Int. J. Curr. Res. Med. Sci. (2022). 8(9): 25-35

International Journal of Current Research in Medical Sciences

ISSN: 2454-5716

(A Peer Reviewed, Indexed and Open Access Journal)

www.ijcrims.com

Original Research Article

Volume 8, Issue 9 -2022

DOI: http://dx.doi.org/10.22192/ijcrms.2022.08.09.005

Duration of Menopause and Status of Iron Parameters in Apparently Healthy Postmenopausal Women in Port Harcourt, Nigeria.

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Abstract

Iron participates in a variety of metabolic processes but potentially toxic in excessive or deficient state. Disorders include iron deficiency, iron overloads or effects on pre and postmenopausal women. This study compared iron status of premenopausal and postmenopausal women in Port Harcourt, Nigeria alongside assessing the effect of duration of menopause on iron status of the subjects. **Materials and Methods**: Apparently healthy 120 postmenopausal women and 120 premenopausal women between 18-65 years participated in the study. Five milliliter venous blood taken from subjects were analysed using Elabscience Sandwich Enzyme-linked immunoassay Monocent Ferritin reagents for serum ferritin and the transferrin kit for serum transferrin. Full blood count comprising haemoglobin (HGB),red blood cell (RBC) count, haematocrit (Hct), and red cell indices was done using SYSMEX KN21 auto-analyzer. Data were analyzed using Graph-Pad Prism 8.0.2 version with $p < 0.05$ as statistically significant. **Results:** The postmenopausal when compared with premenopausal woman, had higher mean values of statistically significantly different ($p>0.05$) in ferritin (209.40 ± 175.30ng/ml versus 63.27 ± 23.78ng/ml), HGB (13.04 ± 2.60g/dl versus 11.65 ± 1.44g/dl), HCT (38.30 ± 6.37% versus 36.13 ± 3.94%), MCH (27.12 ± 2.81pg/cell versus 26.29 ± 2.94pg/cell) and MCHC (33.87 ± 3.75g/dl versus 32.22 ± 1.45g/dl). Transferrin mean value (112.90 ± 109.50mg/dl versus 336.50 ± 104.20mg/dl) was statistically significantly higher in the premenopausal compared to the postmenopausal ($p>0.05$).Mean values of MCV (80.60 ± 6.12fl versus 80.97 ± 8.35fl) and RBC (5.00 ± 3.13$\times 10^{12}$/L versus 4.45 ± 0.60 $\times 10^{12}$/L)was statistically significant different ($p>0.05$) also between post and premenopausal women. Comparing the effect of duration of menopause on the studied parameters in this study, showed no significant difference. Correlation between HGB and HCT ($r =0.883$), MCV and MCH ($r = 0.566$) in postmenopausal women were Strongly positive. High body iron in apparently healthy postmenopausal women in Port Harcourt was observed and supports studies from other parts of the world. The range of iron stores levels in premenopausal and postmenopausal women in Port Harcourt was revealed and duration of menopause was not found to be a causative factor for iron overload.

Keywords: Serum ferritin, transferrin, haemoglobin, haematocrit, red cell indices, premenopausal and postmenopausal.

Introduction

Iron is an essential component of haemoglobin, an erythrocyte (red blood cell) protein that transfers oxygen from the lungs to the tissues. As a component of myoglobin, another protein that provides oxygen, iron supports muscle metabolism and healthy connective tissue. Iron is also necessary for physical growth, neurological development, cellular functioning, and synthesis of some hormones (Wessling-Resnick, 2014). Only a limited number of living things appear to be able to thrive without taking advantage of the capacity of iron to exchange electrons (Golonka *et al*., 2019).

Iron stores in the body exist primarily in the form of ferritin. In the body, small amounts of ferritin are secreted into the plasma. The concentration of this plasma (or serum) ferritin is positively correlated with the size of the total body iron stores in the absence of inflammation. A low serum ferritin value reflects depleted iron stores, but not necessarily the severity of the depletion as it progresses. Normal ferritin concentrations vary by age and sex. Among women, serum ferritin values remain relatively low until menopause and then rises (Gibson, 2005).

