Reproductive Hormonal Profile of Post-menopausal Women in Ebonyi State, Nigeria

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Authors’ contributions

“This work was carried out in collaboration between all authors. Author ECO designed the study, managed the analysis and literature searches and wrote the first draft of the manuscript; author DU performed the statistical analysis, edited the manuscript and managed the literature searches. Authors ABI, OLM, OGO, US, OJC, IIC, UMOA and AA assisted in the analyses of the study and the literature searches. All authors read and approved the final manuscript.

ABSTRACT

Background: Assessment of the concentrations of reproductive hormones during post-menopause has been suggested as a confirmatory test for menopause due to

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irregularities in rise and fall of sex hormones in menopausal transition. In this study, we assessed the concentrations of estrogen, progesterone, follicle stimulating hormone (FSH) and luteinizing hormone (LH) in post-menopausal women in Ebonyi state, Nigeria. In addition we tried to establish if relationships exist between the pituitary hormones and the ovarian (sex) hormones and between the reproductive hormones and age and body mass index (BMI).

**Method:** The study population comprised forty post-menopausal women of mean age 59.6 years and forty young female adult controls of mean age 27.8 years. Blood samples were collected into heparin container and the serum used for the analysis of estrogen, progesterone, FSH and LH using ELISA method.

**Result:** Post-menopausal women had significantly (P<0.001) higher mean serum FSH and LH concentrations compared to their controls. In contrast, the control group had significantly (P<0.001) higher progesterone and estradiol levels compared to the post-menopausal women. There was inverse correlation (P<0.01) between estradiol and FSH levels, but no significant correlations were observed between FSH and Progesterone; LH and Progesterone; and LH and Estradiol respectively. In addition, after adjusting for BMI, the concentrations of LH was significantly associated with age (P<0.01) but FSH, estradiol and progesterone levels were not. Furthermore, estradiol and FSH levels were significantly associated (P<0.001 and P<0.05) with BMI after controlling for age.

**Conclusion:** The present findings underscore the importance of understanding the reproductive hormonal profile of post-menopausal women and the need to consider age and body mass when studying hormonal changes of menopausal women. These data may help clinicians make optimal therapeutic decisions for hormone replacement therapy and life-style changes that may reduce the risk of some of the conditions associated with menopause.

**Keywords:** Follicle stimulating hormone; luteinizing hormone; progesterone; estradiol; menopause; post-menopause.

1. **INTRODUCTION**

Menopause is the cessation of monthly cycle. It announces the end of reproductive phase of a woman's life when the ovaries start decreasing their production of estrogen and progesterone on a permanent basis [1]. Hormonal fluctuation starts about 2 years before menopause and stabilizes from 12 months-2years post-menopause [2,3,4]. Post menopause can be defined as cessation of menstrual flow for a period of 12 months and above [2]. It is therefore more appropriate to declare a woman as menopausal at post-menopause stage. This is because menstrual cycles are usually extremely erratic at early stage thus making hormonal assessment at the early stage of menopause futile. The assessment of reproductive hormones at postmenopausal stage therefore would serve as a confirmatory test for menopause.

Epidemiological studies have reported hormonal involvement in sexual dysfunction during post-menopause. During this period of a woman’s life, there is a decline in sexual interest accompanying relative decline in production of ovarian hormones [5]. A prolonged deprivation of estrogens has been associated with cervical atrophy, reduced production of mucus, atrophic changes, and reduced vaginal lubrication, which can bring about dyspareunia, vaginitis, or vaginismus. These changes can reduce sexual satisfaction and lead to secondary sexual dysfunctions [6].
Changes in sensory perception, systemic blood flow and muscular contractility, are among
the extragenital manifestations of postmenopause. A decline in the production of androgens
often negatively affects sexual desire, sexual fantasies, excitation, and sexual satisfaction
[6-10]. Furthermore, a decline in estrogen levels has been implicated in other changes such
as brittle nails, thinning of the skin, hair loss and generalized aches and pains [11]. The
factors that contribute to the sexual changes that take place during climacterium, and the
suspected role of hormones in these changes, have not yet been elucidated [12,13].

Serum sex hormones have also been linked with the risk of several diseases in
postmenopausal women. For example, estrogen deficiency has been linked with coronary
artery disease, stroke and peripheral vascular disease due to vascular endothelial
dysfunction [14]; osteoporosis, and breast and endometrial cancer [15,16,17]. The role of
endogenous sex hormones in these disease conditions makes the understanding of factors
that influence levels of these hormones increasingly important. This therefore calls for more
studies to elucidate the roles reproductive hormones and other factors such as age and body
weight play in the postmenopausal stage of a woman's life.

