



Cytological and Microbial Investigation of Cervicitis by Endocervical Cytobrushe Smear in Some Iraqi Women Patients

Eman Natiq Naji¹, Intesar N.Khelkal¹, Firyal Naji Aziz², Sahar A.H. AL-Sharqi¹, Sanaa M. Abdul Hussein³

¹Al-Mustansiriah University, College of Science, Biology Department.

²Institute of Medical Technology / Al-Mansour.

³MB Ch B and DGO / Ministry of Health / AL-Numan Hospital.

Abstract

This study is interested with occurrence and evaluation of cervicitis by endocervical cytobrushe smear in a sample of Iraqi women (87) who attending to the obstetrics and gynaecology outpatient clinic in AL-Numan hospital complaining from certain symptoms for a period of 6 months. Samples were obtained from cervical and endocervical area, divided into 2 parts; cytological and microbiological tests have been carried out. Smears were prepared in order to detect inflammation and non inflammation changes in endocervical cells. Microbiological investigation has been performed to identify suspected pathogens; bacterial isolates; *Candida* spp. and *Trichomonas vaginalis*. Results of cytological aspect were shown as increasing in number of lymphocytes and neutrophils indicating that there is an acute inflammation besides clusters of squamous cells in many areas, squamous intraepithelial cells with nuclei were increased in size and shape in addition to containing hyperchromatic, membrane irregularities and thickness have appeared. On the other hand, aggregation of inflammatory cells with numerous squamous cells has been noticed. Microbiological results were represented by detection of several aerobic and anaerobic bacteria accompanied with the infection in addition to *Candida albicans* and *Candida glabrata*. A total of 79 samples have been tested; 44 (55.7%) were positive for culture distributed between acute 29 (36.7%); and chronic; 15 (18.98%). The highest isolation percentages were for *C. albicans* (15.9%), followed by different bacterial species; *Gardnerella vaginalis* (13.6%), *Staphylococcus aureus* (11.3) in acute cervicitis cases, the lowest was for *Lactobacillus acidophilus* (0%) while in chronic cervicitis, the highest percentage was (3.6%) for each of *S. aureus*, *G. vaginalis*, *L. acidophilus* and *C. albicans*.

Keywords: Cervicitis, Endocervical cells, Aerobic & anaerobic bacteria.

Introduction

Sexually Transmitted Diseases (STD) are the commonest infectious diseases of the world and are responsible for vast public health problems. More than 1 million sexually transmitted infections (STIs) are acquired every day worldwide [1].

Cervicitis refers to inflammation of the uterine cervix influences columnar epithelial cells of the endocervical glands as well as the squamous epithelium of the ectocervix. Cervicitis may be due to infectious or non-infectious agents. Acute cervicitis is the result of an infection while chronic cervicitis has a non-infectious source [2]. While cervicitis is considered to arise as a result of sexual transmitted diseases, understanding of its etiology and pathogens is still growing.

Even with highly developed microbiological assays, no causative pathogen is identified in the common of cervicitis cases [3]. Several investigators reported the most important pathogens causing cervicitis as *Neisseria gonorrhoeae* and *Chlamydia trachomatis* but nowadays it is well-known that cervicitis is not caused by these two organisms only [4,5].

The number of assumed aetiological agents concerned in cervicitis is increasing and includes *Mycoplasma genitalium*, Herpes simplex virus, Cytomegalovirus, Bacterial vaginosis and *Trichomonas*. Cervicitis was widespread in female sex workers (FSW) in Peru and was mainly nongonococcal and non-chlamydial in etiology [3]. The possible role of cervicitis in HIV transmission has

been reported [6]. Bacterial vaginosis could lead to cervicitis through a death of bactericidal H₂O₂-producing lactobacilli, low levels of protective vaginal mucins, and increased pro-inflammatory enzymes and cytokines, which in turn may reduce the mucus barrier in cervix [7]. Recently, bacterial vaginosis was associated with cervicitis, in addition to an increased risk of HIV acquisition, undesirable pregnancy outcomes and gynaecological surgery complications [3].

