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Correlation of vaginal pH with FSH levels in menopause

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Abstract

Introduction: Menopause is the physiological cessation of menstruation that denotes a normal human developmental phase in the life of woman. Menopause is suspected with age, cessation of menstruation for more than a year and presence of symptoms. The diagnosis of menopause is confirmed by follicle stimulating hormone (FSH) levels >40 IU/L. Few studies have focused on vaginal pH for menopausal diagnosis. It is a simple, noninvasive and inexpensive method for this purpose.

Aims & Objectives: To correlate vaginal pH with FSH level as marker of menopause.

Material and Method: 200 menopausal women attending OPD of the Department of Obstetrics and Gynecology, Government Medical College and Rajindra Hospital, Patiala were selected randomly. Vaginal pH measurement was done using pH strips. A simultaneous blood sample was taken for FSH level measurement which was done using immuno enzymometric assay. Vaginal pH and FSH levels were correlated as markers of menopause.

Results: According to our study, mean age of attaining menopause came out to be 47.42 ± 3.64 years. Mean vaginal pH in our study was 6.13 ± 0.59 and mean FSH level were 55.73 ± 12.89 IU/L. As FSH levels ≥ 40 IU/L is taken as diagnostic cut off value for menopausal status thus, we concluded that a vaginal pH of ≥ 6.00 can be taken as marker for menopausal status in the absence of any vaginal infection. The sensitivity of pH ≥ 6.00 as marker of menopause came out to be 88%

Conclusion: Vaginal pH is simple accurate and cost-effective tool that can be suggested as a suitable and better alternative to serum FSH estimation for diagnosis of menopause.

Keywords: menopause, vaginal pH, FSH levels

Introduction

Menopause is derived from the Greek word 'men' means month and 'pausis' means cessation [1] Menopause is the physiological cessation of menstruation that denotes a normal human developmental phase in the life of woman [2] It is the objective evidence of ovarian failure. in both the follicular and secretory phases of menstrual cycle. It is defined as the time of cessation and ovarian function resulting in permanent amenorrhea. It takes 12 months of amenorrhea to confirm that menopause has set in and therefore it is a retrospective diagnosis [3]. It is not just cessation of menstruation, it is the depletion of ovarian follicles leading to decrease in ovarian hormones [4].

Mean age of menopause is 51.4 ± 3.8 years [5, 6] The estimated mean age of menopause in Indian woman is 46 years which is less than that of Caucasians [7].

Menopause staging (Ankelesaria) [4]

- Stage 1 : From the earliest perimenopausal symptom to menstrual cessation. The stage can last for 3-5 years.
- Stage 2 : Five years after menopause the stage is further divided into:
 - Stage 2a : From the cessation of menstruation upto 1 year The main symptoms of menopause during this stage are vasomotor instability and urethral syndrome.
 - Stage 2b : From the end of stage 2a upto four years.
 - Stage 3 : From 5 year after menopause upto an indefinite period; probably life time. Further divided into:
 - Stage 3a : Atrophic symptoms
 - Stage 3b : Stage of ischemic heart disease.
 - Stage 3c : Osteoporosis
 - Stage 3d : Very late complications like cerebrovascular changes and Alzheimer's disease.

Physiology

During climacteric ovarian activity declines. Initially ovulation fails, no corpus luteum forms and no progesterone secreted by the ovary. Therefore, the premenopausal menstrual cycles are often anovulatory and irregular. Later, graafian follicles also fail to develop, estrogenic activity is reduced and endometrial atrophy leads to amenorrhea. Cessation of ovarian activity and fall in the estrogen and inhibin levels cause a rebound increase in the secretion of FSH and LH by anterior pituitary gland [8].

Hormonal Levels

Shortly after menopause, one can safely say that there are no remaining ovarian follicles. Eventually there is 10-20-fold increase in FSH and approximately a 3-fold increase in LH reaching a maximal level 1-3 years after menopause, after which there is a gradual but slight decline in both gonadotropins. Elevated levels of both FSH and LH at this time life are conclusive evidence of ovarian failure. FSH levels are higher than LH because LH is cleared from the blood so much faster (initial half-lives are about 20 minutes for LH and 3-4 hours for FSH) and perhaps because there is no specific negative feedback peptide for LH like inhibin [9]. The diagnosis of menopause is confirmed by FSH levels >40IU/mL [10]

Vulvovaginal Atrophy

During perimenopause/menopause, less estrogen may cause the tissues of the vulva and the lining of vagina to become thinner, drier, and less elastic or flexible—a condition known as "vulvovaginal atrophy". Vaginal secretions are reduced, resulting in decreased lubrication. Reduced levels of estrogen also result in an increase in vaginal pH, which makes the vagina less acidic, just as it was before puberty [11-14]

Vaginal pH changes

Prepubertal and postmenopausal vaginal mucus membranes possess an atrophic epithelium with a surface pH of 6.0 to 8.0. At puberty, circulating estrogens increase, resulting in proliferation of vaginal epithelial cells. Glycogen is deposited in the intermediate and superficial epithelial cells of the vagina. Lactobacilli proliferate, causing the enzymatic breakdown of cellular glycogen. Lactic acid and hydrogen peroxide are produced, which lowers the vaginal pH to 3.5 to 4.5. This is considered an indicator for a normal, properly estrogenized vagina [15].

