389–94.

41. Syrja K. Cervical cancer screening in Mediterranean countries: implications for the future. J Clin Cytopathology 2010, 21;359–67.

42. Lo S, Nieminen P, K et al. Large performance variation does not affect outcome in the Finnish cervical cancer screening programme. J Clin Cytopathology 2012, 23;172–80.

43. Both I, Cervical NHS, Programme S, Nhscsp T. Disclosure of cervical cancer audits: how to be honest without making matters worse. J Clin Cytopathology 2013, 24. 73–6.

44. Solomon D, Davey D, Kurman R et al. The 2001 bethesda system. Terminology for reporting results of cervical cytology. JAMA 2002;287:2114–9.

45. Sama D, Cotignoli T, Guerrini L, Maioli P, Sintoni C, Bucchi L Intralaboratory reproducibility of cervical cytology diagnoses in the external quality assurance scheme of the Emilia-Romagna region of Italy. J Clin Pathology.2001.

APPENDICES

Appendix I THE 2001 BETHESDA SYSTEM FOR REPORTING CERVICAL CYTOLOGY

A). Specimen type

Indicate whether the pap smear is a conventional smear or a liquid-based preparation (LBS).

B). Adequacy of the specimen

a).Satisfactory smear- presence or absence of endocervical/transformation zone, quality indicators like inflammation, obscuring blood etc.

Absence of endocervical cells does not disqualify a smear.

Conventional smears need to have approximately 8000-12000 Squamous cells while LBS need approximately 5000 squamous cells to qualify as satisfactory.

b).Unsatisfactory smear- specify reason e.g red blood cells obscuring especially if more than 75% of squamous cells are obscured.

-if a slide is broken beyond repair, its rejected.

- if the request form lacks important details like; patient name etc, its rejected.

-Reject poorly stained smears.

-Reject smears with evidence of air drying/ poor fixation.

C).Interpretation/Results

a). Negative for intraepithelial lesion or malignancy.(NILM)

-Where there is no cellular evidence of neoplasia.

b).Organisms.

-Tricomonas vaginalis, candida spp, bacterial vaginosis, Actinomyces spp, HSV etc.

c). Other non-neoplastic findings. -Atrophy.

- Posthysterectomy glandular cell state.

-Reactive changes associated with inflammation, radiation, IUD use.

d).Others. -Endometrial cells in a woman >40 years.

Epithelial cell abnortmalities

SQUAMOUS CELLS

a).Atypical squamous cells. -of undetermined significance(ASCUS).

-Cannot exclude HSIL (ASC-H).

b). Low grade squamous intraepithelial lesion (LSIL)

c). High grade squamous intraepithelial lesion (HSIL)

-with features suspicious for invasion.

d).Squamous cell carcinoma(SCC)

GLANDULAR CELLS

a).Atypical -Endocervical cells (NOS or in comments)

-Endometrial cells (NOS or specify in comments)

-Glandular cells (NOS or specify in comments)

b).Atypical -Endocervical cells,favor neoplastic

-Glandular cells, favor neoplastic

c).Endocervical adenocarcinoma in situ

d).Adenocarcinoma -Endocervical

-Endometrial

-Extrauterine adenocarcinoma

-Not otherwise specified (NOS)

squamous epithelial abnormalities, not specifically listed in 2001 bethesda terminology

a). Keratinizing lesions
b). Squamous intraepithelial lesions (SIL) - borderline cases
c). SIL with gland involvement

Appendix II PROFORMA FOR REPORTING PAP SMEARS

Study no…….

Patient and clinical information (Indicate as appropriate)

1.Name (initials)……Lab number……

2.Age (In years)……

3. Parity (Where provided or stated)……

4.Contraception use(Where provided or stated)……

5.Clinical history(Where provided or stated)……

……

A).Laboratory Pap smear Report (Review)

Adequacy of the specimen

.....
.....

Interpretation/Results

……
……
……
…….

Investigator……Supervisor……Supervisor……..

Signed……Signed……Signed…….....

Date……Date……Date…….

B). Laboratory Pap smear Report (Primary)

Adequacy of the specimen

.....
.....

Interpretation/Results

……

Appendix III PROFORMA FOR AN ADDENDUM REPORT

Lab Number…… IP/OP Number……..

Patient and clinical information (Indicate as appropriate)

1.Name (initials)……Study Number……

2.Age (In years)…… 3. Parity (Where provided or stated)……

4.Contraception use(Where provided or stated)……

5.Clinical history(Where provided or stated)……

A)Laboratory Pap smear Report (Review)

Adequacy of the specimen

.....

Interpretation/Results

……
……

Investigator……Supervisor……Supervisor……..

Signed……Signed……Signed…….....

Date……Date……Date…….

B) Laboratory Pap smear Report (Primary)

Adequacy of the specimen

.....

Interpretation/Results

.....

……

C)Addendum Report to the Clinician

……
……
Pathologist…… **Sign**…… **Date**……