Menopause, also known as the climacteric, is the time in women's lives when menstrual periods stops permanently, and they are no longer able to bear children (Eunice, 2015) due to loss of ovarian follicular activity. It results in a decrease in estrogen secretion that is responsible for most of the features seen in menopausal women (Thirup, 2003) and it also marks the end of reproductive potential. It is the climax of some 50 years of reproductive aging – a process that unfolds as a continuum from birth through ovarian senescence to the menopausal transition and the post menopause. It occurs between 45 to 55 years.

The term 'menopausal transition' can be used synonymously with the term 'peri-menopause' (Stanzel *et al*., 2020). The period of change in ovarian function from being fertile to becoming infertile, called menopausal transition, is a natural and inevitable change that affects all women. During perimenopause, fewer eggs exist for the ovaries to stimulate, and menstrual periods become irregular. This period of fluctuation can last up to 10 years. Cessation of menstruation marks the later stage of perimenopause. This transition is known to play a major role in the etiology of symptoms such as hot flashes, night sweats, uterine bleeding problems, and vulvovaginal atrophy. Although increased iron as a result of menopause is considered within normal physiologic range, potential health problems in women, as well as in men or neonates, could be linked to increased iron storage, which is normal but not necessarily healthy (Sullivan, 2004). Excessive body iron store causes tissue damage and postmenopausal women are susceptible. Several markers can represent iron stores. The iron parameters analyzed in this research include: serum ferritin, serum transferrin, red blood cell (RBC) count, hemoglobin, hematocrit and red cell indices. There is tendency of elevated body iron stores in post-menopausal women which brings about some menopausal symptoms and diseases. Early and proper diagnosis of body iron stores will allow for improved patient care and health outcomes for older female patients (Sullivan, 2004).

Although previous work has been done on iron stores, the complex evaluation of some of the parameters included in this study and the method of assay used were not considered. Also the assessment of these parameters in pre and postmenopausal women based on duration of menopause was not considered. There is therefore paucity of information on the assessment of these parameters especially in Rivers State, hence the need for this study.

Materials and Methods

Study design and subjects

This study was carried out in Port Harcourt metropolis, Rivers State, Nigeria. Port Harcourt is the capital of Rivers State, Southern Nigeria (South-South geopolitical zone of Nigeria). It lies along the Bonny River, 41 miles (66 kilometer) upstream from Gulf of Guinea. The subjects for this study were recruited from schools, churches, houses and markets.

A total of 240 apparently healthy subjects (aged between 18 and 65 years) were recruited to participate in the study, out of which 120 subjects (test subjects) were postmenopausal women while 120 subjects (control subjects) were premenopausal women who have not reached menopause. A simple random technique was employed for the collection of sample and questionnaire were used.

Ethical Consideration

Ethical clearance was obtained from the Rivers State Ministry of Health,Ethical Committee on Research before carrying out this research.

Inclusion Criteria

Apparently healthy non-pregnant women, within the age range of 18 to 65 years, subjects who were not on any medication / drug for the past two weeks and individuals willing to offer written informed consent were included.

Exclusion Criteria

Pregnant and lactating women, women on hormone therapy for menopause, cigarette smokers, alcoholics, women on iron therapy, women who are suffering from any form of ailment e.g. hypertension, diabetics, cancer and subjects who declined consent were excluded from the study.

Informed Consent

The subjects gave informed consent to blood collection and the use of blood.

Blood Sample Collection

Whole blood samples were collected from the subjects as recommended by the reagent kit used until the required sample size was reached. Venous blood was drawn from antecubital fossa of the subjects with the use of vacutainer. Two (2) ml of venous blood was collected into a glass vacutainer sample bottle containing 0.5ml of 1.2mg/ml dipotassium ethylene diamine tetra-acetic acid (EDTA), and was mixed thoroughly for the estimation of full blood count (FBC). Three (3) ml of the venous blood was also drawn into a plastic non- anticoagulated sample bottle for the analysis of serum ferritin and serum transferrin. Blood samples collected were transported under cold chain from site of collection to Port Harcourt where the samples were analyzed within 24 hours for FBC. Serum was obtained by centrifugation and stored in plain bottle at freezing (-15ºC) temperature till analysis of serum ferritin and serum transferrin.

