

Comparison between Bacterial Vaginosis and Candidiasis in Relation to Estradiol Level and Vaginal pH in Some Infertile Iraqi Women

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Abstract

Background: Female fertility are affected by several factors including microbial and non microbial agents. Microbial infection is one of the most important causes for female infertility. The level of pathogenicity of microbial infections are affected by wide range of factor including age, physiological status, phase of menstrual cycle and race.

Objective: Comparison between bacterial vaginosis (B.V) and candidiasis in relation to Estradiol (E2) level and vaginal pH in some infertile Iraqi women

Methods: The study population was a subset of 109 infertile women attending Institute of Embryo Research and Infertility Treatment at Baghdad University, throughout the period from June till November 2004. Those infertile women were subjected to clinical examination by measuring vaginal pH, vaginal swabs collection to diagnosis of B.V using Amsel clinical criteria beside various micro-biological methods and diagnosis of candidiasis using mycological methods and serum collection from aspirated venous blood at late follicular phase for detection of E2 level.

Result: Forty eight infected infertile women were diagnosed with B.V from 109 infertile women. In those women the Estradiol mean was 41.17 Pg/mL near to lower limit of normal range of E2 level (18-147 pg/mL) and lower than E2

mean of healthy control group 132.5 Pg/mL in this study and most of them 93.75% had vaginal pH greater than 4.5. 24 cases with candidiasis were diagnosed from 109 infertile women. In those women the E2 mean was 183.2 Pg/mL higher than upper limit of normal range of E2 level and higher than E2 mean of healthy control group and candidial infection occur in normal pH range of 3.5 to 4.5

Conclusions: The results of the present study appeared that the hormonal disturbance which was associated with different infertility conditions may be predisposing factor in development of B.V and develop candidiasis among infertile women.

Elevated vaginal pH in infertile women who had B.V could be due to estrogen deficiency while normal pH in candidial infection because estrogen hormone increases cellular glycogen content which favors growth of Lactobacilli that metabolize glycogen to lactic acid and then producing an acidic environment.

Key words: Bacterial vaginosis, candidiasis, Estradiol level, infertile women

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Introduction

The normal vaginal environment is characterized by a dynamic interrelationship between *Lactobacilli acidophilus* and other endogenous flora,

estrogen, vaginal pH and metabolic byproducts of flora and pathogens. Vaginitis develops when the vaginal flora has been altered by introduction of pathogen or by changes in the vaginal environment^(1,2).

Bacterial vaginosis is the most common infectious cause of vaginitis characterized by imbalance of vaginal ecosystem while candidiasis is the second most common cause of vaginitis^(1,3,4). These types of vaginal infections

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differ from others in their relation to estradiol level, cellular glycogen content and the acidity of vaginal fluid ⁽⁵⁻⁷⁾. Hypoestrogenic problems that may be caused by hypothalamic, pituitary and ovarian disorders which are the most common causes of infertility ⁽⁸⁻¹¹⁾ that lead to interruption of estrogen production, produces mucosal atrophy, reduction in cellular glycogen which decreases number of *Lactobacilli* leading to increased vaginal pH which enhances the proliferation of *B.V* related bacteria resulting in vaginal infection with *B.V* ^(1,7,12). Polycystic ovary syndrome (PCO) is one of the most common endocrine disorders and many PCO women have elevated estrogen level ^(13,14) which is one of the most important risk factors of candidal infection ⁽¹⁵⁾, because elevated estrogen induce increase in glycogen content in vaginal epithelial cells which favors growth of yeast cells, as well as favors growth of *Lactobacilli* that metabolize glycogen to lactic acid producing an acid pH of 3.5-4.5 ^(12,16-21). Furthermore, yeast cells possess receptors for estrogen which enhance mycelial formation as well as estrogen was found to reduce the ability of vaginal epithelial cells to inhibit the growth of *C. albican* ⁽²²⁻²⁴⁾.

Patients, Materials and Methods

Study population

One hundred nine infertile women within reproductive age. 72 representing patients group which complaining from vaginal discharge with or without other symptoms and 37 were regarded as control group without any signs and symptoms of vaginal infection were attending Institute of Embryo Research and Infertility Treatment at Baghdad University through the period June till November 2004 were studied. Infertile women were given a self-administered

questionnaire with following information was collected Age, Last menstrual period, Length of cycle, Length of period of last antibiotic treatment, Vaginal discharge and associated symptoms. (Itching, Lower abdominal pain and Dyspareunia) and Infertility duration.

Microbial Examination:

Bacterial vaginosis is diagnosed conventionally when at least three of four Amsel clinical criteria are present including (Thin homogenous discharge, vaginal pH greater than 4.5, fishy odor of vaginal discharge and presence of clue cell) ⁽²⁵⁻²⁷⁾. Accurate diagnosis of vaginal candidiasis depends on culture techniques that will yield correct identification of fungal pathogens ⁽⁴⁾ by using germ tube test which perform by inoculating several colonies into the test substrate (such as fetal bovine serum) and incubating the suspension at 37°C for 3 hours. ⁽²⁸⁻²⁹⁾.

