Original paper

Comparative Study of Pap Smear and Cervical Biopsy Findings

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Abstract

Background: Pap smear is the most important screening test for premalignant and malignant non invasive carcinoma of uterine cervix. Most authorities agree that patients with singinifiatly abnormal pap smear, such as cervical intraepithelial neoplasia (CIN) needs colposcopic evaluation and biopsy, this is especially in high grade cytological abnormalities (CIN II & III) (moderate and severe dysplasia.

Objective: To assess the accuracy of pap smear in detection of premalignant changes of cervical epithelium and correlate the finding of this cytological method with the finding of the biopsy material.

Materials and methods: Aprospective study which includes 100 non-pregnant females aged 16-69 years who had symptoms. The interpretation of cytological results was descriptive done in the Bethesda System terminology 2001 (Solomon and Nayar, 2004), which includes the following entities: NILM , ASC-US, ASC-H, HSIL & LSIL. Also all cases submitted for biopsy and subsequent histopathological examination.

Results & Discussion: Results of pap smear cytopathological examination:

According to Bethesda, the results of NILM, ASCUS, ASC-H, LSIL, HSIL, and SCC were 53% ,4 % , 5% , 24% , 7% , and 2% respectively.

Results of histopathological examination:

Results revealed that benign changes were found in 63 of 100 (63%) which show chronic cervicitis and endocervical polyp , CIN 1 in 20%, CIN 2 in 9%, CIN 3 in 5%, CIS in 1%, and SCC in 2%.

In our study, 10.26% of NILM cytology cases were false negative, which puts us in an intermediate position as compared to many other studies

Conclusion: Pap smear test was found to be equally sensitive to histopathological examination for the early detection of different cervical lesions.

Keywords: Pap smear, cyto-histological correlation , pap accuracy, screening.

List of abbreviations:

SCC : Squamous cell carcinoma
ASC-US: Atypical squamous cell of undetermined sinfificance
ASC-H: Atypical squamous cell that can not exclude high grade lesion
LSIL : Low grade intraepithelial lesion
LSIL-H: Llow grade intraepithelial lesion that cannot exclude high-grade lesion
HSIL : High grade cervical squamous intraepithelial lesion
CIS: Carcinoma in situ

Introduction

Pap smear is the most important screening test for premalignant and malignant non invasive carcinoma of uterine cervix [1,2] .

Most authorities agree that patients with singinifiatly abnormal pap smear, such as cervical intraepithelial neoplasia (CIN) needs colposcopic evaluation and biopsy, this is especially in high grade cytological abnormalities (CIN II & III) (moderate

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and severe dysplasia), however, there are varying opinion on literature regarding recommendation for follow up of mid dysplasia in pap smear. [3,4,5]

It has been established that most cervical cancers can be diagnosed at preinvasive stage with adequate and repetitive cytological screening.[6]

In England cervical cancer is the second most common cancer in women and the leading cause of cancer related death in many developing countries [7].

In fact, the Agency for Health Care Policy and Research (AHCPR) in the US reported that conventional cervicovaginal smears had a sensitivity of 51% and a specificity of 98%.9 [8], Other several studies have reported high false negative rates associated with cervicovaginal smears in screening for cervical cancer, ranging 6.0-22.9%. [9,10,11]

There is a common perception that, because histology is the so-called "gold standard" of diagnosis, non-correlating cytologic results are always wrong. It is important, however, to remember that both colposcopy and directed biopsy are also tests that are subject to variation in sampling, preparation and interpretation error.[1]

Cytology offers certain advantages over histology in the assessment of cervical dysplasia. Cytology provides exquisite nuclear detail, making early nuclear abnormalities easier to appreciate. Also, the morphology of an intact cell in a cytologic preparation, in comparison to that of the sectioned cell in histology, can be of great help in interpreting subtle abnormalities which may be present in histologic preparations. Finally, a broad area is sampled by a good Pap collection, where only a portion of the squamocolumnar junction may be sampled by biopsy.[2]

The Pap test and follow-up biopsy complement one another when both are reviewed and compared together before the biopsy is signed out. The comparison of the two requires extra effort by the pathologist and laboratory staff to assemble all pertinent slides and information for review.[3]

Good histo-cyto correlation is a time-consuming, labor-intensive process. It requires organized record keeping and slide filing capabilities by the laboratory, along with extra efforts by a dedicated laboratory staff to assemble all that is necessary for pathologist review. This type of histology-cytology correlation has been an integral part of value-added services provided by Pathologists for over 40 years.[3]

This study was conducted to assess the accuracy of pap smear in detection of premalignant changes of cervical epithelium and correlate the finding of this cytological method with the finding of the biopsy material, and also detection of human papilloma virus to reduce number of patients that need biopsy and start urgent treatment.

