Association between vaginitis caused by *staphylococcus aureus*, *E.coli* and *candida albicans* and pap smear results

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الخلاصة

ي ه ذه الدراس ة أخ ذت مائ ة مس حة مهبلي ة و مس حة عن ق الـ رحم عش وائيا م ن الس يدات اللات ي راجع ن مستش فى الديواني ة التعليم ي للنس ائية و الاطف ال ف ي محافظ ة القادس ية ، و ك ذلك أخذت عينات من بعض العيادات الخاصة ، العينات أخذت طبعا من النساء المتزوجات ، النس اء الحوامل اللواتي يشكون من نزف مهبلي او النساء اللواتي يستعملن تحاميل مهبلية تم استثناؤهن من ه ذه الدراس ة ، ام ا الفت رة الت ي جمع ت فيه ا العين ات فه ي ب ين تش رين الث اني 2010 و آذار 2011 الدراسة اشتملت على زراعة و عزل معظ م الاحي اء المجهري ة الش ائعة الانتهازي ة الت ي تسبب التهاب المهبل و علاقتها بتغيرات عنق الرحم ، أكثر الاحياء شيوعا و التي تم عزلها ا م ن الس يدات المص ابات بالتهاب المهبل ه ي المكورات العنقودية و العصيات المعوية والفطريات البيضاء و المكورات العقدية و بنسبة 29.7 % ، 17.6 % ، 25.7 % ، 4% على التوالي ..

لقد أظهرت الدراسة أن النساء المصابات بالتهاب المهبل بسبب ه ذه الاحي اء أظهرن تغيرات خلوية نسيجية في مسحات عنق الرحم في 27.2 % ، 46 % ، 21.1 % على التوالي ، بالمقارنة مع العينات المزروعة من المرضى التي أظهرت بكتريا سالكنة طبيعية (و ه م نسبة 11 %) فان نتيجة مسحة عنق الرحم كانت طبيعية.

وهذا يمكن تفسيره بأنه عندما تأثرت تغيرات عنق الرحم بمؤثرات خارجية أدت الى خلايا غير مثالية وكذلك مواد كيميائية تسمى سايتوكين التي تفرز بعد تحفيز رد الفعل المناعي الذي تسببه عن عدوى بكتيرية او التهابات لأسباب أخرى قد تؤثر طفريا وتساهم في تغييرات مسرطنة .

Abstract

In this study, one-hundred vaginal swabs and cervical smears were obtained randomly from women admitted to Al-Diwaniya Maternity and Pediatrics Teaching Hospital in Al-Qadisyia Province; samples from private clinics were also included. The samples were taken from married women. Women who were pregnant, with vaginal bleeding, and who recently have used vaginal suppositories were excluded from the study. The samples were collected from November, 2010 to March, 2011.

The study involved culturing and isolation of most common microorganisms that cause vaginitis and their association with cellular changes of the cervix.

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The most common organisms isolated were *Staphylococcus aureus*(29.7%) , *Escherichia coli* (17.6%),and *Candida albicans* (25.7%). These isolates were associated with cellular changes in (27.2%),(46%), and (21.1%) respectively. In comparison with patients' culture results that revealed normal flora (11%), Pap smear results were normal.
The study conclude that any persistent inflammation of the cervix induced by infectious or non-infectious agents and remain untreated will progress into cervical dysplasia and then into cervical cancer. . This may be explained, when cervical metaplasia disrupted by external influences leads to disordered squamous epithelium called dysplastic epithelia. Also cytokines that are released after stimulation of immune response that is caused by bacterial infections or inflammation of various origins may have mutagenic effect and contribute to carcinogenic changes.

Introduction

Cervical cancer is the second most common cancer in women worldwide and is nearly as common as breast cancer. In developing world, cervical cancer is very much more common than breast cancer. The rate has fallen steeply in recent years because of cervical cytology screening (1).

There are many risk factors for cervical cancer : human Papilloma virus infection, smoking ,HIV infection, Chlamydia infection , stress and stress-related disorders, dietary factors , hormonal contraception, multiple pregnancy, exposure to hormonal drug diethylstilbesterol, and family history of cervical cancer (2),early age at first intercourse and first pregnancy magnified by early use of contraceptives (2,3,4).

