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Study About the Causative Agents of Cervical Infections and Cytopathological Changes in Iraqi Women

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Abstract

Cervical infections are common problems among women, specially of reproductive age, in Iraq ,and are one of numerous risk factors for cervical intraepithelial neoplasia and cervical cancer .

The aim of this study was to investigate the causative agents of cervicitis and their association with cytopathological changes among 67 cases of women, aged from 16 to 60 years, who attended the National Cancer Research Center / University of Baghdad, Iraq , during the period from April to December 2018 .

The age group 36-40 had the highest percentage of cervical infections 13/16 (81.3%) while the age group 56-60 had the lowest percentage 2/6 (33.3%).

Specific microbial inflammations were found in 51/67(76.1%) of the cases, as detected by microbiological examinations, while 49/67(73.13%) were of non-specific inflammations. The main causative agent of cervical infections was *Candida albicans*: 36/67(53.7%) followed by : *Neisseria gonorrhoeae* 5/67(7.5%) , enteric bacteria 6/67(8.9%) and *Trichomonas vaginalis* 4/67(5.9%),.

The cyto-diagnosis , as detected by using pap smears examination, revealed some cases 6/67(8.9%) of suggestive of human papillomavirus HPV(Koilocytotic Atypia) with ASCUS (Atypical Squamous Cells of Undetermined Significance) mixed with *monillia* infections and only two of these cases showed CIN1 (Cervical Intraepithelial Neoplasia) 2/67(2.98%).

The other cytopathological finding was atypical dysplasia of endocervical epithelial cell which showed in cases 18/36(50%) of *monillia* infections, 2/4(50%) of *Trichomonas* infections,3/6(50%) of enteric infections and in 4/5(80%) of *Neisseria* infections .While atypical Metaplasia found in cases 6/36(16.7%) of *monillia* infections ,1/6(16.7) of enteric infections and 1/5(20%) of *Neisseria* infections. There was only 1/36(2.8%) of AGUS (Atypical Glandular Cells of Undetermined Significance) found in case of *monillia* infections. Benign looking of cervical cells , as resulted in pap smear tests , mostly found in cases of *Trichomonas* infections 2/4(50%) , while showed in 2/6(33.3%) of enteric infections and in11/36(30.6%) of *monillia* infections. There were no benign looking cells of cervical in cases of *Neisseria* infections. Meanwhile, no women had adenocarcinoma according to Bethesda classification during this study.

Although there was no incidence of cervical malignant cells seen in our study and only few cases showed CIN1(premalignant disease of the cervix), cytological screening should gain much popularity and should be accessible to all in order to prevent such serious pathological problems.

In Iraq, more attention should be paid for the important causation of cervicitis in women suffering from it because inflammatory and atypical changes on cervical cytology among women tested often indicate the presence of a sexually acquired infection.

Keywords: causative agents, cervical infections, cytopathological findings, Iraqi women.

دراسة حول العوامل المسببة لعدوى عنق الرحم والتغيرات المرضية الخلوية في النساء العراقيات

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الخلاصة

تعد التهابات عنق الرحم من المشاكل الشائعة بين النساء ، خاصة في سن الإنجاب ، في العراق ، وهي واحدة من عوامل الخطر العديدة لأورام داخل عنق الرحم وسرطان عنق الرحم.

ان الهدف من هذه الدراسة هو التحري عن العوامل المسببة لالتهاب عنق الرحم وارتباطها بالتغيرات المرضية الخلوية بين 67 حالة من النساء ، تراوحت أعمارهن بين 16 و 60 عامًا ، اللائي التحقن بالمركز الوطني لبحوث السرطان / جامعة بغداد ، العراق ، خلال الفترة من من أبريل إلى ديسمبر 2018.

كان لدى الفئة العمرية 36-40 أعلى نسبة من التهابات عنق الرحم 16/13 (81.3 %) في حين أن الفئة العمرية 56-60 كان لديها أدنى نسبة 6/2 (33.3 %).

