Correlative Study on the Hepatorenal and Hormonal Alterations in Perimenopausal Women in Enugu, South East of Nigeria

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Abstract: Background: The term perimenopause refers to a woman's shift from a reproductive to a nonreproductive stage. Many hormonal and biochemical changes accompany this transformation. There is insufficient information in the association between hormonal changes, Liver enzymes activities, and renal functions in perimenopause. This study evaluated the hormonal and hepatorenal alterations in perimenopausal and premenopausal women in Enugu State, Nigeria. Methods: A total of 180 apparently healthy women were recruited for this cross-sectional study. 90 perimenopausal women (mean age = 49 years) and 90 premenopausal women (mean age = 29 years) served as test and control subjects. Subjects' anthropometric indices (blood pressure, waist circumference (WC), height, and weight) were measured. five milliliters of fasting blood was collected from the subjects to test for Estradiol (E2), Luteinizing hormone (LH), Follicle stimulating hormone (FSH), Alanine transaminase (ALT), Aspartate transaminase (AST), Alkaline phosphatase (ALP), Protein, Urea, Creatinine, and Fasting blood glucose (FBG) using standard biochemical procedures. Results: Blood pressure, FBG, WC, FSH, LH, ALT, AST, Creatinine levels were substantially higher and Estradiol was lower in perimenopausal women when compared to premenopausal women (P<0.05). There exist significant positive correlations among perimenopausal women in FSH and LH with AST and ALT, Estradiol with protein, Urea vs Creatinine, while Estradiol was negatively associated with ALT, AST, ALP and FSH. Conclusion: Perimenopausal women are likely prone to a higher risk of liver and renal dysfunction. Therefore, the need for regular evaluation of liver and kidney parameters during perimenopause is encouraged.

Keywords: Perimenopause, Hepatorenal Functions, Hormonal Changes, Liver Enzymes, Enugu-Nigeria.

1. INTRODUCTION

Perimenopause is the period when a woman's body naturally transitions to menopause, signaling the end of her reproductive years [1]. Perimenopause is a transition from a reproductive to nonreproductive stage in a woman. This transition is associated with many hormonal and biochemical changes [2]. Perimenopause's observed changes are profoundly founded in hormonal changes, particularly in the level of circulating estrogen. The amount of estrogen in the circulation rises and falls in a predictable pattern during the menstrual cycle and during the peak of procreation [3]. FSH and LH are the two hormones that significantly influence estrogen levels. FSH stimulates the ovaries' follicles, which are fluid-filled sacs that hold eggs, to create estrogen. Estrogen, the principal female hormone, aids in the growth and health of the female reproductive organs, as well as keeping the vaginal area lubricated, stretchy, and blood supplied. During perimenopause, estrogen levels usually decrease [4].

Perimenopauses usually occur from the age of (50±5) years in Caucasians [3] and early 40's to late 50's in Nigeria [5]. In Enugu eastern part of Nigeria, perimenopause usually occurs from mid-40 to late 50's in Igbo women [2]. The clinical symptoms of menopausal transition, such as hot flushes and sleeping problems, night sweats, and vaginal dryness, set in due to the variation in hormones found in perimenopause, mainly due to estrogen deficiency. These symptoms give the individual distress and have an impact on her sexual health [6]. Perimenopause is also known to be associated with hormonal fluctuations due to ovarian depletion, and affects some biochemical changes leading to variations in the organs of the body like the kidney and liver. The kidney is an organ that removes waste and extra fluid from the blood. Protein, urea and creatinine levels could be used as a marker for kidney functioning

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[7]. Most organs are made up of proteins, which also make up enzymes and hormones that regulate physiological activities.

In conditions that promote protein loss, such as kidney disease, total protein may decrease (nephritic syndrome). Creatinine is a metabolite obtained from the high-energy product creatine that is produced in muscle tissue. Healthy kidneys filter creatinine out of the blood, and creatinine exists in the body as a waste product in urine, hence serum creatinine is commonly used as a biomarker of kidney function [8]. A research by Sheng *et al* [7] suggested that female sex hormones have some effect on renal function tests as they recorded increased serum uric acid levels and normal serum creatinine levels in postmenopausal women than in women prior to menopause. Kidney dysfunctioning could lead to stroke, coma and death.

