Demographic and Clinical Study on Patients Referred to The National Cancer Research Center for Pap Smears

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Abstract

A pap test is a simple technique which can detect pre-cancerous and cancerous cells in the vagina and cervix. Cervical cancer is the easiest gynecologic cancer we could   prevent it, with regular screening tests and follow-up this screening may avoid cervical cancer or detact it early.This study aims to estimate cytological changes and precancerus lesions during Pap smear test and visual inspection of the cervix on Iraqi women and determine the relationship with demographic characteristics.   The study included 50 women aged 18-56 years (mean 39 ±10)   in National Cancer of Research Center (NCRC) belong to Baghdad University. These women suffered from genital problems or for checking reproductive health status during the period from 1^{st}December 2016 to 31^{st}April 2018 to perform the grossly inspection of the cervix and take papanacola smear. The results showed that 98% of the women were sexually active, 88% of them in premenapusal period and only (12%) in postmenapusal. Visual inspection of the cervix showed there were (40%) erosion lesions, cytology examination showed (90%) of non specific inflamation, 60% of reactive squamous metaplasia, 26% Koilocytotic atypia, 16% CIN1 or LGSIL. Women how used the contraception 68% and the most of them used pills. The women who had multiple births 26% , 18% had one, and 56 % were nulliparous . The causes of the visit were variable as for   rotine checking 48% or to know the reason of abnormal vaginal discharge 22%. 46% of them were doing this test while 54% of them didn't make it previously. Therefore, we recommend by firm awareness and must perform pap smear screening for sexually active women to prevent precancereus lesions in the cervix and then avoid cervical cancer.

Keywords: Pap smear, Pericancereus lesion , Cervical cancer,Iraqi women health

دراسة ديموغرافية و سريرية للمريضات الوافدات للمركز الوطني الريادي لبحوث السرطان لاجراء فحص لطاخات عنق الرحم

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الخلاصة

اختبار البابا نيكولا تقنية من شأنها كشف الخلايا السرطانية او ماقبل السرطانية في المهبل و عنق الرحم. ان سرطان عنق الرحم اكثر سرطان تناسلي يمكن تجنبه من خلال الاختبارات المسحية المنتظمة والمتتابعة لهذا المسح، اذ من الممكن كشف سرطان عنق الرحم مبكرا. وقد هدفت الدراسة الى تقييم التغيرات الخلوية والافات العيانية ما قبل السرطانية في عنق الرحم خلال اختبار مسحة باب والفحص السريري العياني لمنطقة عنق الرحم في النساء العراقيات لتحديد علاقة هذه التغيرات مع الميزات الديموغرافية. شملت الدراسة

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50 امرأة تراوحت أعمارهن بين (18–56) سنة، ومتوسط (39 ± 10) ارتدن المركز الوطني الريادي لبحوث السرطان – جامعة بغداد وكن يعانين من مشاكل تناسلية أو لغرض اجراء الفحص الدوري ومتابعة الحالة الصحية الإنجابية ابتداءا من بداية كانون الاول 2016 ولغاية اواخراذار 2018 لاجراء الفحص السريري و اختبار مسحة عنق الرحم .

أظهرت النتائج أن 98% من النساء كن نشطات جنسيا، 88% منهم في الفترة الانجابية و 12% فقط في مرحلة ما بعد سن اليأس. أظهر الفحص العياني لعنق الرحم وجود آفات تآكلية (40%) وأظهر الفحص الخلوي 90% من الالتهاب غير المحدد، و 60% من حؤول الحرشف الحركي، و 26% النوات المنتفخة اللانمطية، و 16% CIN1 تنسج تحت الظهارة. وكانت نسبة النسوة اللواتي استخدمن وسائل منع الحمل 68% والاغلبية منهن   ممن استخدمن حبوب منع الحمل. اما ما يخص عدد الولادات فـ 26% كن ممن لديهن تعدد ولادات ، و18%ممن كان لها مولود واحد ، و 56% بلا ولادات . اما سبب الزيارة فقد كان متغيرا ،فمثلا لغرض الفحص الدوري 48 % أو لمعرفة سبب وجود الإفرازات المهبلية غير الطبيعية 22%. 46% منهن كن ممن اجرن هذا الاختبار مسبقا في حين 54% منهن اجرن الاختبار للمرة الاولى. ولذلك ا نوصي بمزيد من الوعي بهذه الاختبار و وجوب اجراء هذا الفحص الدوري للنساء النشطات جنسيا لمنع الآفات ما قبل السرطانية في عنق الرحم وبالتالي تجنب سرطان عنق الرحم.