Materials and methods

Aprospective study which includes a total of 100 non-pregnant females aged 16-69 years who had symptoms such as chronic leucorrhoea, bleeding (including postcoital bleeding and intermenstrual bleeding), or the findings of erosion, an unhealthy cervix, bleeding on touch, in period January 2011-January 2013 in Medical City Teaching Laboratories (Pathology department) in Baghdad, were submitted for both cervical pap smear before treatment and follow up biopsy (punch), and the results were compared in both cytology and biopsy.

Cases are selected according to available complete data including age , clinical feature, and all are examined cytopathologically and histopathologically. So this study includes all symptomatic patients with with abnormality either in pap smear or in biopsy and exclude clinically asymptomatic women .

All cytological smears are stained by Papanicolou method (conventional pap

smear), and we use Bethesda grading system [12] to describe cervical intraepithelial neoplasia (CIN) as shown in table 1, The interpretation of cytological results was descriptive done in the Bethesda System terminology 2001 (Solomon and Nayar, 2004), which includes the following entities: NILM - negative for intraepithelial lesion or malignancy, ASC-US – atypical squamous cells of undetermined significance, ASC-H - atypical squamous cells that cannot exclude high-grade lesion, LSIL - low grade intraepithelial lesion, LSIL-H - low grade intraepithelial lesion that cannot exclude high-grade lesion, HSIL – high grade cervical squamous intraepithelial lesion. also all cases submitted for biopsy and subsequent histopathological examination and divided into six categories include Negative for intraepithelial dysplasia (cervicitis only), CIN1, CIN2, CIN3, CIS and SCC .

Table 1. WHO, CIN and Bethesda terminology

WHO system	**(Dysplasia terminology) CIN terminology**	**Bethesda terminology**
Mild dysplasia	CIN I	Low grade SIL
Moderate dysplasia	CIN II	High grade SIL
Severe dysplasia	CIN III	High grade SIL

We use Papanicolaou staining method for staining the smears submitted for histopathological examination, and the routine Hematoxylin and Eosin method for staining the histopathological sections.

Results

Results of pap smear cytopathological examination:

According to Bethesda, the overall results of cervicovaginal and histologic diagnosis are summarized in Tables 2, 3 & 4. On cytologic diagnosis of cervicovaginal smears, the rates of negative for intraepithelial lesions or malignancy, ASCUS, ASC-H, LSIL, HSIL, and SCC were 58% (58 of 100), 4 % (4 of 100), 5% (5 of 100), 24% (24 of 100), 7% (7 of 100), and 2% (2 of 100), respectively as shown in table 2.

Table 2. Results of pap smear cytopathological examination

Cytological category	No. of cases	%
Negative	58	58%
ASC-US	4	4%
ASC-H	5	5%
LSIL	24	24%
HSIL	7	7%
Scc	2	2%
Total	**100**	**100%**

Results of histopathological examination:

Results revealed that benign changes were found in 63 of 100 (63%) which show chronic cervicitis and endocervical polyp , CIN 1 in 20 of 100 (20%), CIN 2 in 9 of 100 (9%), CIN 3 in 5 of 100 (5%), CIS in 1 of 100 (1%), and SCC in 2 of 100 (2%), as shown in table 3.

For the cytological examination, the true positive 42, true negative was 53, false positive was 10, and false negative was 5, the sensitivity was 89.39 %, the specificity was 84.12%, the positive predictive value was 80.79%, and the negative predictive rate was 91.37%, the accuracy rate was 86.36% .

Excluding 9 ASC (ASCUS and ASC-H), the true negative was 53, true positive was 33, false negative was 5, false positive was 7. The accuracy rate was 87.75% (86/98), the sensitivity was 86.84% (33/38), the

specificity was 91.37% (53/58), the false negative rate was 0.8% (5/58), the false positive rate was 13.20 % (7/53), the positive predictive value was 82.5 % (33/40), and the negative predictive value was 88.33% (53 /60). The accuracy rate, specificity, and positive predictive value increased and the false positive rate decreased when ASC cases were excluded.

