

## **A Study of Papanicolaou Smear Diagnoses in Hawler City**

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### **Abstract**

This study aimed to estimate and analyze the prevalence of different normal, benign and abnormal cellular changes based on cytological diagnosis.

The study included 236 women undergoing cytological exams in obstetric and pediatric hospital in Hawler city. It revealed 4(1.6%) cases of various grades of CIN, 5(2.1%) cases of atypical squamous cells (ASC), 67(28.3%) cases of reactive cellular changes (RCC) and 71(30%) cases of squamous metaplasia. The relation of these conditions to the development of cervical cancer is discussed.

Furthermore, different cervical and vaginal infections were identified by cytological means, including 21(8.8%) cases of Gardnerella vaginalis, 11(4.6%) cases of Candida, 9(3.8) cases of Trichomonas vaginalis, 2(0.8%) cases of viral infection, and 45(19%) cases of nonspecific infection. In some cases more than one lesion is present in the same smear.

### **Introduction**

The cytological appearance of benign, atypical, premalignant, and malignant processes must be thoroughly understood by all those engaged in rendering cytological diagnoses. In addition, cellular changes associated with degeneration, infectious agents, reparative or reactive atypia may be superimposed on benign or malignant processes and must be taken in to account when making a diagnostic decision (Atkinson, 1992).

Cytologic screening for precancerous lesions and cancer of the cervix and subsequent treatments of these lesions have been effective in reducing the incidence and mortality of cervical cancer (Greer, 1997). Screening for cervical cancer is done with a test called the Pap smear, named after George Papanicolaou, a scientist who pioneered the method in the early 1940s (Thomas, 2000). Pap smear is a relatively simple cost-effective, noninvasive screening test that is carried out in conjunction with gynecologic exam at the primary health care level to detect abnormalities that might lead to cervical cancer (Anwar, 1994; Zamani, 1994). Pap test is at present the most essential element in prevention of developing cervical cancer (Elovainio et al., 1997). In countries with organized screening

program for cervical cancer, the incidence rates and mortality have decreased by 60%-90% (Anthony & Peter, 1998). Pap tests are good, but not perfect, their results sometimes appear normal even when a women has abnormal cells of the cervix (Aldrich et al., 2005).

The aim of this study is the recognition of different exfoliate normal and abnormal cellular changes in cervical and vaginal epithelium in various age groups of women and depending on Pap smear reporting form that has been observed to show some point of the screening program of pap smear in Erbil city which was started in a low level from the last three years.

### **Material and method**

This is a retrospective study of some Pap smears performed for women attending obstetric and pediatric in Hawler city, Kurdistan region, Iraq during the year of 2006. A questionnaire containing data about patient's age, occupation, education, if a previous test was done, sexual history, marital status, menstrual cycle history, mode of contraception, clinical finding, and other history All pap smear samples were sent for pathology reports for reporting pap smear results which adopts descriptive diagnoses, (benign cellular changes including infections, reactive changes including inflammation, atrophy, & intrauterine contraceptive device (IUD)related cellular changes, and finally epithelia cell abnormalities including Atypical squamous cells (ASC), and various grade of cervical intraepithelial neoplasia (CIN).

A total number of pap tests in this study were 236 women. A relationship between cervical cytology results and all variable in the questionnaire was estimated.

#### **Methods of cytological diagnoses:**

The normal constituents of the Pap smear comprised normal squamous and glandular epithelial cells, neutrophil polymorphis, some blood and mucin.

#### **Benign disorders of the cervix:**

Inflammation and reactive changes:

Inflammatory changes in squamous cells included slight nuclear enlargement (up to twice the size of an intermediate cell nucleus), bi- or multinucleation, a small perinuclear halo and abundant neutrophil polymorphs in the background.

Reactive changes comprised slight nuclear enlargement as with inflammation in squamous cells and multinucleation with prominent nucleoli in endocervical and metaplastic cells. The chromatin appeared granular to evenly coarse.