Introduction

Prevention, early detection and treatment of the preinvasive cervical lesions, all these are very important in limit cervical cancer. Cytology screening programs can reduce the incidence and mortality of cervical carcinoma in developed countries. On the other hand, cervical carcinoma represents a major public health problem in developing countries. Cervical cancer is the third most diagnosed cancer and the fourth leading cause of death in women worldwide, so cervical carcinoma control methods are an essential way in reducing early death during the most productive period in a woman's life. [1]

The death from cervical carcinoma occurs 50% in the two decades of life from 45 to 65 years with a range from 20 to over 85 years. Studies showed that cervical carcinoma does not increase rapidly from normal epithelium but is existing by more intraepithelial neoplastic changes which are precancerous lesions were termed as cervical intraepithelial neoplasia (CIN). [2- 5] Several reports approved that if these lesions were untreated, up to one third of these precancerous lesions developed to carcinoma. [6, 7] Precancerous changes occur 99.7% in cases of infection with Human papilloma virus (HPV). Oncogenic subtypes of HPV are 16,18,31,33,35,39,45,51,52,56 and 58 but the more malignant sub types are 16 and 18. Multiple sexual partners, younger age at first sexual intercourse, immunosuppression, the changing sexual behaviour and cigarette smoking make as predisposing factors for the HPV persistent infection which leads to cervical cancer. [8, 9]

The Pap test screening (obligatory or voluntary) allows the detection and treatment of precancerous lesions. [10,11] In several western countries, where screening programs have long been applied, cervical cancer rates decreased as more as 65% of the last four decades.[7] Mortality rates have also decreased in high-risk areas, including China, Taiwan, Korea, and India lead to effect screen activities and socioeconomic conditions. [12-15] Decreased cervical cancer rates in the Middle East and other parts of the developing world result to decreased HPV infections and that due to societal disapproval of extramarital sexual activity . [16] The Pap test followed by colposcopy /biopsy and therapy interaction all these are common cytology-based screening, but aren't feasible in most low- and middle income countries because of cost and less healthcare means. However, teaching, and effective screening techniques have been developed for use in these countries, including visual inspection of the cervix, for lesions using either acetic acid or Lugol's iodine as instant prediction then DNA testing for HPV in cervical cell samples to confirm this prediction. [17-19] Vaccination of younger female against HPV-16 and HPV-18 infections is an active new way for reducing cervical cancer but this vaccine very cost and will be a barrier in application in poor countries. [20]

In Iraq cervical cancer is the 12th cancer among women and the 10th among women between 15 and 44 years of age. The annual incidence is 2.1per100,000 and mortality rate of cervical cancer is estimated at 1.4 per 100,000 and there is rising threat to 0.3% for women in all her life.. In the general population of Iraq data is not yet available on the HPV prevalence. In Asia, the region which Iraq belongs, about 2.5% of female in the country are exposure to cervical HPV-16/18 infections during a

period of time and 72% of invasive cervical cancers are documented to HPVs 16 or 18. Non-oncogenic subtypes of HPV, e.g. 6 and 11, lead to low-grade cervical lesions and genital warts.[5,9]
The Iraqi searchers conducted numerous studies about Pap smear test and the main role to detect the reproductive ducts troubles like specific, non-specific infection and cytocologic changes which define as precancerous lesions. These lesions if left long period without treatment, patients contact to high risk to HPV, so these cases must follow up and testing for HPV DNA, colposcopic evaluation and biopsy which prevent cervical cancer. [21- 23] Latest estimates indicate that every year 311 women are diagnosed with cervical cancer and 212 die from the disease [5].

This study aims to estimate cytological changes and precancerus lesions in Pap smear test and visual inspection of the cervix on Iraqi women and determine the relationship with demographic characteristics.

