VAGINAL PH AS A MARKER FOR VAGINITIS AND MENOPAUSAL STATUS

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ABSTRACT

Vaginal PH was determined by universal indicator PH paper in 182 patients seen in Al-Zahara`a Hospital in Najaf city for routine speculum examination. None of the patients were pregnant. Measurements were made of serum levels of follicle – stimulating hormone and estradiol for 81 menopausal patients and vaginal culture were taken for all 182 patients. Vaginal PH was correlated with vaginal cultures and serum follicle – stimulating hormone and estradiol levels by use of statistical analysis. Vaginal PH was elevated in all premenopausal patients with documented bacterial pathogens. Serum estradiol levels showed an inverse and serum follicle – stimulating hormone levels a direct statistical correlation with vaginal PH in postmenopausal patients.

Aim of the study: The study had two objectives : (1) to confirm the elevations of vaginal PH in patients with bacterial pathogens in premenopausal women and (2) to examine the relationship of serum FSH and estradiol levels to vaginal PH in postmenopause patients without bacterial pathogens .

INTRODUCTION

Despite the important implications for women's health and reproduction, little is known about the mechanisms that control and regulate vaginal pH. The prevailing *lactobacillus – Doderlein* theory postulates that the acidic vaginal luminal Ph is produced by cohabituating Doderlein lactobacilli that produce hydrogen peroxide and secret proton, into their immediate environment. [1] A new hypothesis for the regulation of vaginal ph proposes that the luminal vaginal pH is determined by active proton secretion by vaginal epithelial cells through the coordinated action of ion transport mechanisms located in the apical cell membrane. The active net proton secretion occurs constitutively throughout a woman's life but the degree of acidification is estrogen dependant, mostly through the involvement of estrogen receptors [2].

Vaginitis is the most prevalent disorder for which women seek medical assistance [3, 4]. In the presence of vaginitis, an elevated vaginal pH > 4.5 may characterize various conditions such as bacterial vaginosis , trichomonas vaginalis , group B streptococcus , or other pathogenic organisms.The most widely reported such disorder is bacterial vaginosis [3,4]. The use of vaginal pH , amine whiff testing , wet mount , and Gram's stain examination of the vaginal discharge[6] in some combination are among at least seven diagnostic sets of criteria that have been used for diagnosis (Nugent's , spiegel's and Amsel's Diagnostic criteria's)[6] . However , an elevated vaginal pH (5.0 to 6.5) in a normally estrogenized patient is almost always associated with bacterial vaginosis[3,4,7,8] these indirect measures reflect an attempt at cost containment and speeding diagnosis .

Menopause is the physiological cessation of menstruation that denotes a normal human developmental phase in the lives of women [9]. Menopause is the objective evidence of ovarian failure in both the follicular and secretary phases of the menstrual cycle. It begins twelve months after the final menses and it is demonstrated by elevated levels of follicle – stimulating hormone (FSH) and Lutinizing hormone (LH)[10]. The onset of

menopause is reported between 50 and 55 years with an average of 52 years [5]. It is of note that increase in serum FSH , with or without decrease in estradiol , have been shown in women over the age of 40 years yet who continue to cycle regularly. Moreover abrupt fluctuations in serum FSH and estradiol may be also observed with post menopausal levels subsequently returning to the normal reproductive range. [11] Changes in vaginal pH have been observed in premenopausal and postmenopausal women, showing a close relation to ovarian hormones. The normal vaginal pH of a reproductive aged woman is 3.9 – 4.5. During premenopausal years, vaginal luminal pH ranges between 4.5 and 6 , whereas lack of estrogen after the menopause is associated with alkalinization to about 6.5 – 7 . [12] Prepubertal and postmenopausal vaginal mucous membranes possess an atrophic epithelium with a surface pH of 6.0 – 8.0. At puberty, circulating estrogen increase, resulting in proliferation of vaginal epithelial cells. Glycogen is deposited in the intermediate and superfacial epithelial cells of the vagina, and lactobacilli proliferate, causing the enzymatic break down of cellular glycogen. Lactic acid and hydrogen peroxide are produced, which lowers the vaginal pH to 3.5 – 4.5. This is considered an indicator for a normal, properly estrogenized vagina [12]. Vaginal pH can be elevated by bacterial vaginosis , blood , cervical mucus , semen , vaginal medication , and douches. This study presents the results of vaginal pH testing on consecutive premenopausal and postmenopausal patients attending Al.Zahra'a hospital this study suggest that the presence of important aerobic pathogens is likely to be associated with pH values > 4.5 in premenopausal women and also offers preliminary data supporting the hypothesis that pH values > 4.5 in the absence of vaginitis are associated with low estrogen level as in menopause [13, 14].