Viral infection have been strongly associated with cancers (5), also it has been shown that several bacteria can cause chronic infections or produce toxins that disturb the cell cycle resulting in altered cell growth (6). The resulting damage to DNA is similar to that caused by carcinogenic apoptosis. Processes that encourage the loss of cellular control may be tumor initiators (directly causing mutations) or promoters (facilitating mutations), (7).

The immune system is an important line of defense against tumor formation of malignancies that express unique antigens. Certain bacterial infections may evade the immune system or stimulate immune responses

that contribute to carcinogenic changes through the stimulatory and mutagenic effects of cytokins released by inflammatory cells (8). These include reactive oxygen species (ROS), interleukin-8(IL-8), cyclooxygenase-2(COX-2), and nitric acid (NO), (9,10). Chronic stimulation of these substances along with environmental factors such as smoking, or a susceptible host appears to contribute significantly to carcinogenesis (11)

Vaginitis is an infectious or non-infectious inflammation of vaginal mucosa (12).Vaginitis falls in many forms: irritant, hormonal, foreign body, sexually transmitted disease and infective. All types cause a great discomfort to women (13).

Cervical cancer can be prevented by the cervical smear (Paponicolaou ,Pap smear) which is a routine screening test used for the detection of early cervical abnormalities, namely precancerous dysplastic changes of the uterine cervix (14).The regular screening for cervical cancer reduces both the mortality and incidence of cervical carcinoma.Cervical neoplasia develop into invasive cancer after a period of ten years, and this likely to be among women who have escaped screening and proper follow-up.(15,16,17).

The objectives of this study is the isolation and identification of most common pathogens causing vaginitis among women in Diwaniya City/ Iraq.

Detect the presence and the type of cervical changes using the Pap test.

Construct a relationship between these micro-organisms and the corresponding grade of changes.

Materials &Methods

Patients:-

One hundred randomly selected high vaginal swabs and smears were taken from women who visited outpatient clinic in Al-Diwaniya Teaching Hospital for Maternal and Pediatrics and private clinics during the period from November 2010 to March 2011 in Al-Qadisyia Province. Pregnant and menstruating women were excluded from the study, in addition to women that have had recent intercourse, or have used vaginal suppositories or antibiotics.

Women visited the clinics have variable complains such as itching, discharge, lower back pain, infertility, vaginal repair, post caesarian section follow-up and others.

Collection of specimens

Vaginal speculum was used to provide a clear sight of the cervix; swabs were inserted into the posterior fornix, in the upper part of the vagina and rotated there before withdrawing them carefully to avoid any possible contamination. The swabs were placed in Amie's transport media and transported to the laboratory. The specimens for isolation were inoculated on blood agar, chocolate agar, MacConkey's agar, and Sabourad's agar plates. Blood and MacConkey's agar plates were incubated aerobically at 37°C for 24-48 hours ,while the chocolate agar plates were incubated anaerobically at 37°C for 24-48 hours, Sabourad's incubated at 30ºC for 24-48 hours. Cervical smears were taken by Ayer's spatula on ectocervix and endocervix, samples were placed on glass slides labeled with patients number and were fixed in 95% ethanol for one hour, then air dried, stained and screened for abnormal cells (18).

Results

An association between isolated opportunistic micro-organism and Pap smear results were of a high significance (P=0.001) (P≤0.05), as shown in table (1). Culture results of 11% of the patients' revealed vaginal normal flora and normal Pap smear (control group). Also culture results showed (15%) no growth that may be attributed to consumption of antibiotics by patient , or the presence of another causative agent that might need special techniques for their detection such as viruses , Chlamydia ,and other agents. Patients that have positive culture for opportunistic microorganisms were (74%), which is composed of (29.7%) of *S. aureus,*(17.6%) of *E.coli*, (4.0%) of *S. fecalis,*(23%) mixed infections, and *C.albicans* (25.7%).