### **Infections and organisms:**

Infections were accompanied by acute inflammatory cells. The flagellated organisms (*Trichomonas vaginalis*) were oval and pear-shaped with pale basophilic cytoplasm. The flagellum was not visible and indistinct eccentric placed nucleus in some of the positive identification was seen. Features of inflammatory atypia, including nuclear enlargement, cytoplasmic vacuolization and perinuclear halos (Trichomonal effect). The size of it varied from the size of neutrophils to that of parabasal and metaplastic cells. The clinical features of vaginal candidiasis were intense vulvovaginal itching and thick white discharge. Cytologically oval budding yeast forms and pseudohyphae were evident. Most of the fungal elements were basophilic. Herpesvirus infection was identified in one cervical cytology specimen by a large multinucleated squamous cells with molded nuclei that had ground glass chromatin. Infected cells with cytomegalovirus resembled herpetic cells but multinucleation was not present. Squamous or metaplastic cells with high nuclear to cytoplasmic ratio and large intracellular inclusions were characteristic.

### **Atypical squamous cells (ASC):**

ASC had nuclear feature that distinguished from reactive changes but fall short of the criteria for a SILs.

### **Cervical Intraepithelial Neoplasia (CIN):**

The nucleus of CIN I was several times larger than that of a normal intermediate nucleus, the nuclear contour was irregular, and the chromatin was coarse and hyperchromatic. High grade squamous intraepithelial lesions (HSIL) encompass moderate dysplasia (CIN II) and severe dysplasia (CIN III). The cells had enlarged, hyperchromatic, irregular nuclei and moderate to scant cytoplasm depending on the degree of maturation and keratinization.

## **Results**

The study revealed 104(44%) women with normal limits, 123(52%) patients with benign cellular changes, 5(2.1%) patients with atypical squamous cells, and 4(1.6) patients with cervical intraepithelial neoplasia. Cases of with normal limits fall between the ages of 16-70 years, patients with benign cellular changes fall between the ages of 19-70 years, patients with atypical squamous cells fall between the ages of 31-51 years, and patients with cervical intraepithelial neoplasia fall between the ages of 30-49 years.

Summary of some data related to the sampled women are given in (Table 1).

**Table (1): Summary of some data related to the sampled women with normal limits, benign, ASC, and patients with CIN (LSIL and HSIL).**

<b>Pap Smear Results</b>					
<b>Variable</b>		<b>Normal limit</b>	<b>Benign changes</b>	<b>ASCUS and ASCH</b>	<b>CIN(LSIL) and (HSIL)</b>
No. of cases		104	123	5	4
% of cases		44	52	2.1	1.6
Age N(%)	≤20	3(1.2)	5(2.1)	0	0
	21-35	42(17.7)	68(28.8)	2(0.8)	1(0.4)
	36-50	48(20.3)	44(18.6)	1(0.4)	2(0.8)
	≥ 51	11(4.6)	6(2.5)	2(0.8)	1(0.4)
	Mean age	35.8	35.2	39	40
	Age range	16-70	19-70	31-51	30-49
Gravidity N(%)	≤3	32(13.5)	42(17.7)	0	0
	4-7	45(19)	61(25.8)	3(1.2)	1(0.4)
	≥ 8	27(11.4)	20(8.4)	2(0.8)	3(1.2)
	Mean gravidity.	5	5	7.2	8.5
	Gravidity. range	0-16	0-13	4-9	4-13
Parity N(%)	≤3	41(17.3)	53(22.4)	0	0
	4-7	47(20)	59(25)	5(2.1)	2(0.8)
	≥ 8	16(6.7)	11(4.6)	0	2(0.8)
	Mean parity	4.5	4.4	6.6	7.5
	Parity range	0-15	0-12	4-8	4-10
Site of origin No. (%)	Urban	87(36.8)	91(38.5)	3(1.2)	?
	Suburban	12(5)	25(10.5)	0	1(0.4)
	Rural	5(2.1)	7(2.9)	2(0.8)	1(0.4)
Contraception (%)	Non	34(14.4)	23(9.7)	0	0
	Pills	18(7.6)	15(6.3)	3(1.2)	1(0.4)
	IUD	24(10.1)	44(18.6)	3(1.2)	1(0.4)
	Condom	8(3.3)	11(4.6)	0	1(0.4)
	Others	20(8.4)	30(12.7)	3(1.2)	1(0.4)

All the examined women were Moslems, 52% of them belonged to the low economic classes, and 95% of them did not pass the elementary school. Husbands of most of the women had one wife at the time of the study.