SUBJECTS & METHODS

The present study was carried out on 182 patients, 101 premenopause and 81 postmenopause women who were selected randomly from women attending the Gynecology outpatient clinic of Al-Zahra'a hospital in Al Najaf city. History was taken from all the patients , including : name , age , parity , gynecological history involving the past menstrual history (onset of menarchae , regularity , duration and frequency of the period) , contraception , vaginal discharge and dyspareunea , past medical , family and social history . Pelvic examination was done to all the patients by sterile non – lubricating speculum and inspection by bright light. Exclusion criteria from the study were: pregnancy, vaginal medications, and hormonal replacement therapy. An informed consent was obtained from each woman prior to their enrollment in the study. Vaginal pH was measured with universal indicator pH strip after insertion of a non lubricated sterile vaginal speculum , the strip was applied directly to the lateral vaginal wall at the outer third of the vagina until it became wet . Color change of the strip was immediately compared with colorimetric scale and the measurement recorded. Care was taken to avoid cervical mucus and blood, known to affect vaginal pH. Blood samples were obtained by venipuncture within 1 hour of vaginal pH test and assayed for serum FSH and estradiol levels which were determined by Eliza technique and reported in milli international units per milliliters (mlU / ml) and pictogram per milliliter (pg/ml) , respectively . The typical level of FSH in post menopausal women is > 20 mlU/ml . Women were considered to be menopausal if oestradiol value was < 40 pg / ml [5]. All vaginal specimens were collected as a high vaginal swab with use of wood spatula and were sent to hospital laboratory, were aerobic cultures were grown. When the predominant organism recovered was B-hemolytic streptococci, klebsiella or yeast, it has been listed as such. if a combination of such organisms , including Gram – negative aerobic organism such as E – coli were grown , then the patient was deemed as having a " Mixed " infection . Patients were considered to be menopausal if they

had not had a menstrual period in 1 years or if the FSH value was > 20 mlU / ml or oestradiol value was < 40 pg / ml .

STATISTICAL ANALYSIS

Predictive value table were generated by standard techniques [15]. Two issues are involved in the assessment of any diagnostic test: Disease present or absent and diagnostic test result positive or negative from which the sensitivity and the specificity of the test can be calculated.

RESULT

We have four age groups of premenopausal women, we examined their vaginal pH in relation to bacterial pathogen. see table(1).

Of 101 premenopausal pt who had vaginal cultures , 14 cultures grew normal flora,7 yeast ,29 cultures grew B. hemolytic streptococci , 23 klebsiella and 28 mixed aerobic pathogens .

The mean pH of three subgroups growth of aerobic bacterial organisms was significantly higher than that obtained in patients with either normal flora or yeast infection. There was no significant difference in vaginal pH among the three subgroup with bacterial pathogens, and there was no significant difference between the pH in patients with yeast infection and those with normal flora. All 80 patients with positive culture results were asymptomatic apart from thirteen patients were symptomatic. Table (2) shows the predictive value of pH, an elevated pH > 4.5 was 95% sensitive 100% specific , and 95% efficient for the presence of aerobic pathogen (excluding yeast) in premenopausal women .

We have three age groups of postmenopausal women, we examined their vaginal pH in relation to pathogens (table 3).

The result of culture of postmenopausal women shown in (table 4) , the sensitivity was 100% , specificity and efficiency was low .

pH as predictor for estradiol status in postmenopausal women , had sensitivity of 96% , specificity of only 11% and an efficiency accuracy of 69% , these findings demonstrate a positive predictive value of 70% . (Table 5).

When serum FSH was used as a predictor of estradiol status the sensitivity was 98% and efficiency was 70% .

These values demonstrate a positive predictive value of 69%, The mean FSH value for those women with serum FSH ≥ 20 miU / ml and estradiol value < 40 pg / ml was 64. 292 ∓ 26.852 miU/ml (table 6).

The sensitivities of vaginal pH and serum FSH were comparable (p = 0.516) denoting their similarity for diagnosing an estradiol value < 40 pg / ml , which is one of the markers for menopause . Table 7 show the correlation between vaginal pH and FSH and estradiol levels. serum estradiol showed a significant negative correlation with vaginal pH (r = 0.166 , p = 0.069) and FSH (r = 0.372 , p = 0.000 , whereas serum FSH showed a significant positive correlation with vaginal pH (r = 0.351 , p = 0.001) .

We compared vaginal pH , estradiol and FSH of postmenopausal women according to age group: vaginal pH increased with increased age , estradiol level decreased with increased age and FSH increased with increased age (table 8) .