Methods of Assay

1. Automation for full blood count using SYSMEX haematology auto-analyzer, manufactured by KOBE, Japan, model No: KX- 21N.
2. Serum ferritin was done by Enzyme-linked immunosorbent assay (ELISA)
3. Measurements of transferrin was performed using Enzyme-linked immunosorbent assay (ELISA)

Statistical analysis

The generated data were analyzed using Graph-Pad Prism 8.0.2.263 version to obtain mean and standard deviation of the study group. Analysis of variance was used to determine the statistical significance between test group and the control group. Results were presented in tables as means ± standard deviation (M±SD). Sample t-test was used where only two variables were compared and p-value of <0.05 were considered to be statistically significant.

Results

Table 1 shows the socio-demographic characteristics of the subjects. Majority of the control subjects were within the ages of 20-24 years 31(12.92%) and 25-29 years 31(12.92%), also was followed by those within the age range of 30-34 years 15 (6.25%). A further 14 (5.38%) were those within the ages of 35-39 years and 40-44 years. Those above 45 years of age accounted for 11(4.58%) and the least proportion of the study participants in the control group 4(1.67%)

were those within 15-19 years. In the test subjects, majority of the study participants, 58(24.17%) were those within the age range of 55-59 years, a further 44(18.33%) represented those within the age range of 60-64 years and lastly those within the ages of 65-69 years accounted for 18(7.50%).

Table 1: Socio-Demographic Characteristics of the Subjects

Characteristics of the Population		
Age of Premenopausal women	**Number of participant (n)**	**Percentage of the Population (%)**
15 – 19	4	1.67
20 – 24	31	12.92
25 – 29	31	12.92
30-34	15	6.25
35-39	14	5.83
40-44	14	5.83
>45	11	4.58
Mean±SD (30.85±8.64)	**120**	
Total	**120**	**50**
Age of post-menopausal women		
55-59	58	24.17
60-64	44	18.33
65-69	18	7.50
Mean±SD (59.93±3.50)	**120**	
Total	**120**	**50**
Grand Total	**240**	**100**
Duration of Menopause (years)		
1-5	6	5.00
6-10	56	46.67
11-15	47	39.17
>15	11	9.17
Total	**120**	**100**

The result in Table 2 shows the mean and standard deviation offerritin, transferrin, red blood cell (RBC), haemoglobin (HGB), haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) of apparently healthy post- menopausal women (test subjects) compared with the mean and standard deviation of premenopausal women (control subjects). There was significant difference ($p<0.05$), in the mean values of ferritin (209.40 ± 175.30ng/ml versus 63.27 ± 23.78ng/ml), transferrin (112.90 ± 109.50mg/dl versus 336.50 ± 104.20mg/dl), HGB (13.04 ± 2.60g/dl versus 11.65 ± 1.44g/dl), HCT (38.30 ± 6.37% versus 36.13 ± 3.94%), MCH (27.12 ± 2.81pg/cell versus 26.29 ± 2.94pg/cell) and MCHC (33.87 ± 3.75g/dl versus 32.22 ± 1.45g/dl) but there was no statistically significant difference ($p>0.05$) in the mean values of MCV (80.60 ± 6.12fl versus 80.97 ± 8.35fl) and RBC (5.00 ± 3.13$\times 10^{12}$/L versus 4.45 ± 0.60 $\times 10^{12}$/L.

Table 2: Comparison of Ferritin, Transferrin and Complete Blood Counts in Pre and Post-Menopausal women (Test) (Mean ± SD)

