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Assessment of Aromatase in Premenopausal and Postmenopausal Women Attending Rivers State University Teaching Hospital, Port Harcourt, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. Authors BCAE and JGC designed the study, performed the statistical analysis, wrote the protocol and author SAA wrote the first draft of the manuscript. Authors ACA and EI managed the analyses of the study. Author BH managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aim: This study was a cross sectional study aimed at evaluating the concentration of the enzyme aromatase in premenopausal and postmenopausal women in Port Harcourt.
Study Design: The study population comprised of apparently healthy premenopausal and postmenopausal women who were within the age range of 20-79 years. A total of seventy (70)

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participants were voluntarily recruited, who were divided into 2 groups: group A comprising of thirty (30) premenopausal and group B comprising of forty (40) postmenopausal women.
Methodology: An Enzyme-Linked Immunosorbent Assay (ELISA) method was used to analyze the concentration of aromatase in the samples. The statistical analysis was done using GraphPad Prism version 9.4.1.
Results: The results obtained for the respective groups were expressed as mean±SD and statistical significance was determined by a p-value <0.05. The results clearly showed a significant increase in the level of aromatase in postmenopausal women when compared to the premenopausal women.
Conclusion: This finding implies that postmenopausal women have much higher concentration of aromatase than the premenopausal population. Thus, there is an age-related increase in aromatase expression in relation to menopausal status.

Keywords: Aromatase; oestrogen; premenopausal; postmenopausal; ovarian and breast cancer; rivers state.

1. INTRODUCTION

Oestrogens are a class of steroid hormones that are crucial for the healthy physiology and reproduction in females. Oestrogens play crucial roles in the circulatory, nervous, and musculoskeletal systems in addition to the reproductive system [1]. Oestrone (E1), oestradiol (E2), and oestriol (E3) are the three physiological oestrogens that affect women the most [2]. In premenopausal women, 17β-estradiol is the most potent oestrogen produced by the ovaries. It functions as a circulating hormone and has an endocrine effect on distant target organs [3]. In contrast, postmenopausal women tend to produce oestrone, and pregnant women tend to produce oestriol [3]. In premenopausal women, oestrogens are synthesized from androgens by the granulosa cells in the ovaries. In postmenopausal women, oestrogen is produced peripherally by the aromatase enzyme from androgens when the ovaries are no longer functioning [4].

Aromatase is the primary enzyme involved in the process known as aromatization, which transforms androgenic steroids into oestrogens. Aromatase is often referred to as oestrogen synthetase or oestrogen synthase [5]. The enzyme is expressed in a variety of tissues, including skin, endometrium, bone, brain, ovary, testis, placenta, mammary, and adipose tissue. This is because oestrogenic steroid hormones are essential for many crucial physiological processes, including growth, ossification, differentiation, neurologic, and reproductive development. The dynamic maintenance of oestrogen levels is influenced by the age of reproduction [6]. Oestrogens are largely synthesized in postmenopausal women's adipose tissues and other extra-gonadal tissues, such as the brain, bone, breast, and skin, which have the machinery to break down readily available androgen substrates, when ovarian function stops. As a result, these women's levels of circulating oestrogen fall [7]. Although the oestrogens produced in these extra-gonadal tissues may not have a significant physiological effect on the body as a whole, they have a significant physiological effect on the local tissue because of their autocrine or paracrine signaling effects [1].

Aromatase enzyme is the primary source of oestrogen synthesis in postmenopausal women, therefore, inhibiting this specific enzyme causes a significant additional decrease in oestrogen levels. The standard therapy for postmenopausal women with hormone receptor-positive breast cancer is now thought to be aromatase inhibitors [8]. However, the development of resistance to aromatase inhibitors still poses a challenge, particularly in cases of metastatic breast cancer as clinical resistance to aromatase inhibitors may render breast cancer therapy ineffective. High oestrogen levels are linked to an increased risk of breast cancer, and long-term oestrogen exposure raises the risk of breast cancer [9]. The enzyme aromatase is the rate-limiting enzyme in the synthesis of oestrogen [10]. As a result, it's reasonable to believe that this enzyme, aromatase is involved in both the development and treatment of hormone-dependent breast cancer.

