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Abnormal Papanicolau Smear and Colposcopic Findings, How Much They Correlate?

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Abstract: **Background:** Cervical cancer is a significant health issue and a leading cause of death among women worldwide. However, the disease is preventable through early detection and appropriate treatment. Screening tests like Pap smear can identify early cervical epithelial changes, while colposcopy can detect invasive or preinvasive lesions for biopsy and further management. Implementing robust screening programs is crucial in preventing cervical cancer.

Aim of the study: To estimate the accuracy of pap smear in the estimation of abnormal findings in comparison to colposcopy findings.

Patients and methods: a cohort study conducted in the Department of Obstetrics and Gynecology at Al-Elwiya Maternity Teaching Hospital / Baghdad during a period from the 1st of June 2022 till the 1st of June 2023. The study included non-pregnant women who attend the gynecological clinic for a pap smear, and the results showed abnormal pap smear findings. All participants with abnormal pap smears underwent colposcopic examination. Comparison of the result of pap smear with colposcopy and with histopathology, and comparison of colposcopy results with histopathology results, to estimate and compare the accuracy of both pap smear and colposcopy.

Results: the total cases were 117 case, 78 women had abnormal pap smear. Nulliparity, and secondary smoking were higher in cases of high-grade and cervical cancer cases. The accuracy of pap smear in detecting low-grade cervical interepithelial neoplasia was 82.1% with sensitivity and specificity of 92.1% and 72.5% respectively. With false positive rate of 6.4%. While the accuracy of colposcopy in the prediction of low-grade cervical interepithelial neoplasia (in comparison to histopathological test) was 91% with sensitivity and specificity of 91.9% and 90.2%. The predictive ability was much better using colposcopy (87.1% and 93.6% for sensitivity and specificity respectively).

Conclusion: Pap smear results correlate with colposcopy and histopathological results with high sensitivity in detecting low and high grade cervical intraepithelial neoplasia.

1. Introduction:

1.1 Background:

Cancer of the cervix is an increasing health problem and an important cause of mortality in women worldwide. Cervical cancer is a preventable disease due to the long preinvasive stage. Early detection and appropriate treatment are possible if robust screening is implemented. Early cervical epithelial changes can be identified by a Pap smear test, which is the primary screening test for detection of precancerous cervical intraepithelial neoplasia and the early stage of invasive cervical cancer[1].

1.2 Epidemiology:

Cervical cancer is the second most common malignant tumor among women worldwide, second only to breast cancer. Human papillomavirus (HPV) infection, especially high-risk human papilloma virus (HR-HPV) persistent infection, is a prerequisite for the development of cervical intraepithelial neoplasia (CIN) and cervical cancer(2).

The incidence of cervical cancer is rising worldwide. The difference in incidence between developing and developed countries, where cervical cancer cases have been significantly reduced, is large. In developing countries, the burden of cervical cancer is still high. According to the World Cancer statistics, >80% of all the cervical cancer cases are found in developing and low-resource countries, because of a lack of awareness and difficulty in running cytology-based screening programs(3).

According to International Agency for Research on Cancer – one of world health organization agency, in Iraq the total cases of cervical cancer at 2020 was 704 cases (over five years), the estimate prevalence of cervical cancer in 2020 was 3.5 cases per 100,000 population, while 286 new cases was diagnosed at that year with estimated incidence was 2.2 cases per 100,000 population, and mortality from cervical cancer reach 1.5 per 100,000 (total death in 2020 was 193 cases)(4)

1.3 Risk factors for CIN and subsequent Cervical cancer

Several risk factors for cervical cancer are linked to exposure to the HPV(5). Invasive cancer development process could prolong up to 20 years from the precursor lesion caused by HPV. Table 1 shows the risk factors for HPV and CIN. However, there are also other numerous risk factors (such as reproductive and sexual factors, behavioral factors, etc.) for cervical cancers which include sexual intercourse at a young age (<16 years old), multiple sexual partners, smoking, high parity, and low socio-economic level(6).

Table 1: Risk factors of CIN.

Demographic risk factors
➢ Ethnicity (Latin American countries, U.S. minorities)
➢ Low socioeconomic status
➢ Increasing age
Behavioral risk factors
➢ Early coitarche
➢ Multiple sexual partners
➢ Male partner with multiple prior sexual partners
➢ Tobacco smoking
➢ Dietary deficiencies
Medical risk factors
➢ Cervical high-risk human papillomavirus infection
➢ Exogenous hormones (combination hormonal contraceptives)
➢ Parity
➢ immunosuppression
➢ Inadequate screening

1.3.1 Sexually transmitted infections (STI)

HPV The primary cause of pre-cancerous and cancerous cervical lesions is infection with a high-risk or oncogenic HPV types. Most cases of cervical cancer occur because of infection with HPV16 and 18. High-risk types, especially HPV16, are found to be highly prevalent in human populations. The infection is usually transmitted by sexual contact, causing squamous intraepithelial lesions. Most lesions disappear after 6−12 months due to immunological intervention. However, a small percentage of these lesions remain and can cause cancer(5).

Permanent infection with one of the high-risk types of HPV over time leads to the development of cervical intraepithelial neoplasia (CIN). The major mechanisms through which HPV contributes to carcinogenesis involve the activity of two viral oncoproteins, E6 and E7, which interfere with major tumor suppressor genes, P53 and retinoblastoma. In addition, E6 and E7 are associated with changes in host DNA and virus DNA methylation. Interactions of E6 and E7 with cellular proteins and DNA methylation modifications are associated with changes in key cellular pathways that regulate genetic integrity, cell adhesion, immune response, apoptosis, and cellular control(7).

Human immunodeficiency virus (HIV) The risk of developing infection from high-risk HPV types is higher in women with HIV. The results of the studies on the relationship between HIV and cervical cancer suggested a higher rate of persistent HPV infection with multiple oncogene viruses, more abnormal Papanicolau (Pap) smears, and higher incidence of CIN and invasive cervix carcinoma among people with HIV. Women infected with HIV are at increased risk of HPV infection at an early age (13−18 years) and are at high risk of cervical cancer. Compared with non-infected women, HIV positive patients with cervical cancer are diagnosed at an earlier age (15−49 years old)(8).

1.3.2 Reproductive and sexual factors

Sexual partners Factors relating to sexual behavior have also been linked to cervical cancer. One study found that an increased risk of cervical cancer is observed in people with multiple sexual partners. Moreover, many studies have also suggested that women with multiple sexual partners are at high risk for HPV acquisition and cervical cancer. The association remained exist even after controlling for the status of HPV infection, which is a major cause of cervical cancer. Also, early age at first intercourse is a risk factor for cervical cancer(9).

Oral contraceptive (OC) pills OC pills are known to be a risk factor for cervical cancer. In an international collaborative epidemiological study of cervical cancer, the relative risk in current users increased with an increase in the duration of OC use. It has been reported that the use of OC for 5 years or more can double the risk of cancer. And in a multi-center case-control study, among women who tested positive for HPV DNA, the risk of cervical cancer increased by 3 times if they have used OC pills for 5 years or more. In addition, a recent systematic review & meta-analysis also suggested that OC pills use had a definite associated risk for developing cervical cancer especially for adenocarcinoma. Oral contraceptives (birth control pills) have been associated with an increased risk of cervical cancer, although the overall risk is still considered low. The reason for this increased risk is not entirely clear, but several mechanisms have been proposed.

