Original Study

Study of Estradiol, Testosterone and DHEA-S Status in Women After Surgical Menopause

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Abstract

Aims and Objectives : The study was done to assess the hormones namely Estradiol, Testosterone, and Dehydroepiandrosterone-sulphate (DHEAs) one day before operation and on ninth post-operative day following surgical menopause. *Materials and Methods :* This is a cross sectional observational study. The study was done amongst the thirty four women aged between 40-48 years with functioning uterus and at least one ovary, not using any exogenous hormone preparations affecting ovarian function for last three months and having at least one menstrual period in three previous months were included in this study. They had under gone hysterectomy with bilateral salpingo-oophorectomy due to non-ovarian pathology. Fasting venous blood samples were taken one day before operation and on ninth post-operative day of surgical menopause Serum concentration of estradiol, testosterone, and DHEA-S were determined. *Results :* The circulating estradiol level decreased significantly ($p = 0.043$) from 161 pg/ml pre-operatively to 108 pg/ml. on ninth post-operative day after surgical menopause. In spite of reduction in mean testosterone level from 0.11 ng/ml. to 0.09 ng/ml. following surgical menopause, which is statistically insignificant ($p = 0.247$).There was no significant difference between the serum DHEA-S level before and after surgical menopause. A significant positive correlation was observed between pre-operative circulatory levels of DHEA-S with that of estradiol while there was absence of any significant co-relations between any of the other pairs of values. *Conclusion :* The circulating estradiol level decreased significantly on ninth day after surgical menopause and significant positive correlation between pre-operative circulatory levels of DHEA-S with that of estradiol, but there was no significant co-relation between post-operative circulating estradiol with that of DHEA-S. Testosterone did not show any significant relation with estradiol neither in pre-operative period nor in post-operative condition.

Keywords

surgical menopause, estradiol, testosterone, DHEA-S

Introduction

Menopause is defined as permanent cessation of menstruation occurs at the average age of 52 yrs (Massachusetts study) and diagnosed retrospectively after a minimum of 1 yr. of amenorrhoea. Most Indian studies show the age of menopause to be 48 yrs. This is not a central event but rather primary ovarian failure. When both the ovaries are removed during hysterectomy those hormones get suddenly interrupted and their levels fall resulting in symptoms of early menopause[1]. This is termed as surgical menopause. Surgical menopause occurs in women who have not yet had natural menopause, women who had surgery on their ovaries or have had a hysterectomy, despite retention of their ovaries, experiencing early menopause. After menopause symptoms appear in

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almost all women. Principal health concerns of menopausal women include vasomotor symptoms uro-genital atrophy, osteoporosis, cardiovascular disease, cognitive decline and sexual problems. Till today in most cases menopause is diagnosed retrospectively based on menstrual history and age without relying on laboratory status[2].

Surgical menopause differs from natural menopause. The physiological changes which result in final menstrual period (FMP) can start 10 yrs prior to this. Hormonal changes continue long after the FMP. This episode of dynamic neuro-endocrine change is characterized by 'the climacteric' from the Greek ward 'Klimax'-ladder that is the climb to the menopause. But in case of surgical menopause the hormonal profile changes abruptly experienceing menopausal symptoms more intensely. Such women face more severe hot flushes, which are more, frequent and last longer. These women have also been documented to be at increased risk of heart disease and osteoporosis[3]. Women with surgical menopause are younger than with natural menopause. They feel more depressed than other women. There are no certain documented reasons for these severities. Further, women with surgical menopause are still recovering from a major surgery when it begins. Thus they have to heal both physically and mentally to adjust to it.

After surgical menopause some women may be given estrogen immediately after surgery in order to prevent the changes, especially hot flushes. However, use of estrogen is controversial and is not recommended for women with cardio-vascular disease or with a high risk of developing cardiovascular disease. After surgical menopause in young women libido is decreased. In addition she suffers from depression, headache and lethargy. Thus came the replacement with testosterone, which can alleviate these symptoms. This improves quality of life (as per recommendations of the International Menopause society). Androgen co-therapy with estrogen should be administered at the lowest does for the shortest time that meets the treatment goal.