**Benign cellular changes:**

There were 123(52%) patient of Benign cellular changes including 54(44%) patients with cervicovaginal infection, 21(17%) patients with squamous metaplasia and reactive cells. Combined infection, squamous metaplasia and reactive cells were found in 20(16.2%) patients (Table 2).

**Table (2): Detailed cytological results of smears contained squamous metaplasia (Sq. M) and reactive cells (R. C)**

Results	Sq. M	R.C
Pure conditon	31	28
S. M & R.C	21	21
NSI	3	2
Gardrerella v.	7	8
Candida a.	2	3
Trichomonas v.	2	1
Viral infection	1	1

Cervicovaginal infection was the most common finding in patients with benign cellular changes. It was found in 74(31.3%) patients, including 45 (19%) cases of nonspecific infection and 43(18.2%) cases with specific infection. Among the specific infections, there were 21(8.8%) cases of Gardnerella vaginalis, 11(4.6%) cases of Candida, 9(3.8%) cases of Trichomonas vaginalis, 2(0.8%) cases of viral infection (one case of HSV and one case of CMV), and 45(19%) cases of nonspecific infection. Some of the cases of cervicovaginal infection showed mixture of two or more infections (Table 3).

**Table (3): Number and types of single and combined cervicovaginal infections.**

Infec*	NSI	Gard	Can.	Tri.	Vir.
NSI	33	7	4	1	-
Gard.	7	11	2	1	-
Can.	4	2	4	1	-
Tri.	1	1	1	6	-
Vir.	-	-	-	-	2

\*Three of these cases contained atypical squamous cells.

**Atypical squamous cells (ASC)**

5(2.1%) of the total of 236 cases showed atypical squamous cells in their smears, including three patients with atypical squamous cells of undetermined significance (ASCUS)and two patients with atypical

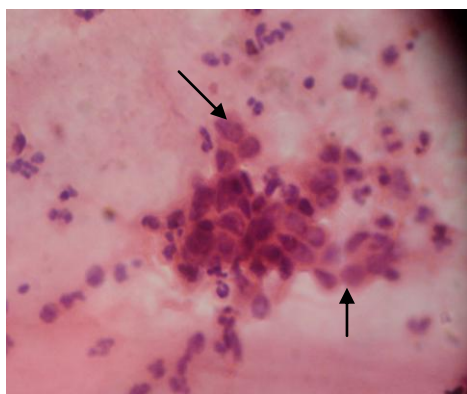
squamous cells-cannot exclude HSIL(ASCH). Combined ASC with reactive cells were present in three cases (Table 4).

**Table(4) : Detailed cytological results for smears with cervical dysplasia (CIN) and atypical squamous cells.**

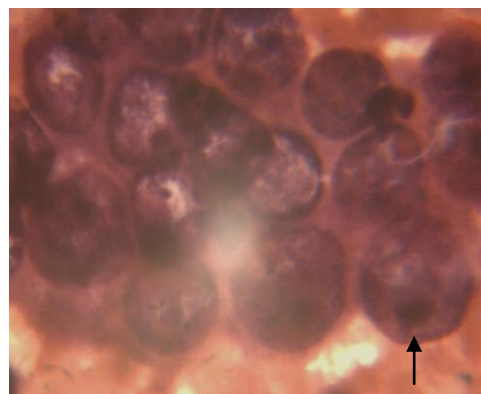
Cellular abnormalities <sup>14</sup>	Inflam	Infect	R. C	Sq. m	Blood
ASC-US	+++	+	+	+	-
ASC-US	+++	-	-	+	-
ASC-US	+++	-	-	-	-
ASC-H	++	-	+	-	-
ASC-H	Erosive	+	+	+	-
Mild Dysp.	+++	-	-	-	+
Mild Dysp.	+++	-	-	-	-
Moderate Dysp.	++	-	-	-	-
Severe Dysp.	+++	-	-	+	-