Cervicitis has been described as the female counterpart of urethritis in male [5]. It can progress to pelvic inflammatory disease (PID) with severe reproductive complications, even in asymptomatic conditions [8]. In developing countries where tuberculosis is endemic; tuberculosis cervicitis is similar to cervical cancer [9]. The current search aim to assess the incidence of cervicitis (inflammatory and non-inflammatory) and describe the cytological changes and most important microbial contributory agents.

Materials and Methods

The population studied consisted of 87 nonpregnant women of reproductive age (age range 30-50 year/median age 35 year) presenting to the obstetrics and gynaecology outpatient clinic in AL-Numan hospital in Baghdad from the first of November 2016 to the end of March 2017. Patients were selected based on a detailed standard questionnaire regarding the reasons for attendance, age, symptoms (vaginal discharge, vaginal bleeding, vaginal itching, irritation of the external genitals, pain during

intercourse, bleeding or spotting after sexual intercourse or between periods, a burning sensation during urination, lower back or abdominal pain, pus-like (purulent) discharge with an unpleasant odor, accompanied by intense vaginal itching or abdominal pain or other of the symptoms above, contraceptive method).

Samples Collection

The best time to collect samples is when the woman is not menstruating for three days before the tests and avoid the following because these might interfere with the interpretation of the test more difficult (intercourse, vaginal douches, vaginal medications, vaginal contraceptives). An instrument called a speculum was inserted into the vagina to hold the vaginal walls apart, thus permitting an inspection of the cervix and vaginal walls for redness, irritation, unusual discharge, or sores.

The physician has collected a sample for an endocervical cytobrush smear (cervical brush Figure 1), a sample of mucus and cells was obtained from the cervix (the part of the uterus that extends into the vagina) and endocervix (the opening of the cervix) using a small cervical brush and turn 360 degrees this allows removal of the surface cells of the whole of the squamocolumnar junction. The sample was rinsed into a vial containing phosphate-buffer saline solution (0.15 M, pH 7.3) and transport media without any solidifying agents then, sent to the laboratory for microbiological and cytological examination in the same hospital.



Figure 1: Cervical brush

Samples Preparation

Before any of the following tests were done, the samples have mixed well and gently by vortex each sample was separated into two parts in clean sterile tubes.

Cervical Cytology

In the laboratory the endocervical cytobruche smear collection fluid along with collected material was centrifuged at 1500 rpm for 10 min, then supernatant was discarded and direct smear was prepared from cell button and fixed in cytofixative with equal part of 95% ethyl alcohol and ether for at least 20 minutes and then staining was done by a standard Papanicolaou method.

According to revised Bethesda system the smears that showing inflammatory or non inflammation changes that were included in this study, while unqualified smear and the smears showing any atypical morphology like atypical cells of undetermined significance (ASCUS), mild dysplasia and moderate or severe dysplasia were excluded [10;11].

Detection and Identification of Microorganisms

Women with or without inflammatory changes on their endocervical cytobruche smear were tested as follows:

The specimens collected from patients were inoculated onto appropriate culture media for standard aerobic, microaerophilic (5-10% of CO₂) and anaerobic bacteria all of them were incubated at 37°C for 24h, 48h and 72h respectively. The positive cultured samples were tested for the presence of different aerobic bacteria, microaerophilic bacteria and *Candida* species by general culture morphology, biochemical tests and germ tube test for *Candida spp.*

The isolated pathogens were finally identified by using the automated system VITEK 2 (BioMerieux, Marcy l'Etoile, France). On the other hand the further diagnosis for anaerobic bacterial isolates were done by using API 20A (BioMerieux, France).

A wet mount as well as a gram-stained smear was examined under microscope to screen for *T. vaginalis* and to obtain valuable

information about the microorganisms (yeasts and bacteria) present as well as for the diagnosis of bacterial vaginosis according to [12] and [13]. Statistical analysis: statistical package for the social science system version SPSS 16. Variables are accessible as total numbers and percentage, the nominal categorical data between the groups compared using Chi-squared test as appropriate. $P < 0.05$ was considered statistically significant.