Therefore, it is very much pertinent to have a study to assess the sex steroid hormonal concentration namely estradiol testosterone and dehydroepiandrosterone – sulphate (DHEA-S) before and after oophorectomy in surgically menopausal women.

With this idea based on available literature and having permission from Institutional Ethics Committee and informed written consent from the patient I assessed sex steroid hormones namely testosterone, estradiol and DHEA-S concentration one day before and on ninth post operative day of surgical menopause.

So the aim of the study was to assess the sex steroid hormones estradiol, testosterone and DHEA-S before and after in oophorectomy in surgically menopausal women and to compare their concentration and to find out whether there was any association exists between circulating estradiol with testosterone and DHEA-S levels.

Materials and Method

This was a cross sectional observational study. I included the patients who attended Gynaecology outpatient department of Sambhu Nath Pandit Hospital, Kolkata - 700 020 on Wednesday for one year period from Oct 2009 to Sept 2010. The concentration of circulating steroid hormones were determined at Biochemistry Laboratory of Medical College & Hospitals, Kolkata.

Women with age 40-48 yrs with functioning uterus and at least one ovary, not using exogenous hormone preparations affecting ovarian function for last 3 months and having at least one menstrual period in the three previous months were included in the study. These patients had undergone hysterectomy with bilateral oophorectomy due to fibroid uterus, adenomyosis, endometriosis or chronic pelvic pain.

Those patients taking any hormonal preparation due to any cause or having ovarian pathology excluded from the study. Sample size – thirty four patients were included in this study.

The necessary permission from hospital ethics committee was obtained for the study. The procedure of study was explained in details to patients individually. Valid written informed consent was obtained from each patient prior to inclusion in study.

Morning fasting 5ml venous blood sample was collected one day prior to oophorectomy from antecubital vein without any additives by an acceptable venepuncture technique as first sample. The sample was allowed to clot at room temperature. After separating serum if the specimen was not tested immediately, it was refrigerated at 2°C – 8°C. If storage period was anticipated more than three days, the

specimen was frozen at 20°C avoiding repeated freezing – thawing of the samples. As the specimen containing precipitate may give inconsistent test results, such specimen was centrifuged and any sample demonstrating gross haemolysis or turbidity was discarded. Another blood sample was collected in a same manner as stated above on 9th post-operative day following oophorectomy of the same women.

The samples were tested for estradiol, testosterone, and DHEA-S at Biochemistry Laboratory of Medical College & Hospitals, Kolkata. **ESTRADIOL** – Quantitative measurement of estradiol in sample serum was done by EIAgen estradiol kit which is a direct solid phase enzyme immunoassay in TECAN SEMI AUTOMATED ELISA READER.

TESTOSTERONE – Testosterone was determined in TECAN SEMI AUTOMATED ELISA READER in a solid – phase enzyme immunoassay with Microwell testosterone EIA Kit by Syntron Bioresearch, Inc., 2774 Loken Average West Carisbad,CA92008,USA.

DHEA-S – Sulphated form of dehydroepiandrosterone i.e., DHEA-S was determined in serum by direct immuno-enzymatic assay by EQUIPAR diagnostic kit code no. 74050A. The machine used was TECAN SEMI AUTOMATED ELISA READER.

All reagents, standards and samples were brought at room temperature before the analysis.

The serum concentration of the hormones namely estradiol, testosterone, and DHEA-S – were obtained both preoperatively one day before operation and on 9th post operative day of the same patient.

The study end point was summarized by standard descriptive statistics with SPSS software.

Results

The data was collected tabulated and analysed for various parameters and compared.

Table 1
Mean, Standard Deviation (SD) and p value of Estradiol, Testosterone, and DHEA-S in pre-operative and post-operative conditions

Hormones	Mean ± SD		p value
	Pre operative (n=34)	9th day Post operative (n=34)	
Estradiol pg/ml	161.2 ± 85.4	107.9 ± 55.77	0.043*
Testosterone ng/ml	0.11 ± 0.11	0.09 ± 0.10	0.247
DHEA-S µg/ml	1.43 ± 0.81	1.18 ± 0.83	0.101

* Statistically significant.