Study Groups	Parameters								
	Age (years)	**Ferritin (ng/mL)**	**Transferrin (mg/dL)**	**RBC ($x10^{12}$/L)**	**HGB (g/dL)**	**HCT (%)**	**MCV (fl)**	**MCH (pg/cell)**	**MCHC (g/dL)**
Premenopausal women (Control Subjects) (n=120)	30.85±8.64	63.27±23.78	336.50±104.20	4.45±0.60	11.65±1.44	36.13±3.94	80.97±8.35	26.29±2.94	32.22±1.45
Post-Menopausal Women (Test Subjects) (n=120)	59.93±3.50	209.40±175.30	112.90 ±109.50	5.00±3.13	13.04±2.60	38.30±6.37	80.60±6.12	27.12±2.81	33.87±3.75
T value	34.18	8.532	16.20	1.906	5.142	3.166	0.3885	2.237	4.508
P value	<0.0001****	<0.0001****	<0.0001****	0.0578	<0.0001****	0.0017**	0.6980	0.0262*	<0.0001****
Remark	S	S	S	NS	S	S	NS	S	S

Key: RBC= red blood cell, HGB= haemoglobin, HCT= haematocrit, MCV= mean corpuscular volume, MCH= mean corpuscular haemoglobin and MCHC= mean corpuscular haemoglobin concentration. S=Significant($p<0.05$); NS= Not Significant ($p>0.05$), ****= $p<0.0001$, shows Significance Level.

Table 3: Effect of Duration of Menopause on Ferritin, Transferrin and Complete Blood Counts in Post-menopausal women

Duration of Menopause (years)	Parameters								
	Age (years)	Ferritin (ng/mL)	Transferrin (mg/dL)	RBC ($x10^{12}$/L)	HGB (g/dL)	HCT (%)	MCV (fl)	MCH (pg/cell)	MCHC (g/dL)
1-5 (n=6)	55.00±0.00[a]	92.70±51.44	32.02±25.94	4.33±0.62	11.95±1.57	36.37±4.86	84.45±5.33	27.68±1.72	32.80±0.51
6-10 (n=56)	57.21±1.44[b]	194.70±168.40	114.90±103.60	5.36±4.50	13.10±2.34	38.15±6.36	80.48±6.11	27.01±2.75	34.08±3.66
11-15 (n=47)	62.77±1.88[c]	219.60±171.00	111.00±104.10	4.72±0.84	13.03±3.95	38.33±7.06	80.48±6.11	27.47±2.75	33.86±4.06
>15 (n=11)	64.36±1.57[d]	304.30±233.20	155.30±119.80	4.75±0.48	13.39±2.82	39.70±4.23	77.06±7.03	25.75±3.65	33.46±4.02
F value	148.4	2.210	1.679	0.4933	0.4229	0.3631	2.274	1.237	0.2604
P value	<0.0001****	0.0907	0.1754	0.6876	0.7369	0.7798	0.0838	0.2997	0.8538
Remark	S	NS	NS	NS	NS	NS	NS	NS	NS

Key: RBC= red blood cell, HGB= haemoglobin, HCT= haematocrit, MCV= mean corpuscular volume, MCH= mean corpuscular haemoglobin and MCHC= mean corpuscular haemoglobin concentration.

Within each parameter, mean ±SD with different superscript a, b, c and d is significantly different at $p<0.05$, S=Significant; NS= Not Significant ($p>0.05$), ****= $p<0.0001$, shows Significance Level. The superscripts a, b, c and d shows that mean ± SDs are significant from each other after Tukey's multiple test of comparison.

Table 4: Correlation of Age, Parity, Duration of Menopause, Ferritin, Transferrin and Complete Blood counts in Postmenopausal women

	Age (years)	Duration of menopause (years)	Parity	Ferritin (ng/mL)	Transferrin (mg/dL)	RBC ($x10^{12}$/L)	HGB (g/dl)	HCT (%)	MCV (fl)	MCH (pg/cell)	MCHC (g/dL)
Age (years)	**1**										
Dur (years)	-0.002	1									
Parity	-0.168	0.279	1								
Ferritin (ng/mL)	0.156	0.147	-0.053	1							
Transferrin (mg/dL)	0.063	0.026	-0.150	0.213	1						
RBC ($x10^{12}$/L	-0.036	-0.022	-0.047	-0.073	0.045	1					
HGB (g/dL)	0.027	-0.035	-0.263	0.032	-0.013	0.055	1				
HCT (%)	0.037	-0.010	-0.242	-0.093	0.005	0.051	**0.883**	1			
MCV (fl)	-0.089	0.155	0.169	0.014	0.102	-0.090	-0.306	-0.250	1		
MCH (pg/cell)	-0.090	0.011	-0.109	-0.006	0.048	-0.017	**0.422**	0.305	**0.566**	1	
MCHC (g/dL)	-0.040	-0.030	-0.137	0.016	-0.095	0.044	**0.595**	0.435	-0.224	0.400	1

Key: RBC= red blood cell, HGB= haemoglobin, HCT= haematocrit, MCV= mean corpuscular volume, MCH= mean corpuscular haemoglobin and MCHC= mean corpuscular haemoglobin concentration.
The numbers in bold represents the correlated variables.

