Levels of IL-1β and IL-8 in Iraqi women with Bacterial vaginosis and Trichomoniasis

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Abstract

In order to identify and isolate the microbiological causes of the main genital tract infections and investigate the levels of proinflammatory cytokines IL – 8 and IL - 1β in vaginal fluid of women with bacterial vaginosis and trichomoniasis compared with healthy control group, a study was carried out on 164 women aged from 16 to 46 years, within reproductive age . Different laboratory methods were done to identify and isolate the microbiological causes of genital tract infection. Results demonstrated that the most common genital tract infection was bacterial vaginosis which detected in 48 women (37.5 %), followed by candidiasis in 42 (32.8 %), trichomoniasis in 38 (29.7 %), chlamydial infection in 28 (21.9 %) and gonococcal infection in 6 (4.7 %). Our study demonstrated the increase levels of proinflammatory cytokines IL – 8 and IL - 1β in bacterial vaginosis and trichomoniasis, the mean level of IL – 8 in bacterial vaginosis was 62.26 (pg\ml) SD +\- 31.12 and in trichomoniasis 59.45(pg\ml) SD +\- 20.23 compared with healthy control group 19.96 (pg\ml) SD +\-9.13, which detect highly significant relationship, the mean level of IL - 1β in bacterial vaginosis was 464.28 (pg\ml) SD +\- 448.663, in trichomoniasis 489.59 (pg\ml)SD +\-476.927 compared with healthy control 91.55 (pg\ml) SD +\- 42.313, which showed highly significant relationship

Keywords: IL-1β , IL-8, vaginosis , trichomoniasis.

Introduction

Genital tract infections (GTI), including sexually transmitted infections (STIs) represent a major public health problem in women. Vaginitis is the most common

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gynecologic complaints [1]. Vaginitis develops when the vaginal flora has been altered by introduction of a pathogen or by changes in the vaginal environment that allow pathogens to proliferate.

Bacterial vaginosis previously referred to as Gardnerella vaginitis or nonspecific vaginitis can be asymptomatic in up 50% of women [2]. It is the most common clinical vaginitis and occurs when the normal lactobacillus, which produces hydrogen peroxide and keeps the vaginal PH acidic, is overgrown by other components of vaginal flora, typically anaerobes such as Gardnerella or Mycoplasma. Although it is not sexually transmitted, it is more common in women with multiple or new partners [3].

Trichomoniasis occurs in approximately three million women annually. *Trichomonas vaginalis*, a small motile protozoan, is not part of normal vaginal flora. It is sexually transmitted and frequently occurs in the presence of other infections. It occurs in 13% to 25% of women attending gynecologic clinics and 7% to 35% of women attending clinics for sexually transmitted diseases. Although sexual contact is the primary mode of transmission [4].

A number of infections and conditions that involve the vaginal environment are commonly seen in women with gynecological disorders. These include Candidiasis, sexually transmitted diseases (STDs), cervicitis and pelvic inflammatory diseases (PID). Cervicitis may be accompanied by purulent discharge which may be a symptom of infection with gonorrhea or chlamydia [5,6].

Most of the available data focus on the innate immune response. Interleukin (IL-1β), sometimes referred to as master cytokines is almost always measured in studies of innate immune response. The consequence at the present time is that BV induces a low grade proinflammatory state in the vagina as reflected by elevated of IL-1β, IL-6 and tumor necrosis factor alpha (TNF-α). Moreover, successful treatment of BV with antibiotics lowers the level of these cytokines to baselines [7].

IL-8 is a major chemotactic factor for neutrophil; therefore, the striking absence of these cells in the BV may be explained, at least in part, by the lack of this cytokine in the vaginal fluid of women with BV. IL-8 is low or absent under normal conditions but highly inducible by a wide range of extra cellular stimuli, such as the proinflammatory cytokine IL-1 [8]. The present research aim to isolate and identify the microbiological causes of the main infections of genital tract and investigate the levels of proinflammatory cytokines IL-8 and IL-1β in vaginal fluid of women with genital tract infections compared with healthy control.

Materials and methods

Vaginal samples were taken from 128 women with abnormal vaginal discharge with or without any other symptoms compared with 36 healthy women without any signs or symptoms of genital tract infections or abnormal vaginal discharge and free of pathogen. Physical examination, vaginal and endocervical swabs were taken from every women under study and vaginal fluid taken from all women and kept in container contain phosphate buffer solution PBS and frozen under -20c°.

Five milliliters venous blood samples were taken from all women to identify specific IgG and IgM antibodies of chlamydia trachomatis.