One theory is that the hormones in oral contraceptives may promote the growth of the human papillomavirus (HPV), which is a significant risk factor for cervical cancer. Oral contraceptives may also affect the immune system's ability to clear HPV infections, allowing the virus to persist and progress to cervical cancer.

Another proposed mechanism is that oral contraceptives may affect the cervical mucus, making it more hospitable to HPV and other infections. Additionally, long-term use of oral contraceptives may lead to changes in the cervical cells, making them more susceptible to cancer-causing agents. This study concluded that use of OC pills is an independent risk factor in causing cervical cancer(10).

1.4 Pathophysiology:

1.4.1.1 Oncogenesis

Most women readily clear HPV, but those with persistent infection may develop preinvasive dysplastic cervical lesions. From such lesions, squamous cell carcinoma of the cervix typically arises at the squamocolumnar junction. In general, progression from dysplasia to invasive cancer requires several years, although times can vary widely. The molecular alterations involved with cervical carcinogenesis are complex and not fully understood. Carcinogenesis currently is suspected to result from the interactive effects among environmental insults, host immunity, and somatic-cell genomic variations. Increasing evidence suggests that HPV oncoproteins may be a critical

component of continued cancer cell proliferation. Unlike low-risk serotypes, oncogenic HPV serotypes can integrate into human DNA (Figure 1)[11].

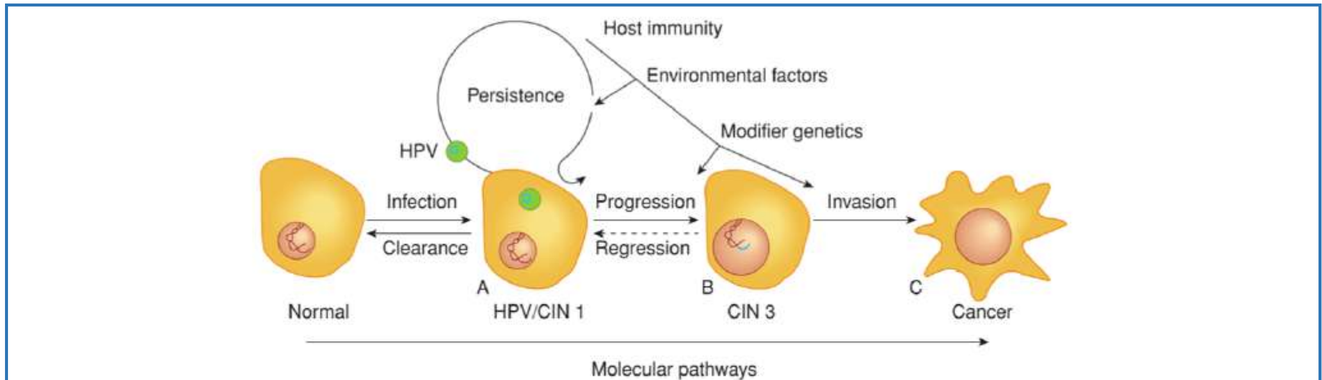


Figure 1: Critical end points lie on the spectrum of cervical dysplasia[11].

A. This initial point shows the cell at risk due to active HPV infection. The HPV genome (blue ring) exists as a plasmid, separate from the host DNA.

B. The clinically relevant preinvasive lesion, cervical intraepithelial neoplasia 3 (CIN 3) or carcinoma in situ (CIS), is an intermediate stage in cervical cancer development. The HPV genome has become integrated into the host DNA, resulting in increased proliferative ability.

C. Interactive effects between environmental insults, host immunity, and somatic cell genomic variations lead to invasive cervical cancer.

As a result, with infection, oncogenic HPV's early replication proteins E 1 and E2 enable the virus to replicate within cervical cells. These proteins are expressed at high levels early in HPV infection. They can lead to cytologic changes detected as low-grade squamous intraepithelial (LSIL) cytologic findings from Pap testing. Amplification of viral replication and subsequent transformation of normal cells into tumor cells may follow. Specifically, the viral gene products E6 and E7 oncoproteins are implicated in this transformation (Figure 2). E7 protein binds to the retinoblastoma (Rb) tumor suppressor protein, whereas E6 binds to the p53 tumor suppressor protein. In both instances, binding leads to degradation of these suppressor proteins. The E6 effect of p53 degradation is well studied and linked with the proliferation and immortalization of cervical cells[7].

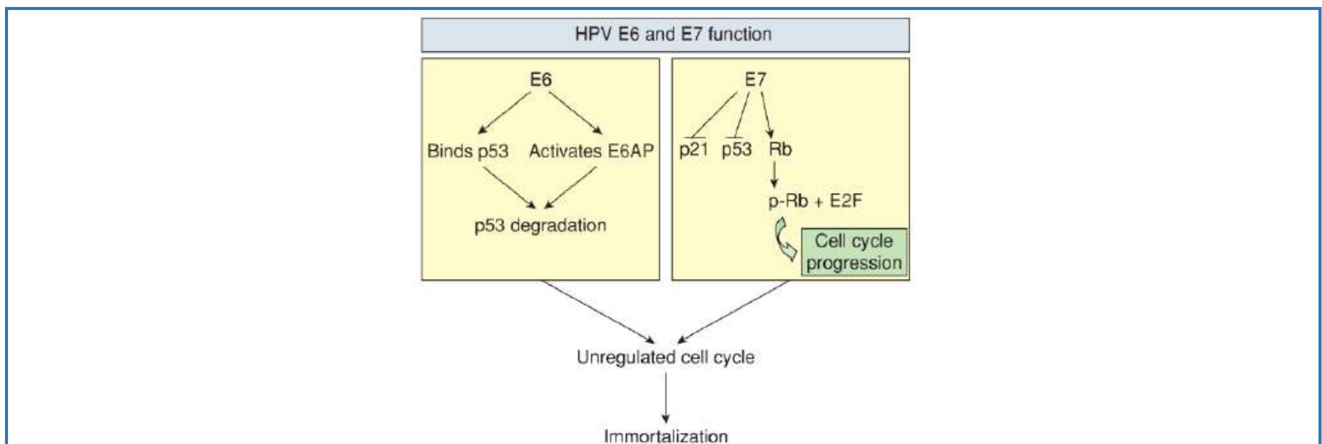


Figure 2: Effects of E6 and E7 oncoproteins[11].

On the left, viral oncoprotein E6 directly binds p53 and activates E6AP to degrade p53 tumor suppressor protein. On the right, E7 oncoprotein phosphorylates retinoblastoma tumor suppressor protein, resulting in release of E2F transcription factors, which are involved in cell cycle progression. E7 also downregulates p21 tumor suppressor protein production and subverts p53 function. The cumulative effect of E6 and E7 oncoproteins eventually results in cell cycle alteration, promoting uncontrolled cell proliferation

1.5 Diagnosis:

1.5.1 Symptoms

Some women diagnosed with this cancer are asymptomatic. In others, early-stage cervical cancer may create a watery, blood-tinged vaginal discharge. Intermittent vaginal bleeding that follows coitus or douching also can be noted. As a malignancy enlarges, bleeding typically intensifies, and occasionally, a woman presents with uncontrolled hemorrhage from a tumor bed(12).