The endocrine feedback loops that provide for integrated function among the organs of the
hypothalamic-pituitary-gonadal axis are paramount to reproductive potentials in reproductive
aged women [18]. However, the interactions between the pituitary hormones and the ovarian
hormones during the post-menopause stage are not well defined. Similarly there is paucity of
information on the hormonal profile of post-menopausal Nigerian women. Furthermore, we
believe that the roles which reproductive hormones and other factors such as age and body
weight play in the postmenopausal stage of a woman's life need to be elucidated. In view of
these facts, we aimed at estimating the concentrations of reproductive hormones—estrogen
(estriol), progesterone, follicle stimulating hormone and luteinizing hormone in
postmenopausal women in the present study. In addition we tried to establish if there are
significant interrelationships between the pituitary hormones and the ovarian (sex) hormones
on one hand, and whether relationships exist between these reproductive hormones and age
and body mass index (BMI) on the other hand.

2. METHODOLOGY

2.1 Subjects

The study population comprises forty apparently healthy post-menopausal women (mean
age of 59.6 years) and forty healthy reproductive controls (mean age of 27.8 years). These
participants were randomly selected from residents of Onicha Local Government Area of
Ebonyi State, Nigeria. Subjects were selected based on the results of a structured
questionnaire, body mass index measurement (computed as weight in kilogram divided by
height in meters squared), resting blood pressure measurements and blood chemistry tests.
Subjects were included in the study if they were non-smokers, non-alcoholics, non-obese
(BMI < 30 Kg/m²), non-diabetics, non-hypertensive, apparently without chronic systemic
disorders or secreting pituitary tumors and not taking medications that could affect hormonal
functions or taking hormonal treatment in the previous three months. Their consents were
sought, ethical issues were referred accordingly and they participated voluntarily. Withdrawal
at any time was permitted. The post-menopausal group included those within 12 months and
above into menopause, while the control group included those not menstruating or ovulating
at the time of sample collection and who were sure of their last menstruation date. The
ovulation period was then calculated based on the date of when the last menstruation started (usually between 12-16th day). Since all women do not follow similar menstrual pattern, we allowed a space of 11-21st day of their last menstrual period to accommodate variations in menstrual patterns. Women who were not up to 12 months into menopause were excluded from the postmenopausal group, while reproductive women who were not sure of the date of their last menstrual period (LMP) were excluded from the control group.

2.2 Sample Collection and Laboratory Analysis

5ml of blood was collected into a heparin bottle, centrifuged and the serum was used for laboratory analysis. The serum samples were obtained and analyzed during morning hours. The entire hormonal assay was done using the principle of peroxidase-conjugated sandwich ELISA (enzyme linked immunosorbent assay) by Diagnostic Automation, Inc, Calabasas. The final colour developed was measured spectrophotometrically at 450 nm using IPHIPPERION-3.0

2.3 Statistical Analysis

Data was expressed as mean and standard deviation. Comparative analysis involving two continuous variables was done using independent sample T-test and analysis of covariance (ANCOVA). Correlation between two variables was done using multivariable linear regression analysis. Statistical significance was set at p<0.05. All statistics were done using SPSS for windows (version 16.0)

3. RESULT

Table 1 shows the age and anthropometric measurements of the post-menopausal women and their controls. Independent sample t-test indicated that postmenopausal women were significantly (P<0.001) older than the controls. Similarly, postmenopausal women indicated higher body weight and body mass index compared to their controls. No significant difference was observed in height of both groups.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Control (n=40)</th>
<th>Post-menopausal women (n=40)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>27.80 ± 5.83</td>
<td>59.60 ± 8.41</td>
<td>0.000</td>
</tr>
<tr>
<td>Height (meters)</td>
<td>1.69 ± 0.07</td>
<td>1.70 ± 0.07</td>
<td>0.431</td>
</tr>
<tr>
<td>Weight (Kg)</td>
<td>57.63 ± 5.68</td>
<td>64.73 ± 8.47</td>
<td>0.000</td>
</tr>
<tr>
<td>Body Mass Index (Kg/m2)</td>
<td>20.05 ± 1.08</td>
<td>22.23 ± 2.74</td>
<td>0.000</td>
</tr>
</tbody>
</table>

Table 2 indicates the mean serum progesterone, estradiol, follicle stimulating hormone and luteinizing hormone concentrations in post-menopausal women and their normal control. Independent sample t-test indicated that post-menopausal women had significantly higher (P<0.001) mean serum FSH and LH concentrations but significantly lower (P<0.001) progesterone and estradiol levels compared to their controls. The significant differences observed between postmenopausal women and their controls persisted even after controlling for age and BMI using analysis of covariance (ANCOVA). Our data further revealed that postmenopausal women had progesterone level declined by 74%, estradiol
level decreased by 93%, FSH increased by 86% while LH increased by 81% compared to the control subjects.