The liver is one of the body's primary organs that perform diverse functions essential for life [9]. Perimenopause, which is associated with a deficit of estrogen, deficit of estrogen promotes proliferation of stellate cells, fibrogenesis, mitochondria dysfunction, decline in immune response and disarray in the balance between antioxidants formation and pathology, oxidative stress [10]. All these factors lead to liver diseases, many of the liver enzymes like AST, ALT and ALP are increased in liver disease and necrosis [11] so assaying these liver enzymes will act as diagnostic biomarkers for assessing necrosis of the liver cells. The enzyme aspartate transaminase is required for energy production and is found in two forms: mitochondrial and cytoplasmic. A spike in mitochondrial AST in the blood is significantly predictive of tissue necrosis in myocardial infarction and chronic liver disease, and it is more markedly raised in people with liver cirrhosis [11]. Alkaline phosphatase is an enzyme found in the cells that line the liver's biliary ducts. It is also found in the small intestine's mucosal epithelium, the kidney's proximal convoluted tubules, bone, liver, and placenta. Cirrhosis, hepatitis, and congestive heart failure all cause a little increase in ALP [10- 11].

The perimenopause phase has received little attention, despite the fact that it is a time when hormonal fluctuations cause several of the biochemical changes including several health problems. In perimenopause, these changes in health and physiological changes may contribute to a higher prevalence of metabolic syndrome and cardiovascular events [2].

2. MATERIALS AND METHODS

2.1. Research Design and Sample Technique

The study was carried out in Enugu metropolis. Enugu, Southeast of Nigeria. The study was a cross-sectional study which involved ninety perimenopausal (45-55years $\overline{X}$= 49 years) and ninety premenopausal (25-35 years, $\overline{X}$= 29 years) women living in Enugu metropolis. The premenopausal women's hormone samples were collected during the early follicular phase (menstrual phase, day 3-day 5) of their menstrual cycle, but the perimenopausal women's sample collection day was not specified due to anomalies in their menstrual cycle. All samples were obtained in the morning and while fasting. Five milliters of venous blood were drawn from each participant, for blood glucose testing, 1ml was poured into a fluoride oxalate vial and the remaining blood was placed in a plain tube, allowed to coagulate, centrifuged, and the serum recovered was used for hormonal and biochemical examination within one week of the sample collection. At the beginning of the study, the participants filled structured questionnaires and their clinical and demographic information were recorded. The results obtained were analysed statistically using SPSS version 22-computer software at 95% confidence level.

2.2. Sample Size Determination

The sample size used in this study was obtained using the formula of Naing *et al* [12]

Equation: Sample size N = $\dfrac{Z^2\,P\,(1-P)}{D^2}$

A prevalence rate of 12.1% according to Adeoye *et al,* [13] was used

Z = statistics for the level of 95% confidence interval (1.96)

P = prevalence

D = Desired degree of accuracy; which is α= 0.05

Sample size, N = $\frac{(1.96)^2\ P\ (1-P)}{D^2}$

$N = \frac{3.842 \times 0.121\ (1-0.121)}{0.05 \times 0.05}$

$N = \frac{3.842 \times 0.121\ (0.879)}{0.0025}$

$N = 163.4$

The study was then expanded to 180 women to increase the sample size's power.

2.2.1. Inclusion Criteria

Healthy perimenopausal women (45-55) years with irregular menstruation as test subjects. These women have hot flashes, mood changes and irritability. The premenopausal women (25-35) years having regular menstruation were also included as control subjects.

2.2.2. Exclusion Criteria

All women with irregular menstruation from puberty, pregnant women, HIV positive, known diabetes, hypertensive, hypothyroidism, hysterectomy and postmenopausal women were all excluded.