Table 3 shows the effect of duration of menopause and it was observed that postmenopausal women who have been into menopause for more than 15 years had the highest mean age value 64.36±1.57, this was followed by women who have been into menopause for 11-15 years (62.77±1.88), 57.21±1.44 for those who have been into menopause for 6-10 years and (55.00±0.00)· for those who are 5 years into menopause. These values were found to be statistically significant (p=148.4, p<0.0001).However, there was no significant difference (p<0.05) in all the studied parameters in postmenopausal women.

The correlation pattern of age, parity, ferritin, transferrin, RBC, HCT, HGB, MCV, MCH and MCHC in postmenopausal women is shown in table 4. There was no correlation between age vs duration of menopause (r = -0.002), positive weak correlation was observed between duration of menopause vs parity (r = 0.279). No correlation was observed between parity vs ferritin (r = - 0.053). Positive weak correlation exists between ferritin vs transferrin (r = 0.213). There was no correlation between transferrin vs RBC (r = 0.045) and RBC vs HGB (r = 0.055). Strong positive correlation was observed between HGB vs HCT (0.883), negative weak correlation exists between HCT vs MCV (r = -0.250), strong positive correlation was observed between MCV vs MCH (r = 0.566) and MCH vs MCHC showed a weak positive correlation (r = 0.400).

Discussion

Biochemical and haematological assessment of iron status in postmenopausal women is necessary as this group of women are already at risk for increased body iron stores due to permanent cessation of menstrual bleeding. An understanding of the risk factors, clinical presentation, and management of these common menopausal symptoms allow for improved patient care and health outcomes for older female patients (Sullivan, 2004). The result of the present study clearly demonstrates that normal ferritin concentrations vary by age and sex in apparently healthy postmenopausal women. Among women, serum ferritin values remain relatively low until menopause and then rises (Gibson, 2005). This study revealed a statistically significant higher mean serum ferritin concentration in postmenopausal women compared with premenopausal women. This could be due to iron increase as a result of decreasing menstrual periods (Millan & Kirchhoff, 1992; Zacharski *et al*., 2000). Post-menopausal women conserve iron as menstrual cycles and periods discontinue, since the major route of iron loss is by blood loss, this may account for the increase in serum ferritin of post-menopausal women. This result agrees with previous work reported by Zacharski *et al*., (2000) in which there is significant difference in serum ferritin in postmenopausal women as against the control. It also agrees with another study by Sullivan and Erhabor *et al*. (2017) which reported that serum ferritin levels were significantly higher in post-menopausal women compared with pre-menopausal women. Ferritin is the major storage form of iron in the body and it is an acute phase reactant protein (ARP).It is essential for making new red blood cells (RBCs) and haemoglobin. Small quantities of ferritin are also present in human serum and are elevated in conditions of iron overload and inflammation.

Also, postmenopausal women had significantly decreased value of serum transferrin than premenopausal women suggesting accumulation of iron stores due to the permanent cessation of menstrual flow. This is in line with the report of Okafor *et al*. (2019), which affirmed high value of transferrin saturation value in post-menopausal women compared to pre-menopausal women. Transferrin saturation of less than 20 % indicate iron deficiency, while transferrin saturations of more than 50% suggests iron overload. Transferrin is the transporter protein for iron and its concentration can be determined by immunological methods (WHO & CDC, 2016). Both total iron binding capacity (TIBC) and transferrin rise in iron deplete states (iron deficiency anaemia) and fall in inflammatory and iron overload disorders. This study revealed that there was no statistically significant difference in

the mean values of red blood cell (RBC) in postmenopausal women and premenopausal women in relation to their age, as compared within the ages of these groups of women. This result contradicts previous work carried out by Obeagu *et al*. (2016) in which premenopausal women were found to have increased red blood cells as compared to postmenopausal women.