تم العثور على التهاب جرثومي معين في 67/51 (76.1 %) من الحالات ، كما تم الكشف عنها بواسطة الفحوص الميكروبيولوجية ، في حين كان 67/49 (73.13 %) من التهاب غير محدد. العامل المسبب الرئيسي لعدوى عنق الرحم كان المبيضات البيضاء *Candida albicans*: 67/36 (53.7 %) يتبعها: *Neisseria gonorrhoeae* 5/67 (7.5 %) ، والبكتيريا المعوية *enteric bacteria* 67/6 (8.9 %) ، و *Trichomonas vaginalis* 4/67 (5.9 %) ،. تم الكشف عن التشخيص الخلوي باستخدام فحص مسحة عنق الرحم ، عن بعض الحالات المقترحة للاصابة بفيروس الورم الحليمي البشري 67/6 (8.9 %) لوجود (*Koilocytotic Atypia*) مع ASCUS (خلايا غير نمطية من الخلايا الحرشفية ذات مغزى غير محدد) ممزوجة بالتهابات المونيليا من هذه الحالات أظهرت CINI (الأورام الظهارية داخل عنق الرحم) 67/2 (2.98 %).

اما الاكتشافات الخلوية الأخرى فهي خلل التسنج الشاذ للخلايا الظهارية في بطانة عنق الرحم والتي أظهرت في الحالات 36/18 (50 %) من التهابات المونيليا *monillia* ، 4/2 (50 %) من عدوى المشعرات *Trichomonas* ، 6/3 (50 %) من الالتهابات البكتيرية المعوية *enteric bacteria* وفي 5 / 4 (80 %) من الالتهابات النيسيرية *Neisseria*. وعلى الرغم من التحول الشاذ للخلايا والذي وجد في الحالات 36/6 (16.7 %) من التهابات المونيليا *monillia* ، 6/1 (16.7 %) من الالتهابات البكتيرية المعوية *enteric bacteria* و 5/1 (20 %) من الالتهابات النيسيرية *Neisseria*، كان هناك فقط 36/1 (2.8 %) من AGUS (الخلايا الغدية غير نمطية من أهمية غير محددة) حيث وجدت في حالة الالتهابات المونيليا *monillia*. المظهر الحميد للخلايا العنقية، والذي تم فحصه من خلال مسحات عنق الرحم، وجد في اغلب حالات عدوى المشعرات 4/2 (50 %) ، بينما أظهر في 6/2 (33.3 %) من الالتهابات البكتيرية المعوية *enteric bacteria* و 36/11 (30.6 %) من التهابات المونيليا *monillia*. لم يكن هناك خلايا حميدة من عنق الرحم في حالات الالتهابات النيسيرية *Neisseria*. وفي الوقت نفسه ، لم يكن لدى النساء سرطان غدي وفقًا لتصنيف بيتسدا خلال هذه الدراسة. وعلى الرغم من عدم وجود حالات للخلايا الخبيثة في عنق الرحم في هذه الدراسة ، ووجود فقط حالات قليلة أظهرت CINI (مرض ما قبل الخبيث لخلايا عنق الرحم) ، لكن يجب أن يكون للفحص الخلوي انتشار واسع و يكون في متناول الجميع من أجل منع حدوث مثل هذه المشاكل الامراضية الخطيرة. في العراق ، ينبغي إيلاء المزيد من الاهتمام للتحري عن المسببات الرئيسية لعدوى عنق الرحم لدى النساء اللائي يعانين من ذلك لأن التغيرات الالتهابية والغير طبيعية على الخلايا في عنق الرحم بين النساء اللائي تم اختبارهن تشير في كثير من الأحيان إلى وجود عدوى مكتسبة جنسيًا.

Introduction

Cervicitis is the most common infection of all gynecological disorders affecting women at some point in their lives [1]. When lower genital tract infection spreads to the upper genital tract this may lead to ectopic pregnancy , chronic pain in the pelvic area and infertility, [2]. women of reproductive

age may be found to have large numbers of bacteria such as Enterobacteriaceae, Streptococci, Staphylococci as well as Lactobacilli which are mostly associated with healthy state [1]. There are some factors may disturb the vaginal pH such as the using of antibiotic which disrupts the Lactobacillus, the acidic pH is no longer maintained and pathogenic bacteria may grow [3] leading to inflammation in vagina that may transfer to the cervix causing cervical infection[4].

The most common causes of cervical infections are: bacteria such as *Chlamydia trachomatis* and *Neisseria gonorrhoea*, fungi such as *Candida albicans*, parasites such as *Trichomonas vaginalis*, and viruses such as *herpes simplex* [5]. The infection with human papilloma virus (HPV) has been found to be an important factor in the development of pre-invasive and invasive types of cervical cancer [6]. Although the high rate of cervical cancer, women lack knowledge about this disease and HPV infection. In addition, the diagnostic cervical screening assays are still restricted [7].

