ORIGINAL ARTICLE

# THE SIGNIFICANCE OF THE PAPANICOLAOU SMEARS IN DIAGNOSIS OF SQUAMOUS INTRAEPITHELIAL LESION. A CYTO HISTOPATHOLOGICAL STUDY

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## ABSTRACT

**OBJECTIVE:** To assess the frequency of intraepithelial lesion in Pap smear and to correlate between the cytopathological and histopathological findings this study were done.

**STUDY DESIGN:** A prospective observational study.

**PLACE & DURATION:** From September 2008 to June 2010 at Azadi Teaching Hospital Duhok City.

**METHODOLOGY:** Cytopathological and histopathological analysis of 136 specimens of cervix diagnosed between 2008 and 2010 at Azadi teaching hospital in Duhok city. Initial cytopathological interpretation was performed based on the Bethesda 2001 criteria. Formalin-fixed, paraffin-embedded tissues were reviewed for confirming histopathological diagnosis, presence of atypia, koilocytosis and changes related intraepithelial lesion.

**RESULTS:** The mean age was 36 years. Out of the 136 cases analyzed. Cytologically includes (n=8) 6% cases of high grade squamous intraepithelial lesion (HSIL), (n=17) 12.5% were low grade squamous intraepithelial lesion(LSIL), (n=77) 56.5% were atypical squamous cells of undetermined significance (ASCUS), (n=34) 25% were negative for intraepithelial lesion and malignancy (NILM) . Histopathologically 2 were squamous carcinoma, 6 were CIN2 and CIN3, (n=37) 27.3% were CIN1 and (n= 91) 66.9% were negative for neoplasia. Koilocytosis was observed in 49.

**CONCLUSIONS:** Women with a Pap diagnosis of NILM, ASCUS and LSIL appear to be less likely to harbor a high-grade lesion than those diagnosed with HSIL alone.

The association between cytological (NILM & ASCUS) and histopathological findings is variable and does not show that compatibility. This study showed the high frequency of koilocytosis in both cytology and biopsy which may reflects the high incidence of HPV infection.

**KEYWORDS:** HPV, Cervical cancer, Atypia, Koilocytosis, Neoplasia

## INTRODUCTION

The frequency of intraepithelial neoplasia of the cervix has been debated in studies originating from different parts of the world, but no substantial evidence has been generated on northern of Iraq subjects, that correlate between cytology and histopathology.

Invasive squamous carcinoma of the cervix results from the progression of preinvasive precursor lesions called cervical intraepithelial neoplasia (CIN) or dysplasia. CIN is histologically graded into mild dysplasia (CIN 1), moderate dysplasia (CIN 2) or severe dysplasia (CIN 3). Not all of these lesions progress to invasive cancer; many mild and moderate lesions regress. A further categorization, the Bethesda system, is based on cytological findings: Atypical squamous cells of undetermined significance (ASCUS) or cannot rule out low-grade squamous intraepithelial lesions (LSIL), LSIL (consisting of cytological atypia and CIN 1), and high-grade squamous intraepithelial lesions (HSIL), primarily include CIN 2, CIN3 or carcinoma in situ[1].

The Bethesda System (TBS) for reporting cervical or vaginal cytological diagnoses was introduced in 1988 and revised in 1991 to establish uniform terminology and standardize diagnostic reports. In addition, it introduced a standardized approach for reporting if an individual specimen is adequate for evaluation[2-6].

Several studies were conducted to identify the etiological factor of cervical carcinoma and found that human papilloma virus (HPV), particularly types 16 and 18, as the main cause of cervical cancer and its

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precursor lesions [7]. These two viral types were found in more than 60% of a large series of cases of invasive cervical cancers reported from 22 countries around the world [8-12]

Both incidence and mortality from cervical cancer have sharply decreased in a number of large populations following the introduction of well-run screening programs [1-6] In Iceland, the mortality rate declined by 80% for more than 20 years, and in Finland and Sweden by 50% and 34%, respectively. Similar reductions have been observed in large populations in the United States and Canada. Reductions in cervical cancer incidence and mortality were proportional to the intensity of screening [1-9]. Mortality in the Canadian provinces was reduced most remarkably in British Columbia, which had screening rates two to five times those of the other provinces [13].