Bacteriological and mycological studies: gram staining of vaginal discharge smears was performed for the diagnosis of bacterial vaginosis or vaginitis (mycosis). Aerobic and anaerobic culture media were also inoculated. Parasitological studies: Gram staining and fresh examination between slide and cover slide were made to observe the morphology and mobility of *trichomonas vaginalis*.

The IL-1β and IL-8 determination were carried out by enzyme amplified sensitivity immune assay (EASIA) kit from Bio source Nivelles Belgium.

Statistical methods used statistical tables including observed frequencies with their percentage, summary statistics of the readings distribution (mean, SD, SE, minimum and maximum).

Results

This study shows that the incidence rate of GTIs with different microorganisms among females attending consultation of Baghdad teaching hospital for gynecology and obstetric were 48 (37.5 %), Candidiasis 42 (32.8 %), Trichomoniasis 38 (29.7 %), Chlamydiosis 28 (21.9 %) and Gonorrhea 6 (4.7 %) shown in table (1).

The result of different laboratory methods to identify the common causes of genital tract infections found many combined infections in the patients.

Table (2) show the Co – infection among the females which showed that in trichomoniasis was found lactobacilli in 2 patients (5.26 %), Bacterial vaginosis in 4 (10.52 %), Candidiasis in 4 (10.52 %), Chlamydiosis in 8 (21.04 %), gonorrhea in 2 (5.26 %), Chlamydia and β – hemolytic streptococcus in 2 (5.26 %).

Table 1. The incidence rate of genital tract infections in (128) patients

Type of infection	N	Incidence rate	Comparison of significant	
			P-value	Sig.
Bacterial vaginosis	48	37.5	0.00	HS (P<0.01)
Candidiasis	42	32.8		
Trichomoniasis	38	29.7		
Chlamydiosis	28	21.9		
Gonorrhea	6	4.7		

In Bacterial vaginosis was found lactobacilli in 7 (14.58 %), Candidiasis in 2 (4.16 %), Chlamydiosis in 6 (12.48 %), gonorrhea in 4 (8.32 %). *E coli* in 6 (12.48 %), candidiasis and *E coli* in 6 (12.48 %), *Staph. aureus* in 2 (4.16 %), *Chlamydia* and *Klebsiella* spp. in 2 (4.16 %), β – hemolytic *streptococcus* in 2 (4.16 %), *proteus* spp. and *E coli* in 2 (4.16 %).

In Candidial infection was found lactobacilli in 13 (30.95 %), *Chlamydia* in 4 (9.52 %), *E coli* and *Klebsiella* spp. in 2 (4.76 %). In Chlamydial infection lactobacilli was found in 2 (7.14 %).

The mean level of IL – 8 (pg/ml) in (36) healthy control group was 19.96 (SD)+/- 9.13, in (38) trichomoniasis patients was 59.45 (SD) +/- 20.32 (pg/ml) and in (48) bacterial vaginosis patients was 62.26 (SD) +/- 31.12 (pg/ml) which showed highly significant relationship between the level of IL – 8 in healthy control and trichomonal and bacterial vaginosis infections ($p<0.01$), also showed no significant relationship between trichomoniasis and bacterial vaginosis ($p>0.05$), these results showed in Table (3)**.**

The mean level of IL - 1β (pg/ml) in (36) healthy control group was 91.55 (SD)+/- 42.313 (pg/ml), in (48) bacterial vaginosis patients was 464.28 (SD) +/- 448.663 (pg/ml) and in (38)trichomoniasis patients was 489.59 (SD) +/- 476.927 (pg/ml), which showed highly significant relationship between the level of IL - 1β (pg/ml) in healthy control and bacterial vaginosis and trichomonal infections ($P < 0.01$), also showed no significant relationship between bacterial vaginosis and trichomoniasis ($P > 0.05$), these results showed in Table(4).

Table 2. o – infection in patients with different genital tract infections

Type of infection	Co-infection	Total Number	Co-infection Number	Percentage %
Bacterial vaginosis	Lactobacilli	48	7	14.58
	Candida		2	4.16
	Chlamydia		6	12.48
	Gonorrhea		4	8.32
	E.coli		6	12.48
	Candida &E.coli		6	12.48
	Staphylococcus aurous		2	4.16
	Chlamydia & Klebsiella spp.		2	4.16
	β-hemolytic streptococcus		2	4.16
	Proteus spp. & E.coli		2	4.16
Trichomoniasis	Lactobacilli	38	2	5.26
	Bacterial vaginosis		4	10.52
	Candida		4	10.52
	Chlamydia		8	21.04
	Gonorrhea		2	5.26
	Chlamydia & β-hemolytic streptococcus		2	5.26
Candidiasis	Lactobacilli	42	13	30.95
	Chlamydia		4	9.52
	E.coli & Klebsiella spp.		2	4.76
Chlamydiosis	Lactobacilli	28	2	7.14
	Candida		2	7.14
Total		156	85	