Material and methods

Fifty women referred to National Cancer Research Center (NCRC) - Baghdad University. These women had genital health problems or to check health status by routine examination from 1st December 2016 to 31st march 2018. Information about Pap smear importunes and its purpose was explained for all participants. The study included women who were sexually active and did not undergo hysterectomy. Demographic variables and database were taken like age, parity, sexually transmitted diseasing replacement therapy, history of contraception, menopausal status, and smoking. All these data were entered in statistic program (SPSS V.22). All patients perform a pelvic examination and Pap smear sampling by the Ayre's spatula or a brush. Slide smears of patients were taken at a clinic by visual inspection of the cervix and vagina, and then were stained in the cytology laboratory in (NCRC). Cervical Pap smears were examined by the pathologist in the Department of Pathology in (NCRC).

For cytological diagnosis, Papanicolaou Stain applied as [24] by wet method:

Before staining all slides fixed immediately in 95% ethanol for One-third to half an hour and then the slides were dipped in descending concentrations of ethanol, each for 10 dips, including 95%, 80%, 70% and 50% ethanol. After that, the slides were immersed for 10 times in distilled water, followed by 1-2 min staining with Hematoxylin. extreme stain on slides was separated by running tap water shortly. afterwards, ascending concentrations of ethanol (50%, 70%, 80% and 95%) were done to dip the slides for 10 times in each concentration. Then, the slides were put in Orange-G stain for 2 min, followed by 10 dips in 95% ethanol. Eosin stain was also used to stain the samples for 2 min.. Later, the slides were dipped in 95% ethanol for 10 dips, and this was frequented for another 10 dips in another 95% ethanol. Then, 100% ethanol was used for 10 dips. The slides were shortly put in a 50:50 mixture of absolute ethanol: Xylene for 5 min. at last, the slides stayed in Xylene for 20 min to be mounted with DPX (mixture of distyrene, a plasticizer, dissolved in xylene). Then, the slides were examined in a light microscope in 40X magnification.

Results
Table 1- Demographic characteristic of the patients

feature	No.	%
Occupation		
House keeper	13	26
Employed	37	74
Total	50	100
Marital status		
Married	49	98
Widowed	1	2
Total	50	100
ways of .contraception		
With out contraception	23	46
Pill	12	24
Condom	8	16
Coltus interruptus	2	4
Loop	5	10
Total	50	100
Contraception		
Yes	27	68
No	23	32
Total	50	100
Menstrual period		
Regular	37	74
Irregular	13	26
Total	50	100
Cause of visit		
Checking	24	48
Post-coital bleeding	10	20
Dyspareunia	4	8
Irregular vaginal bleeding	1	2
Abnormal vaginal discharge	11	22
Total	50	100
Number of births		
0	20	40
1	11	22
2	13	26
3	3	6
4	2	4
5	1	2
Total	50	100
Have pap smear before?		
Yes	23	54
No	27	46
Total	50	100

   In Table-1 the results appeared that women ages ranged from 18 to 56 years old with an average of approximately 39 (±10) years, most of them were employers 74%,. The married women were 98 % and the usage of contraceptives was 54% for all methods but the most favourite method was pills 44% of women which used the contraceptives. Most women were regular menstrual period 74%. The main reason to visit clinic was to check-in 48%, followed by the abnormal vaginal discharge 22%, and the women who post-coital bleeding 20. %. Nulliparous women were 40%, the highest number of women who had 2 children 26% and the less how had 5 children 2%. The women who performed this examination before was 54% and who didn't was 46%.

In Table-2 the average age of women was 39 yr. in range (18-56). 86% were in premenopausa, while 14% in postmenopausa. The youngest in postmenopausal age was 38 years old and the oldest was 55 years old. Their ages at marriage ranged between 12 to 43, mean 25 years old (± 6.3) Std. Deviation. Their ages at the first birth were (15 -40), mean (20.7) years old (±7) Std. Deviation.