In our study, there was a significant increase in the haemoglobin (Hb) concentration of postmenopausal women compared to premenopausal women. This could be due to dehydration in this group of women because as one ages, these receptors may become less sensitive to water changes, making it harder for them to detect thirst. Hb is based on whole blood and is therefore dependent on plasma volume. It is elevated during dehydration than in normovolemic state. This supports the report of Obeagu *et al*. (2016) which showed that there was an increase in the Hb level of menopausal women when compared with that of the premenopausal women, though not significant. The findings of this study also showed that there was an increase in the haematocrit (HCT) level in postmenopausal women compared to the control. The variation could be due to changes in the body physiology with ageing such as altered metabolism, increased blood pressure, cessation of menstrual bleeding etc. Furthermore, it could also be due to nutritional/dietary intake of some nutrients, environmental and or genetic makeup. Poor nutrition resulting in vitamin B12 and folic acid deficiency in old age could cause early haematological changes during ageing (Padalia *et al*., 2014). Our study is consistent with Kharab (2008), which indicated that higher values for HCT were found. In this study, it was observed that mean corpuscular volume (MCV) showed no significant difference between the postmenopausal women and the control group. The values in both groups were within the normal range of individuals in the locality of the study. Previous studies by Obeagu *et al*. (2016), contradicts the result of this work and suggested that there was a significant difference in the mean values of postmenopausal women than the control group.

This study also revealed that there was a statistically significant increase in the mean corpuscular haemoglobin (MCH) and the mean corpuscular haemoglobin concentration (MCHC) of postmenopausal women when compared with that of the premenopausal women. This may be caused by macrocytic anaemia due to vitamin B12 or folate deficiency. Most of these women may be vegetarians as their diet contains little or no vitamin B12. Furthermore, the incidence of vitamin B12 and folate deficiency, often manifested by macrocytosis, increases with age (Kauwell *et al*., 2000). This is in line with previous studies by Obeagu *et al*. (2016) which indicated that the increase in the MCH and MCHC with menopause is usually noticed in women who were at least 10 years postmenopausal. Mean Corpuscular Hemoglobin (MCH) quantifies the amount of hemoglobin per red blood cell and is calculated by dividing the hemoglobin by the red blood cell count while Mean Corpuscular Hemoglobin Concentration (MCHC) indicates the amount of hemoglobin per unit volume and is calculated by dividing the haematocrit (Hct) by the concentration of red blood cell count. In comparing the effect of duration of menopause on age, ferritin, transferrin, RBC, HGB, HCT, MCV, MCH and MCHC, it was observed that only age showed significant variation. All the parameters analyzed showed no statistically significant difference suggesting that duration of menopause did not exert any significant influence in the studied parameters.

The findings of this study also showed that a strong positive correlation exists between MCV and MCH ($r = 0.566$) in postmenopausal women. This suggests larger number of erythrocytes which confirms some evidence that the life span of red blood cells Is shorter in the elderly, and as a result, the percentage of red blood cells released from the bone marrow increases progressively with age. Furthermore, the incidence of vitamin B12 and folate deficiency, often manifested by macrocytosis, increases with age (Kauwell *et al*., 2000).

In conclusion, the result of the present study showed that high body iron amount was more frequent in apparently healthy postmenopausal women studied in Port Harcourt as observed in studies in other parts of the world. This study revealed the iron stores levels in premenopausal and postmenopausal women in Port Harcourt. Also duration of menopause in this study was not found to be a causative factor for iron overload.

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How to cite this article:
Ohahuru, Vivian C., Koate, Baribefe B.D & Ken-Ezihuo, Stella U. (2022). Duration of Menopause and Status of Iron Parameters in Apparently Healthy Postmenopausal Women in Port Harcourt, Nigeria. Int. J. Curr. Res. Med. Sci. 8(9): 25-35.
DOI: http://dx.doi.org/10.22192/ijcrms.2022.08.09.005