Table 3. Mean level of IL-8 (Pg/ml) among patients with B.V & trichomoniasis compared with healthy control

studied groups	N	Mean	SD	Std. Error	Mini.	Maxi.	Comparison of significant
Apparently healthy control	36	19.96	9.13	1.52	0.00	37.33	-
Bacterial Vaginosis	48	62.26	31.12	4.49	27.72	185.29	0.00 HS (P<0.01)
Trichomoniasis	38	59.45	20.23	3.28	31.97	113.60	0.00 HS (P<0.01)
Total	122						
Trichomoniasis Vs Bacterial Vaginosis				0.578 NS (P>0.05)			

Table 4. Mean level of IL-1β (Pg/ml) among patients with B.V & trichomoniasis compared with healthy control

studied groups	N	Mean	SD	Std. Error	Mini.	Maxi.	Comparison of significant
Apparently healthy control	36	91.55	42.313	7.05	32.12	193.52	-
Bacterial Vaginosis	48	464.28	448.663	64.75	120.01	2144.24	0.00 HS (P<0.01)
Trichomoniasis	38	489.59	476.927	80.61	65.47	2154.66	0.00 HS (P<0.01)
Total	122						
Trichomoniasis Vs Bacterial Vaginosi				0.769 NS (P>0.05)			

Discussion

Genital tract infections (GTIs) caused by numbers of microorganisms which usually are transmitted through sexual contact. Pelvic inflammatory disease (PID), most frequently a complication of GTIs, have been shown to be an immense problem in developed countries. GTI in both the developed and developing countries constitute enormous health problem [9]. Vaginal and cervical infections are the most common women's health problem, and have been increasingly linked to a growing array of serious health risks. The female genital tract provides a satisfactory environment for many pathogenic microorganisms, and multiple infections are therefore common [10]. The common mucosal immune system contributes to immune defense in the female genital tract. Cervicovaginal secretion contain high concentrations of intrinsic immune components (e.g., complement and defenses) and immunoglobulin that are the first line of defense against invasion by pathogens [11], additional protection is provided by cellular immune components, many different cytokines have been detected in the cervical and vaginal secretions of adult women [12]. However, little is known about changes in local cytokine concentrations in response to microbial infection of the genital tract.

This study shows that the incidence rate of GTIs with different microorganisms among females attending consultation of Baghdad teaching hospital for gynecology and obstetrics were 48 (37.5 %), Candidiasis 42 (32.8 %), Trichomoniasis 38 (29.7 %), Chlamydiosis 28 (21.9 %) and Gonorrhea 6 (4.7 %).

Women often seek medical care for vaginal complaints many times; the cause of the complaint is misdiagnosed by the women and / or her provider. These vaginal complaints may be related to infections, which when misdiagnosed or mistreated, can lead to more severe problems.

The results of culture and smears of vaginal and endocervical swabs taken from females attendant the consultation of Baghdad teaching hospital for (gynecology and obstetrics) show that females were subjected to our study infected with different microorganisms and no one of the women with abnormal vaginal discharge with no of any microorganisms. Our results showed that the most common infection among women was Bacterial vaginosis, which represent the highest rate of infection (37.5 %). Many studies in the world demonstrated that BV. is currently the most common cause of vaginitis in pregnant and non pregnant women [13; 14; 15; 16;17]. All these studies are in agreement with our study that shows BV. is the most prevalent form of vaginal disturbances in women. BV. has been associated with many gynecological and obstetric complication such as cervicitis, pelvic inflammatory disease, urinary tract infections, endometritis, post operative infections, salpingitis, premature rupture of the membranes, preterm delivery [16]. Taking into consideration the occurrence rate of BV. and its connection with numerous gynecological and obstetric sequelae, and taking into account that the diagnosis of BV. is quick, simple and inexpensive.

The second most common cause of genital tract infections in our study was Candidiasis which represents 32.8 %. Millions of women worldwide continue to suffer from vulvovaginal candidiasis, which is second only to anaerobic bacterial vaginosis [18; 14; 17] and all these result accepted with our study.