Table 2. Reproductive hormone levels compared between post-menopausal women and their controls

<table>
<thead>
<tr>
<th>Variables</th>
<th>Control</th>
<th>Post-menopausal women</th>
<th>P-value (unadjusted)*</th>
<th>P-value (adjusted) **</th>
</tr>
</thead>
<tbody>
<tr>
<td>Progesterone (ng/ml)</td>
<td>1.9 ± 2.23</td>
<td>0.5 ± 0.41</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>Estradiol (Pg/ml)</td>
<td>111.1 ± 14.4</td>
<td>7.7 ± 3.76</td>
<td>0.000</td>
<td>0.001</td>
</tr>
<tr>
<td>FSH (MIU/ml)</td>
<td>9.6 ± 4.43</td>
<td>68.1 ± 40.62</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>LH (IU/L)</td>
<td>10.2 ± 6.16</td>
<td>53.1 ± 11.95</td>
<td>0.000</td>
<td>0.000</td>
</tr>
</tbody>
</table>

*Independent sample t-test; ** Analysis of covariance (ANCOVA).

Table 3 shows relationships between pituitary hormones and sex hormones in postmenopausal women. After controlling for age and BMI, Pearson’s multivariable regression analysis indicated lack of significant associations between FSH and Progesterone; LH and Progesterone; and LH and Estradiol respectively. In contrast, significant inverse correlation (P<0.01) was observed between FSH and estradiol.

Table 3. Age and BMI adjusted relationships between pituitary hormones and ovarian (sex) hormones in post-menopausal women

<table>
<thead>
<tr>
<th>Variables</th>
<th>Correlation coefficient</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FSH vs. Progesterone</td>
<td>-0.226</td>
<td>0.214</td>
</tr>
<tr>
<td>FSH vs. Estradiol</td>
<td>-0.572</td>
<td>0.001**</td>
</tr>
<tr>
<td>LH vs. Progesterone</td>
<td>0.245</td>
<td>0.176</td>
</tr>
<tr>
<td>LH vs. Estradiol</td>
<td>0.042</td>
<td>0.818</td>
</tr>
</tbody>
</table>

**Significant (P<0.01).

Linear regression analysis indicated significant correlation between age and estradiol (P<0.01) and LH (P<0.001). In contrast, no significant associations were observed between age and progesterone and FSH. After controlling for BMI, the initial correlation observed in age vs. estradiol disappeared, while that of age vs. LH persisted (Table 4).

Table 4. Relationship between age and reproductive hormones in post-menopausal women

<table>
<thead>
<tr>
<th>Age vs.</th>
<th>Unadjusted linear correlation</th>
<th>Bmi-adjusted correlation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Coefficient</td>
<td>P-value</td>
</tr>
<tr>
<td>Progesterone</td>
<td>-0.120</td>
<td>0.499</td>
</tr>
<tr>
<td>Estradiol</td>
<td>-0.471</td>
<td>0.005**</td>
</tr>
<tr>
<td>FSH</td>
<td>0.270</td>
<td>0.123</td>
</tr>
<tr>
<td>LH</td>
<td>0.570</td>
<td>0.000***</td>
</tr>
</tbody>
</table>

**Significant (P<0.001); ***P<0.01.

Linear regression analysis indicated significant correlation between BMI and estradiol (P<0.001) and FSH (P<0.05) and LH (P<0.05). However, no significant association was observed between BMI and progesterone. After controlling for age, the correlation
disappeared in BMI vs. LH but remained in BMI vs. estradiol (P<0.001) and in BMI vs. FSH (P<0.05; (Table 5).

Table 5. Relationship between BMI and reproductive hormones in post-menopausal women

<table>
<thead>
<tr>
<th>Bmi vs.</th>
<th>Unadjusted linear correlation</th>
<th>Age-adjusted correlation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Coefficient</td>
<td>P-value</td>
</tr>
<tr>
<td>Progesterone</td>
<td>0.092</td>
<td>0.606</td>
</tr>
<tr>
<td>Estradiol</td>
<td>0.691</td>
<td>0.000***</td>
</tr>
<tr>
<td>FSH</td>
<td>-0.430</td>
<td>0.011*</td>
</tr>
<tr>
<td>LH</td>
<td>-0.350</td>
<td>0.042*</td>
</tr>
</tbody>
</table>

***Significant (P<0.001); * P<0.05.

4. DISCUSSION

In this study, post-menopausal women had significantly lower estrogen and progesterone levels while follicle stimulation hormone and luteinizing hormone levels were significantly higher when compared to the control group. This result was expected since it has been established that during menopause, there are very low levels of sex hormone (estrogen, testosterone and progesterone) and high levels of FSH and LH in the blood stream in response to depleted ovarian follicles [19]. During this period, the ovaries stop responding to FSH and LH but the anterior pituitary keeps releasing FSH and LH. Our findings agree with previous studies [3,20-24] which showed a significantly higher FSH and LH levels and significantly lower estradiol and progesterone levels in postmenopausal older women compared to their younger reproductive controls.