Vaginal pH can also be elevated by bacterial vaginosis, cervical mucus, semen, vaginal medication and douches. In the absence of vaginitis, vaginal pH of 6-7.5 strongly suggests menopause.

Aims and Objectives

The present study was conducted with following aims and objectives:

- To study the vaginal pH as marker of menopause.
- To evaluate FSH levels as marker of menopause.
- To correlate vaginal pH estimation with FSH levels as marker of menopause.

Materials and Methods

This prospective study was conducted in the department of Obstetrics and Gynecology of Government Medical Colleges and Rajindra Hospital, Patiala. 200 women attending OPD and fulfilling the inclusion criteria were selected randomly. An informed consent was taken from each woman prior to their enrolment in the study.

Inclusion criteria

- Women with age ≥ 40 years and who had their last menstrual period one year back or more

Exclusion criteria

- Pregnancy and lactational amenorrhoea
- Vaginal medications and douches
- Sexual intercourse within previous three days
- Women on HT
- Genital malignancy.

Those women who had vaginitis were treated and pH was measured after 3 weeks.

A thorough menstrual, sexual, medical and family history was taken including vasomotor complaints, urogenital complaints and psychosocial factors. The findings were recorded in proforma.

Vaginal pH measurement

It was done using pH strips which measures pH across range of 2 to 10.5. Principle: The pH paper is impregnated with the indicator dye Nitrazine (Phenaphthazine). The color of the paper changes from pink at pH 2 to dark blue at pH 10. Changes in color shades occurs with pH increments from pH 2 to 10.5. The pH of vaginal fluid is determined by comparing the color of pH paper that has come in contact with a fluid sample to a standard color chart provided with each roll of pH paper.

Procedure: During vaginal examination, after insertion of a non-lubricated sterile vaginal speculum, 4 cm of the micro range pH strip was applied directly to lateral wall of vagina at lower third of it until it became wet. Color changes of the strip were immediately compared with the calorimetric scale and measurements were recorded. Care was taken to avoid cervical mucus, blood and other substances such as semen and lubricating jelly, known to affect vaginal pH.

Serum FSH level measurement

A simultaneous blood sample was taken for FSH level measurement. Follicle Stimulating hormone (FSH) is a glycoprotein consisting of two subunits with an approximate molecular mass of 35,500 Daltons. The α -subunit is similar to other pituitary hormones (LH, TSH, CG) while the β -subunit is unique. The β -subunit confers the biological activity to the molecule.

Principle: The measurement was done by *Immunoenzymometric Assay*. The FSH levels were measured in IU/L. The results were analyzed at the end of study.

Observations

The mean age of subjects in our study was 52.64 ± 7.57 years with minimum age of 40 years and maximum age of 75 years. Majority of the subjects were from urban area (73.5%). There were 157 (78.5%) subjects who had natural menopause and 43 (21.5%) subjects who had surgical menopause.

The commonest complaint of all the subjects were related to sleep disturbance (28%) followed by muscle/joint pains (26.5%), hot flushes (11%), emotional problems (16%), lethargy (14%) and dyspareunia (4.5%). Majority of subjects in present study were vegetarian by diet (88.0%). Mean age of attaining menopause came out to be 47.42 ± 3.64 years in our study.

The average vaginal pH of subjects in all age groups in the study

was 6.13 ± 0.59 and the distribution of pH value in relation to age is shown in Table No.1. On statistical analysis, the difference regarding pH values in age groups was highly significant

($p < 0.001$). We conclude that as age advances, the pH value increases.

Table 1: Distribution of subjects according to pH value in relation to age

Age (in years)	No. of cases	pH 5 (%)	pH 6 (%)	pH 7 (%)	pH (Mean±S.D.)
40-49	66	14 (21.2)	52 (78.8)	0 (0)	5.79±0.41
50-59	83	10 (12.0)	59 (71.1)	14 (16.9)	6.05±0.53
≥60	51	0 (0)	15 (29.4)	36 (70.6)	6.71±0.46
Total	200	24	126	50	6.13±0.59

The mean FSH levels of subjects in all age groups in the study came out to be 55.73 ± 12.89 IU/L. Only 9% of subjects had FSH < 40 IU/L and majority (91%) had FSH above 40 IU/L. (Table No. 2)