The peripheral conversion of androgens to oestrogens in adipose tissues and other extra-gonadal tissues by the aromatase enzyme is the main source of oestrogen synthesis in postmenopausal women [11]. However, in

premenopausal women, aromatase is confined to the granulosa cells in the ovaries and is produced under the influence of the gonadotropins- follicle stimulating hormone (FSH) and luteinizing hormone (LH) [12]. Therefore, it makes sense that postmenopausal women will have high levels of aromatase due to the presence of high levels of oestrogen. Since aromatase is a crucial enzyme in the production of oestrogens, this makes postmenopausal women more likely to get breast cancer. Aromatase is hence commonly dysregulated in breast cancers [13].

According to Castro-Piedras et al. [14], aromatase expression can be controlled transcriptionally by hormones, cytokines, growth factors, non-protein coding RNAs, and signaling molecules. More so, by post-translational modifications like phosphorylation [15], and more recently, acetylation [16]. Numerous malignancies, particularly breast cancer, have been linked to oestrogen overproduction and aromatase dysfunction (overexpression of aromatase) [17,18,19]. Aromatase is the only known vertebrate enzyme that can aromatize a six-membered ring; aromatase is, therefore, the sole source of oestrogen in the body [20]. Atypical aromatase expression in breast cells or the surrounding adipose stromal cells, especially in postmenopausal women, may have a significant effect on breast tumor management because aromatase is the enzyme responsible for the synthesis of oestrogens.

Therefore, since there is a spaucity of data on the concentration of aromatase enzyme in both premenopausal and postmenopausal women, and since postmenopausal women have a higher chance of developing breast cancer, the present study intended to compare the extent of expression of aromatase in these (postmenopausal) women to those in premenopausal women.

2. MATERIALS AND METHODS

2.1 Study Subjects

A total of 80 women (both premenopausal and postmenopausal) who were apparently healthy, attending Rivers State University Teaching Hospital between August and September, 2022 for their routine health checks were recruited. The participants that were selected for this study were between 20 -79 years of which 30 were postmenopausal women and 40 premenopausal women.

2.2 Inclusion and Exclusion Criteria

Those included in the study were apparently healthy women attending the Rivers State University Teaching Hospital for medical checkups without any medical challenges between the ages of 20 = 70 years. Those excluded were women on cancer treatment or related therapy or hormonal replacement therapy. More so, those with an illness like malaria, typhoid fever, HIV, tuberculosis, diabetes, secondary hypertension and other cardiovascular disorders, thyroid disorders, and other hormonal disorders like hyperandrogenism were excluded. In addition, those excluded include, those that did not give their consent orally and in written forms.

2.3 Ethical Consideration, Consent, and Clearance

A well-structured questionnaire was given to participants to obtain vital information. All relevant information, like the purpose and methodology of the experiment, was explained to study participants beforehand, and informed consent was obtained. The study protocol was approved by the Ethical Review Board of Rivers State University Teaching Hospital, Port Harcourt with approval number RSUTH/REC/2023315.

2.4 Specimen Collection and Preparation

Approximately 5 ml of whole blood samples were collected from participants into plain bottles. The specimens were allowed on the bench to clot at room temperature of 25^{o}C and thereafter retraction and spun at 3500rpm for 10 minutes to obtain serum. The sera were stored at -20˚C until the time of analysis. Before the assay, the frozen samples were slowly thawed and centrifuged to remove the precipitates.

2.5 Laboratory Assay of Aromatase

The serum samples were analyzed for the in vitro quantitative detection of human aromatase (ARO) concentration using the Enzyme Linked Immunosorbent Assay (ELISA) kit.