Table 2-Reproductive status with Age stages

Reproductive status and Age	N0.	Range	Mean	Std. Deviation
Age	50	18-56	39	10
Age at marriage	50	12-43	25	6.3
Age at pregnancy	30	14-39	20.7	7
Age at first child birth	30	15-40	21.5	6.8
Age at menopause	7 (14%)	38-55	47	5.5
Age at premenopausa	43(86%)	18-50	32	2.2

Table 3-Visual inspection and cytological changes of cervix

| Clinical and cytological findings	No.	%
Colposcopical findings:		
No lesion	25	50
Erosion	23	46
Genital warts	2	4
Microscopic examination: Cyto- pathological		
Reactive squamous metaplasia	30	60
Koilocytotic atypia(Possibility of Human Papilloma Virus infection)	13	26
CIN1or LGSIL(Cervical Intraepithelial Neoplasia or Low grade squamous intraepithelial lesion)	8	16
Clusters & sheets of benign- abnormal exfoliated endometrial cells	7	14
Endometrial hyperplasia	6	12
Atypical squamous cells of undetermined significance (ASC-US)	5	10
Atrophy	4	8
Microscopic examination :Inflammation		
Non specific inflammation (vaginitis, cervicitis, endocervicitis)	45	90
Moniliasis (spores or hyphi of *candidia albecanus*) , *Trichomonus vaginitis*	9	18
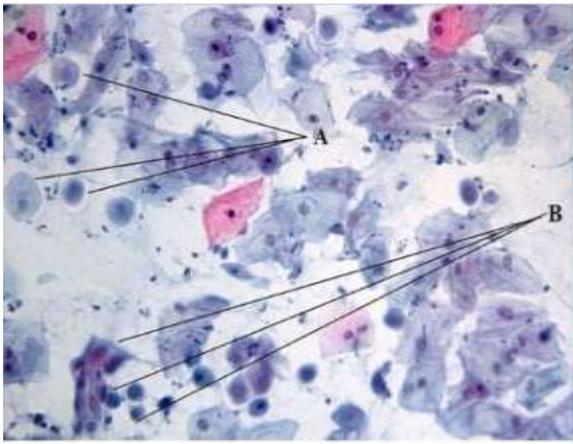
Figure 1- included microscopic view of pap smear: (A) the left reactive squamous metaplasia, (B) the endocervical glandular dysplasia (i.e., CIN I or LGIL according to Bethesda Terminology).
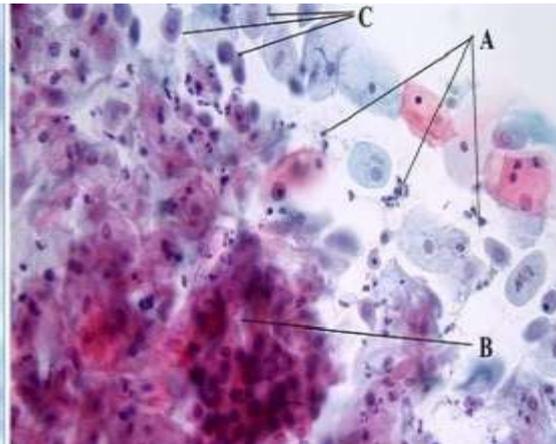
Figure 2 - (A) Cervical smear shows a sever degree of non -specific cervicitis extending to the endocervix, (B), numerous sheets of benign- -looking abnormally exfoliated endocervical cells evidence of minima atypia within (C)squamous and endocervical cells
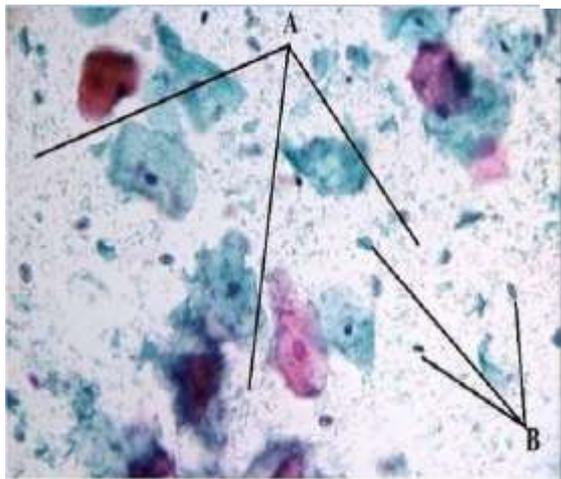