Our finding also established that estradiol level decreased by 93%, progesterone decreased by 74% while LH increased to 5-folds and FSH increased to 7-folds in post-menopausal women compared to control subjects. Our finding is in strong agreement with the work of Henrich et al. [25] which showed that mean FSH was seven times higher in post-menopause compared to the reproductive stage. The present data also agrees with some studies [26,27] which showed that estradiol in menopausal women decreased by more than 90% and LH levels increased 4- to 5-fold compared with those of younger, reproductive controls. The present findings underscore the importance of understanding the hormonal levels of the hypothalamic-pituitary-gonadal axis in postmenopausal stage of a woman’s life in order to make optimal therapeutic decisions for hormone replacement therapy and life-style changes that may reduce the risk of some of the conditions associated with menopause in women.

The interactions between the pituitary hormones and the ovarian hormones during the postmenopausal stage are not well defined. Similarly there is paucity of information on the interrelationships between the hormones of these two endocrine organs. In the present study, there was significant inverse correlation between FSH and estradiol after adjustment for age and BMI. However, no significant correlations were observed between FSH and progesterone, LH and estradiol, and between LH and progesterone respectively. A previous study by Ausmanas et al. [22] showed an inverse correlation between estradiol and FSH levels in postmenopausal Asian women thus agreeing with the present finding. The inverse correlation of estradiol and FSH levels suggests that estradiol still affects the pituitary FSH output at the postmenopausal state. It also suggests the possibility that FSH may be used to indicate the total estrogen status of postmenopausal women. Furthermore, the observation of significant relationship between FSH and estradiol and the lack of such association
between LH and estradiol emphasizes the complexity of the hypothalamic-pituitary-ovarian regulatory system and suggests that LH and FSH are modulated independently at the level of pituitary.

We assessed the extent of relationship between age and the reproductive hormones. A linear regression analysis indicated that age positively correlated with LH but inversely correlated with estradiol, whereas progesterone and FSH levels were not significantly associated with age. However, after controlling for BMI, the correlation between age and estradiol disappeared thus suggesting that the influence of age on estradiol is accounted for by changes in BMI. On the other hand, the correlation between age and LH persisted after controlling for BMI. A previous study [22] has reported significant relationships between age and LH and estradiol but not FSH in post menopausal women, thus agreeing with the present data. Vermeulen et al. [28] reported inverse correlation between age and estradiol in postmenopausal women. However, other studies [29,30,31] indicated lack of significant relationship between age and estradiol (estrogen). In contrast to our findings, Reyes et al. [31] have demonstrated significant correlation between age and FSH in post-menopausal women. We could not find any study on the relationship of progesterone and age in postmenopausal women. The present findings underline the need to consider age when determining hormonal changes and its associated disease risks and sexual changes in postmenopausal women.

Our data further revealed that body mass index was independently related to estradiol and FSH. Estradiol increased, while FSH declined with increase in BMI. These findings concurred with a previous study [32] which reported positive correlation between BMI and estrogen in post-menopausal women. Similarly, Ausmanas et al. [22] reported significant associations between BMI and three of the hormones estradiol, FSH and LH in post-menopausal women. Furthermore, Vermuelin et al. [28] reported that estradiol was significantly correlated with the degree of adiposity and fat mass. In another study involving both normal and obese post-menopausal women, Klinga et al. [33] reported that FSH inversely correlated with body weight while estradiol did not change with changes in body weight. The present data which showed positive correlation between estrogen and body mass index is compatible with the hypothesis that after menopause, excess fat mass increase estrogen concentration through the aromitisation of androgens in adipose tissue. These findings further reiterate the need to put into consideration body mass when determining the role of reproductive hormones in sexual changes and disease conditions associated with post-menopausal state in women.

5. CONCLUSION

In conclusion, the present study indicated that post-menopausal women had significantly lower estrogen and progesterone levels and higher FSH and LH levels compared to the control group. There was inverse correlation between estradiol and FSH levels, but no significant correlations were observed between FSH and Progesterone; LH and Progesterone; and LH and Estradiol respectively. In addition, after adjusting for BMI, the concentrations of LH was associated with age but FSH, estradiol and progesterone levels were not. Furthermore, estradiol and FSH levels were significantly associated with BMI after controlling for age. These findings underscore the importance of understanding the reproductive hormonal profile of menopausal women and the need for more detailed studies on the biological significance of hormonal changes with age and body weight. These data may help clinicians to make optimal therapeutic decisions for hormone replacement therapy.
and life-style changes that may reduce the risk of some of the conditions associated with menopause in women.

CONSENT

All authors declare that written informed consent was obtained from the patient (or other approved parties) for publication of this case report and accompanying images.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES


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