**Cervical intraepithelial neoplasia (CIN):**

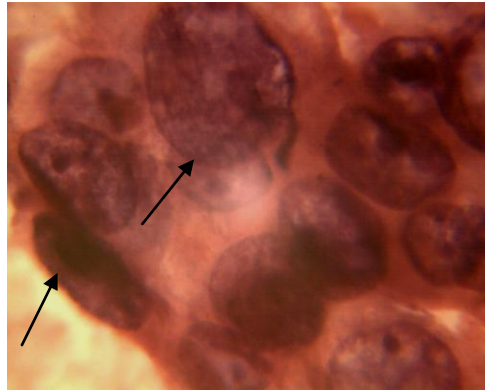
There were 4(2.1%) patients with CIN, including 2 patient with CIN I,one patient with CIN II, and one patient with CIN III, and their smears showed dysplastic cells ranging as mild, moderate and severe dysplasia respectively (Fig 1,2,3).One smear contained fresh blood (Table 4).



**(Fig. 1): A mildly dysplastic cells is seen with an enlarged nucleus and irregular nuclear margins and abnormal chromatin (H and E : x100).**



**(Fig. 2): This is a cluster of moderately moderate dysplastic cells with high nuclear:cytoplasmic ratio with an enlarged hyperchromatic nucleus (H & E: x1000).**



**(Fig. 3): This is a cluster of severity dysplastic cells has abnormal clumped, cleared chromatin and delicate cytoplasm. The nuclear margins are irregular with prominent nucleoli (H and E: x1000).**

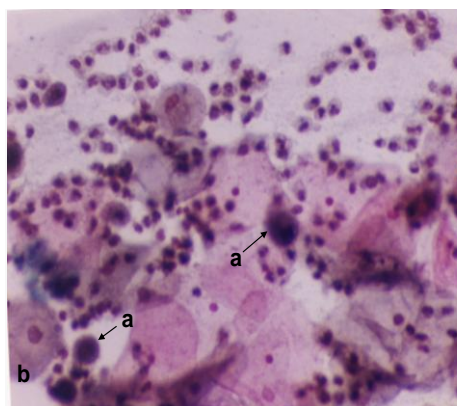
### **Discussion**

Data, on prevalence rates of cervical lesions shows 4(2.5%) CIN of varies degrees in which included one case of CIN I, one case of CIN II, and 2 patients of CIN III. Various studies have shown prevalence rates for overall abnormal cytological diagnoses for screening programs in different countries. In two studies in Holland, rates of abnormal Pap tests, including all abnormal diagnoses, were (0.54%) and (0.8%) respectively (Elias et al., 1983; Graaf et al., 1988). While in Thailand (1.2%) of abnormal cytological diagnoses was recorded (Chou & Chen, 1989). Low ratio of abnormal cervical cytology have been recorded from regularly screened population in such countries. Most of our examined women are in a low standard socially and uneducated, nearly all of them were taking the pap smear for the first time and the follow up for cervical cytology test has never been done before for these women in which these are may be some points of reasons why the ratio of CIN increased in comparison with the other research results which are mentioned above. More recently, a study in a rural Costa Rican population showed a prevalence rate of abnormal Pap tests of 3.7% CIN of varies grades( Herrero el at., 2000). In American study, the prevalence rates of abnormal results were 3.2%, 3.0%, and 2.7%, among blacks, whites, and Hispanics respectively, according to the Bethesda System (Benard et al., 2001). The research using the Bethesda System has shown a higher prevalence rate than the current study and others. In fact, the Bethesda System includes atypical squamous cells of undetermined significance, atypical glandular cells of undetermined significance, and HPV cytological diagnoses, which increase the overall prevalence of abnormal results (Kurman & Solomon, 1994).

If we count or better to say consider our atypical squamous cells pap smear results with CIN as in the Bethesda system, we find out that the ratio of abnormalities increases from 1.6% to 3.7% which is almost close to the

results of the above mentioned researchers which follow the Bethesda system to analyze the prevalence of cervical smear abnormalities.