1.5.2 Physical Examination

Precancerous changes in cervix usually cause no signs or symptoms, and abnormal pap test results are often the first sign that some cells in the cervix are abnormal. This is why it is important to have regular pap tests. If early cell changes develop into cervical cancer the most common signs include vaginal bleeding between periods, menstrual bleeding that longer or heavier, pain during intercourse, bleeding after intercourse, pelvic pain, and recurrent vaginal discharge(11).

1.6 Screening:

1.6.1 Background:

In the 1940s, cervical cancer was a principal cause of death for women of childbearing age in the United States. Dr. George Papanicolaou was a Greek immigrant who initially began his academic career studying the reproductive cycles of guinea pigs. After moving to the United States, he held a position in the anatomy department at Cornell University. He changed his focus of study to human physiology and began collaborating with gynecological pathologist Dr. Herbert Traut. While working together at Cornell University, they published Diagnosis of Uterine Cancer by the Vaginal Pap Smear in 1943. This significant work detailed how normal and abnormal vaginal and cervical cells could be viewed under a microscope and how they should be classified. Not long after that, the Pap Smear became the gold standard in cervical cancer screening, and it is still the primary screening tool for cervical cancer today(13).

1.6.2 Papanicolaou Smear

Cytological evaluation of cervical biopsy is the primary tool to diagnose cervical cancer. Although Papanicolaou (Pap) tests are performed extensively to screen for this cancer, this test does not always detect cervical cancer. Specifically, Pap testing has only a 53- to 80-percent sensitivity for detecting high-grade lesions on any given single test. Thus, the preventive power of Pap testing lies in regular serial screening(3). Moreover, in women who have stage I cervical cancer, only30 to 50 percent of single cytologic smears obtained are read as positive for cancer. Hence, Pap testing alone for evaluation of a suspicious lesion is discouraged. Instead, these lesions are directly biopsied with Tischler biopsy forceps or a Kevorkian curette. When possible, biopsies are taken from the tumor periphery, as central portions often contain only necrotic tissue, which will fail to yield a histologic diagnosis. Moreover, biopsies ideally include underlying stroma, so that invasion, if present, can be assessed. If abnormal Pap testing results are noted, colposcopy is often performed, and adequate cervical and endocervical biopsies are obtained. In some cases, cold knife conization is needed for this(11).

1.6.3 Pathophysiology related to the use of pap smear

Almost all cervical cancers are either squamous cell carcinoma or adenocarcinoma. The major steps known to be necessary in cervical carcinogenesis include HPV infection, HPV persistence, progression to dysplasia, and invasion. Steps in the reverse direction are possible, including clearance of HPV infection and regression or resolution of precancerous lesions. Steps of regression and clearance are quite common, making most cervical HPV infections transient and self-limited. It has been shown that approximately 67% of HPV infections will be cleared without intervention within 12 months and over 90% will clear within 2 years. Traditionally, it has been thought that a long-lasting HPV infection causes cervical intraepithelial neoplasia (CIN) in a slow, progressive, and consecutive way; from HPV infected normal tissue to CIN1 (low grade), CIN2 (moderate grade), CIN3/CIS (high grade), and finally cancer. However, recent data suggests that CIN1 may not be necessary for the development of CIN3 and that CIN3 could evolve directly from normal epithelium infected by HPV as described by a “molecular switch” model. In this model, the severity

of dysplasia is determined by the degree of methylation of certain genes, and this might not progress in a linear fashion. As such, clinically relevant CIN3 may develop rapidly after HPV infection(14).

Therefore, all CIN1 lesions and most CIN2 may not be precursor stages of cervical cancer, but rather the changes of a productive HPV infection. It may then take a decade or more to develop invasive cervical cancer from CIN3. Currently, the standard treatment recommendations following diagnosis of CIN1 include monitoring for progression, whereas treatments for CIN2 and CIN3 include cryotherapy, thermosablation, loop electrosurgical excision procedure (LEEP) and cold knife conization (CKC)(15).

1.6.4 The Papanicolaou Smear and Cytology-Based Cervical Cancer Screening

The American College of Obstetricians and Gynecologists (ACOG) joins ASCCP and the Society of Gynecologic Oncology in endorsing the U.S. Preventive Services Task Force (USPSTF) cervical cancer screening recommendations (16), which replace ACOG Practice Bulletin No. 168, Cervical Cancer Screening and Prevention, as well as the 2012 ASCCP cervical cancer screening guidelines (17). The adoption of the USPSTF guidelines expands the recommended options for cervical cancer screening in average-risk individuals aged 30 years and older to include screening every 5 years with primary high-risk human papillomavirus (hrHPV) testing. Primary hrHPV testing uses high-risk HPV testing alone (no cytology) with a test that is approved by the U.S. Food and Drug Administration (FDA) for stand-alone screening. Consistent with prior guidance, screening should begin at age 21 years, and screening recommendations remain unchanged for average-risk individuals aged 21–29 years and those who are older than 65 years, as shown in Table 2. Management of abnormal cervical cancer screening results should follow current ASCCP guidelines(18, 19).

Table 2: USPSTF Recommendations for Routine Cervical Cancer Screening.

Population	Recommendation
Aged less than 21 years	No screening
Aged 30-65 years	any one of the following: ➢ Cytology alone every 3 years ➢ FDA-approved primary hrHPV testing alone every 5 years ➢ Co-testing (hrHPV testing and cytology) every 5 years
Aged greater than 65 years	No screening after adequate negative prior screening results
Hysterectomy with removal of the cervix	No screening in individuals who do not have a history of high-grade cervical precancerous lesions or cervical cancer

1.6.5 Screening Options

There are now three recommended options for cervical cancer screening in individuals aged 30–65 years: primary hrHPV testing every 5 years, cervical cytology alone every 3 years, or co-testing with a combination of cytology and hrHPV testing every 5 years Table 2. All three screening strategies are effective, and each provides a reasonable balance of benefits (disease detection) and potential harms (more frequent follow-up testing, invasive diagnostic procedures, and unnecessary treatment in patients with false-positive results). Data from clinical trial, cohort, and modeling studies demonstrate that among average-risk patients aged 25–65 years, primary hrHPV testing and co-testing detect more cases of high-grade cervical intraepithelial neoplasia than cytology alone, but hrHPV-based tests are associated with an increased risk of colposcopies and false-positive results(20).