Table 2
Correlation coefficient and level of significance of testosterone, and DHEA-S with estradiol in preoperative and postoperative conditions.

Co-relation with estradiol	Pre operative	9th day post operative
Testosterone (ng/ml)		
Pearson correlation co-efficient	0.238	0.380
Significance	0.285	0.073
DHEA-S (µg/ml)		
Pearson correlation co-efficient	0.482	- 0.029
Significance	0.020*	0.894

* Statistically significant.

Discussion

The present study was undertaken to find out the change in levels of circulatory estradiol, testosterone, and DHEA-S in women undergoing surgical menopause and to assess whether any association exists between circulating estradiol with Testosterone and DHEA-S.

Several studies showed that there were a significant drop in circulating estradiol after surgical menopause[4]. Korse CM *et al* have demonstrated that this drop in serum estradiol level is more rapid in oophorectomized women than those in natural menopause[5]. Significantly they found that mean estradiol levels declined 1.4 pmol/l per year in menopausal women, but the estradiol level declined @ 11.1 pmol/l in women with surgical menopause.S. Muttkrishna *et al* in their study on ovarian pituitary feedback loop in women demonstrated that serum estradiol level fell rapidly within first six hours of surgery and then stabilized after ward until the end of the study period of three days[6].Similar results from other studies demonstrated that oophorectomy results in a sudden drop in circulating endogenous estrogen, the primary source of it being the ovary[5].

Baglietto L *et al* reported that fat mass may increase estrogen concentration through aromatization in peripheral tissues in post-menopausal women [7]. Langhlin Ga *et al* in the Rancho Bernardo study observed that total estradiol levels to be lower in bilaterally oophorectomized women[8]. In the present study circulating estradiol level decreased significantly ($p<0.05$) from 161pg/ml pre-operatively to 108 pg/ml on the ninth day after surgical menopause. The findings of the present study thus corroborates well with the observations of previous studies. Alteration in circulating level of testosterone after surgical menopause was investigated in the present study. In this study in spite of a reduction in mean testosterone level from 0.11 ng/ml to 0.09 ng/ml, this decrease was found to be statistically insignificant ($p>0.05$). This apparently contradicted with findings from other studies. Kores C M *et al* have observed a decrease in testosterone level in oophorectomized women but not in naturally menopausal women[9]. They have attributed it to the post menopausal activity of the ovaries continuing to secrete testosterone even after cessation of estradiol secretion during natural menopause. They surmised that these results demonstrate that post menopausal ovary remains an ongoing site of testosterone production throughout the lifespan of older women. Other studies investigated the effect of extraneous factors on testosterone levels in postmenopausal women. Chan MF *et al* have found that physical activity levels were inversely associated with circulating concentration of testosterone, post-menopausal women with greater physical activity seems to have lower endogenous testosterone concentration[10]. The absence of any significant decrease in circulating testosterone level before and after surgical menopause observed in the present study may be attributed to the presence of certain confounding variables described to influence serum testosterone. They include the range of physical activity of the subjects, race and ethnicity or due to use of contraceptive hormonal preparations. Further stratification of the study population along these lines would have been helpful. It may be surmised that though, after surgical menopause, the ovaries cease to be the site for testosterone secretion, circulating testosterone levels may not alter significantly due to its synthesis and secretion by fat cells from adrenal hormones. Whereas several reports indicated that the post menopausal ovary in naturally menopausal women had the ability to secrete androgens[11,12,13]. Couzinet *et al* recently demonstrated that in the absence of adrenal function, postmenopausal women averaging 12 years after menopause had no detectable circulating androgens and that their post menopausal ovaries were devoid of gonadotropin receptors and steroidogenic enzymes. These observations suggest that post menopausal ovary as early as five years after menopause is not a source of androgens[14]. The parallel changes in DHEAS and testosterone reported by Lasley BL *et al* supported the concept that testosterone was derived from an adrenal, not from ovarian source and suggested that the adrenal might be a primary source of androgen production after menopause. They postulated that ethnicity, psychosocial, lifestyle and genetic factors might influence possible adrenal weak androgen axis activation during the latter stages of the menopausal transition and into post menopause[15]. Alteration in circulating DHEA-S level has been investigated. Sheideler *et al*[16] reported a failure of DHEA-S to exhibit a persistent decline during menopausal transition using the non-human primate as a model for the human menopausal transition. Earlier reports from animal models, parallel to reports in human populations, were either cross-sectional or did not focus on either gender or ovarian stage-specific differences[17]. Cross sectional data had revealed an increased variance in circulating DHEA-S in older female but not in male. When Shiedeler *et al* analyzed