Table 3. Results of histopathological examination

Histological Category	No. of cases	%
Negative	63	63%
CIN1	20	20%
CIN2	9	%9
CIN3	5	5%
CIS	1	1%
Scc	2	2%
Total	**100**	**100%**

Table 4. Results of cytological and histopathological examination

Cytological category / Histological category	**Negative**	**ASC-US**	**ASC-H**	**LSIL**	**HSIL**	**Sqcc**	**Total**
Negative	53	2	1	7	0	0	63
CIN1	3	1	1	14	1	0	20
CIN2	2	1	2	2	2	0	9
CIN3	0	0	1	1	3	0	5
CIS	0	0	0	0	1	0	1
Sqcc	0	0	0	0	0	2	2
Total	58	4	5	24	7	2	100

The accuracy of cytologic diagnosis according to grade:

In comparative analysis of cytologic and histologic diagnosis according to the grade of cytologic diagnosis, such as the LSIL and HSIL groups, including HSIL and SCC.

The sensitivity of the LSIL and HSIL groups was 77 % (14 of 18) and 61% (8 of 13), respectively.

The false positive rates were 29 % (7of 24) in the LSIL group and 0% (0 of 9) in the HSIL group. The positive predictive values were 60 % (14 of 21) in the LSIL group and 100% (8 of 8) in the HSIL group. The sensitivity of the

HSIL group was lower than the LSIL group, but the false positive rate was lower in the HSIL group than the LSIL group. These analyses had been performed, except for ASC (table 5).

Table 5. The accuracy of cytologic diagnoses according to grade

	LSIL	HSIL or grater than HSIL
Sensitivity	77%	61%
False positive rate	29%	0%
Positive predictive value	60%	100%

Results for detection of HPV:

Of 100 cases 5 were positive for HPV changes by pap smear and 93 were negative, in biopsy all positive cases in pap smears are also positive in biopsy , but 2 were negative in pap and are positive in biopsy as shown in table 6 & 7.

Table 6. Results for detection of HPV

Diagnosis	Pap	Biopsy
HPV changes	5/100	7/100

Table 7. Results for detection of HPV

		HPV by biopsy		Total
		negative	positive	
HPV by Pap	Negative	93	2	95
	Positive	0	5	5
Total		93	7	100

Results according to age:

As shown in table 8, according to age most patients in this study were between 30-39 years which accounts for about 31% , followed by 28% for 20-29 years , 18% for 40-49, 12% for 50-59 years, 7% for 60-69 years and 4% for 10-19 years.

Table 8. Age distribution of patients

Age	No. of patients	%
10-19	4	4%
20-29	28	28%
30-39	31	31%
40-49	18	18%
50-59	12	12%
60-69	7	7%
total	100	100%

Results according to main complain of the pateints :

Most pateints presented with vaginal discharge 46%, while 28% presented with abnormal vaginal bleeding, 18% presented with lower abdominal pain, and 8% presented with dysuria table 9.

Table 9. Distribution according to main complain

Main complain	No.	%
Pain	18	18%
Vaginal discharge	46	46%
Bleeding	28	28%
Dysuria	8	8%

Relationship between age and pap smear results:

Table 10. realtionship between age and pap smear results

Pap results / Age	Negative	ASC-US	ASC-H	LSIL	HSIL	Scc	Total
10-19	3			1			4
20-29	17	2	1	7			28
30-39	18		2	8	1	1	31
40-49	9	1	1	5			18
50-59	5	1	1	3	1	1	12
60-69	6				2		7
total	58	4	5	24	7	2	100

By applying T-test there was no significant association found between age and pap smear findings with a p value of 0.653.