1.7 Colposcopy:

This outpatient procedure examines the lower genital tract, primarily the cervix. The colposcope provides a bright light source and variable magnification through an optical lens system or higher

solution digital imaging. Colposcopy requires special training, which encompasses terminology, lesion identification and grading, and biopsy techniques, Table 3[11]. It is primarily indicated for the evaluation of abnormal cervical cancer screening tests, signs, or symptoms of cervical cancer, and surveillance of treated or untreated CIN. Its primary goal is to identify invasive or preinvasive lesions for biopsy and subsequent management. Prior to colposcopic examination, a woman's medical history is reviewed, and indications for colposcopy are confirmed. Urine pregnancy testing is performed if clinically indicated. Colposcopic examination is optimally timed to avoid menses but is not delayed if there is a visible cervical lesion or abnormal bleeding, or if the patient is unlikely to return. In cases of severe cervicitis or other pelvic infection, treatment may be indicated before performing biopsies or endocervical curettage. However, abnormal cervical discharge without an identified pathogen may be a cancer indicator[21].

Table 3: Clinical considerations directing colposcopy[11].

Clinical objectives
Provide a magnified view of cervix, vagina
Identify cervical squamocolumnar junction
Detect lesions suspicious for neoplasia
Direct lesion biopsy
Clinical Indications
screening result
Grossly visible cervical lesion
Unexplained lower genital tract bleeding
Unexplained vaginal discharge
Contraindications: none
Relative contraindications
Upper or lower reproductive tract infection
Uncontrolled, severe hypertension
Uncooperative or overly anxious patient

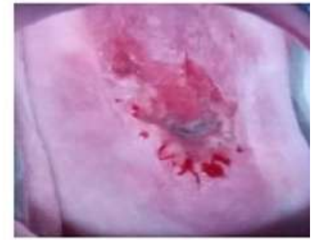
1.7.1 Maneuver of colposcopy

A bimanual examination precedes colposcopy to detect cervical enlargement or fixation suggesting malignancy, evidence of intrauterine pregnancy, or pelvic tenderness suggesting genital tract infection. Vaginal speculum selection and placement should provide optimal inspection of the cervix and upper vagina. Solutions used during colposcopy are normal saline, 3- to 5-percent acetic acid, and Lugol iodine. Because high-grade lesions are fragile, these solutions are applied by gently dabbing with a saturated swab or sponge or by spray-bottle misting. To begin, normal saline helps remove discharge and cervical mucus and allows initial assessment of vascular patterns and surface contours. A green (red-free) light filter adds contrast to aid vascular pattern visualization[22].

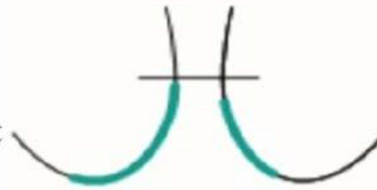
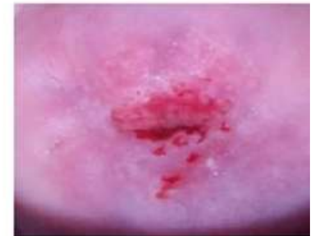
Acetic acid 3- to 5-percent solution is mucolytic and exerts its whitening effects, termed aceto whitening, presumably by reversibly denaturing cellular proteins. This causes neoplastic areas to appear denser compared with the normal surrounding epithelium, and lesions assume varying hues of transient whiteness. Several minutes may be needed for this effect to fully develop. Lugol iodine solution stains mature, glycogen-rich squamous epithelium a dark purple-brown color in reproductive-aged women with normal serum estrogen levels. Due to incomplete maturation, dysplastic epithelium has a lower glycogen content, fails to fully stain, and appears yellow. Lugol iodine application is particularly useful when abnormal tissue is not identi1ied using acetic acid. It is also used to define the limits of the active TZ, as cells of immature squamous metaplasia are glycogen-poor and do not stain as strongly as mature squamous epithelium. Lugol solution should not be used in patients allergic to iodine, radiographic contrast, or shellfish[23].

First, general assessment determines if the entire cervix and the SCJ are fully visualized. Nearly all cervical neoplasia lies within the TZ and adjacent to the SCJ. There are three types of transformational zone and explained in Figure 3.

Type 1
- **Completely ectocervical**
- **Fully visible**
- **Small or large**



Type 2
- **Has endocervical component**
- **Fully visible**
- **May have ectocervical component which may be small or large**

Type 3
- **Has endocervical component**
- **Is not fully visible**
- **May have ectocervical component which may be small or large**

Figure 3: Types of transformation zone.

Within a dysplastic lesion, the most severe disease tends to be at its proximal (cephalad or upper) limit. Thus, complete colposcopic visualization of the cervix, SCJ, and upper limits of all lesions is essential to exclude invasive cancer and determine disease severity. When the SCJ or lesions extend into the endocervical canal, an endocervical speculum can aid in their complete visualization(24).

1.7.2 Lesion Grading

With colposcopy, normal squamous epithelium of the cervix appears featureless, pale pink. and smooth. Blood vessels lie below this layer and therefore are not visible or are seen as a fine capillary network. The mucin-secreting columnar epithelium appears red due to its thinness and close proximity of underlying blood vessels. Its villous appearance derives from epithelial peaks and crypts. A lesion's acetowhiteness, margins (borders), vascular patterns, size, and location relative to the SCJ help indicate the most abnormal epithelium(25), Table 4 shows the colposcopic features of both CIN and cervical cancer.

Table 4: Colposcopic impressions of CIN and cancer(11).

Colposcopic Feature	Low-Grade	High-Grade	Cancer
Margins/contour	Condylomatous Papillary Irregular Geographic Flat or raised	Sharp Flat contour Rolled Peeling Internal margin Ridge sign Fused columnar villi	Exophytic/gross tumor Irregular surface Necrosis/ulceration
Acetowhite change	Thin	Thick/dense	May not be acetowhite

	Translucent Rapid fading	Rapid appearance Slow fading Variegated red/white Cuffed crypts	
Vascular patterns	Fine patterns	Coarse patterns	Atypical vessels
Iodine staining	Positive/Partial	Negative	Negative

1.7.3 Limitations of colposcopy:

Although colposcopy is a valuable tool for identifying abnormal cervical cells and guiding biopsy, there are several limitations associated with the procedure. Some of these limitations include:

- Operator dependency: The quality and accuracy of a colposcopy exam depend on the operator's experience and skill level. A less experienced operator may miss subtle abnormalities or misinterpret findings, leading to a false-negative result.
- Invasive and uncomfortable: The procedure can be invasive and uncomfortable for the patient, which may lead to anxiety and discomfort. Additionally, some patients may find the procedure embarrassing or distressing.
- Limited view: The colposcope provides a magnified view of the cervix, but it has a limited field of view. Therefore, the operator may miss abnormalities outside the scope of the exam.
- False positives: Colposcopy may also lead to false-positive results, where abnormal areas are identified that are not actually cancerous or precancerous. This can lead to unnecessary biopsy and treatment, which can cause physical and emotional harm to the patient.
- Cost and availability: Colposcopy require specialized equipment and trained operators, which can be expensive and may not be readily available in some areas. This can limit access to screening and diagnostic services for some women[26].

1.8 AIM OF THE STUDY:

To estimate the accuracy of pap smear in estimation of abnormal findings in comparison to colposcopy findings

2. Patients And Methods:

2.1 Study design, setting and data collection time

This is a cohort study conducted in the Department of Obstetrics and Gynecology at Al-Elwiya Maternity Teaching Hospital / Baghdad during a period from the 1st of June 2022 till the 1st of June 2023. In outpatient clinic for cervical screen.