Evidence is presented of an increasing incidence of vulvovaginal candidiasis, the cause of which is unclear, but this increase is probably the result of multiple factors including wide spread abuse of antibiotic, and most important inadequate vaginal therapy (19). Some women never experience vulvovaginal candidiasis, other have infrequent episodes. Two fundamental questions face investigators: the mechanism was by asymptomatic colonization converts to symptomatic disease and the elusive explanation for frequent recurrences of vulvovaginal candidiasis, although several factors have been identified as predisposing to recurrent vulvovaginal candidiasis (pregnancy, oral contraceptives, exogenous hormones, antibiotics, diabetes mellitus, etc.)

Trichomoniasis is the third most common cause of vaginitis and genital tract infections in our study and this result in accordance with other studies [20;17]. Trichomonads are transmitted sexually also like other pathogenic organisms *Neisseria gonorrhoeae* and *Chlamydia trachomatis*.

T. vaginalis is the most common pathogen that is sexually spread in women [21], because many men are asymptomatic and may not seek for medical care, these men facilitate spread of *Trichomonas vaginalis* to women through sexual transmission [22].

Our study showed the increase concentration of IL – 8 and IL - 1β in both Trichomoniasis and Bacterial vaginosis in compared with the concentration of both cytokines in healthy women who had no signs or symptoms of infection or inflammation. These results in agreement with many studies. Basso *et al*., [23] showed in his study on gyneco – obstetric infection, the levels of IL – 8 and IL - 1β increased in vaginal secretions of women who had Bacterial vaginosis and vaginitis compared with the levels of both cytokines in healthy control group.

Shaio *et al.*, [24] demonstrated that membrane components of *T. vaginalis* induce IL – 8 productions by monocytes. This was the first study to report the ability of *T. vaginalis* to induce IL – 8 secretion by neutrophils, also Jae – Sook Ryu *et al*., [25] that the IL – 8 produced by stimulation with live was found *Trichomonas vaginalis* by neutrophils and importance of contact between neutrophils and *T. vaginalis* for IL – 8 production. Adherence between neutrophils and *T. vaginalis* was seen in vaginal smears; trichomonads were found fused with polymorph nuclear leukocytes in most trichomoniasis patients. Although neutrophils were confirmed to strongly induce IL – 8 productions after neutrophils were stimulated with *T. vaginalis* also vaginal epithelial cells produce IL – 8 early in infection with *T. vaginalis* [26].

Bacterial vaginosis has traditionally been considered a non inflammatory condition, but these findings clearly demonstrate that this condition is associated with inflammatory changes. Many studies demonstrated the increase of local cytokines in the cervicovaginal secretion in women with bacterial vaginosis.

Yudin *et al*., [27] demonstrated that the cervical levels of IL - 1β, IL – 8 and IL – 6 decreased after treatment and cure of bacterial vaginosis in both the oral and vaginal treatment.

Mattsby – Baltzer *et al*., [28] demonstrated that BV. is associated with increased levels of IL - 1β in the lower genital tract of pregnant women and suggest that the ability of BV. associated endotoxins to induce cytokine production in monocytic cells may partly explain the increased IL - 1β levels. Wasiela *et al*., [29] show that in vagina of women with B.V the levels of proinflammatory cytokines IL - 1β, IL – 1, IL – 8 and IL – 6 rise. These findings is in agreement with our study. B.V status causes a dramatic increase of IL - 1β concentrations showing that the innate immune system is reacting strongly and trying to fight abnormal microbial colonization [30].

The high IL – 8 levels may be responsible for the presence of vaginal leukocytes in vaginal discharge of women with B.V due to co infection with other genital tract infections such as candidiasis and cervicitis due to gonococcal and chlamydial infections that showed inflammatory sings. Also, several studies demonstrated that *Candida albicans*, *Neisseria gonorrhoeae* and *Chlamydia trachomatis* have been reported to induce IL – 8 production by vaginal epithelial cells in the infection [31;32]. On the basis of generally accepted notion, high levels of IL - 1β should be paralleled by high levels of induced chemokines such as IL – 8 [33]. IL - 1β induces IL – 8 production [34] and activates neutrophils [35]. Also, Yu *et al*., [36] demonstrated that IL – 8 enhances the release of other inflammatory cytokines such as IL – 1. These results accepted with our study and may be explain the high levels of both cytokines in response to bacterial vaginosis and trichomoniasis, also neutrophils has been considered to be primarily responsible for inflammatory changes and IL – 8 would be a powerful stimulus for the invasion of neutrophils and their degranulation. On the basis of the present study together with several studies, we hypothesize that after infection with trichomoniasis and bacterial vaginosis chemokines secreted such as IL – 8 and IL - 1β. Both cytokines could be useful as evolutive markers of infection and high concentration of both cytokines in bacterial vaginosis and trichomoniasis patients are experiencing an activated innate immunity status, to fighting the damaging effect of abnormal vaginal colonization and pathogens.

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