longitudinal data from daily profiles of urinary hormone metabolites to explain the increased variance, there was a rise in DHEA-S during menopause transition. Thus these observations suggest a rise in DHEA-S concentration during menopausal transition. DHEA-S seems to be converted primarily to testosterone and androstenedione in both men and women[18]. Lasley *et al*[15] have observed concordant circulating DHEA-S levels with testosterone in terms of age-related patterns of decline. This suggested that DHEA-S production is associated with testosterone production via peripheral conversion in older women. The present study was undertaken with the hypothesis that circulating DHEA-S might be elevated after surgical menopause. It was however observed that there was no significant difference between the serum DHEA-S level before and after surgical menopause. This finding has to be evaluated in the light of other factors described to be modulating the DHEA-S level. One such factor which seemingly modified DHEA-S was Body Mass Index (BMI). In general higher BMI have been found to be associated with lower DHEA-S levels[19]. Lifestyle differences, including smoking, could also account for variation in the pattern of circulating DHEA-S. Other studies have reported the effect of stress in influencing the pattern of DHEA-S expression[20,21,22]. In the present study, perceived stress of surgical menopause might have presumably played a significant role in modulating DHEA-S expression. Also, ethnic difference among the subjects might be contributing factor for variability of DHEA-S in the menopausal women. In this respect, it is also pertinent to note that McTiernan A *et al* observed higher DHEA-S concentration was associated with use of non-steroidal anti-inflammatory agents (NSAIDs)[23], a drug which might, at any rate, be presumed to have been used at least in a subgroup of surgical menopause patients for relief of post-operative pain.Assessment of correlations, if any, between circulatory estradiol and that of testosterone, and DHEA-S was performed in the present study. A significant positive correlation was observed between preoperative circulatory levels of DHEA-S with that of estradiol, while there was absence of any significant correlations between post-operative circulating estradiol with that of DHEA-S. More over the other hormone estimated in the study such as testosterone did not show any significant relation with estradiol neither in pre-operative period nor in post-operative condition.Estradiol is synthesized directly from testosterone by peripheral aromatization in both males & females. The immediate precursor of testosterone is androstenedione which itself is synthesized from DHEA and not DHEA-S. DHEA-S is basically an adrenal originated hormone. This indicates that the positive correlation observed is a normal physiological phenomenon.Theca cells, which are present in the ovary are the source of androstenedione and testosterone. After removal of ovary there is a loss in supply of androstenedione synthesized from DHEA in the ovary, resulting in loss of correlation which was existant in the pre-oophorectomized state.