Results and Discussion

This study has been carried out on 87 patients age ranged (30-50) years attending to obstetrics and gynaecology outpatient clinic in AL-Numan hospital in Baghdad from the first of November 2016 to the end of March 2017 suffering from symptoms that described in table 1.

All of 87 patients were separated into two groups (Table 1) first one ranged (30-40) years while the second one ranged (41-50), in the current search we found that the greatest of patients number belonged to age group 40-51 years which they were 35 (40.2%) and 24 (27.5%) with negative inflammatory and positive inflammatory changes respectively while 7 (8%) of infected women have a typical cell morphology in the cytological examination so they excluded from this study.

On the other hand the lowest number was found in the other age group (30-41) separated into two parts 11 (12.6%) and 9 (10.6%) with negative and positive inflammatory cases respectively and only one woman has a typical cell morphology so she was excluded.

The majority relentless of vaginal discharge that accompanied with other symptoms like vaginal itching & irritation, burning sensation during urination and lower back or abdominal pain were found in 28 (32.2%) of no inflammatory cases and 7 (8%) in positive inflammatory cases followed by lower back or abdominal pain in 9 (10.3%) and 19 (21.8%) in negative and positive inflammatory cases.

All excluded cases women 8 (9.1%) were accompanied symptoms includes vaginal bleeding, burning sensation during urination and lower back or abdominal pain as listed in Table 1.

Table 1:Symptoms and CytologicalCriteria of study group

| Patient characteristics Total count (87) | Endocervical cytobrush smear | | |
|--|---|---|--|
| | Cytological examination | | |
| | Negative inflammatory changes No.(%) | Positive inflammatory changes No.(%) | A typical cell morphology excluded No.(%) |
| Age groups/year | | | |
| 30-40 | 11(12.6) | 9(10.6) | 1(1.1) |
| 41-50 | 35(40.2) | 24(27.5) | 7(8) |
| Symptoms | | | |
| 1-Vaginal discharge | 6(6.9) | 13(14.9) | 0(0) |
| 2-Vaginal bleeding | 0(0) | 0(0) | 0(0) |
| 3-Vaginal itching & irritation | 3(4.4) | 7(8) | 0(0) |
| 4-Pain during intercourse | 6(6.9) | 5(5.7) | 0(0) |
| 5-Bleeding or spotting after sexual intercourse or between periods | 2(2.3) | 7(8) | 0(0) |
| 6-Burning sensation during urination, | 8(9.2) | 17(19.5) | 0(0) |
| 7-Lower back or abdominal pain | 9(10.3) | 19(21.8) | 0(0) |
| 8-Pus-like (purulent) discharge with an unpleasant odor | 2(2.3) | 9(10.3) | 0(0) |
| Accompanied symptoms (1,3 and 7) | 4(4.6) | 9(10.3) | 0(0) |
| Accompanied symptoms (1,3 ,6 and 7) | 28(32.2) | 7(8) | 0(0) |
| Accompanied symptoms (2,6and 7) | 0(0) | | 8(9.1) |

As shown in Fig.2 ; provocative changes was detected on a endocervical cytobrush stained smear included the presence of cells with enlarged nuclei, pyknosis or karyorrhexis, perinuclear halos, and vacuoles , increased numbers of polymorphonuclear leukocytes or neutrophils and parabasal cells

with generalised eosinophilia of the cells were recorded, the presence of epithelial cells covered with blue-stained coccoid bacteria on the stained cervical smear together with a decreased number or a lack of lactobacilli represented findings suggestive of bacterial vaginosis.