Clinical examination

The external genitalia are first inspection for erythema, edema or excoriation ^(11, 30). Non-lubricated bivalve speculum was inserted into the vagina for inspection of vaginal and cervical erosion and color of mucus. Vaginal discharge was also inspected for its characters ⁽³¹⁻³²⁾.

Vaginal pH

pH measurement was easily carried out by clipping a short piece of pH paper (range pH 1-14) to a forceps and dipping it into the vaginal discharge in the lateral fornices, ⁽³³⁾. The color was then compared to the colors and corresponding pH values on a standard chart.

Sampling collection

Serum collection

Before vaginal examination, about five mL of venous blood sample was

collected from each woman at cycle day 12.

The blood was delivered into a sterile screw plastic tube, and then centrifuged at 3000 RPM for 5 minutes the serum was then collected into another sterile tube and was kept in deep freeze at -20°C for estimation of estrogen level.

Swabs Collection

Three sterile cotton tipped swabs were used to collect vaginal discharge from the posterior fornix^(32,34,35). Two swabs were used for cultivation. One blood agar and chocolate agar were incubated anaerobically at 37°C. The second blood agar MacConkey's and Sabouraud's agar were incubated

aerobically at 37°C for 72 hour. The third swab was used for the preparation of Gram's stain, Whiff test and KOH preparation by rolling this swab on two clean glass slides first smear was mixed with a drop of 10% KOH for Whiff test then examine for detection of Candida organisms and the second smear was fixed by heat and then stained according to the Gram's stain procedure⁽²¹⁾.

Estrogen (E₂) assay

Estrogen level of the frozen serum of infertile women was estimated by VIDAS Estradiol 11 (E₂ 11) kit-from biomerieux sa-France.

Table 1: show the ranges of expected normal values of E₂ (from biomeriex Sa).

Normal subjected female	E ₂ range, pg/mL
- Follicular phase	18 – 147
- Pre-ovulatory peak	93 – 575
- Luteal phase	43 – 214
- Menopause	< 58

Note:

E₂ 11 is an automated quantitative test for use on the VIDAS instruments for the quantitative measurement of 17β estradiol in human serum or plasma (lithium heparinate), using the (ELFA) technique.

Statistical methods

The Statistical methods that were used in study to analysis the results of this study include Chi-square test and ANOVA test

Result

Vaginal pH as predictor for candidiasis and bacterial vaginosis in infertile women

The results of vaginal pH testing in both patients and control groups are shown in table (2), which showed highly significant (P<0.001) relationship

between B.V and vaginal pH when compared to the control group 45/48 (93.75%) of infertile women who had B.V were found to have vaginal pH equal or above 4.5 versus 31/37 (83.8%) of healthy control group had vaginal pH less than 4.5. While no significant relationship between high vaginal pH and candidial infection compared to the control group, however 18/24 (75%) of infertile women with candidial infection had vaginal pH less than 4.5.

Table 2: Vaginal pH as predictor for for candidiasis and bacterial vaginosis in infertile women

Type of vaginal infection	Vaginal PH				Chi-square Test p-value
	< 4.5		>4.5		
	N	%	N	%	
Bacterial vaginosis N=48	3	6.25	45	93.75	P<0.001 HS
Candidiasis N=24	18	75	6	25.0	P>0.05 NS
Normal N=37	31	83.8	6	16.2	*

***P-value in relation to normal group **NS: Non significant**

*****HS: High significant **** N: number of patient**

The relation between E₂ mean and incidence of different types of vaginal infection among infertile women

The different effects of E₂ level on occurrence of B.V and candidiasis among infertile women are described in table (3). Highly significant difference (P<0.001) was demonstrate between both B.V and Candidiasis and healthy control group in E₂ mean and between each other . The E₂ mean of 48 infertile women who had B.V was 41.17 Pg/mL

±4.15 lower than E₂ mean of 37 healthy infertile women 132.5 Pg/mL±11 and E₂ mean of candidiasis cases, while E₂ mean of 24 infertile women with candidiasis was 183.2 Pg/mL ±32.6 higher than E₂ mean of control group and E₂ mean of B.V. From the above results,B.V occurred at lower E₂ level (mean 41.17 Pg/mL) while candidial infection occurred at higher E₂ level (mean 183.2 Pg/mL)

Table (3) The relation between E₂ mean and incidence of different types of vaginal infections among infertile women

Type of vaginal infection	N	E2 Mean ± S.E.M
Bacterial vaginosis	48	41.17 ± 4.15
Candidiasis	24	183.2 ± 32.6
Normal	37	132.5 ± 11.0

* ANOVA test P<0.001 High significant

Date are Mean ± SEM

N: number of patient

H.S: High significant

Discussion

The recurrent hormonal treatment and personal hygiene of infertile women were considered.