In Iraq, the most common location of cancer in women are breast and cervix according to the current data obtained from the Iraqi Ministry of Health and WHO [8]. However, death rates due to such disease can be reduced if early detection method, such as cervical screening method, is applied during early stages [9]. The advantages of cervical screening, which is based on Papanicolaou smear test (Pap test), are well established; applying of this routine test has given rise to decrease the prevalence of such type of cancer [10].

In different parts around the globe, an increased awareness has been driven toward cervicitis due to their important public health and socio economic problems [11]

In Iraq, Unfortunately, insufficient attention has been paid for the important causation of cervicitis in women who are suffering from it [12]. Therefore, our objective is to investigate the prevalence of different causative agents of cervicitis and find out their association with different cytopathological changes of the infected cervix among samples of Iraqi women.

Materials and methods

A total of 67 cervical samples were taken from women aged from 16 to 60 years old, suffering from cervicitis, attending the National Cancer Research Center / University of Baghdad, Iraq, during the period from April to December 2018. Cytological evaluation was carried out on satisfactory cervical smears. All samples were investigated for the presence of pathogenic microorganisms by using different microbiological tests.

For microbiological examination, specimens were collected from the cervix of patients using sterile cotton swabs. The swabs were inserted 1cm in the cervical canal and peripheral cervix wall, and then rotated with ring motion for at least 2-5sec. to allow the absorption of the exudates before withdrawing the swab. Direct microscopical examination was done using wet preparation technique for the detection of *Trichomonas vaginalis*. Gram stain was used to identify bacteria, clue cells and extra or intra-cellular diplococci such as *Neisseria spp.* [13]. Specimens also were examined by culturing onto different types of media such as (blood agar, chocolate agar and MacConkey agar.) for the identification of other causes such as gram positive or gram negative bacteria and these diagnoses were confirmed by using different biochemical tests. *Candida albicans* was detected by wet mount examination, gram staining, culturing method onto Sabouraud dextrose agar and confirmed by germ tube test [14].

Pap smear technique was used for cytological examination. The smears were collected by a broom brush with appropriate instructions to the women [15]. The pointed tip of the brush was introduced through the external and the squamocolumnar junction was scraped by rotating the brush to 360°. The smear being transferred directly and evenly spread onto a microscope slide and the slide immediately fixated in 95% ethanol. The slides were stained for cytological diagnosis, according to the method of Papanicolaou stain [16]. The cytopathological changes of the cervical smears were diagnosed by the staff of pathologists in our research center. The cytological changes observed in the cervical smears were graded according to The Bethesda system of 2001.

Pregnant women and post hysterectomies' women were excluded from the study. Women also were excluded from the study if they had administered medications such as antibiotic or antifungal treatment since their smear had been taken.

Results

Cultures and cervical cytology samples were obtained from 67 cases of women. In about 51/67(76.1%) of the cases were of specific inflammations, as detected by microbiological examination.

The main causative agents among patients with cervicitis was *Candida albicans* :36/67(53.7%). Other causative agents were distributed as: *Trichomonas vaginalis* 4/67(5.9%), *Neisseria gonorrhoea* 5/67(7.5%) and enteric bacteria 6/67(8.9%) as shown in Table-1.

The age group 36-40 had the highest percentage of infection 13/16 (81.3%) while the age group 56-60 had the lowest 2/6 (33.3%) Most of women were at age group 36-40years 16/67(23.9%) who infected with sexual transmitted diseases, i.e. 3/16(18.8%) infected with *Neisseria* as seen in Table-1.