2.3. Ethics Approval

Ethical approval was duly obtained from Research Ethics Committee of University of Nigeria Teaching Hospital, College of Medicine, Ituku Ozalla, Enugu State, (Ref no: UNTH/CSA/329/VOL.5), Informed consent was also obtained from each of the participants before enrollment into the study.

2.4. Measurements of Anthropometric Indices

2.4.1. Blood Pressure

Participants' blood pressures were measured using a standardized automatic blood pressure monitor. After 30 minutes of relaxation, in a sitting position, two recordings were made from the left arm of participants. The two readings were taken at five-minute intervals, and the analysis was based on the average of the two data.

2.4.2. Waist Circumference and Body Mass Index

Individuals' weight, height and WC were all measured. A soft tape was used to measure the waist circumference of standing subjects midway between the lowest rib and the iliac crest. The body weight was measured in kilograms using a standardized bathroom weighing scale and the heights were measured in meters while standing. By dividing weight in kilograms by height in meters squared (kg/m^2), the BMI was calculated.

2.5. Biochemical Analysis

The glucose oxidase method [8] was used to determine the fasting blood glucose level. Luteinizing hormone (LH), Follicle Stimulating Hormone (FSH) and Estradiol (E_2) were tested using the Enzyme immunoassay (kit) method [14].

Determination of serum ALT and AST were carried out using Reitman-Frankel colorimetric method [15]. ALP was assayed using Phenolphthalein monophosphate substrate method [16]. Urea determination was done using

Berthelot urease method [17]. Creatinine was determined using Jaffe kinetic method [18] and protein was estimated using Biuret method [8].

2.6. Statistical Analysis

At a 95% confidence level, statistical analysis was performed using the Statistical Package for Social Sciences (SPSS) version 22 computer software. The mean and standard deviation of the results were calculated. Results were expressed as mean$\pm$ standard deviation (SD). Independent t-test was used to determine significant differences between means, and correlations to obtain the relationships between parameters.

3.RESULTS

Table 1: Biophysical and Anthropometric parameters in perimenopausal and premenopausal women

Groups	SBP (mmHg)	DBP (mmHg)	BMI (kg/m²)	WC (cm)
Perimenopausal women N= 90	122 ± 2.0	83 ± 5.1	30.1 ± 5.1	87 ± 0.7
Premenopausal women N= 90	116 ± 6.0	76 ± 3.0	30.0 ± 4.7	78.1 ± 0.4
P- value	P< 0.0001*	P< 0.0001*	P = 0.944	P< 0.0001*

Values are presented as Mean ± SD
* significant values

Table 1 shows the biophysical and anthropometric parameters of perimenopausal and premenopausal women. The SBP (122 ± 2.0 mmHg), DBP (83 ± 5.1 mmHg) and WC (87 ± 0.7 cm) is significantly higher in mean values in perimenopausal women when compared with premenopausal SBP (116 ± 6.0 mmHg), DBP (76 ± 3.0 mmHg), and WC (78.1 ± 0.4 cm) (p< 0.0001). However, the BMI of perimenopausal women (30.1± 5.1) did not differ significantly from that of premenopausal women (30.0 ± 4.7) (p> 0.05).

Table 2: The levels of LH, FSH, E_2 and FBG in perimenopausal and premenopausal women

Groups	FBG (mmol/l)	E_2 (pg/ml)	LH (miu/ml)	FSH (miu/ml)
Perimenopausal women N= 90	5.5 ± 10.1	50.52 ± 8.0	45.1 ± 7.0	36.77 ± 7.7
Premenopausal women N= 90	5.3 ± 5.7	74.68 ± 11.5	20.0 ± 2.6	10.36 ± 8.0
P-value	P< 0.0201*	P< 0.0001*	P< 0.0001*	P< 0.0001*

Values are presented as Mean ± SD
* significant values

Table 2 shows the mean ± SD of fasting blood glucose (FBG), estradiol (E_2), LH, and FSH of perimenopausal and premenopausal women. The result shows significantly higher levels of FBG (5.5 ± 10.1 mmol/l), LH (45.1 ± 7.0 miu/ml), FSH (36.77 ± 7.7 miu/ml) and a lower E_2 (50.52 ± 8.0 pg/ml) in perimenopausal women compared to premenopausal women FBG (5.3 ± 5.7), LH (20.0 ± 2.6), FSH (10.36 ± 8.0 miu/ml) and E_2 (74.68 ± 11.5 pg/ml) (P< 0.05).