To assess the frequency of intraepithelial lesion in Pap smear and to correlate between the cytopathological and histopathological findings in our setup.

## METHODOLOGY

A prospective study includes cytopathological and histopathological analysis of random sampling of 136 specimens diagnosed between September 2008 and June 2010 at Azadi teaching hospital in Duhok city. Pap smear used to detect early intraepithelial changes in the cervix using Papanicolaou stain. Initial cytopathological interpretation was performed based on the Bethesda 2001 criteria. The cytology results were concurrent with cervical biopsy (punch biopsy) with cytology or after cytology (all cases were verified by histology). The hematoxylin-eosin stained

**Table – I: Indications for cytohistopathological test (n=136)**

| Symptoms | No. of patients | % |
|---|---|---|
| Discharge with cervical erosion | 87 | 64.0% |
| Pain | 27 | 19.8% |
| Bleeding | 22 | 16.2% |
| Total | 136 | 100% |

sections were reviewed to confirm the diagnosis. The data collected were age of patient, presentation which included lower abdominal pain, vaginal discharge and colopscopy findings where the cervical erosion was the main finding (Table – I). Thus, this study was undertaken on symptomatic patients. The main symptoms included vaginal discharge, bleeding (spontaneous and post coital bleeding) and pain. Inadequate sampling and cases with cytology only (without biopsy) were excluded from this study

## RESULTS

The patients in this study presented clinically with different symptoms (Table – I) with a mean age of 36 years. Out of the 136 cases analyzed, cytologically, (n=8) 6% were high grade squamous intraepithelial lesion (HSIL), (n=17) 12.5% were low grade squamous intraepithelial lesion(LSIL), (n=77) 56.5% were atypical squamous cells of undetermined significance (ASCUS), (n=34) 25% were negative for intraepithelial lesion and malignancy (NILM) (Table - II).

Histopathological findings: (n=2) 1.4% were invasive squamous carcinoma, (n=6) 4.4% were CIN2 and CIN3, (n=37) 27.3% were CIN1 and (n= 91) 66.9% were negative for neoplasia (Table - III). Koilocytosis was observed in 49 cases.

The correlation between cytological and histological findings is highlighted in (Table - IV)

Statistical methods include chi-square and t-statistics were calculated to measure the association between different parameters mainly a comparison between cytology and biopsy.

**Table – II: Cytology of Pap smears (n=136)**

| Cytology Findings | No. of patients | % |
|---|---|---|
| *NILM | 34 | 25.0% |
| **ASCUS | 77 | 56.5% |
| ***LSIL | 17 | 12.5% |
| ****HSIL-including carcinoma | 8 | 6.0% |
| Total | 136 | 100 |

*NILM = negative for intraepithelial lesion and malignancy.
**ASCUS = atypical squamous cells of undetermined significance.
***LSIL=low grade squamous intraepithelial lesion.
****HSIL high grade squamous intraepithelial lesion.

**Table – III: Histopathology of cervical biopsies (n=136)**

| Histopathology | No. of patients | % |
|---|---|---|
| Inflammation | 91 | 66.9% |
| *CIN1 | 37 | 27.3% |
| CIN2 | 4 | 3.0% |
| CIN3 | 2 | 1.4% |
| Carcinoma | 2 | 1.4% |
| Total | 136 | 100% |

**Table – IV: The correlation between cytological and histological findings (n=136)**

| Cytopathological interpretation | No. and percent of cytology | Negative | CIN1 | CIN2 | CIN3 | Invasive carcinoma | Correlation |
|---|---|---|---|---|---|---|---|
| NILM* | 34 (25%) | 28 | 5 | 1 | 0 | 0 | 83% |
| ASCUS** | 77 (56.5%) | 61 | 14 | 1 | 0 | 0 | 80% |
| LSIL*** | 17 (12.5%) | 1 | 16 | 0 | 0 | 0 | 94% |
| HSIL**** | 8 (6%) | 0 | 2 | 2 | 2 | 2 | 75% |
| Total | 136 (100%) | 91 | 37 | 4 | 2 | 2 | 83% |

*NILM = negative for intraepithelial lesion and malignancy.
**ASCUS = atypical squamous cells of undetermined significance.
***LSIL=low grade squamous intraepithelial lesion.
****HSIL high grade squamous intraepithelial lesion.