DISCUSSION

In assessing the status of the vaginal ecosystem the hydrogen ion concentration (pH) of the vagina is perhaps the most significant predictor of its status and that " three simple procedures can be performed in the office to characterize the vaginal ecosystem : pH determination , whiff test and microscopic examination of Gram stained vaginal discharge[5] . Platz. Christensen et al. have stated: "the occurrence of clue cells and an increased pH of the vaginal fluid were utilized as indications of bacterial vaginosis [16] The clinical consequences of an elevated vaginal pH have been recently amplified.

Women with a vaginal pH > 4.5 and a Gram stain score > 7 on a scale of 0 to 10 were considered to have bacterial vaginosis[8,17] . Hillier et al. reported that bacterial vaginosis is associated with preterm delivery of low – birth – weight infants, independent of other recognized risk factors. Ernest et al.[18] reported that among 115 women at high risk for a low birth weight infant those with a mean vaginal pH > 4.5 had a three fold increased risk of premature rupture of the membranes compared with those with a mean pH $\leq$ 4.5 . These studies along with a study by krohn et al. [8] and the current American college of obstetricians and Gynecologists technical bulletin [3] on vaginitis, all stress the pivotal importance of the vaginal pH level for the diagnosis of bacterial vaginosis . This study supports the fact that the presence of potentially pathogenic bacteria from the vagina result in an elevated vaginal pH (5.0 to 6.5) [3, 4, 7,8,18] . The observation that the presence of *G.vaginalis* precedes the development of bacterial vaginosis warrants consideration of it's treatment even in asymptomatic individuals. In normal fertile women, lactobacilli maintain the normally acidic vaginal pH that protect the vagina against colonization by potential pathogens through several mechanisms . First, the maintenance of a low pH is of direct importance as reported by Stamey et al. [19] . Who observed that colonization of the vaginal introitus with E-coli is rarely noted at a vaginal pH below 4.5 Second , some strains of lactobacilli produce hydrogen peroxide that prevent vaginal colonization with pathogens [20] . Finally , fragments of *lactobacillus* cell walls prevent the attachment of *E-coli* to epithelial cells , perhaps by blocking potential sites of attachment [21] In menopausal women two factors may influence vaginal pH : menopausal status and the presence of potentially pathogenic organisms . Cultures were obtained to adjust for estradiol status and culture results. Therefore it is not surprising that the false – positive rate were high. However, the false – negative rates were 0%, demonstrating that vaginal

pH is still useful. This is supported with caillouette et al. study [17]. For this reason alone vaginal pH should become a routine test during most speculum examinations [17]. Patients during serum estradiol transition or menopause and patient who have become noncompliant as a result of side effects of Hormonal replacement therapy could do self testing for vaginal pH. This could become as a routine examination as self – testing of urine and blood for diabetes, breast self examinations, or self testing of blood pressure for hypertension. The goal is to achieve patient cooperation and compliance, resulting in a vaginal pH of 4.5, with relief of menopausal symptoms and side effects. Hence, exclusion of vaginitis is essential for the vaginal pH to reflect the state of the menopausal vagina. In the current study, both vaginal pH and serum FSH showed similar sensitivity (p = 0.516) in predicting oestradiol levels < 40 pg / ml. Caillouette et al. [17] assessed vaginal pH using Nitrazine pH paper and serum FSH among 172 post menopausal women . The reported sensitivity of vaginal pH in predicting oestradiol status was 88% and the positive predicting value was 96%, which is in accordance with our results. Amal Z Azzam et al. [22] in Alexandria university reported similar sensitivity (p = 0.32) in predicting oestradiol levels < 40 pg/ml, which support our study. Roy et al. [23], reported that in the absence of vaginitis , a vaginial pH > 4.5 indicated menopause and that the sensitivity of vaginal pH (74%) was similar to serum FSH (68%) in establishing the diagnosis of low oestradiol level . Similar results were also reported in the study of Baksu et al.[24] . In the present study, a significant inverse correlation was observed between serum oestradiol level and both vaginal pH and serum FSH, which is in agreement with previous studies [17,23]. Vaginal pH as a marker for the serum estradiol level demands further investigations. The numbers of menopausal women in this report are too small a number to draw definite conclusions. Should this proposition be validated by

larger studies, a powerful screening tool will have been established. This tool could assist the physician or clinical assistant in checking the vaginal estradiol effect and, as a result, establish proper estrogen dosing. In summary a vaginal pH of 6.0 to 7.5, in the absence of potentially pathogenic aerobic bacteria appears to be a reasonable marker of estradiol status for most menopausal patients. A vaginal pH of 5.0 to 6.5 in a well estrogenized patient (premenopausal or menopausal) appears to be a reasonable marker for the presence of potentially pathogenic aerobic bacteria. Further investigations will be required to delineate the appropriate algorithms for selection of patients for culture and antibiotics therapy or hormonal therapy adjustments. In consideration of all that has been said, vaginal pH testing appears to be that cost – effective, "low – tech "diagnostic tool.