Conclusion

The study was done to observe the alteration in levels of circulatory estradiol, testosterone and DHEA-S in women undergoing surgical menopause and further to assess whether any association exists between circulating estradiol with testosterone and DHEA-S.In this study circulating estradiol level decreased significantly (p=0.043) from 161 pg/ml preoperatively to 108 pg/ml on the ninth day after surgical menopause. This finding corroborates well with previous observations done by several workers.The circulating mean testosterone level also decreased from 0.11 ng/ml to 0.09 ng/ml. But this decrease was found to be statistically insignificant (p=0.247). This was probably due to physical activity of the subjects, race and ethnicity. Further study along this line may be helpful. After surgical menopause ovarian production of testosterone ceases, the circulating testosterone levels may not alter significantly due to its synthesis and secretion by the fat cells from adrenal hormones. There was no significant difference of circulating DHEA-S level before and 9th day after surgical menopause. Body mass index (BMI), smoking habit or the stress due to surgery itself may have presumably played a significant role in modulating the DHEA-S expression. Also use of NSAIDS (Non steroidal anti inflammatory drugs) following operation may take part in non-significant difference of circulating DHEA-S before and after 9th postoperative day of surgical menopause. Those factors may be included in future study. A significant positive co-relation was observed between pre operative circulatory levels of DHEA-S with that of estradiol, but there was no significant co-relation between post-operative circulating estradiol with that of DHEA-S. Moreover, the other hormone estimated in the study such as testosterone did not show any significant relation with estradiol neither in pre-operative period nor in post-operative condition.

PATIENT'S PROFILE AND COLLECTION OF DATA

Name : ……. Age : ……..…….
Marital status : …….. Parity : ……..……..
Social status …
Address ……..……
……
Date of admission : ……
Chief complaint : ……...
H/O Present illness : ……..
Menstrual History: a) Age of onset of first period ……
b) Regularity of cycle …… c) duration ……
d) Length of cycle …..…… e) L M P ……..……..
Obstetric history: No. of living children Boys …..…. Girls ……
Immunization : …… Last issue : ……..……
Pregnancy events : ……..……...……
Method of delivery : ……..……
Puerperium : ……..……
Abortion : ……..… Spont : …..…… Induced : ……..……
Past medical history : ……
Past surgical history : ……
Family history : ……
Personal history : ……...……
EXAMINATION : GEN. & SYST ……..….. Built ……..……
Nutrition : ……..…… Stature : ……..……. Pallor : ……
Jaundice : …….. Teeth, gum & tonsil : ……
Neck glands : …….. Edema : ……
CVS & Resp. System : ……..……
Pulse : …….. BP : ……..……
GYNAE. Examination : ……..… Breast : ……
Abdominal. Inspection : ……...……
Palpation : …….. Percussion : ……..……
Auscultation : ……
PELVIC Examination : …… Inspection : ……
Vaginal Examination : ……...……
Rectal examination : ……..……
Recto vaginal examination : ……..……
INVESTIGATIONS : ……
Operation contemplated : ……..……
Postoperative period : ……
Date of collection of blood : ……
Pre operative period : ……
Post operative period : ……..……