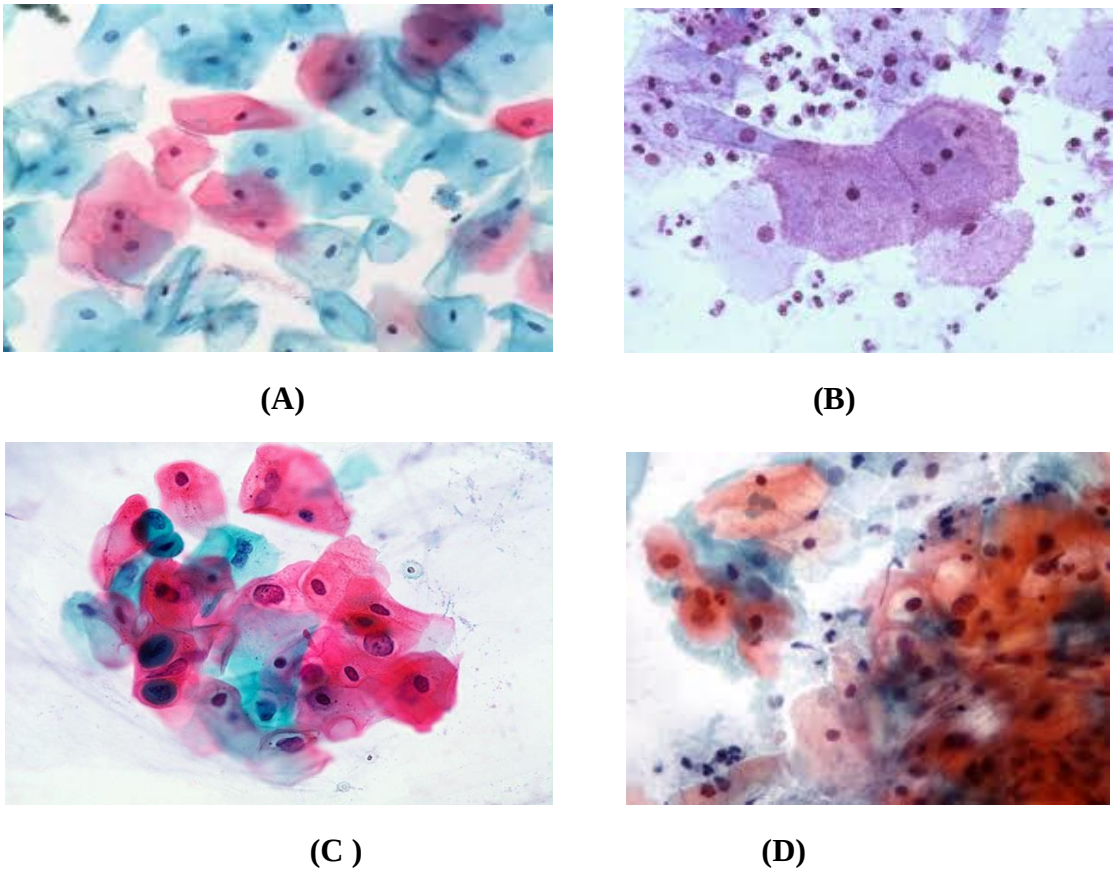
Realtionship between main complain and pap smear results

Pain and vaginal discharge were found to be the most common complaints among

different lesions of the cervix and most of the signs and symptoms were not specific, Bleeding and pain were the main signs that were found to be associated with more advanced lesions, however, none of the signs were specific.

Table 11. realtionship between main complain and pap smear results

pap smear results / Main complain	Negative	ASC-US	ASC-H	LSIL	HSIL	Scc	Total
Pain	15	0	1	0	2	0	18
Vaginal discharge	29	3	2	12	0	0	46
Bleeding	8	0	1	12	5	2	28
Dysuria	6	1	1	0	0	0	8
Total	58	4	5	24	7	2	100