CONCLUSION

Measurement of vaginal pH is simple, effective, and inexpensive for screening purpose. A vaginal pH of 4.5 is consistent with a pre menopausal serum estradiol level and the absence of bacterial pathogens. An elevated vaginal pH in the 5.0 – 6.5 range suggest a diagnosis of either bacterial pathogen or decreased serum estradiol . In patients with an elevated pH in premenopausal period vaginal culture should establish the diagnosis . In the absence of bacterial pathogens a vaginal pH of 6.0 to 7.5 is strongly suggestive of menopause .

TABLES

Table (1) Correlation of age group of premenopause with vaginal pH and bacterial pathogen

Age groups Years	No.	Vaginal PH No (%)		Bact. Patho. Growth
		≤ 4.5	>4.5	No(%)
15-20	21	7(33.3)	14(66.7)	15(71.43)
21-30	31	11(35.48)	20(64.15)	24(77.42)
31-40	39	4(10.26)	35(89.74)	32(82.05)
41-45	10	3(30)	7(70)	9(90)

Table (2) : Ph as predictor for positive β -hemolytic streptococci , Klebselia or mixed aerobic organisms in premenopausal women.

	Test + (ph >4.5)	Test – (PH ≤ 4.5)	Total	
Disease +	76	4	80	Sensitivity 95%
Disease -	0	14	14	specificity 100%
Total	76	18	49	

Positive predictive value 100% , false –positive rate 0%, negative predictive value 77%, false –negative rate 23% , efficiency 95%.

Table (3). Correlation of age groups of postmenopausal women with pathogens.

Age groups	No.	Vaginal PH No. (%)		Bact. Patho.Growth
		≤ 4.5	>4.5	
Less than50yrs	21	3(14.29)	18(85.7)	10
51-60yrs	38	2(5.26)	36(94.7)	3
More than 60	22	0(0)	22(100)	1

Table (4) : PH as predictor for positive β -hemolytic streptococci, Klebselia or mixed aerobic organisms in postmenopausal women.

	Test + (ph >4.5)	Test – ($PH \leq 4.5$)	Total	
Disease +	14	0	14	Sensitivity 100%
Disease -	62	5	67	specificity 7%
Total	76	5	81	

Positive predictive value 18% , false –positive rate 82%, negative predictive value 100%, false –negative rate 0% , efficiency 23%.

Table (5) : PH as predictor for estradiol status in postmenopausal women

	Test + (ph >4.5)	Test – ($PH \leq 4.5$)	Total	
Low estrogen ($E_2 < 40 pg/ml$)	53	2	55	Sensitivity 96%
High estrogen ($E_2 \geq 40 pg/ml$)	23	3	26	specificity 11%
Total	76	5	81	

positive predictive value 70%, false-positive rate 30% , negative predictive value 60%, false-negative rate 40%, efficiency 69%.

Table (6) : FSH as a predictor of oestradiol status in Postmenopausal women

	$Fsh \geq 20 mlu/ml$	$Fsh \leq 20 mlu/ml$	Total	
Low estro($E_2 < 40 pg/ml$)	51	1	52	Sensitivity 98%
High estro ($E_2 \geq 40 pg/ml$)	23	6	29	specificity 21%
Total	74	7	81	

positive predictive value 69%, false-positive rate 31% , negative predictive value 86%, false-negative rate 14% , efficiency 70% .

Table (7) Correlation between vaginal pH and FSH; and oestradiol levels in menopausal women

Ostradiol	Vaginal pH	Ostradiol
r-value	-0.166	
p-value	0.069NS	
FSH		
r-value	0.351**	-0.372**
p-value	0.001	0.000

r: Pearson Correlation Coefficient
** Correlation is significant at the 0.01 level

Table (8) Comparisons of Vaginal pH, estradiol and FSH of postmenopausal women according to age groups.

Parameters	Age groups		
	Less than50yrs	51-60yrs	More than60yrs
Vaginal pH	5.548±0.820	6.250±0.795 a(0.002)**	6.659±0.808 a(0.000)**
Oestradiol level(pg/ml)	84.091±26.146	64.761±30.557 a(0.031)*	46.943±31.752 a(0.000)** b(0.018)*
FSH level (mIU/ml)	48.882±27.075	67.887±37.698	73.748±52.794 a(0.044)*

significant at the 0.05 level, ** significant at the 0.01 level, a=differ from group 1, b=differ from group

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