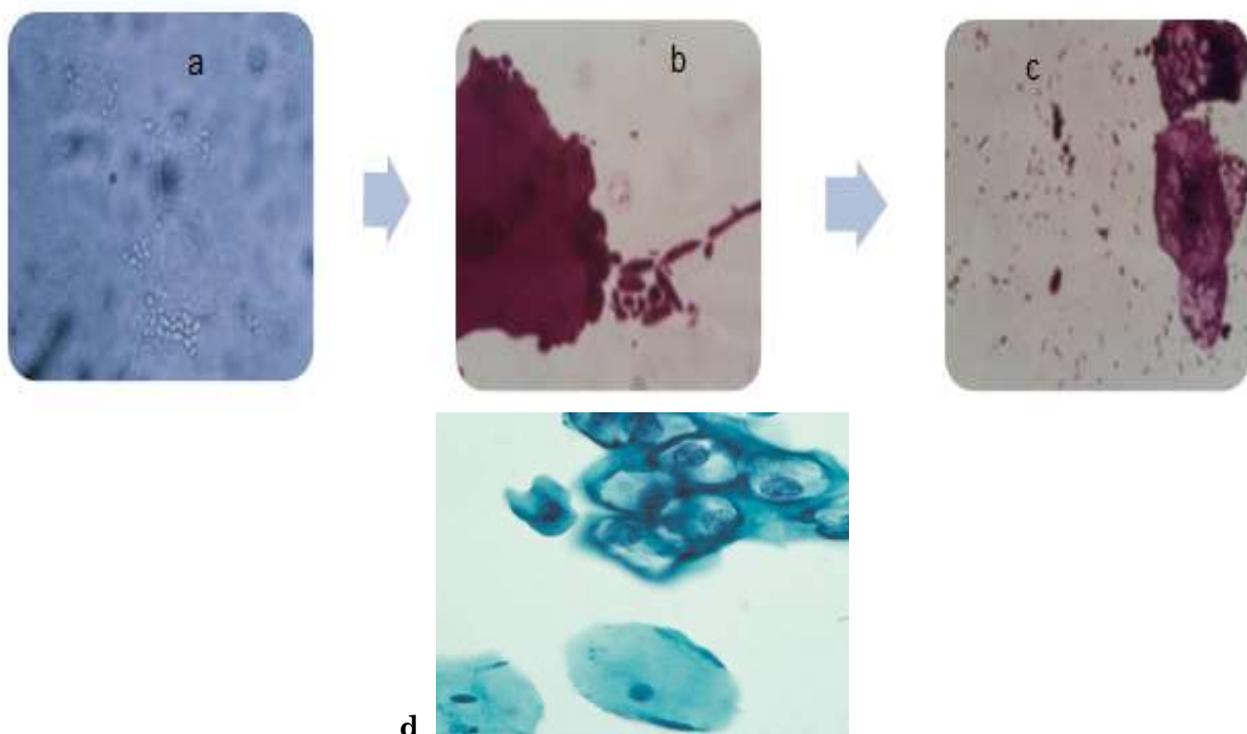


Figure 2: Inspection of genitals, women endocervical cytobrush smear to screen for normal(Negative and positive inflammatorychanges) and or abnormal cervical cell

a: Wet mount preparation and b: gram stain reaction :showed candida yeast cell and pseudomycellium adhering to a surface of normal epithelial cells (cervical candidiasis + inflammation)c:Gram stain reaction showed bacterial cell a aggregation and adhering to a surface of normal epithelial cells (Bacterial vaginosis+ inflammation). d: Normal and abnormal cervical cells Cytological endocervical brush smear was characterised by Increased number of lymphocytes and

neutrophils indicating that there is an acute inflammation (Figure3a). There was cluster of squamous cells in many area (Figure 3b) also squamous intraepithelial cells with nuclei were increased in size and shape, and contain hyperchromatic also, membrane irregularities and thickness (Figure 3c).