At any rate, prevalence rates of cervical intraepithelial lesions, regardless of classification, present widely varied values. This may be attributed to cervical cancer screening, diverse collection techniques, quality of samples, and especially differences in diagnostic criteria. In addition, all prevalence rates mentioned are crude rates that do not consider the difference in age distribution for each population. Due to low number of patients with CIN in this study, no statistical analysis could be performed. However, the mean age for patients with CIN in the present study (40 years) is remarkably similar to that reported by (Parkin, 1997), higher than that reported by (Morgolis et al., 1999), and lower than that reported by (Kim et al., 1992). Our cases of CIN were in a low socioeconomic condition, they were married early and became pregnant before being 19 years old, and they were multi-pregnant ranged as 3-12 children. All these reasons are some points of risk factor of developing cervical intraepithelial neoplasia. However, exactly how these risk factors are connected to CIN is unknown (Wright et al., 2002) and (Hoffman & Martino, 2004). Other risk factor for developing CIN appears to be higher after exposure to HPV that cause genital warts, however the exact cause of CIN is unknown (Hoffman & Martino, 2004). Research work has shown that dyskaryotic cells may arise from atypical cells. Four carefully designed prospective studies of women with cervical cytologic atypia found underlying CIN in 11% to 16% of cases, even when a subsequent smear was normal (Maier & Schultenover, 1986; Spitzer et al., 1987 and Davis et al., 1987). On cytological examination of the cervix, atypical squamous cells (ASC) display cellular abnormalities more marked than simple reactive changes, but not full criteria for CIN(SIL) (Fig.4) (Solomon et al. , 2002).



**(Fig. 4): a- Atypical squamous cell, ASC-H with the nuclei of these atypical squamous cells are three-four times the size of normal intermediate cell nuclei thus increasing of nuclear cytoplasmic ratio. b-An intermediate cell has a indistinct perinuclear inflammatory type (PAP stain, x100).**



A common cause of ASC pap smear results are minor infection, cervical inflammation, repeat trauma as a result of multiple pregnancy, and hormonal causes (Kiviat et al., 1985). 4/5(80%) and 3/5(60%) ASC pap smear results in this study showed inflammation and infection respectively (Table 4). 3/5(60%) of the total of the patients with ASC used combined oral contraceptive pills and all of them have many children ranged as 6-9 (Table 1). Combined ASC, infection, immature metaplastic cells, atrophy, reactive changes were recorded in one postmenopausal patients.

The interpretation of cervical-vaginal smears from postmenopausal patients can be challenging. Extensive inflammation, parabasal cells with organophilic cytoplasm, nuclear variations secondary to drying, and degeneration associated with atrophic vaginitis may result in cellular changes falsely interpreted as squamous atypia and/or a more severe lesion. Immature metaplastic and reparative changes are another cause of false-positive results ( Jovanovic et al., 1995).

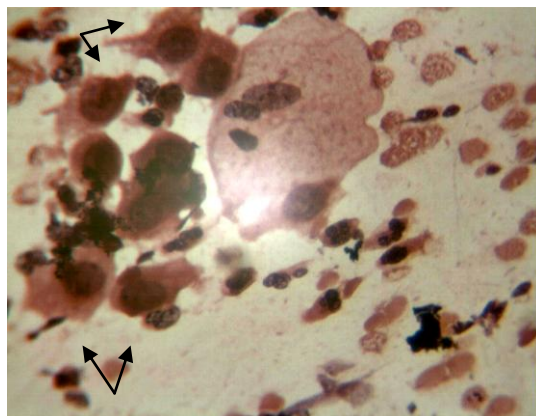
The discovery rate of ASC in this study was (2.1%). It is more or less similar to that which was reported in the cytological surveyed population in Cambridge (Diaz & Kabawat, 1999). Mount and Papillo, 1999 have reported a higher ASC rate up to 9% in Northern New England.

All the examined women for the study in Northern New England were aged between 10-19 years. The high rate of ASC pap smear results may reflect a high level of sexual activity among the adolescent girls, because ASC pap smear results for this population are often or more often caused by sexually transmitted human papilloma virus (HPV) (Mount & Papillo, 1999).