Cytological evaluation was carried out on 67 satisfactory cervical smears. The cyto-diagnosis revealed 49/67(73.13%) of non-specific inflammations which include some cases of suggestive of human papillomavirus HPV(Koilocytotic Atypia) with ASCUS (Atypical Squamous Cells of Undetermined Significance) mixed with *monillia* infections were found in 6/67(8.9%) and only two 2/67(2.98%) showed CIN1 (Cervical Intraepithelial Neoplasia) . The other cytopathological finding was atypical dysplasia of endocervical epithelial cell which was showed in 18/36(50%) of *monillia* infections , 2/4(50%) of *Trichomonas* infections ,3/6(50%) of enteric infections and in 4/5(80%) of *Neisseria* infections .While atypical Metaplasia found in 6/36(16.7%) in *monillia* infections ,1/6(16.7) in enteric infections and 1/5(20%) in *Neisseria* infections .There was only one case 1/36(2.8%) of AGUS(Atypical Glandular Cells of Undetermined Significance) of cytological changes in *monillia* infections. Benign looking of cervical cells mostly found in *Trichomonas* infections 2/4(50%) , in enteric infections 2/6(33.3%) and in *monillia* infections 11/36(30.6%) .While there was no benign looking cells of cervical in *Neisseria* infections, see Table-2. Meanwhile, no women had adenocarcinoma found in this study, according to Bethesda classification. The distribution of the causative agents according to age group shown in Table-1

Table 1-causative agents of cervical infections and age group of women infected

Causative agents	Age group									Total&%
	16-20	21-25	26-30	31-35	36-40	41-45	46-50	51-55	56-60	
Candida albicans	1	6	1	6	8	3	3	0	2	30 44.8%
Monillia+koilocyte atypia	0	0	0	1	2	1	1	1	0	6 8.9%
Trichomonas	0	0	0	1	0	1	0	2	0	4 5.9%
Neisseria	0	0	1	0	3	0	0	1	0	5 7.5%
Enteric bacteria	1	0	1	3	0	0	1	0	0	6 8.9%
No growth	0	2	2	3	3	0	1	1	4	16 23.8
Total of the column	2	8	5	14	*16	5	6	5	6	67 100%

Table 2-cytopathological findings on cervical examination of different types of cervical infections

Cytopathological Findings on Cervical Examination of monillia infections	No.	%
Atypical dysplasia of endocervical epithelial cells	18	50%
Atypical Metaplasia – inflammatory induced and ASCUS** / Koilocytotic Atypia***	4	11.1%
Atypical Metaplasia – inflammatory induced and ASCUS** / Koilocytotic Atypia*** with CIN1	2	5.6%
AGUS*	1	2.8%

benign looking of endocervical cells	11	30.6%
Total no. of monillia infection	36	100%
Cytopathological Findings on Cervical Examination of trichomonas infections	No.	%
Atypical dysplasia of endocervical epithelial cells	2	50%
Atypical Metaplasia – inflammatory induced	0	0%
AGUS*	0	0%
ASCUS** / Koilocytotic Atypia***	0	0%
CIN I	0	0%
Benign looking cells	2	50%
Total no. of trichomonas infections	4	100 %
Cytopathological Findings on Cervical Examination of enteric infections	No.	%
Atypical dysplasia of endocervical epithelial cells	3	50%
Atypical Metaplasia – inflammatory induced	1	16.7%
AGUS*	0	0%
ASCUS** / Koilocytotic Atypia***	0	0%
CIN I	0	0%
Benign looking of cervical cells	2	33.3%
Total no. of enteric infections	6	100%
Cytopathological Findings on Cervical Examination of Neisseria infections	No.	%
Atypical dysplasia of endocervical epithelial cells	4	80%
Atypical Metaplasia – inflammatory induced	1	20%
AGUS*	0	0%
ASCUS** / Koilocytotic Atypia***	0	0%
CIN I	0	0%
Benign looking cells	0	0%
Total no. of Neisseria infections	5	100%

*Atypical Glandular Cells of Undetermined Significance

**Atypical Squamous Cells of Undetermined Significance

*** Suggesting Human Papilloma Virus infection by Cytology

In some cases the actual microbiological organism can be identified on the slide or will produce a distinctive cytopathic effect such as dysplastic cells (Figure -1) allowing the cytotechnologist to make a diagnosis on the infective organism such as *Trichomonas vaginalis* (Figure-2) and *Candida albicans*(Figure-3)