Figure 3 -Cervical smear shows (A) dysplastic cells and,(B) positive for lactobacilli infection,these bacilli are associated with cytolysis. Clinically this may be associated with an odourless white vaginal discharge.
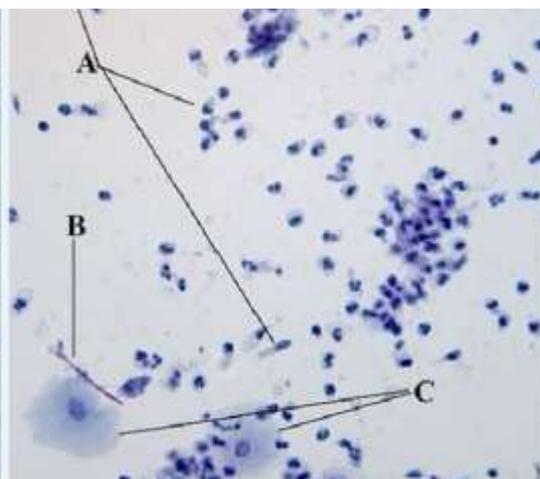
Figure 4-Cervical smear shows (A) positive for *Trichomons vaginalis*, (B) hyphi of *Candida albicans*, (c)dysplastic squamous cells
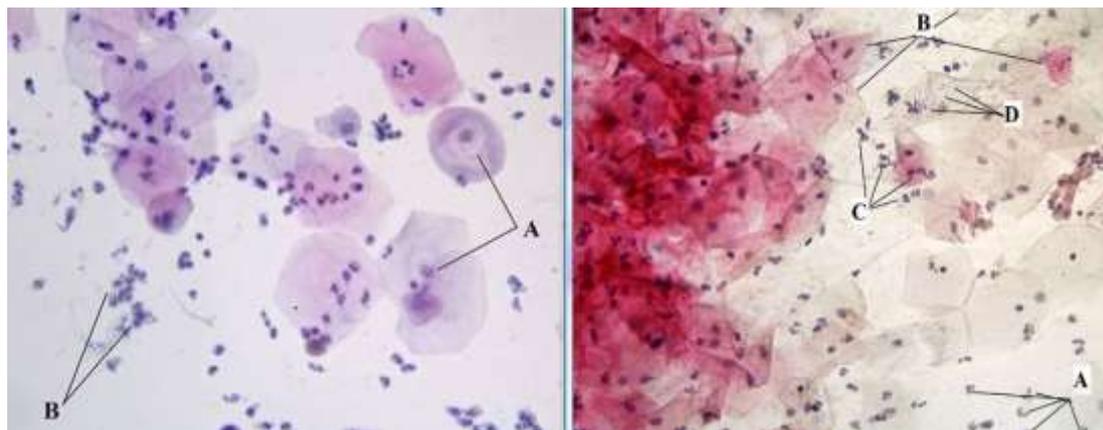
Figure 5-Cervical smear shows (A) an evidence of koilocytotic atypia which mean possibility of Human Papilloma Virus infection and(B). sever degree of non – specific cervicitis

Figure 6-Pap smear of woman in 22 years old shows (A) heavy acute inflammatory cells infiltration, (B), clusters of mild atypical and desquamated endocervical epithelial cells changes, C)positive for *Candida albicans* (,(D growth of normal vaginal flora) were affirmed in bacteriological examination.