(A) **(B)**

(C) **(D)**

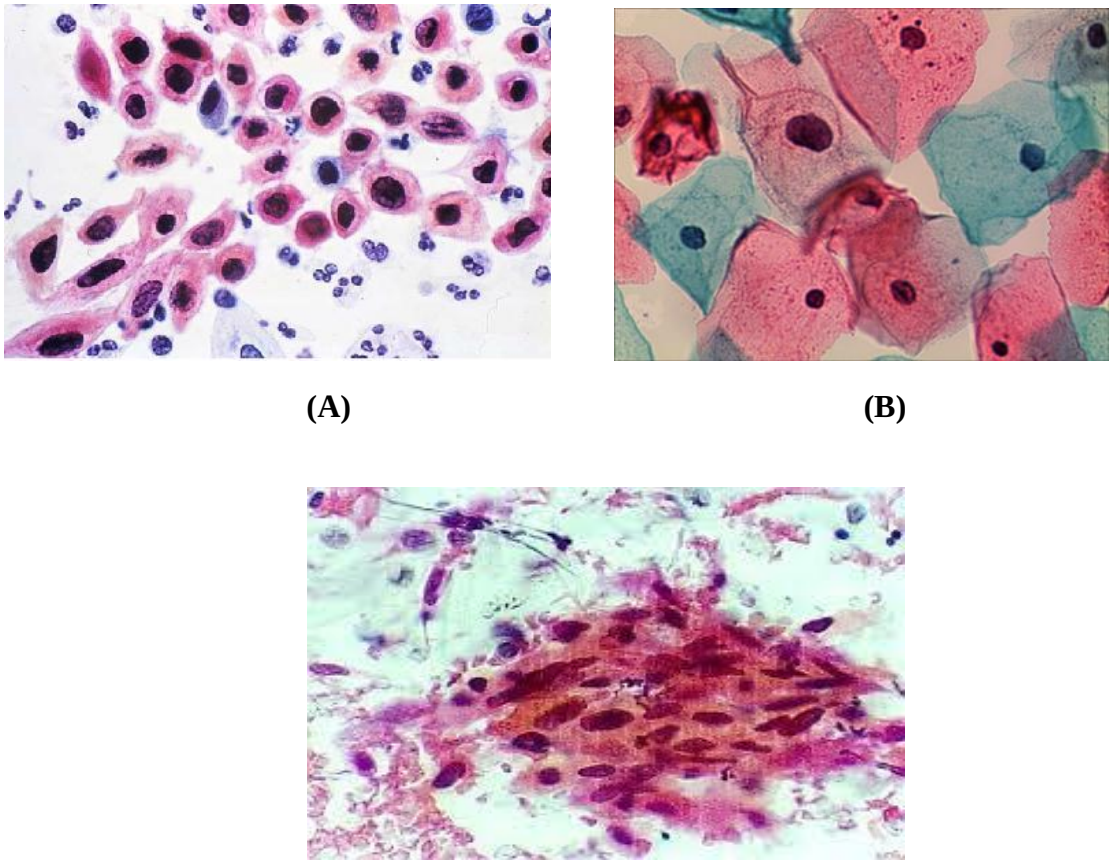
Figure 1. (A) Normal pap smear. (B) Pap smear showing chrnoic cervicitis. (C) Pap smear showing LSIL. (D) Pap smear showing LSIL with HPV changes**.**

Discussion

The cervicovaginal smear is considered to be a good method to evaluate the status of uterine cervical epithelium with low cost and easy methodology. Thus, this method has been widely used as a screening test for epithelial neoplasms of the uterine cervix in population-based cancer screening programs and a biannual cervicovaginal smear is recommended for women over the age 30 years by the national cancer screening program.[13]

The major problem with cervicovaginal smears is the rate of false negative cytology, which has been reported to range between 6 and 55% in several studies.[16-19]

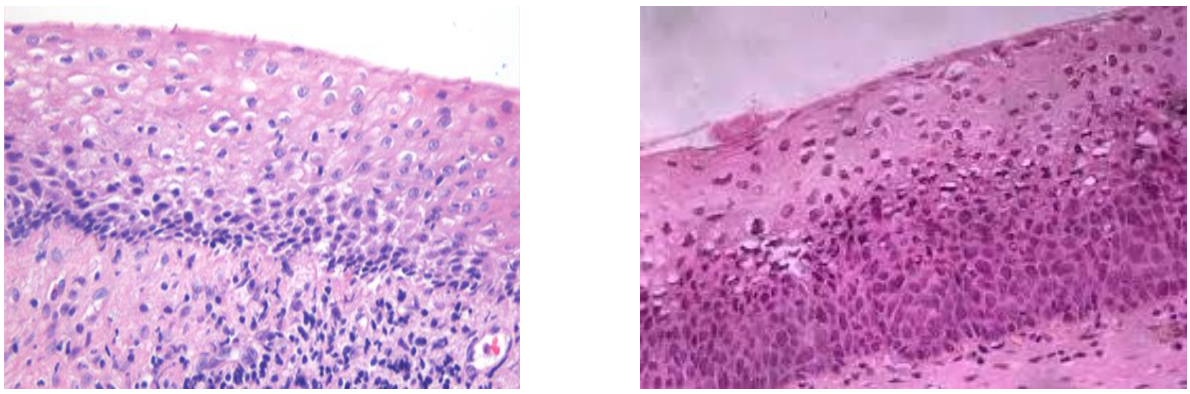
In our study, 10.26% of NILM cytology cases were false negative, which puts us in an intermediate position as compared to many other studies [20,21]