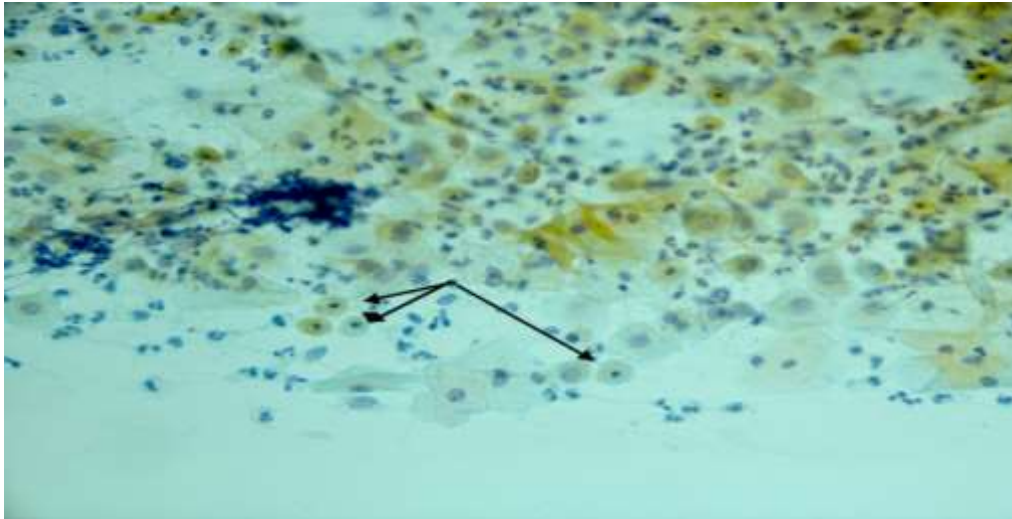


Figure 1-(dysplastic cells) Papanicolaou stain of pap smear of cervix (x40).

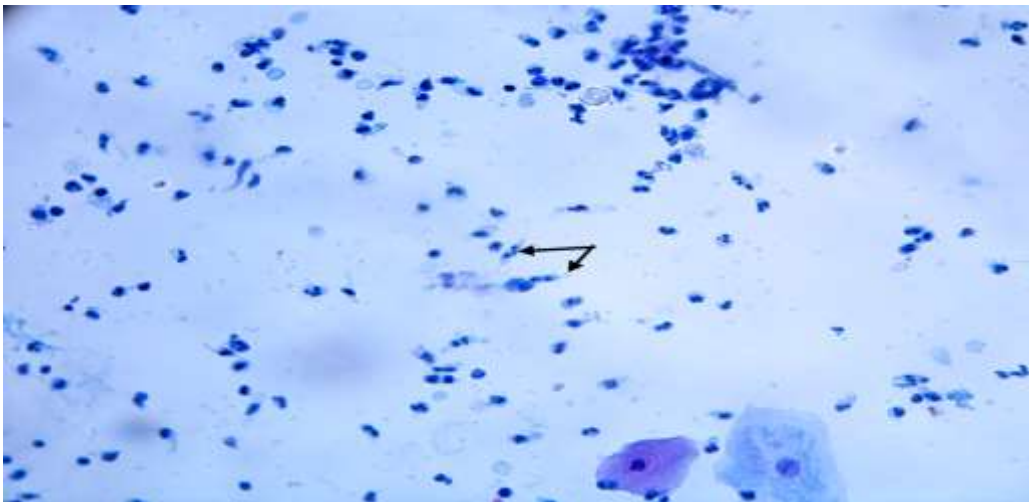


Figure 2-(Trichomonas) Papanicolaou stain of pap smear of cervix (x40).