2.2 Study patients and Inclusion criteria

The study included 117 women who attend to cervical screen clinic for pap smear, and results showed abnormal pap smear finding.

2.3 Exclusion criteria

- Pregnant patients.
- Known cases of cervical cancer.
- Normal pap smear (39 cases from the total number)

2.4 Data collection tools

The data were arranged on a questionnaire paper which was designed for the study including the following information:

- Patient age.
- Gravida and Parity.

- Past medical and surgical history.
- Indication for pap smear (screening or genitourinary abnormality)
- This questionnaire included measuring weight and height to calculate Body Mass Index level, which is calculated by weight in (kilograms) divided by the square of height in (meters). Weight and height are measured by the same scale for all the subjects.
- The patients were instructed that (pap smear preparation) no vaginal medication or douches 48 hours before the procedure, no sextual intercourse at least 24 hours before procedure. The procedure should be ten days after LMP.

Cases undergone per vaginal examination, speculum examination, after that wooden spatula introduced into the vagina and rotated through ectocervix and posterior vaginal fornix, after that the swab applied over glass slid and treated with 95% alcohol fixation and sent for laboratory cytological analysis. After 10- 14 days the results of abnormal pap smear were obtained. All participants with abnormal pap smear undergone colposcopic examination (using video path WelehAllyn, USA): patient in lithotomy position per vaginal examination and speculum examination after that positioning of the colposcope (that had attached camera that linked to screen) few inches from valval and turn the light source on, and observe through vagina the cervix, removal of secretion using cotton swab applying aceto white solution and wait for few minutes for estimation abnormal area, which undergo biopsy.

Comparison the result of pap smear with colposcopy and with histopathology, to estimate and compare the accuracy of both pap smear and colposcopy.

2.5 Maneuver of sampling

Full visualization of the cervix is essential to detect gross lesions and collect adequate specimens. Speculum placement should be as comfortable as possible. A thin coating of water-based lubricant can be smeared on the outside of the speculum blades without compromising Pap test quality or interpretation. Touching the cervix prior to performing Pap testing is avoided, as dysplastic epithelium may be inadvertently removed by minimal trauma. Discharge covering the cervix may be carefully absorbed by a large swab without contacting the cervix. Vigorous blotting or rubbing can theoretically cause scant cellularity or a false-negative Pap test result. When indicated, additional cervical sampling to detect infection should follow Pap test collection. Sampling of the transformation zone at the squamocolumnar junction (SCJ) adds substantial sensitivity of the Pap test. Techniques are adapted and sampling devices are chosen according to SCJ location, which varies widely with age, prior obstetric laceration, and hormonal status[27].

2.6 Cytology Collection

Three types of plastic devices are commonly used to sample the cervix: the spatula, broom, and endocervical brush (cytobrush) as shown in Figure 4. A spatula samples the ectocervix. An endocervical brush samples the endocervical canal and is used in combination with a spatula. A broom samples both endo- and ectocervical epithelia simultaneously but can be supplemented by an endocervical brush. Wooden collection devices and cotton swabs are no longer recommended due to their inferior collection and release of cells. A cervical Ayers spatula with its saddle-shaped end is positioned to best fit the ectocervical contour, straddle the SCJ, and sample the distal endocervical canal. It firmly scrapes the cervical surface while completing at least one full rotation. After the spatula sample is obtained, the endocervical brush is inserted into the endocervical canal until the outermost bristles remain visible just within the external os. This prevents inadvertent sampling of lower uterine segment cells, which can mimic atypical cervical cells. To minimize bleeding, the brush is used after the ectoccrvix has been sampled and is rotated only one-quarter to one-half turn. If the cervical canal is very wide, the brush can be directed around the canal perimeter to contact all surfaces[11].

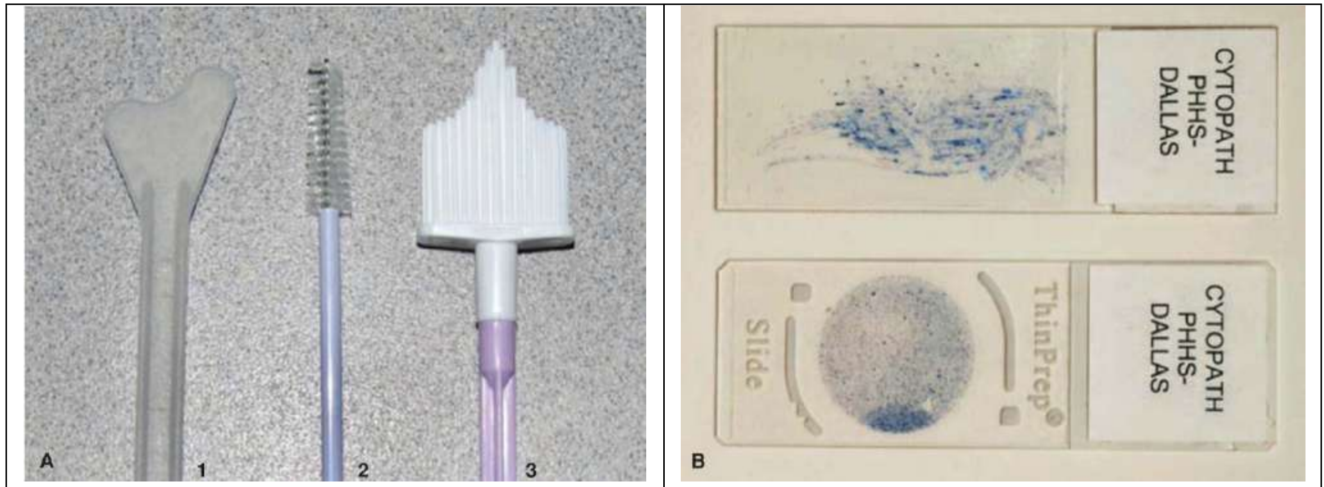


Figure 4: Cervical cytology[11].

A. Cervical cytology collection devices: (1) Plastic spatula. (2) Endocervical brush. (3) Plastic broom.

B. Pap test preparations.

2.7 Ethical considerations and official approvals

formal consent was obtained from each patient prior to collecting data, and information was anonymous. Names were removed and replaced by identification codes. All information is kept confidential in a password secured laptop and data used exclusively for research purposes.

Administrative approvals were granted from Council of Iraqi Board of Medical Specialization. Also, Approval of the Department of Obstetrics and Gynecology at Al-Elwiya Maternity Teaching Hospital.

2.8 Statistical analysis

All data were introduced into Microsoft Excel 16 and statistical analysis were conducted using IBM-SPSS (USA Chicago) and data were presented in the form of number, percentage, mean, standard deviation (SD) and presented in the form of tables, charts, or graphs.

Testing of the level of significance of the categorical data was conducted using Chi square or Fisher exact test while continuous variables were tested using student t test or Mann Whitney u test when appropriate.