On the other hand, aggregation of inflammatory cells with numerous squamous cells (Figure 3d)

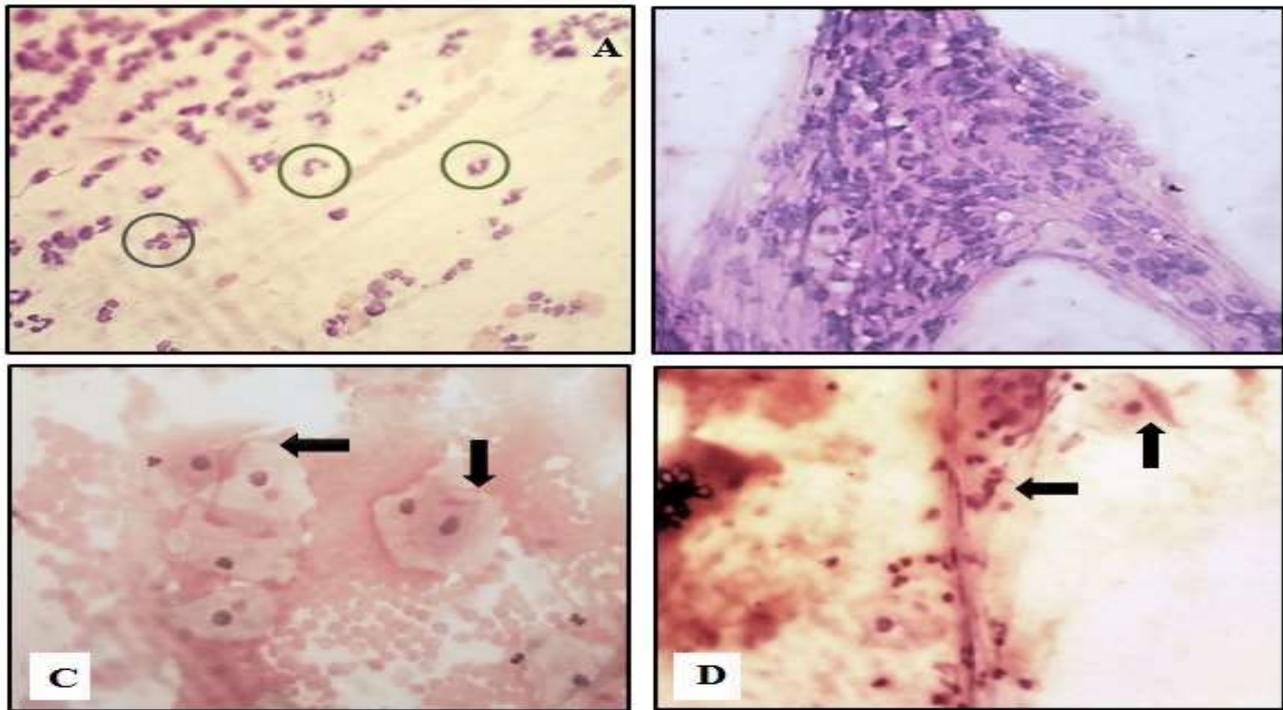


Figure. 4Endocervical cytobrushe smear showing,A:Increase number of lymphocytes (blue ring) and neutrophils (green ring) indicate that there is an acute inflammation.B:Cluster of squamous cellsC:squamous intraepithelial cells with nuclei are increase in size and shape, and contain hyperchromatic(arrow) also, membrane irregularities and thickness (head arrow), D: Aggregation of inflammatory cells (arrow) with numerous squamous cells (head arrow).

Results of the microbial examination were shown in table .2 & figure.5 as following ; 44 /79 (56%) were given positive culture ;15(32.6%) with not provocative cases while 29(87.9%)were coincident with inflammatory cases. The inflammation on endocervical cytobrushe smear extensively decreased percentage of negative cultures (p<0.001) and increased percentage associated with positive cultures (p<0.001). Identification results of cervicitis causative agents have showed that bacterial vaginosis was more repeatedly in the two tested groups ; bacterial isolates and Candidasp. were

accountable for all cervicitis cases. Bacteria were distributed as; aerobic isolates ;S.aureus(6.8%,11.3%) ,E.coli (2.3%,4.5%),K. pneumoniae (0%,2.3%) ,P.aeruginosa (0%,4.5%) whereas microaerophilic isolates ;H.ducreyii (2.3%,9.1%) ,G.vaginalis (6.8%,13.6%),Strep. pyogenes (0%,4.5%) while anaerobic bacteria ; Peptostreptococcus anaerobicus(0%,2.3%), B.fragilis (2.3%,6.8%) , Lactobacillus acidophilus(6.8%,0%)in addition to C.albicans (6.8%,15.9%) &C.glabrata(0%,2.3%)as well as mixed (0%,11.4%).