## DISCUSSION

This study was undertaken because squamous cell carcinoma, the most common type of malignancy affecting females in many countries, evolves from cervical intraepithelial neoplasia (CIN) while cervical adenocarcinoma develops from cervical glandular intraepithelial neoplasia (CGIN) [14]. The Pap smear remains the golden standard for detection of cervical intraepithelial lesion.

Among 136 women participated in the study. The NILM and ASCUS groups there were (6/35=17%) and (15/77=20%) cases showed in histology CIN1 and CIN2; collectively they constitute 18.75% (Table - IV).

This diversity (correlation) between results of Pap smear and biopsy indicates the presence of false negative results on Pap smear, especially in group NILM and this reflects importance of collecting samples, interpretation and adequacy of smear. The TBS 1991 reported the adequacy of cervical cytology preparations in three categories: "satisfactory," "unsatisfactory," and "satisfactory but limited by," included factors such as the lack of transformation zone components and the presence of partially obscuring factors (i.e., blood or inflammation). This category was confusing to some clinicians and prompted unnecessary repeat testing [2, 3].

It has been shown that the presence of endocervical cells as a quality indicator of adequate sampling increases the detection of cervical abnormalities [15], however, other studies [16,17] have not demonstrated that a lack of transformation zone components in otherwise negative specimens indicates a higher risk of subsequent detection of histologic high-grade squamous intraepithelial lesions (HSIL).

Regarding the ASCUS which is an interface between a negative smear and presence of true dysplasia (15/77=20% cases showed CIN1 and CIN2) we can't consider this result as false negative but it may highlight the explanations of this diversity between smears and biopsy.

There was good correspondence between Pap smears reflected (LSIL & HSIL) and histopathological findings, with complete absence of false positive. But the striking result is the high rate of CIN1 (37; 27.3%) which is a disquieting sign about the incidence of dysplasia and carcinoma in this locality highlighting

the importance of screening test.

The overall results show 83% of correspondence and the remaining 17% of incompatibility occurred within the NILM & ASCUS groups due to false negative results.

The rate at which invasive cancer develops from CIN is usually slow, measured in years and perhaps decades [18]. This long natural history provides the opportunity for screening to effectively detect this process during the preinvasive phase, thus allowing early treatment and cure. About 70% of ASCUS and CIN 1 lesions regress within 6 years, while about 6% of CIN 1 lesions progress to CIN 3 or worse. About 10% to 20% of women with CIN 3 lesions progress to invasive cancer [18-21].

The leading etiologic factor in the development of preinvasive and invasive cervical cancer is infection with specific types of human papilloma virus (HPV). Many studies conducted worldwide confirmed the correlation between HPV and cervical carcinoma transmitted by sexual contact. Thus women not sexually active rarely develop cervical cancer whilst sexual activity at an early age with multiple sexual partners is a strong risk factor. About 95% of women with invasive cervical cancer have evidence of HPV infection [22-29]. The early sign of HPV infection is koilocytosis which has been seen in 49 ( 36 %) and this need to be proved by immunohistochemistry specific for HPV or polymerase chain reaction (PCR). Many women with HPV infection, however, never develop cervical cancer; thus this infection is necessary but not sufficient for development of cancer [30].

## CONCLUSIONS

1- Women with a Pap smear diagnosis of NILM, ASCUS and LSIL appear to be less likely to harbor a high-grade lesion than those diagnosed with HSIL alone.

2- The association between cytological (NILM & ASCUS) and histopathological findings is variable and does not show this compatibility. Thus, follow up of symptomatic women with NILM & ASCUS by repeated cytology at four to six months interval is recommended.

3- This study showed the high frequency of koilocytosis in both cytology and biopsy which may reflects the high incidence of HPV infection.

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