Table 3: The levels of AST, ALT and ALP in perimenopausal and premenopausal women

Groups	AST(IU/L)	ALT (IU/L)	ALP (IU/L)
Perimenopausal women N= 90	19.93 ± 6.6	16.04 ± 5.9	24.58 ± 0.4
Premenopausal women N= 90	15.92 ± 5.3	10.40 ± 5.0	23.78 ± 4.8
P- value	0.012*	0.001*	0.229

Values are presented as Mean ± SD
* significant values

Table 3: shows the serum values of AST, ALT, and ALP in perimenopausal and premenopausal women. This result shows significantly higher values of AST (19.93 ± 6.6 IU/L), ALT (16.04 ± 5.9 IU/L) in perimenopausal women compared to premenopausal AST (15.92 ± 5.3 IU/L), ALT (10.40 ± 5.0 IU/L) (P< 0.05). There was no significant difference (P> 0.05) in ALP (24.58 ± 0.4 IU/L; 23.78 ± 4.8 IU/L) in both perimenopausal and premenopausal women respectively.

Table 4: The serum levels of Urea, Creatinine and Protein in Perimenopausal and Premenopausal women

Groups	Urea (mmol/l)	Creatinine (µmol/l)	Protein (g/l)
Perimenopausal women N= 90	5.10 ± 15.0	83.36 ± 15.0	64.83 ± 4.4
Premenopausal women N=90	4.71 ± 1.5	74.74 ± 16.4	67.04 ± 7.4
P-Value	0.264	0.034*	0.135

Values are presented as Mean ± SD
* significant value

Table 4: Shows the serum values of Urea, Creatinine, and Protein in Perimenopausal and Premenopausal women. The result shows significantly higher values of creatinine (83.36 ± 15.0 µmol/l)) in perimenopausal women compared to premenopausal creatinine (74.74 ± 16.4 µmol/l)) while there was no significantly different in Urea (5.10 ± 15.0mmol/l, 4.71 ± 1.5mmol/l) and protein (64.83 ± 4.4g/l, 67.04 ± 7.4g/l) in both perimenopausal and premenopausal women respectively.

Table 5: The relationship between hormonal parameters (FSH, LH, E_2) and hepatorenal parameters (AST, ALT, ALP, Urea, Creatinine, Protein) in perimenopausal women

Parameters	(r) Pearson	P- values
FSH vs AST	0.220	0.023*
FSH vs ALT	0.241	0.015*
FSH vs ALP	0.044	0.635
FSH vs Urea	0.057	0.726
FSH vs Creatinine	- 0.021	0.900
FSH vs Protein	0.117	470
LH vs AST	0.224	0.022*
LH vs ALT	0.211	0.021*
LH vs ALP	0.056	0.543
LH vs Urea	0.096	0.294
LH vs Creatinine	-0.045	0.628
LH vs Protein	0.111	0.228
E_2 vs AST	-0.482	0.002**
E_2 vs ALT	-0.501	0.001**
E_2 vs ALP	-0.228	0.012*
E_2 vs Urea	-0.014	0.930
E_2 vs Creatinine	-0.135	0.408
E_2 vs Protein	0.325	0.041*
Urea vs Creatinine	0.367	0.020*
FSH vs E_2	-0.774	0.000**
AST vs ALT	0.846	0.000**