Table-3 and all Figures-(1, 2, 3, 4, 5 and 6) appeared the pathogenic results according to Bethesda Terminology, most smears of cases showed a severe degree of non-specific cervicitis / endocervicitis 90% with reactive squamous metaplasia 60%. Mild koilocytotic like atypia is observed with mild dysplasia (27%).Some smear showed sever chronic cervicitis/ endocervicitis with atypical changes in endocervical & benign-looking epithelial& metaplastic cells in favour of CIN1 which ranged between ASCUS 10% to LSIL or LGSIL 17%with possibility of polyp. Other Smears showed infiltration by clusters of squamous epithelium cells indicate different degree of maturation, some with degenerative changes. Some cases were showing evidence of cellular changes consistent with tissue repair. Other cases were showing sever degree of non specific cervicitis extending to the endocervix or specific cervicitis like Moniliasis (spores or hyphi of *Candida albicans*) alone or with *Trichomons vaginalis*

Discussion

The Papanicolaou (Pap) smear screening test started in the United States in 1941, to detect early cervical cancer and reduce incidence and mortality as a result of this cancer. Finally this technique with other like colposcopy inspection with acetic acid or Lugol's iodine or DNA testing for HPV have become a typical models for diagnosis and screening. In spite of the Pap smear is administered to asymptomatic patients, rather than diagnostic tests (confirm or refute the expected disease). [23]
Cancer of the crevice is the second most common cancer among women worldwide after breast, with 83% of cases occurring in developing countries. HPV infection is detected in 99.7% of the cervical cancer cases. [8,9] Although the infection recovers without any treatment in most women, the women with persisting HPV infection are at risk for CIN II, III and cervical cancer. Reports of test sensitivity and specificity vary significantly, in a systematic review conducted by Alwan et al., sensitivity of Pap smear was found 91%, and specificity was found 100%. [3, 25] In this study most women were married (98%) and without partner were only (2%). This means sexual activity has a major role in the exposure of the female reproductive system to many health problems. [26]

In this study the occupation, or regularity of menstrual cycle didn't be a significant difference. 68% of the women were taking contraceptives and the major type was pills. The women who suffer from abnormal vaginal discharge (22%) and post coital bleeding (20%). Most of previous studies which agreed with this study, found there was a significant correlation between abnormal Pap smears with hormonal contraception, post coital bleeding and vaginal discharges that make the woman requires referring a clinic and checking her cervix by macro and micro tests. The vaginal secretions, abdominal pain abnormalities and itching are the most important signs of cervical inflammation. [19,27]

In this study there were only mild and moderate unhealthy problem like erosion 46%, genital warts 4%. In microscopic examination, there were non-specific vaginitis, cervicitis, endocervicitis 90% extending to the endocervix with reactive squamous metaplasia and atypical cellular changes 70% (CIN1and SIL) 16% which can be termed as precancerous lesions. These cases can treated with little cost and short time, which necessary, treated as soon as possible, to prevent progression to advance stage of neoplasia or cervical cancer. [23]In this study, 18% showed severe degree of cervicitis / endocervicitis with *Candida albicans* and *Trichomons vaginalis*. So Pap smear could diagnose some microbiological agents in addition to *Candida albicans* and *Trichomons Vaginalis,* such as actinomyces spp., Herpes Simplex or HPV. [28] Several studies in Iraqi hospitals found the incidence of cervical cancer is very low like other islamic countries, but if this cancer occurs will develop to advance stage and prognoses very poor survive. [29]

In this study observed of mild koliocytotic atypia in squamous cells with mild dysplasia (26%), thus Possibility of HPV infection cannot be excluded or probably inflammatory-induced ,immediate follow up as that might cause a series of alteration to carcinoma when untreated.[30] There are many potential risk factors have been influenced the rate of cervical cancer, including marital and sexual history, smoking, gynecologic and obstetric events, infectious agents specially HPV, oral contraceptive, occupational factors, immunosuppression, and dietary habits. Recent studies indicate the age at first intercourse, number of pregnancies, and the numbers of sexual partners are effectively risk factors [5, 7].

According to last studies pap smear covers only mini number of women, from which a very small proportion with low social standing but in this study 54% of the women were underwent to this test previously which considered as a positive condition. In the absence of a well-organized screening program, a special approach is required to increase women's awareness to attend clinics and hospitals to confirm Pap smear [30].

Conclusion:

We conclude that Pap smear is one of the most important methods of early detection of cervical cancer, which requires follow-up to detect genital problems, especially cancer and pre-cancerous lesions. If lesions and the infection treated, prevention occurs and stopped the development of a serious pathology problem. We strongly recommend that sexually active women should undergo routine cervical smear screening because sexually active women are more contact to infect than others

Acknowledgements

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