References

1. Siddle N., Sarrel P., White head M. — The effect of hysterectomy on the age at ovarian failure, identification of a subgroup of women with premature loss of ovarian function and literature review. *Fertil Steril*. **47**:94-100, 1987.
2. Burger H.G., Dudby E.C., Hopper J.L., *et al*. — Prospectively measured levels of follicle stimulating hormone, estradiol and dimeric inhibins during the menopausal transition in a population based cohort of women. *J. Clin. Endocrinol Metab*. **84**;4025-4030, 1999.
3. Dewhurst's Textbook of obstetrics and Gynaecology. 7th edition.
4. Al-Azzawi, Palacios S. — Hormonal changes during menopause. *Maturitas*. **63**(2) : 135-137. E pub 2009, Apr 15, Jun 20, 2009.
5. Abu-Taha M., Rins C., Hermenegildo C. *et al*. — Menopause and ovariectomy cause a low grade of systemic inflammation that may be prevented by chronic treatment with low doses of oestrogen or Losartan. *J Immunol*. **183**(2) : 1393-1402 E pub. 2009 Jun 24, Jul 15, 2009.
6. S. Muttukrishna, S. Sharma, D.H. Barlow *et al*. — Serum inhibins, estradiol, progesterone and FSH in Surgical menopause: a demonstration of ovarian pituitary feed back loop in women – *Human Reproduction*. Vol **17**, No. 10 pp 2535-2539, 2002.
7. Baglietto L., English D.R., Hopper J.L. *et al* — circulating steroid hormone concentrations in post menopausal women in relation to body size and composition *Brest Cancer Res Treat*. May 29, 2008.
8. Langhlin G.A., Barrett – Contrer E., Kritz-silverstin D. *et al*. —Hysterectomy, oophorectomy, and endogenous sex hormone levels in older women, the Rancho-Bernardo study, *J. Clin. Endocrinol. Metab*. **85**; 645-651, Feb. 2000.
9. Korse C.M., Bonfrer J.M., Von Beurden M. *et al*. — Estradiol and testosterone levels are lower after oophorectomy than after natural menopause – Tumour Biol. 2009; 30(1) : 37-42 E pub. 2009 Feb. 5.
10. Chan M.F., Dowsett M., Folkerd E. *et al*., usual physical activity and endogenous sex hormones in post-menopausal women; The Europian prospective investigation into cancer – Norfolk population study. *Cancer Epidemiol. Biomarkers prev*. **16**(5); 900-905, May 2007.
11. Longcope-C. — Metabolic and blood production rates of estrogen in post-menopausal women. *Am. J. Obstet. Gynecol*. **111** : 778-781, 1971.
12. Vermeulen A. — The hormonal activity of the post-menopausal ovary. *J. Clin. Endocrinol. Metab*. **42** : 247-253, 1976.
13. Adashi E.Y. — The climacteric ovary as a functional gonadotropin-driven androgen-producing gland. *Fertil Steril*. **62** : 20-27, 1994.

14. Conzinet B., Meduri G., Lecce M.G. *et al.* — The Post-menopausal ovary is not a major androgen producing gland. *J. Clin. Endocrinol Metab.*, **86**; 5060, 2001.
15. Lasley B.L., Santoro N., Randlof J.F. *et al.* — The relationship of circulating dehydroepiandrosterone, testosterone and estradiol to stages of the menopausal transition and ethnicity, *J. Clin. Endocrinol. Metab.* **87**;3760, 2002.
16. Shideler S.E., Gec N.A., Chen J. — Estrogen and progesterone metabolites and follicle stimulating hormone in the aged macaque female. *Biol Reprod.* **65** : 1718-1725, 2001.
17. Kemmitz J.W., Roecker E.B., Haffa A.I.M. *et al.* — Serum dehydroepiandrosterone sulphate concentration across the life span of laboratory-housed rhesus monkeys. *J. Med. Primatol.* **29** : 330-337.
18. Wolf O.T., Neumann O., Hellhammer D.H. *et al.* — Effects of a two week physiological dehydroepiandrosterone substitution on cognitive performance and well-being in healthy elderly men and women. *J. Clin. Endocrinol Metab.* **82** : 2363-2367, 1997.
19. Davison S.L., Bell R., Donath S. *et al.* — Androgen in adult females; changes with age, menopause and oophorectomy – *J. Clin. Endocrinol Metab.* **90**(7); 3847-3853 E pub. Apr. 12, 2005, July, 2005.
20. Bromberger J.T., Meyer P.M., Kravitz H.M. *et al.* — Psycho logic distress and natural menopause, a multiethnic community study. *Am. J. Ablic Health.* **91** : 1435-1442.
21. Gold E.B., Stemfeld B., Kelsey J.L. *et al.* — The relation of demographic and life style factors to symptoms in a multi-ethnic population of 40-55 year old women. *Am. J. Epidemiol.* **152** : 463-473.
22. Gold E.B., Bromberger J., Crawford S. *et al.* — Factors associated with age at menopause in a multi-ethnic population of women. *Am. J. Epidemiol.* 865-874, 153.
23. Mc Tiernan A., Wu L., Barnabei V.M. *et al.* — Relation of demographic factors, menstrual history, reproduction and medication use to sex hormone levels in post-menopausal women – *Breast Cancer Res. Treat.* **108**(2) : 217-231, Mar 2008. E pub. May 22, 2007.

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