3. Results:

One hundred seventeen women had undergone pap smear. Thirty-nine case were had normal pap smear and were excluded from the study. Abnormal result found in 78 women who had various complains, all the participants that underwent pap smear examination and showed abnormal finding further investigations requested, which include human papilloma virus detection, colposcopy, and biopsy.

The pap smear result showed that the most common abnormality is low grade squamous intraepithelial lesion (LISL), as shown in Figure 5.

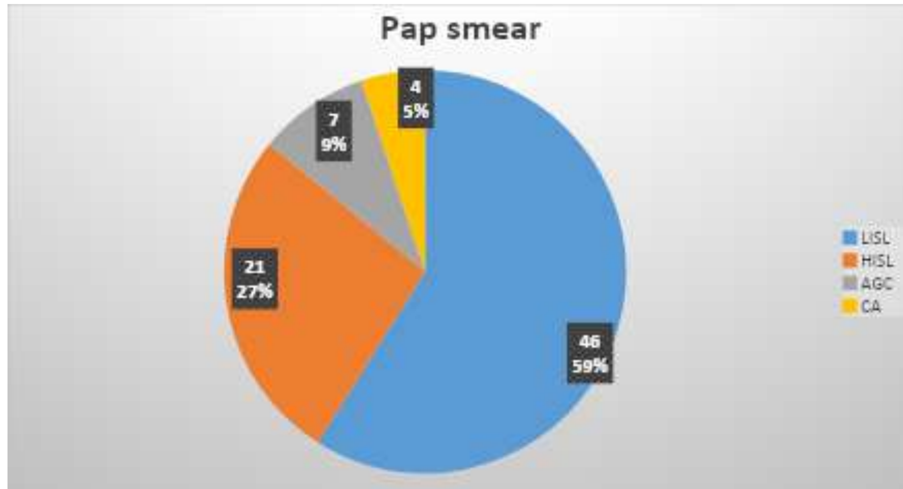


Figure 5: Pap smear results.

The mean age was not different (0.536) between the groups as shown in

Figure 6: Age distribution according to pap smear results.

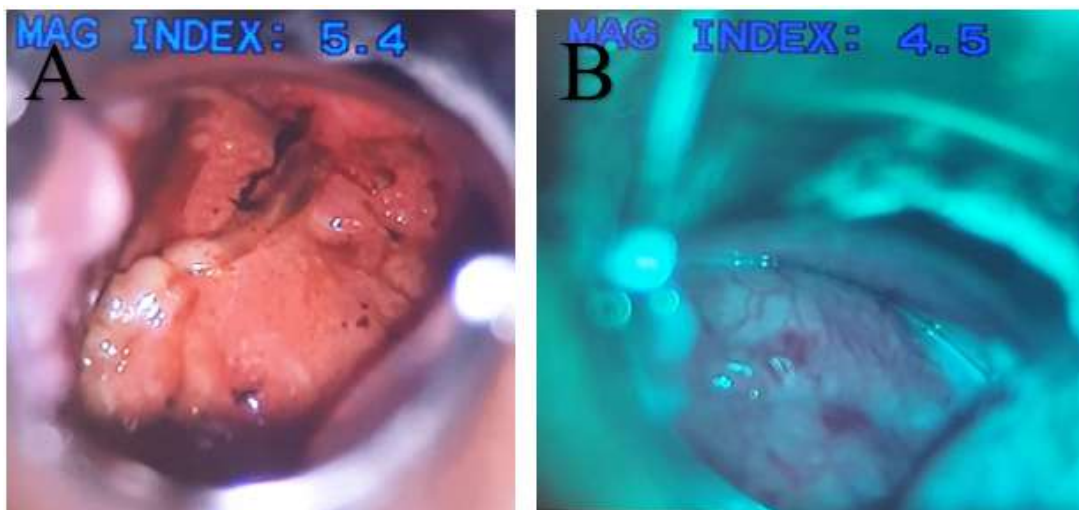


Figure 7: Colposcopy shows CIN1 after application of aceto white.

A: Plane colposcopy.

B: Green filter.

Regarding risk factors, we found that 75% of cancer cases were null parous, and 61.9% of HSIL cases were null parous, this difference was statistically significant. Secondary smoking was also different between the groups. The other risk factors showed no statistical difference as illustrated in Table 5.

Table 5: Patient characteristics.

Variables		LISIL No. (%)	HSIL No. (%)	AGC No. (%)	CA No. (%)	P value
Parity	Null	12 (26.1)	13 (61.9)	1 (14.3)	3 (75)	**0.001**
	Multiparity (1-3)	8 (17.4)	7 (33.3)	3 (42.9)	1 (25)	
	Grand multiparity (>3)	26 (56.5)	1 (4.8)	3 (42.9)	0 (0)	
Education	Illiterate	4 (8.7)	1 (4.8)	0 (0)	2 (50)	0.241
	Primary	22 (47.8)	12 (57.1)	4 (57.1)	2 (50)	
	Secondary	8 (17.4)	3 (14.3)	2 (28.6)	0 (0)	
	Higher	12 (26.1)	5 (23.8)	1 (14.3)	0 (0)	
Occupation	Housewife	41 (89.1)	19 (90.5)	7 (100)	4 (100)	0.730
	Employee	5 (10.9)	2 (9.5)	0 (0)	0 (0)	
Regularity of menstrual cycle	Irregular	36 (78.3)	19 (90.5)	6 (85.7)	3 (75)	0.646
	Regular	10 (21.7)	2 (9.5)	1 (14.3)	1 (25)	
smoker	Yes	7 (15.2)	3 (14.3)	0 (0)	1 (25)	0.662
	No	39 (84.8)	18 (85.7)	7 (100)	3 (75)	
Secondary smoker	Yes	9 (19.6)	8 (38.1)	4 (57.1)	3 (75)	**0.027**
	No	37 (80.4)	13 (61.9)	3 (42.9)	1 (25)	

The causes of presentation were mainly for post coital bleeding, as shown in

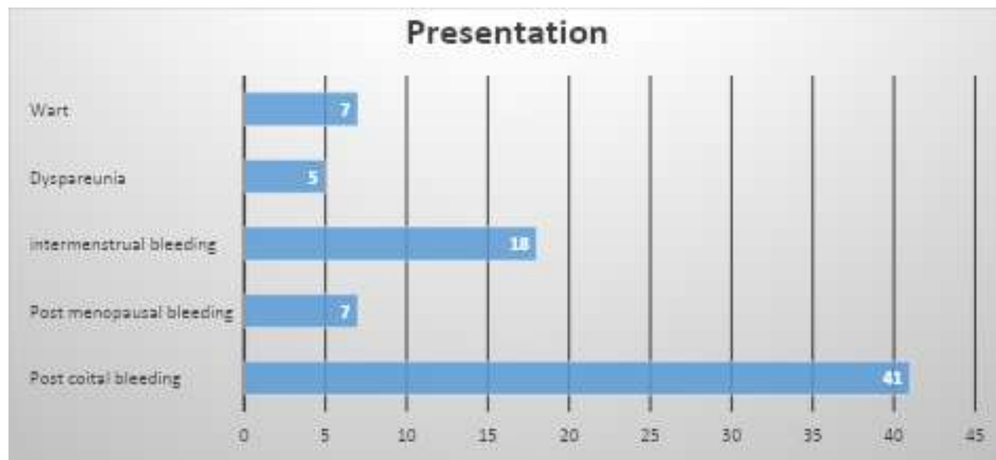


Figure 8: Presentation.