Table 2: Distribution of Microbial Pathogens in the Study

| Culture results Total No. (79) | No Inflammation Total No. (46) | Inflammation Total No. (33) | P value |
|-----------------------------------|-----------------------------------|--------------------------------|---------|
| Negative cultures | 31(67.4) | 4(12.1) | 0.001 |
| Positive cultures | 15(32.6) | 29(87.9) | 0.001 |
| Aerobic bacterial isolates | No(%) # | No(%) | |
| <i>Staphylococcus aureus</i> | 3(6.8) | 5(11.3) | |

| | | | |
|---|--------|---------|--|
| <i>Pseudomonas aeruginosa</i> | 0(0) | 2(4.5) | |
| <i>Klebsiella pneumonia</i> | 0(0) | 1(2.3) | |
| <i>Escherichia coli</i> | 1(2.3) | 2(4.5) | |
| <i>Microaerophilic bacterial isolates</i> | No(%) | No(%) | |
| <i>Haemophilus ducreyji</i> | 1(2.3) | 4(9.1) | |
| <i>Gardnerella vaginalis</i> | 3(6.8) | 6(13.6) | |
| <i>Streptococcus pyogenes</i> | 0(%) | 2(4.5) | |
| <i>Anaerobic bacterial isolates</i> | No(%) | No(%) | |
| <i>Peptostreptococcus anaerobicus</i> | 0(%) | 1(2.3) | |
| <i>Bacteroides fragilis</i> | 1(2.3) | 3(6.8) | |
| <i>Lactobacillus acidophilus</i> | 3(6.8) | 0(%) | |
| <i>Candida species</i> | No(%) | No(%) | |
| <i>Candida albicans</i> | 3(6.8) | 7(15.9) | |
| <i>Candida glabrata</i> | 0(0) | 1(2.3) | |
| Mixed culture | 0(0) | 5(11.4) | |

The percentage was calculated from the total positive bacterial cultures=44out of the79 women involved in this study