2.6 Statistical Analysis

Statistical analysis of data was performed using GraphPad Prism version 9.0.2. Descriptive

statistics such as Mean and Standard Deviation were employed while inferential statistics like unpaired t-test, and ANOVA were used. Statistical significance is defined as a p-value of less than 0.05 at 95% confidence interval.

3. RESULTS

3.1 Socio-Demographic Characteristics of the Premenopausal and Postmeno-pausal Population

The socio-demographic characteristics of the premenopausal women indicate that 80% were between 20-29 years, 15% were within the age range of 30-39, and 5% were 40-49 years. Of these subjects, 22.5% experienced menarche between 7-11 years, 52.5% between 11-12 years of age, and 25% had menarche after 14 years. In addition, 22.5% of participants had children, and 77.5% of participants had never had children. Of the 22.5% that had their offspring, 44.44% had their first child between 20-24, and 55.56% between 25-29 years. About 2.5% use control pills, 7.5% are smokers, and 32.5 % consume alcohol regularly. No member of the population was taking hormone replacement therapy (Table 1).

When the socio-demographic characteristics of postmenopausal women are considered, 3.33% were below 40 years and above 70 years, 16.67% were within the age range of 40-49, 60% between the age range of 50-59, and 16.67% between 60-69 years. In terms of menarche and the onset of menopause, 26.67% had menarche between 11 and 12 years of age, and 73.33% had menarche after 14 years; while 23.33% were menopausal at less than 45 years, 40% between 46-50, and 36.67% between 51-55 years. About 16.67% of had their first child before the age of 20, 33.33% between 20-24, 43.3% between 25-29; and 6.67% gave birth after 30 years. None of the participants had taken birth control pills or hormone replacement therapy, and there were neither smokers nor alcohol consumers among the population (Table 2).

3.2 Results of Aromatase Levels in Postmenopausal and Premenopausal Women

The comparative result of aromatase concentration in postmenopausal and premenopausal women indicated significantly higher values in the postmenopausal women at $p<0.05$ (Table 3). When different age groups of the premenopausal women were considered, no significant differences were observed between the age groups of 20-29, 30-30, and 40-49 (Table 4). However, significantly different values were obtained when different age groups in postmenopausal women were considered. Significantly higher values were observed in the age group 60 -69 compared with 50-59 and 40-49 and similarly, significantly higher values were seen in the 50-59 age group compared with the 40 -49 age group (Table 5).

Table 1. Socio-demographic characteristics of the premenopausal population

Characteristics	Number (N)	%	X^2	*P* value
Age Range (Years)				
20-29	32	80	149.3	<0.0001
30-39	6	15		
40-49	2	5		
Age at Menarche (Years)				
7 – 11	9	22.5	37.03	<0.0001
12 – 13	21	52.5		
14 or older	6	15		
Smoking and Alcohol Consumption				
Smokers	3	7.5	169.0	<0.0001
Non-Smokers	37	92.5		
Alcohol Consumers	13	32.5		
Non-Alcohol Consumers	27	67.5		
Childbirth Status				
Had Children	9	22.5	135.1	<0.0001
Never Had Children	31	77.5		
Birth Control Pills (20-24)	1	2.5		

Table 2. Socio-demographic characteristics of the postmenopausal population

Characteristics	Number (N)	%	X^2	*P* value
Age Range (years)				
30-39	1	3.33	158.1, 4	<0.0001
40-49	5	16.67		
50-59	19	63.33		
60-69	4	13.33		
70-79	1	3.33		
Age at Menarche (years)				
12 – 13	8	26.67	44.18	<0.0001
≥14	22	73.33		
Age at Menopause (years)				
< 45	7	23.33	7.146	0.0281
46-50	12	40		
51-55	11	36.67		
Childbirth Status				
Had Children	29	96.67		
Never Had Children	1	3.33	173.0	<0.0001
Age at 1st childbirth (years)				
<20	5	16.67	45.01	<0.0001
20 – 24	10	33.33		
25 – 29	13	43.33		
>30	2	6.67		

Table 3. Comparative results of aromatase level in postmenopausal and premeno pausal women