All participants underwent HPV testing 65% of the cases were HPV positive as shown in Figure 9. The majority of carcinoma cases and HSIL cases and all atypical glandular cells tested positive for human papilloma virus, as shown in Table 6.

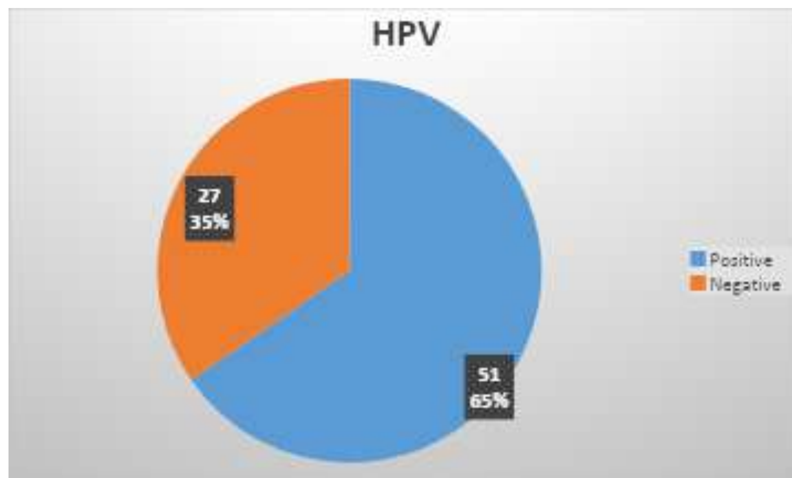


Figure 9: Human papilloma virus testing.

Table 6: Distribution of HPV cases.

HPV	LSIL	HSIL	AGC	CA	P value
	No. (%)	No. (%)	No. (%)	No. (%)	
Positive	19 (41.3)	20 (95.2)	7 (100)	3 (75)	<0.0001
Negative	27 (58.7)	1 (4.8)	0 (0)	1 (25)	

The positive HPV was significantly higher in HSIL and CA than LSIL.

The results of colposcopy showed that 8 cases (10.3%) of abnormal pap smear had normal findings in colposcopy, as shown in Figure 10.

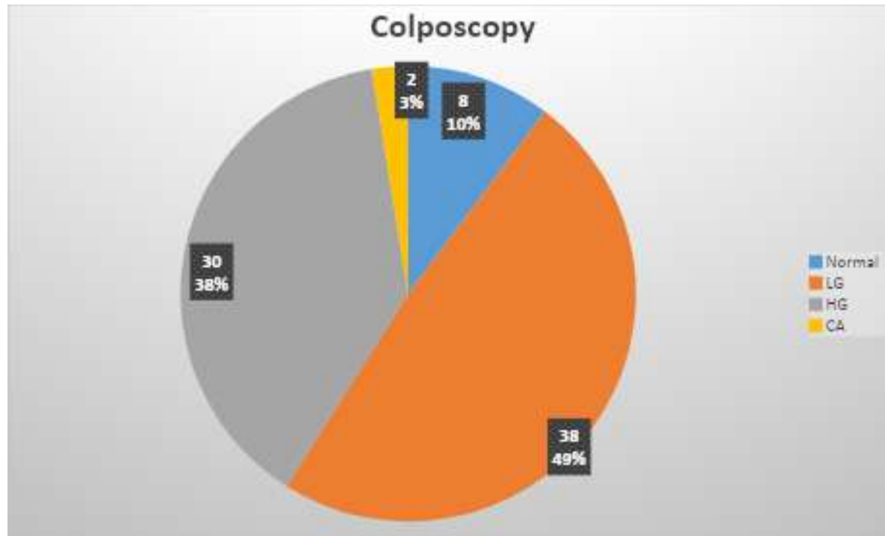


Figure 10: Colposcopy findings.

The distribution of cases was shown in Table 7. The distribution of colposcopy findings according to pap smear was significantly different. Five cases who were diagnosed to have LSIL, one case diagnosed as having HSIL, and two cases of AGC, were found to have normal colposcopy findings (false positive rate 6.4%). Two cases of those diagnosed as having carcinoma were found to be of high-grade CIN on colposcopy.

Table 7: Distribution of colposcopy findings according to pap smear.

Colposcopy	LSIL	HSIL	AGC	CA	P value
	No. (%)	No. (%)	No. (%)	No. (%)	
Normal	5 (10.9)	1 (4.8)	2 (28.6)	0 (0)	<0.0001
LG (CIN1)	35 (76.1)	3 (14.3)	0 (0)	0 (0)	
HG (CIN2 & 3)	6 (13)	17 (81)	5 (71.4)	2 (50)	
CA	0 (0)	0 (0)	0 (0)	2 (50)	

The accuracy of each finding of pap smear were examined for prediction of colposcopy finding of equivalent severity (i.e., the accuracy of LSIL in pap smear to have low grade CIN (CIN1) in colposcopy, and so on), as shown in Table 8.

Table 8: Predictive ability of Pap smear according to colposcopy findings.

Predictor	LSIL	HSIL	CA
Sensitivity	92.1	56.7	100
Specificity	72.5	91.7	97.4
Positive predictive value	76.1	81	50
Negative predictive value	90.6	77.2	100
Accuracy	82.1	78.2	97.4
Odd ratio	30.75	14.39	N/A
95% confidence interval	7.83-120.83	4.11-50.34	N/A

The results of histopathology were illustrated in Figure 11

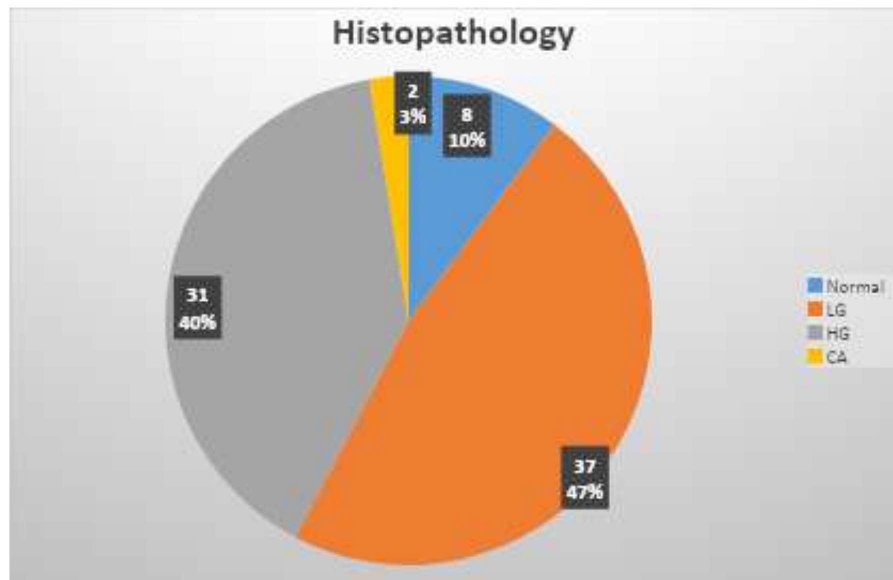


Figure 11: Distribution of histopathology results.