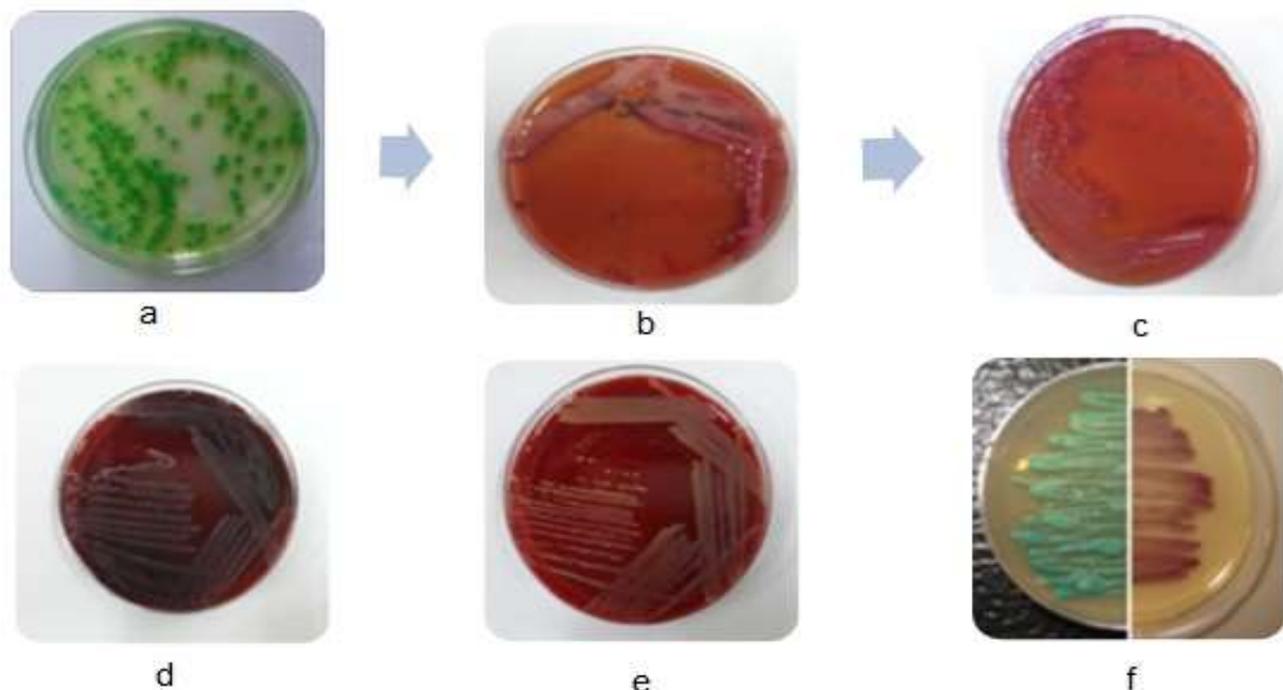


Figure 5: Colonies morphology for some of bacteria and candida species isolated from some cytobruche smear positive cultures.

a: *P. aeruginosa* on *Pseudomonas chromagar*/b:*Klebsiella pneumoniae* on *MacConkey agar*/c: *Bacteroides fragilis* on *MacConkey agar*/d: *Streptococcus pyogenes* on *blood agar*/e: *Haemophilus ducreyji* on *blood agar*/f: *Candida spp* growing on *Candida chromogen agar* (left: The color of *C. albicans* colonies is green, while in the right:*C. glabrata* colonies were appeared dark Pink).

Jeanne and Marrazzo, 2011 [7] have suggested that cervix is never sterile and therefore culture will always reveal organisms. Taylor, 2014 [5] has recommended combinations of clinical manifestations include (mucopurulent discharge from the cervix, cervical friability) and microscopic findings in order to increase the accuracy of cervicitis diagnosis.

The results of this study were in accordance with the study of [14], particularly what concerning moniliasis, or *C. albicans* isolation as well as several pathological changes in squamous epithelial layer (Fig.4-a,b,c), they reported that among the total of 300 satisfactory pap smears, pathological changes were found in 209 (67.7 %) while the rest 91 (30.3%) did not show any abnormality. More than one type of pathology were found in some cervical smears.

The prevalence of different pathological changes revealed by cytological diagnosis of 302 pap smear were: Non-specific cervicitis 188(62.7%), Squamous metaplasia 23 (7.7%), Moniliasis 11 (3.7%), Squamous dysplasia (mild and moderate) 7 (2.3%), *T. vaginalis* 4 (1.3%), HPV infection 4(1.3%), IUCD changes 4 (1.3%) and 2 smears (0.7%) were unsatisfactory and excluded from the study.

Ismailet *al.*,2015 [15] have been investigated cervical cytological lesions incidence in patients at Erbil city, 1763 patients were screened , they documented mild and moderate cervicitis were found 47 (2.7%) and 164 (9.3%) in patients respectively . Carcinoma of cervix were detected in women of ≥ 50 years old; all carcinoma cases were associated with sever cervicitis.

In the study of [2] in Nigeria,they reported acute cervicitis is prevalent, and *C. trachomatis* the major cause of cervicitis in commercial sex workers CWR and cervicitis appears more frequent in young CSWs between (15–20) years.

While in the study of (3) in Peru they have documented that the major causes of cervicitis in female sex workers FSW are *N.gonorrhoeae* and *C.trachomatis*, less commonly, *M.genitalium*, *T.vaginalis* and *Herpes simplex* Virus (HSV) , but in our study we have found the primary pathogens are group of aerobic and anaerobic ,Gram positive and negative in addition to *Candida spp.* As in table .2 and figure .3 .