Parameter	Postmenopausal (n = 30)	Premenopausal (n = 40)	P Value	Remark
Aromatase (ng/mL)	4.45±3.36	0.27±0.18	< 0.0001	S

S=Significant at P=.05

Table 4. Comparative results of aromatase level in postmenopausal women at different age groups (years)

Parameter	40-49 (yrs)	50-59(yrs)	60-69(yrs)	F value	P value	Remark
Aromatase (ng/mL)	$0.70±0.78^a$	$5.05±3.38^b$	$7.34±0.16^c$	6.62	0.0049	

PostHoc: Values in the row with different superscripts (a. b, c) differ significantly at P=.05

Table 5. Comparative results of aromatase level in premenopausal women at different age groups (years)

Parameter	20-29(yrs)	30-39(yrs)	40-49(yrs)	F value	P value	Remark
Aromatase (ng/mL)	0.27±0.17	0.24±0.25	0.35±0.07	0.27	0.76	NS

NS=Not Significant at P=.05

4. DISCUSSION

The findings discussed here outline the variations in aromatase expression between premenopausal and postmenopausal women as well as the effects of menopause (and aging) on aromatase expression in healthy women.

The significantly higher value of aromatase level in postmenopausal women compared to premenopausal women (Table 3) indicates that menopause causes an enhanced expression of aromatase in the blood and probably tissues. This could be a result of the major hormonal changes induced by menopause or the change in

hormones inducing the menopause. The higher level of aromatase could also be linked to lowered physical activities, weight gain and higher BMI, and increased presence or activities of adipocytes during the menopausal transition. Our result is in agreement with the finding from Brown et al. [21] who reported that aromatase activity is higher in postmenopausal women than premenopausal women. However, our aromatase value in postmenopausal women is slightly lower than the result from the study on postmenopausal and premenopausal women by Enas et al. [22].

The values of aromatase increased from 0.70±0.78 ng/mL, 5.05±3.38 ng/mL, to 7.34±0.16 ng/mL for the different age groups of 40-49 years, 50-59 years, and 60-69 years respectively (Table 4) indicates an age-dependent increase in aromatase levels. In other words, the expression of aromatase is also signaled by age, thus making age a critical player in aromatase expression in the blood and tissue. This result supports the findings of Brown et al. [21], who concluded that menopausal status and age are strongly related to an increase in aromatase expression. These results further suggest that in postmenopausal women, the synthesis of aromatase occurs in several extra-ovarian locations such as adipose tissue, and skin. This age-dependent expression of aromatase is further supported by the results observed in premenopausal women. Their results indicated no significant difference in aromatase levels across the age groups 20-29 years, 30-39 years, and 40-49 years (Table 5).

The results obtained further suggest that for most women in their active reproductive age, their aromatase level may remain steadily low, increasing gradually as aging processes. The steadily low values of aromatase in premenopausal women could be due to the production of aromatase majorly from the granulose cells of the ovaries under the regulation of gonadotropins (follicle stimulating Hormone and Luteinizing Hormone) unlike in postmenopausal where production is from extra-ovarian sources. A similar finding was also documented by Shaheenah et al. [23], who demonstrated low expression of aromatase in the blood samples of premenopausal women. Ben-Chioma and Elekima [24], in related studies, have documented the need for trace elements supplementation (especially selenium) among females with a family history of breast cancer to help eradicate or reduce the impact of adiposity on the generation of free radical and aromatase, especially in postmenopausal women.

5. CONCLUSION

There is an age-related increase in aromatase expression in relation to menopausal status and therefore, estimation of aromatase concentration in premenopausal and postmenopausal women should be recognized as a routine test in some conditions.

CONSENT

All relevant information, like the purpose and methodology of the experiment, was explained to study participants beforehand, and informed consent was obtained.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee of Ethical review Board of Rivers State University Teaching Hospital, Port Harcourt with approval number RSUTH/REC/2023315and have therefore been performed by the ethical standards laid down in the 1964 Declaration of Helsinki."

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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