Table (1) Association between Culture results and Pap smear results

Culture results		Pap smear result					Total
		Normal	MIR	ASCUS	CIN I	CIN II	
No growth	Count	4	11	0	0	0	15
	% within culture result	19.0%	19.0%	0.0%	0.0%	0.0%	38.0%
	% within Pap smear result	26.7%	73.3%	0.0%	0.0%	0.0%	100.0%
N.F.	Count	11	0	0	0	0	11
	% within culture result	52.4%	0.0%	0.0%	0.0%	0.0%	52.4%
	% within Pap smear result	100.0%	0.0%	0.0%	0.0%	0.0%	100.0%
Staph aureus	Count	0	16	3	3	0	22
	% within culture result	0.0%	27.6%	30.0%	14.3%	0.0%	71.9%
	% within Pap smear result	0.0%	72.7%	13.6%	13.6%	0.0%	100.0%
E. coli	Count	0	7	4	2	0	13
	% within culture result	0.0%	12.1%	40.0%	9.5%	0.0%	61.9%
	% within Pap smear result	0.0%	53.8%	30.8%	15.4%	0.0%	100.0%
Mixed infection	Count	0	12	1	3	1	17
	% within culture result	0.0%	20.7%	10.0%	14.3%	4.8%	81.0%
	% within Pap smear result	0.0%	70.6%	5.9%	17.6%	5.9%	100.0%
C. albicans	Count	6	9	2	2	0	19
	% within culture result	28.6%	15.5%	20.0%	9.5%	0.0%	90.5%
	% within Pap smear result	31.6%	47.4%	10.5%	10.5%	0.0%	100.0%
S. fecalis	Count	0	3	0	0	0	3
	% within culture result	0.0%	5.2%	0.0%	0.0%	0.0%	5.2%
	% within Pap smear result	0.0%	100.0%	0.0%	0.0%	0.0%	100.0%
Total		21	58	10	10	1	100

(P=0.001) MIR= Mixed Inflammatory Reaction; ASCUS= Atypical Squamous Cell of Undetermined Significance; CIN I= Cervical Intraepithelial Neoplasia I; CIN II= Cervical Intraepithelial Neoplasia II

Staph aureus isolated in 22% of the patients, Pap smear results showed 72.7% of patients with MIR and 27.2% with mild dysplasia. Recent studies have suggested that membrane damaging Staphylococcal exotoxins, such as α-toxin, facilitate TSST-1 penetration across vaginal tissue by cytotoxic and proinflammatory disruption of mucosa .Vaginal inflammation is caused by interleukin -8 (IL-8), a proinflammatory chemokine that attracts polymorphonuclear leukocytes (19). In this study *E. coli* was associated with MIR in 7⁄13 (53%) of the patients and dysplasia in 6⁄13 (46%).

Escherichia coli is considered a resident bacteria in intestine, but numerous strains of these bacteria have ability to initiate disease when bacteria travel from its original place (20).Once in deeper tissues, their ability to persist and cause injury is little understood except for the action of LPS endotoxin and the species known to produce exotoxins or capsules (21).

E.coli also release CNF (cytotoxic necrotizing factors) cycle stimulator that triggers G1-S transition and induce DNA replication .The number of cells does not increase. The cells become multinucleated instead, perhaps by toxins ability to inhibit cell differentiation and apoptosis (22, 23).

Mixed infections showed 29.4% of patients with mild to moderate dysplasia and 12⁄17 (70.6%) with MIR.

Twenty one percent of patients with *Candida albicans* infection showed dysplasia and 47.4% showed MIR. Candidiasis may be predisposed by the use of antibiotics, uncontrolled diabetes, contraceptives, immunosuppression, corticosteroid therapy, thyroid or endocrine disorders. The results are summarized in table (2):

Table (2) Distribution of Women According to Culture and Pap smear results

Culture Results	**No.of patients (%)**	**Pap Smear Results**	
		No. (%) MIR	**Dysplasia**
No growth	15/100(15%)	11⁄15(73%)	0%
Normal Flora	11/100(11%)	0%	0%
Staph aureus	22/74(29.7%)	16/22(72%)	27.2%
E.coli	13⁄74(17.6%)	7/13(53%)	46%
Mixed infection	17/74(23%)	12/17(70.5%)	29.4%
C.albicons	19/74(25.7%)	9/19(47.4%)	21.1%
S.fecalis	3/74(4%)	3/3(100%)	0%

MIR= Mixed Inflammatory Reaction

Conclusion

The most common opportunistic organisms that have been isolated and chosen for the study were *Staphylococcus aureus ,E. coli* and *Candida albicans*. Inflammation of the cervix caused by infectious or non – infectious agents (allergy, hormones, trauma) and persist for a period of time may progress into cellular changes and then into cervical cancer if neglected.

Recommendations

1- Promote a national cervical screening campaign.

2- Initiate educational programs for women, discussing the importance of personal hygiene, nutrition and routine clinical visits for check-up in decreasing incidence of cervical cancer.

3-Study other pathogenic microorganisms isolated from the vagina and their association with cervical cancer risk factors among women in Al-Diwaniya City.

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