The highest isolation percentages were for *C.albicans* (15.9%), *G.vaginalis* (13.6%), *S.aureus* (11.3) respectively in acute cervicitis cases,the lowest was for *L.acidophilus* (0%) while in chronic cervicitis ,the highest percentage was (3.6%) for each of *S.aureus*, *G.vaginalis*, *L.acidophilus* and *C.albicans*. These differences could be attributed to variations in different factors of patient group (age, physiological condition,hormonal changes, nutrition, administration of drugs, intelligence,different contraceptive methods, pregnancy, birth ,abortion).

Islamic countries from the best societies that religious people have rules and instructions that restrict and prevent STD so cervicitis cases are in low percentages comparable to other countries. Rare bacterial isolates have been reported in our study (result which was different from previous local and worldwide studies) such as *H. ducreyii*, *G.vaginalis*, *S. pyogenes* and *B. fragilis* in addition to yeast *C.glabrata*,those isolation rates may be attributed to the high accuracy of sampling from the exact region of endocervix.

Chlamydia infection was detected in 34 (17%) of the total 200 cases surveyed in the study of [16] at Brazil besides a high prevalence of

colonisation of the cervix ; 4.0% for *N. gonorrhoeae*, 0.9% for *M. genitalium*, 21.9% for *M. hominis*, 38.4% for *Ureaplasma spp.*. Similar finding were showed in the study of [17] on 679 women also they indicated ; women with *C. trachomatis* had a higher frequency of both PID (18.3% vs 4.9%, $P < .001$) and cervicitis (33.4% vs 22.3%, $P < .001$) than women with *M. genitalium*. Our results were in agreement with the findings of [18]; they established that wet preparation in chlamydia positive cases revealed the increased numbers of PMN (>10 /hpf) were statistically not significant in symptomatic group ($p > 0.194$), but significant in the asymptomatic group ($p < 0.036$), also we noticed significant rise in neutrophile cells and elevation in polymorphonuclear leukocytes numbers in inflammation and irritation cases as swown in Fig. 4 a.

Although we have no Chlamydia isolates in our study but other bacterial isolates which causes these symptoms. Al-Mosawi, 2009 [19] was found from study was achieved on 120 women attending the outpatient clinic of the obstetric and gynecology department of Basrah General Hospital, that ; Mycoplasmal species: *M. fermentans*, *U.urealyticum*, *M.hominis* and *M.penetrans* were more frequently distributed in women who were complaining of vaginal discharge followed by urethral abnormal and itching ,the isolation rates of some bacterial species; *E.coli* (2.5 %) and *P.aeruginosa* recovered (0.8%) in 1 case only as a mixed infection with *S.epidermidis*,while in our study (4.5) for each. *N.gonorrhoeae* cause cervicitis beginning 2 to 7 days after sexual exposure which progress later to Pelvic inflammatory disease [20] Qasim and Ali, 2014 [21] have reported the advantage of serological test in the diagnosis of *Herpes simplex* virus type 2 infection in 104 (21-53 years) women presented with cervicitis with no clinically apparent genital ulcer or blisters,who were referred to the Maternity Teaching Hospital and private laboratories in Erbil city, Kurdistan region, Iraq , all Pap smear results showed features of cervicitis but without viral cytopathic herpetic changes.

Conclusion

We concluded from the present search that the report of inflammatory changes on the endocervical cytobrushe smear increases the

recognition and the occurrence rate of cervicitis and genital infections especially in asymptomatic women mainly if the results of both cervical cytological and microbiological tests were accompanied with the clinical symptoms like vaginal discharge type, burning sensation during urination and lower back or abdominal pain. In addition the isolation of different bacterial types and *Candida* spp in all positive cultured cases 56% concert a good way for bacterial vaginosis and candidiasis discovery mainly in the asymptomatic patients. We suggest to use this method as substitute broadcast method of cervical cancer instead of cervical Pap test.

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