On the other hand, under diagnosing epithelial abnormalities as repair is a source of false-negative pap test results (Colgan et al., 2000).

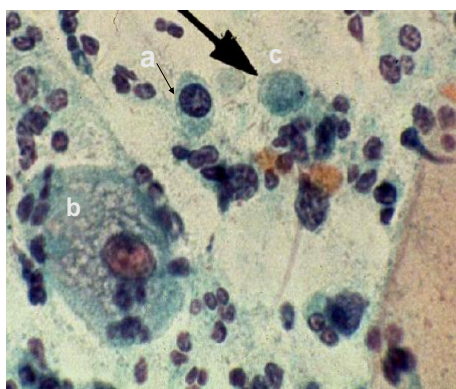
Longitudinal studies of patients with reparative or reactive changes in cervical smears have shown an increase risk of developing SIL in subsequent years (Chang et al., 1996; Soofer & Sedaway, 1997). It has been stated that reactive or reparative changes are more likely to be mistaken for malignancy than SIL (Colgan et al., 2000).

Reactive pap test, classified in the Bethesda System as "negative for diagnostic epithelial abnormalities", contain cellular changes intermediate between those in "negative-within the normal limits" cases and those in ASC or AGC"(Fig 5) (Kurman & Solomon, 1994).



**(Fig. 5): Reactive changes: The nuclear margins are smooth and their chromatin are bland. The nuclear enlargement can be interpreted as due to reactive changes (H& E, x400).**

Reactive cellular changes associated with inflammation, atrophy, radiation, IUD, and other (Atkinson, 1992). In the current study, (57.1%) cases of the total of 67 patients of reactive cellular changes used IUD for longer than two years and (79%) of them had moderate, severe, and erosive cervicitis. The other benign cellular changes in this study are associated with different infectious agents in which they are common sexually transmitted and non-sexually transmitted infections. In the present study, non specific infections (NSI) was the most common infection of the cervix and vagina followed by *Gardnerella vaginalis*, *Candida*, *Trichomonas vaginalis* and viral infection. Highest ratio of NSI in the current study is similar to the results that reported in Mosul city (Hasan, 1989). Although Pap smear can not diagnose the infective agents in this group of lesions, yet exclusion of other specific agents is helpful for planning other investigations which are more efficient in identifying the causative microorganisms. Infection is the most common cause of acute inflammation, although a specific bacterial agent is frequently not identified. Symptomatic cases of acute cervicovaginitis are often associated with gram negative rod *Gardnerella vaginalis*. The organism may be apparent in large confluent sheets, often encrusting the cytoplasm of more mature squamous cells (clue cell) (Atkinson, 1994 ; Atkinson, 2004 and McKee, 2004) (Fig.6).



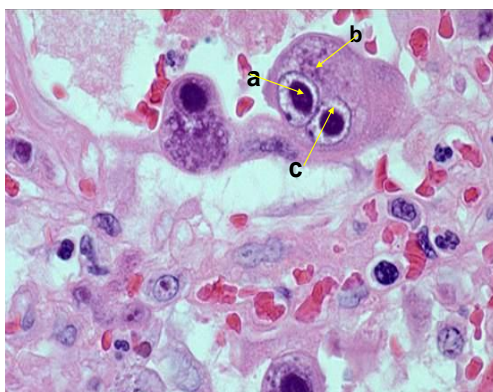
**(Fig. 6): a-Atypical squamous cell (ASCUS): This small cell shows a high nuclear/ cytoplasmic ratio, yperchromasia and smooth nuclear margin. b- Bacterial vaginosis: The organisms of bacterial vaginosis are seen adherent to the epithelial cell. c-Trichomonas vaginalis: the pear shape and eccentrically placed nuclei.This image shows organism are 2-3 times the size of polymorphs (PAP stain, x400).**

*Gardnerella vaginalis* was the most common infection among the specific infections. This is similar to that mentioned by (Atkinson, 2004). Examination of Gram stained vaginal secretions for bacterial vaginosis with clinical findings of vaginal pH and odor test are considered the diagnostic gold standard (Atkinson, 2004).The presence of candidiasis and trichomoniasis was highly associated with abnormal cytologic findings, particularly those indicative of inflammation (McKee,2004). Trichomoniasis is a parasitic sexually transmitted infection (Fig.6).