Vaginal pH as predictor for candidiasis and bacterial vaginosis in infertile women.

Measurement of vaginal pH is useful, effective and inexpensive for screening purposes. In the present study, table (2) shows highly significant relationship ($P < 0.001$) between vaginal pH and B.V. The pH value in 93.75% of infertile women who had B.V was greater than 4.5. This result is in agreement with other studies who depend on Amsel clinical criteria which have considered vaginal pH ≥ 4.5 as one of four criteria to confirm the diagnosis of B.V^(27, 36-38). The failure of any of the following three endocrine glands hypothalamus, pituitary and ovary lead to inhibition of estrogens production by ovary in women within reproductive age^(8, 9, 11). On the other hand, estrogen deficiency in menopausal women lead to elevated vaginal pH, this is due to lack of glycogen content and disappearance of *Lactobacilli*⁽³⁹⁾. Therefore, the two states are comparable. As well as, anaerobic bacteria that are associated with B.V produce organic amine, which raise vaginal pH^(17, 33, 40). Our data suggest that the elevated vaginal pH in infertile women who had B.V could be due to estrogen deficiency and presence of amines, which are produced by anaerobic bacteria that are responsible for further increase in vaginal pH in those women.

Only 16.2% of healthy control group had vaginal pH ≥ 4.5 this may be due to either recent sexual intercourse or douching or touching cervical mucus^(41, 42).

This study found no significant correlation between vaginal pH and candidial infection when compared to the control group, because 75% of infertile women with candidiasis and 83.8% of healthy control group had vaginal pH less than 4.5. Thus, the results of this study are in good

accordance with many studies demonstrating candidial infection occur in normal pH range from 3.5 to 4.5^(11, 43-45).

Many recent studies emphasized that some infertile women with polycystic ovary, and other causes of hyperandrogenemia have elevated or normal level of estrogens^(9,13,14,46). It was reported that estrogen increases cellular glycogen content which favors growth of *Lactobacilli* that metabolize glycogen to lactic acid producing an acid pH of 3.5-4.5^(12,16-19).

The result of our study suggests that the normal vaginal pH in some infertile women infected with candidiasis may be due to elevated estrogen level and heavy colonization of vagina by *Lactobacilli*.

The relation between E₂ level and occurrence candidiasis and bacterial vaginosis among infertile women

The relation between serum estrogen (E₂) level and occurrence of candidiasis and bacterial vaginosis among infertile women are shown in table (3). The results of this study demonstrated that the E₂ mean of 48 infertile women who had B.V was 41.17 Pg/mL near to lower limit of normal range of E₂ level at follicular phase. From the above result, one can conclude two things, these women were more likely to have hypoestrogenic problems, these problems may be caused by hypothalamic or pituitary or ovarian disorders which are the most common causes of infertility⁽⁸⁻¹¹⁾. The other thing is B.V occurred at low estrogen level, the reason of this state is explained by many recent studies who reported estrogen depletion caused by castration, aging or other causes that lead to interruption of estrogen production, produces mucosal atrophy, reduction in cellular glycogen which decreases number of *Lactobacilli*

leading to increased vaginal pH which enhances the proliferation of *B.V* related bacteria resulting in vaginal infection with *B.V* ^(1,7,12). The results of this study suggest that the hypoestrogenic state which is associated with some infertility conditions could play a role in development of *B.V* among those infertile women. In the present study, serum E_2 level was found higher than the upper limit of normal range of E_2 level at follicular phase in 24 infertile women who had candidiasis 183.2 Pg/mL this may be due to endocrine disorders which is one of the most important risk factors of candidial infection ⁽¹⁵⁾. Elevated estrogen induce increase in glycogen content in vaginal epithelial cells which favors growth of yeast cells ^(20, 21).

Furthermore, yeast cells possess receptors for estrogen which enhance mycelial formation as well as estrogen was found to reduce the ability of vaginal epithelial cells to inhibit the growth of *C. albican* ⁽²²⁻²⁴⁾.

Polycystic ovary syndrome is one of the most common endocrine disorders and many infertile women with this syndrome have elevated estrogen level ^(13, 14).

From the above results of this study it was concluded that elevated estrogen level in some infertile women may encourage colonization of *Candida spp.* in vagina leading to candidiasis

From table (3) it can be also observed that E_2 mean of infertile women with *B.V* was lower than E_2 mean of healthy women which were regarded as control group which was within normal range of E_2 level also E_2 mean of infertile women with candidiasis was higher than E_2 mean of those healthy women.

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