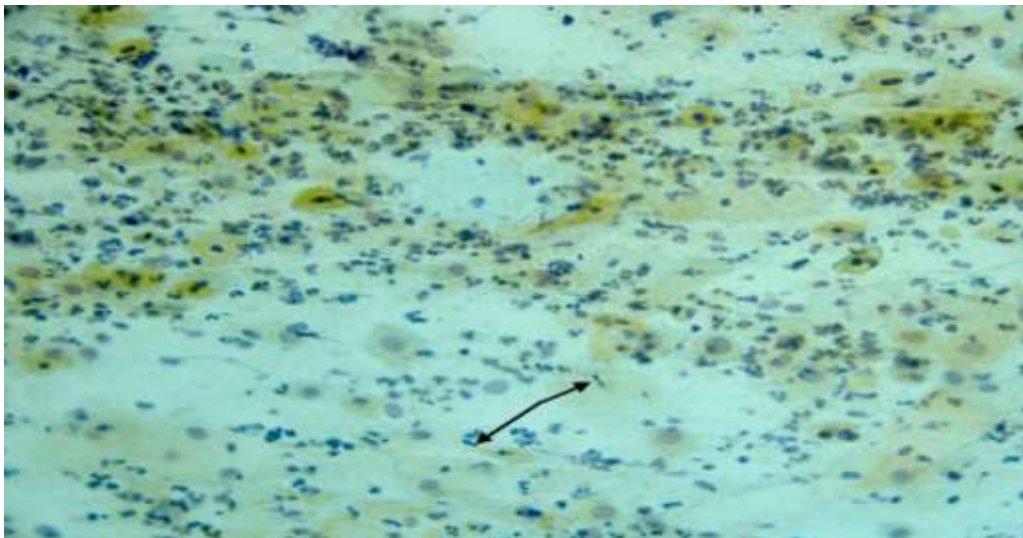


Figure 3-(candidiasis) Papanicolaou stain of pap smear of cervix (x40).

Discussion

In Iraq, cervical infections are common problems among women, specially of reproductive age. There are numerous risk factors for cervical intraepithelial neoplasia and cervical cancer such as

cervical microbial infection [17,18].The presence of sexually acquired infections are often linked with inflammatory changes in the cytology of the cervix [14]

This study investigated the causative agents of cervicitis and their association with cytopathological changes among 67 cases of Iraqi women. The age group of(36-40) had the highest percentage of cervical infections 13/16 (81.3%) as found in a previous research [19] while the age group (56-60) had the lowest 2/6 (33.3%).

Specific microbial inflammations were found in high percentage 51/67(76.1%) of the cases, while 49/67(73.13%) were of non-specific inflammations.

The main causative agent of cervical infections was *Candida albicans* :36/67(53.7%), followed by: *Neisseria gonorrhoeae* 5/67(7.5%) , enteric bacteria 6/67(8.9%) and *Trichomonas vaginalis* 4/67(5.9%).These findings are disagreed with one previous study in Najaf city(Iraq) [20] which resulted that bacterial infection of cervix were 58.4% and only 23.4% were of *Candida albicans* . There are many factors such as immune status, hygiene, level of education and level of infection among patients may also have a profound influence on the ability of *C. albicans* to cause cervicitis.

The cyto-diagnosis revealed some cases 6/67(8.9%) of suggestive of human papillomavirus HPV(Koilocytotic Atypia) with ASCUS mixed with *monillia* infections and only two of these cases showed CIN1 2/67(2.98%) These are the most serious cases found in this study, due to the probability of HPV infection, and that inflammatory changes on cervical cytology smears were linked with developed risk of having a sexually acquired infection. These inflammatory changes often masked underlying premalignant disease of the cervix [14].

Across Iraq, although the incidence of cervical cancer is in the downward trend but remains to be a significant public concern for women in the country. An expert in the field of medicine has shown that cervical malignant disease can be avoided among women, especially when detected earlier [21].

The other cytopathological finding was atypical dysplasia of endocervical epithelial cell which showed in cases 18/36(50%) of *monillia* infections, 2/4(50%) of *Trichomonas* infections,3/6(50%) of enteric infections and in 4/5(80%) of *Neisseria* infections .While atypical Metaplasia found in cases 6/36(16.7%) of *monillia* infections ,1/6(16.7) of enteric infections and 1/5(20%) of *Neisseria* infections.There was only 1/36(2.8%) of AGUS found in case of *monillia* infections.

Benign looking of cervical cells mostly found in cases of *Trichomonas* infections 2/4(50%), while showed in 2/6(33.3%) of enteric infections and in11/36(30.6%) of *monillia* infections. There were no benign looking cells of cervical in cases of *Neisseria* infections.

Risk factors of such infections (sexually transmitted diseases) include younger age, separated, using non-barrier contraception, or a change of sexual partner within the past year. In other study ,Low-income women were found to be at high risk of developing cytopathological changes, which is attributable not only to the higher prevalence of risk factors in this population but also to the lack of regular health clinic visits[22].

In Iraq, more attention should be driven toward the crucial causation of cervicitis in women who are suffering from it because inflammatory changes on cervical cytology among women tested often indicate the infection with sexually transmitted diseases.

Although there was no incidence of cervical malignant cells seen in our study and only few cases showed CIN1(premalignant disease of the cervix) , this agreed with one previous study [23] , cytological screening should gain much popularity and should be accessible to all in order to prevent development of such serious pathological problems.

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