(A) **(B)**

(C)

Figure 2. (A) Pap smear showing HSIL. (B) Pap smear showing ASCUS. (C) Cervical smear showing malignant squamous epithelial cells consistent with SCC



(A) **(B)**

Figure 3. (A) Histopathological section from cercix shows CINI. (B) Histopathological section from cervix shows CIN II

Allard et al. have reported that CIN lesions are not randomly distributed across cervix and there is a predilection for the locations anterior and posterior to the cervical os [22].

This can also explain why some cases cytologically are false-negative, whereas biopsy materials contain abnormal cells.

It is well-known that, in the United States, at least 10% of cervical smears diagnosed as negative from cytotechnologist are needed to be re-screened by a pathologist or a qualified cytotechnologist [23,24].

Another problem is the interpretation of postmenopausal smears with marked atrophic changes. It has been recently shown that cyto-morphological features favoring CIN III in postmenopausal smears include increased number of abnormal single cells with high nuclear/ cytoplasmic ratio accompanied by an irregular nuclear membrane [25]. Furthermore, nuclear enlargement, abnormal chromatin pattern with a granular background can also be seen in reactive changes and may lead to an interpretation error[26]. However, other benign hormone induced cellular changes may be diagnosed as ASC. Similarly, cases of atypical reparative changes and atypical parakeratosis may be interpreted as ASC favour dysplasia depending on cell size, nuclear/cytoplasmic ratio and the number of abnormal cells [27]. Those criteria should be remembered when examining a Pap smear.

Cellular morphology may be altered by a variety of iatrogenic factors such as radiotherapy, ablative procedures and instrumentation ([26,27]. These changes may increase both falsepositive and negative interpretations.

In contrast to our study and many other studies, astudy done in 1996 by GPS Yeoh and KW Chan found that pap smear is an inaccurate diagnostic test.[28]

The causes of high false negative rates can be grouped as follows:

1. sampling error committed during the sampling procedure from patients, i.e., sample preparation of the slide in the physician's office and slide manipulation in the laboratory.
2. interpretation error committed by the pathologist and cytotechnicians during diagnosis and screening.

The causes of false negative cases differ among the existing studies. Most studies have reported that sampling errors are more frequent than interpretation or screening errors[29,30,31] ,but in one study, it has been suggested that 50-90% of false negatives may be due to the limitation of vigilance and recognition in screening [29].

The cervical cancer prevention effort, worldwide have focused on screening sexually active women using cytology smear and treating precancerous lesion thus by decreasing the incidence and mortality from cervical cancer. The diagnosis is made by screening an asymptomatic population, the test in use are cervical cytology, and histological examination of the biopsy material added by numerous technique such as Cervicography and assessment of HPV DNA type. [32].

In most developed countries, women are advised to have their first test soon after becoming sexually active and subsequently every 1-5 years. The current recommendation of the American Cancer Society, National Cancer Institute,

American College of Obstetrics & Gynaecologists and others [12, 14] is that all women who are sexually active above the age of 18 years should have annual pap smear for 3 years.[33,34]

The rate of dysplasia in our study,for HSIL cytology interpretation is similar to other international studies [21], but higher than other reports [35,36].

In our study there was no significant association between age and pap smear finding , in other study they found that malignancy is less among the older age group.[37]

Pain and vaginal discharge were found to be the most common complaints among different lesions of the cervix and most of the signs and symptoms were not specific, Bleeding and pain were the main signs that were found to be associated with more advanced lesions. Pradhan P also found the similar findings.[37]

Conclusion

In this stuy , the screening techniques by pap smear are significant enough to reduce the incidence of preventing carcinomas like cervical carcinomas, Pap smear test was found to be equally sensitive to histopathological examination for the early detection of different cervical lesions. However, it is advised to perform biopsy if any abnormalities are detected in pap smear for correlation and confirmation.

Acknowledgment

We dedicate the sub staff and technicians in Baghdad Medical City, in the histopathological unit, and the staff in college of medicine in Kerbala university.

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