Table 2: Distribution of subjects according to FSH levels in relation to age

Age (in years)	No. of cases	FSH <40 (IU/L)		FSH ≥40 (IU/L)		FSH (IU/L) (Mean±SD)
40-49	66	7	10.6%	59	89.4%	50.16±9.9
50-59	83	11	13.3%	72	86.7%	54.8±13.07
≥60	51	0	0%	51	100%	64.45±11.55
Total	200	18		182		55.73±12.89

The difference regarding mean FSH levels in all age groups came out to be highly significant ($p < 0.001$). When we tried to correlate the serum FSH levels and pH levels with respect to age, we concluded that as age advances, pH values and FSH levels increase. (Table No. 3)

Table 3: Correlation of serum FSH and pH with respect to age

Age (in years)	No. of cases	pH (Mean ± SD)	FSH (IU/L) (Mean±SD)
40-49	66	5.79±0.41	50.16±9.9
50-59	83	6.05±0.53	54.8±13.07
≥60	51	6.71±0.46	64.45±11.55
Total	200	6.13±0.59	55.73±12.89

Table 4: Correlation of vaginal pH with serum FSH levels

No. of cases (%)	FSH (IU/L)	pH (Mean± SD)
18 (9%)	<40	5.67 ±0.59
182 (91%)	≥ 40	6.23 ±0.54

18 subjects (9%) with FSH levels < 40 IU/L had mean pH 5.67 ± 0.59 and 182 subjects (91%) with FSH levels ≥ 40 IU/L had mean pH 6.23 ± 0.54 . As serum FSH levels ≥ 40 IU/L is taken as diagnostic cut off value for menopausal status. So, from above findings we conclude that a vaginal pH of ≥ 6.00 can be taken as marker for menopausal status. (Table No. 4). The overall sensitivity of FSH ≥ 40 IU/L as indicator of menopause was found to be 91% and pH ≥ 6.00 as indicator of menopause was found to be 88%.

Discussion

Many menopausal women experience irregular menses and array of symptoms long before they meet the definition of menopause. Cessation of ovarian activity and fall in estrogen levels cause rebound increase in FSH levels. The low estrogen levels leads to atrophic vagina and rise in vaginal pH. During menopause, two factors influence vaginal pH: menopausal status and the

presence of potentially pathogenic organisms. Therefore, exclusion of vaginitis is essential for the vaginal pH to reflect the status of menopausal vagina. In our study we have measured pH only after treating the vaginal infection.

Table 5: Comparison of age at menopause

Various authors	Mean age of menopause (in years)
Madhukumar S <i>et al.</i> (2012)	49.7
Borker SA <i>et al.</i> (2013)	48.26
Panda S <i>et al.</i> (2014)	47.8 ± 4.1
Singh A <i>et al.</i> (2014)	46.24 ± 3.38
Present study	47.42 ± 3.64

In the present study the mean age of attainment of menopause was 47.42 ± 3.64 which is comparable to the studies done by Madhukumar S *et al.*, Borker SA *et al.*, Panda S *et al.* and Singh A *et al.* [16-19] (Table No. 5)

Table 6: Vaginal pH value in different studies

Various authors	No. of patients	Age Group (in years)	Mean Vaginal pH
Yoruk P <i>et al.</i> (2006)	50	47-70	5.9 ± 0.95
Moradan S <i>et al.</i> (2010)	200	45-65	6.6 ± 0.5
Panda S <i>et al.</i> (2014)	173	31-60	5.3 ± 0.7
Present Study	200	40-75	6.13 ± 0.59

The mean pH of subjects in the present study was 6.13 ± 0.59 comparable to studies done by Yoruk P *et al.* [20] and Moradan S *et al.* [21] but is different from study conducted by Panda S *et al.* [18] because it included subjects with comparatively earlier age group. (Table No. 6)

Table 7: Serum FSH levels in various studies

Various studies	No. of cases	Age Group (in years)	Mean FSH levels (in IU/L)
Yoruk P <i>et al.</i> (2006)	50	47-70	53.3 ± 32.8
Vahidroodsari F <i>et al.</i> (2010)	103	31-95	80.79 ± 6.42
Panda S <i>et al.</i> (2014)	173	31-60	46.5 ± 11
Present Study	200	40-75	55.73 ± 12.89

The mean FSH of subjects in the present study was 55.73 ± 12.89 IU/L which is similar to study done by Yoruk P *et al.* [20] and comparable to study by Panda *et al.* [18] and lower than the study done by Vahidroodsari *et al.* [22] as he took women of later age group (till 95 years) as well.

Conclusion

We observed from our study that as age advances, vaginal pH value and serum FSH levels increase. As FSH levels ≥ 40 IU/L is taken as diagnostic cut off value for menopausal status, so we

concluded that vaginal pH of >6.00 can be taken as marker for menopausal status. It is a simple, accurate and cost-effective tool that can be suggested as a suitable alternative to serum FSH measurement for the diagnosis of menopause.

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