*Weak significant correlation,   ** Strong significant correlation

In Table 5: The hormones that were measured (FSH, LH, and E_2) were correlated with the levels of the hepatorenal parameters (AST, ALT, ALP, Urea, Creatinine, Protein) separately in perimenopausal women. The results showed a significantly positive correlation between FSH and AST (r = 0.220; p=0.023), FSH and ALT (r= 0.241; p= 0.015), LH and AST (r= 0.224; p= 0.022), LH and ALT (r= 0.211; p= 0.021), E_2 and protein (r= 0.325; p=0.041), Urea and Creatinine (r= 0.367; p= 0.020), AST and ALT (r= 0.846; p= 0.000). A negative correlation also exist between E_2 and AST (r= - 0.482; p= 0.002), E_2 and ALT (r= - 0.501; p= 0.001), E_2 and ALP (r= - 0.228; p= 0.012), E_2 and FSH (r= - 0.774; p= 0.000). There was no significant correlation with other parameters.

4. DISCUSSION

Assessment of some hormones and biochemical parameters frequently during perimenopause helps to monitor and prevent possible symptoms because of hormonal and biochemical changes due to winding down of ovaries [2]. In a cross-sectional examination of perimenopausal and premenopausal women, this study looked at some biochemical (hepatorenal) and hormonal (FSH, LH, E2) changes. Perimenopausal women have significantly greater systolic and diastolic blood pressure than premenopausal women, according to the findings. Age-related arterial stiffness, increased pulse pressure, decreased sex hormone (estrogen), and increased sensitivity to dietary sodium chloride are all factors that contribute to an increase in blood pressure [1- 2]. All of these factors play a role in the development of hypertension. This study's findings are consistent with those of Kow Nanse Arthur *et al.* [19], who found an increase in both SBP and DBP in Ghanaian menopausal women and also in perimenopausal Igbo women in Enugu, Nigeria according to Ikegwuonu *et al,* [1-2].

Perimenopausal and premenopausal women's BMI and WC were also determined. When perimenopausal women were compared to premenopausal women, the WC of perimenopausal women was found to be considerably higher. Perimenopausal and premenopausal women had similar BMIs. This could be due to estrogen insufficiency and changes in fat distribution, both of which are linked to menopause. This finding is consistent with the findings of Tende *et al,* [20] on menopausal Zaria women in Northern Nigeria. In both perimenopausal and premenopausal women, the BMI result showed no significant change. This observation is in line with previous studies [1-2] who reported no significant changes in BMI between premenopausal and perimenopausal women in Enugu State, Nigeria.

Table 2 of this study revealed that perimenopausal women had considerably higher mean FBG levels than premenopausal women. This could be due to a decrease in blood dehydroepiandrosterone sulfate (DHEAS) levels as well as decreased insulin sensitivity with age [21]. DHEAS is a hormone that helps to minimize visceral fat storage and insulin resistance. This finding is in line with the previous study by [19], which demonstrated significantly higher fasting blood glucose in menopausal Ghanaian women. The pituitary hormone (FSH and LH) and sex hormone (E_2) levels were evaluated. In comparison to premenopausal women, perimenopausal women had significantly greater FSH and LH levels and lower estradiol (E2) levels. This finding concurs with a previous study [2] that reported decreased E_2 levels with increases in FSH and LH of women in menopause transition. This is expected in perimenopausal women due to ovarian follicle depletion, which causes the ovaries to stop responding to FSH and LH. The anterior pituitary continues to release FSH and LH in the absence of negative feedback, raising their concentration in the bloodstream as estradiol level falls.

Table 3 of this study revealed that perimenopausal women had considerably higher levels of AST and ALT than premenopausal women. This higher AST and ALT levels in the perimenopause could be due to the decline in estrogen level, which is associated with menopausal transition. Estrogen suppresses stellate cell proliferation and fibrogenesis in the liver; it also has protective effects on mitochondrial structure and function, slows cellular senescence, boosts innate immunity, and promotes antioxidant effects on the liver [9]. This finding is in line with the work of Kim and colleagues [22], which demonstrated that advancement in age, have a positive relationship with aspartate and alanine aminotransferases.