According to histopathology result, the predictive ability of pap smear (in regard to histopathological results) showed high sensitivity and specificity for invasive cancer, while low- and high-grade CIN were had less accuracy, sensitivity, and specificity, as shown in Table 9.

Table 9: Predictive ability of pap smear according to histopathology result.

Predictor	LSIL	HSIL	CA
Sensitivity	89.2	51.6	100
Specificity	68.3	89.4	97.4
Positive predictive value	71.7	76.2	50
Negative predictive value	87.5	73.7	100
Accuracy	78.2	74.4	97.4
Odd ratio	17.76	8.96	N/A
95% confidence interval	5.20-60.71	2.80-28.71	N/A

The colposcopy had much better sensitivity, specificity and accuracy in prediction of low-grade, high-grade CIN and invasive cancer in comparison to pap smear, as shown in Table 10,

Table 10: Predictive ability of colposcopy findings

Predictor	LG	HG	CA
Sensitivity	91.9	87.1	100
Specificity	90.2	93.6	100
Positive predictive value	89.5	90	100
Negative predictive value	92.5	91.7	100
Accuracy	91	91	100
Odd ratio	104.83	99	N/A
95% confidence interval	21.86-502.74	25.56-476.7	N/A

4. Discussion

Cervical cancer is the second most common cancer and the second most common cause of death from cancer in women of reproductive age worldwide[28]. The gold standard for diagnosing HSIL and cervical cancer is a combination of colposcopy, cytology, and colposcopy-directed biopsy. Using colposcopy enables experienced examiners to detect suspicious lesions[29].

The current study included patients who had abnormal pap smear findings. Cases were further assessed using colposcopy and biopsy to estimate the accuracy of both pap smear and colposcopy.

The most common type of cervical abnormality was LSIL, similarly found by Gardella et al(30) who found that low grade intraepithelial neoplasia is the most common type of presentation by pap smear.

The age was not different according to the findings of pap smear, Akinfolarin et al(31) found that advancing age, associated with higher incidence of abnormal pap smear result, the current study included women who already had abnormal pap.

The parity distribution showed significantly higher rate of cancer, and HSIL in nulliparous women. This finding was similar to what found by Choi et al(32) and Khatoon et al(33).

The socioeconomical factors (level of education and occupation) were not different regarding to Pap smear results, while Kashyap et al(34) in their case control study found that women with poor socioeconomical background (low level of education, rural area, unemployed) were had higher risk of having abnormal pap smear results, the difference from the current study that in their study they included cases of normal pap smear and abnormal pap smear, while the current study only cases of abnormal pap smear included.

The regularity of menstrual cycle was not alter in according to the abnormality of pap smear, although the regularity of menstrual cycle regarding result of pap smear was not investigated in previous literature, Okodo et al(35) found that sample collected at early secretory phase was associated with higher rate of false negative results. Thus cases of irregular menstruation should be carefully examined as suggested by Okodo et al(35) to improve detection rate.

History of smoking was not different according to Pap smear results, Sugawara et al(36) found that smoking is a risk factor for cervical cancer, this difference in results may be attributed to that only eleven cases were smokers in the current study, and this may increase the rate of false negative result.

Secondary smokers had significantly higher rate of HSIL and cervical cancer, this result was found similar to the study conducted by Jiang et al(37).

The presentation of the cases was mainly for post-coital and intermenstrual bleedings. Cohen et al(38) and Wan et al(39) had found that the most common presentation of cervical dysplasia, and cervical cancer were post coital bleeding and intermenstrual bleeding.

The rate of human papilloma virus infection was significantly higher in cases of HSIL and cervical cancer. Similar result found by Raj et al(40), this may be attributed due to its action on the activation of proto oncogenes that lead to evading apoptosis with further development of dysplasia and carcinoma, as stated by Tang et al(41).

The colposcopy findings showed that 10.8% of abnormal pap smear results, those cases had normal colposcopy findings (false positive) which is lower than what had been found by Cobucci et al(42) found the false positive rate to be 16.5%. the rate of false positive could give us an idea about the over investigation that could happen in the investigation. To note that Cobucci et al(42) had examined the accuracy of pap smear in screening program, while in this study we are examining the accuracy of pap smear in only abnormal pap smear results, and this could be the reason for lower false positive result we found. Also, it is important to notice that 13% of LSIL found by pap smear actually had HG CIN in colposcopy, this highlights the additive value of colposcopy to pap smear result.

The accuracy of pap smear (when compared to colposcopy findings) (table 8) in detecting LSIL was 82.1% with sensitivity and specificity of 92.1% and 72.5% respectively, while when comparing pap smear results with histopathological findings the accuracy decreased to 78.2% with sensitivity and specificity of 89.2% (table 9) and 68.3% respectively. While the accuracy of colposcopy in prediction of low-grade CIN was 91% with sensitivity and specificity of 91.9% and 90.2% (table

10), these findings highlight the importance of conducting colposcopy as it had better accuracy than pap smear. Cobucci et al[42] found that sensitivity and specificity of pap smear (according to histopathology) in screening program was 93% and 73% respectively in cases of low grade CIN. While Nkwabong et al[43] found that the sensitivity and specificity of pap smear in cases of abnormal pap smear was 55.5% and 75% respectively. To this we note the difference in predictive ability when we examine only abnormal pap smear results, this highlights the importance of pap smear as screening tool, with abnormal results could be further investigated by colposcopy.

The sensitivity for high grade CIN by pap smear was lower than the low-grade CIN (56.7% Vs 92.1%) but associated with higher specificity 91.7% (table 8), when comparing this result with histopathology slight decline in predictive ability was found 51.6% and 89.4% (table 9) for sensitivity and specificity respectively. The predictive ability was much better using colposcopy (87.1% and 93.6% (table 10) for sensitivity and specificity respectively). These results also highlight the importance of colposcopy in abnormal pap smear result (in other words pap smear could not replace colposcopy in diagnosing cervical abnormality).

Cobucci et al[42] in the cases of screening pap smear found the sensitivity of pap smear in detection of CIN 2 or higher grade was associated with 64% sensitivity, and 84% specificity, but they found the positive predictive value, and negative predictive value of 99% for each which highlight the importance of the use of pap smear as screening tool.

5. Conclusion & Recommendations

Conclusion

- Pap smear results correlate with colposcopy and histopathological results with high sensitivity in detecting low and high grade cervical intraepithelial neoplasia.

Recommendations

- The use of pap smear as a screening tool could be associated with early detection of cervical abnormalities.
- We recommend the use of colposcopy in cases of abnormal pap smear results, to improve the accuracy and detection rate.
- This study could have an important role in guiding larger study that investigate the cases of screening pap smear to estimate the accuracy and effectiveness of screening program.
- Better health education to general population about the benefit of pap smear in early detection of cervical cancer.
- HPV vaccine should be recommended as it is safe and effective.

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