Cytological smears of nearly all of the patients with Candidiasis and Trichomoniasis in the present study were showed acute inflammation in their smears. Most of the patients with Candidiasis in the current study used IUD, only three of them took the pill. The predisposing factors seem to be hormonal, associated with changes in the balance of cell types in the lining epithelium of the Vagina (Georgopapadaku & Walsh, 1994). On the other hand, the finding ratio of Candida (Hasan, 1989) and trichomonas (Stary et al., 1991) were lower than that reported by the current investigation.

From these few points that mentioned above, we concluded that Pap smear is not a diagnostic test, but it is a screening test. Reading error, sampling and technique errors in handling the smear may go error diagnoses or the true results may be undetected. In general Pap smear can not be used to diagnoses sexual transmitted disease (STDs). However the finding of trichomonal organisms in smears should prompt a repeated culture and direct microscopy of vaginal wet mount (Peterson, et al., 1995).

Herpes simplex viral infection shows typical cellular changes including giant cells with multinucleation, nuclear molding, and "ground-glass" chromatin. It is also accompanied by inflamed and reactive endocervical cells (Mckee, 2004). This corroborates our finding. Viral infection in the current study is not common. Its ratio was lower than that reported by (Morse et al., 2005). This is because of the sources of the selected women in the present study and some social factors in Islamic society. In cytological examination, human cytomegalovirus (HCMV) infection can not be implied unless typical HCMV-infected cells like owl's-eye cells are present (Fig.7). However, such cells are not always observed in HCMV-infection cases (Takeuchi et al., 2004). Characteristic of virus can be seen and identified in the Pap smear, even in an asymptomatic patient. But the most accurate diagnostic technique is to isolate the virus on tissue culture cells by inoculation (Vora, 1977).



**(Fig. 7) :( owl' s-eye cell): This case indicates cytomegalovirus (CMV)-associated intracytoplasmic inclusion in the intermediate squamous cell of a cervical smear. a-nucleus of the intermediate cell, b-its cytoplasm and c-the viral inclusion body (H &E: x400).**

The absence of endocervical or metaplastic cells does not render the smear inadequate or suboptimal. Their presence denotes sampling of the squamocolumnar junction, where most dysplasias are believed to arise (Hasan, 1989). Absence of endocervical cells were encountered in cervical smears of 16(6.7%) women. The absence of endocervical cells was attributed to inadequate sampling as there was no satisfactory explanation for their absence.

Although this not a population based study, the results represent a few sample of the user Kurdish women. Therefore, these estimates should be close to the actual values of a lower-income population and could provide a reference for planning, assessment, and follow-up of cervical cancer screening activities.

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## دراسة في تشخيص مسحة البابينيكولا في مدينة أربيل

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### الخلاصة

تهدف الدراسة الى تقييم و تحليل تفشي و انتشارمختلف التغييرات الخلوية الحميدة الطبيعية و التغييرات غير الطبيعية بناءا على التشخيصات الخلوية. تضمنت الدراسة ٢٣٦ امرأة خاضعة للفحص الخلوي في مستشفى الولادة والاطفال في مدينة أربيل. كشفت الدراسة عن ٤ (١٦%) حالات من الثدن الحرشفي و ٥ (٢١%) حالات من الخلايا اللانمطية و ٤٢ (١٧%) حالة من التغييرات الخلوية المتفاعلة و ٥٠ حالة من التنسج الحرشفي. وفضلاً عن ذلك فقد كشف الفحص الخلوي عن حالات مختلفة من ألتهابات عنق الرحم و المهبل والتي تضمنت ٢١ (٨%) حالة من الألتهاب بالغاردينيللا المهبلي و ١١ (٤%) حالة من داء المبيضات و ٩ (٣%) حالة من مشعرات المهبلي و حالتين من الألتهابات الفيروسية و ٤٥ (١٩%) حالة من الاتهاب اللانوعي. في بعض الحالات كانت هنالك أكثر من آفة مرضية في المسحة الواحدة.