Furthermore, the Urea and Creatinine levels of perimenopausal women were slightly higher and significantly higher, respectively when compared to premenopause. This observation agrees with the work of Gault *et al* [23], who also observed significantly higher creatinine levels in perimenopausal Caucasian women. This observation in Creatinine was attributed to ageing as well as higher blood pressure and blood glucose levels seen in the perimenopausal women of this study, which could increase the chance of having urologic health issues [24]. The protein level of the perimenopausal women was lower, although not statistical significant compared to the premenopausal women. This finding could be linked to its higher affinity for estrogen since steroidal estrogens have a low solubility in the blood and their association with serum protein reduces clearance and tightly regulates the bioactive fraction [25].

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This study also evaluated the relationship between hormonal parameters (FSH, LH, E_2) and hepatorenal parameters (AST, ALT, ALP, Urea, Creatinine, Protein). There exist significant positive correlations between FSH vs AST, ALT; LH vs AST, ALT; E_2 vs Protein, Urea vs Creatinine and AST vs ALT. Negative correlations exist between E_2 vs AST, ALT, ALP and FSH vs E_2. These findings are in line with previous works [26- 27] which reported a dwindling of estradiol level with increases in age, LH and FSH due to the liver's ability to compensate for the hormonal imbalance. Again, Santoro [28] reported that a progressive loss of ovulatory function may have a negative feedback mechanism on the anterior pituitary gland, thereby stimulating an increase in FSH and LH with a concomitant decrease in E_2, which can impair its protective benefits on the liver, causing an increase in the levels of liver enzymes AST, ALT and ALP in the blood. This association observed between the hormonal parameters which are perimenopausal indices (FSH, LH, E_2) and that of hepatorenal parameters (AST, ALT, ALP, Urea, Creatinine, Protein) could be a strong indication that perimenopause affects the liver enzymes as well as kidney parameters.

CONCLUSION

This study shows that there are changes in some hormonal parameters (FSH, LH, E_2) as well as liver and kidney parameters among the perimenopausal women. These changes in liver enzymes (liver biomarkers) and changes in kidney parameters among perimenopausal women could predispose them to liver pathology and renal dysfunction.

Key Messages

Hepatorenal functions should be determined frequently in perimenopause to prevent metabolic syndrome associated diseases and hepatorenal dysfunctioning in menopause.
Perimenopause phase has been understudied, yet this is the period that many biochemical changes and derangements in health start developing.
These derangements in health lead to disease state in menopause as well as in post menopause.

Limitations

This study did not include electrolytes level measurement among the kidney function parameters because the researchers believe that urea and creatinine measurements are better biomarkers of Kidney function.

Ethics Approval and Consent to Participate

Ethical approval was duly obtained from Research Ethics Committee of University of Nigeria Teaching Hospital, College of Medicine, Ituku Ozalla, Enugu State, (Ref no: UNTH/CSA/329/Vol.5). Informed consent was also obtained from each of the participants before enrollment into the study.

Human And Animal Rights

No animals were used in this research. All humans research procedures followed were in accordance with the standards set forth in the Declaration of Helsinki principles of 1975, as revised in 2008 (http://www.wma.net/en/20 activities/10ethics/10helsinki/).

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Author's Contributions

I.C.I: Conceived of the study, carried out the laboratory analysis and drafted the manuscript.

I.J A: Participated in conception and design of the study, involved in recruitment of the participants and statistical analysis.

I.E. A: Drafted the manuscript, participated in analysis and interpretation of data.

I.E.U: Participated in laboratory analysis and interpretation of data.

J. C. O: Laboratory analysis and statistical analysis.

B. C.O: Laboratory analysis and recruitment of participants.

N. S.N: Laboratory analysis and data interpretation.

C. B. M: participated in laboratory analysis and data interpretation

S. O. E: Drafted the manuscript and participated in statistical analysis

All authors read and approved the final manuscript.

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