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ORIGINAL RESEARCH

Alterations in Haematological and Clotting Profile of Post-Menopausal Women in Benin City, Nigeria

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Abstract

Background: The cessation of ovarian functions at menopause and the accompanying decline in the production of ovarian steroid hormones creates a unique set of health concerns for women. Reductions in sex steroid levels, particularly oestrogen, have been associated with various diseases and conditions, including bleeding disorders, coronary heart disease (CHD), osteoporosis, cognitive dysfunction, urinary incontinence, hot flushes, and mood changes, among others.
Objective: To determine changes in haemorheological and clotting profile in post-menopausal women.
Methods: Two hundred participants comprising one hundred and fifty post-menopausal women and fifty healthy pre-menopausal control subjects were studied. The investigations carried out include whole blood viscosity, plasma viscosity, fibrinogen concentration, Prothrombin time (PT), Activated partial thromboplastin time with kaolin (APTTK) levels and complete blood count using standard methods.
Results: The mean age ($p = 0.01$), platelet count ($p = 0.013$), neutrophil ($p = 0.03$), neutrophil to lymphocyte ratio ($p = 0.045$) and platelet to lymphocyte ratio ($p = 0.044$) in postmenopausal women were significantly higher while lymphocyte count ($p = 0.004$) was significantly lower in postmenopausal compared to premenopausal women. Similarly, plasma oestradiol ($p = 0.001$), plasma viscosity ($p = 0.03$), relative blood viscosity ($p = 0.03$), whole blood viscosity ($p = 0.03$) and PTTK ($p = 0.04$) were significantly lower among postmenopausal women compared to premenopausal control subjects.
Conclusion: Relative plasma viscosity correlated positively with age. There were significantly lower levels of haemorheological and clotting profile in post-menopausal women. These changes may be due to age or a decline in circulating oestrogen levels.

Keywords: Coagulation, Female, Menopause, Nigeria, Oestradiol, Plasma viscosity, Post-menopause.

Introduction

The moment in a woman's life following menopause is called post-menopause. A woman is post-menopausal when she has not had her menstruation for an entire year. [1] Menopausal symptoms, such as hot flashes, can cease for most women during this stage. [2, 3] Hormone

replacement therapy has been associated with lower rates of cardiovascular diseases in post-menopausal women. [4, 5] The underlying metabolic basis for the reduced vascular risk has been explained, in part, by favourable changes in low-density lipoprotein (LDL), high-density lipoprotein (HDL), and thrombotic markers such as plasminogen activator inhibitor, fibrinogen, and D-dimer. [6 - 8]

A few researchers have also reported changes in post-menopausal women's coagulation factors. The investigation of coagulation factors in post-menopausal women revealed that fibrinogen levels rise within six weeks of hormone replacement therapy (HRT), and antithrombin III levels fall, leading to a thrombogenic state. In another report, fibrinogen and LDL cholesterol, generally recognised risk markers of cardiovascular disease, were favourably influenced by raloxifene therapy on healthy post-menopausal women. [9] Furthermore, in investigating the effect of oestrogen-progesterone hormonal replacement therapy on blood coagulation and fibrinolysis in post-menopausal women, prothrombin time (PT) was low. The shortening of PT observed in post-menopausal women, and other study groups was attributed to the increase in factor VII. However, there was no observable change in activated partial thromboplastin time (APTT) and thrombin time (TT). [10]

Post-menopausal stage of life in women is associated with a low oestrogen level. Menopausal symptoms also accompany this decrease in oestrogen. Previous research has also reported that post-menopausal women are at increased risk of developing conditions like atherosclerosis, coronary heart disease, osteoporosis, and cancer. [11, 12] These conditions were more complicated in women who smoked cigarettes. [13] Despite these reports, there is a lack of information on the haemorheological changes and clotting profile of post-menopausal women in Nigeria. This study aimed to determine the changes in haemorheology and clotting profile in a cohort of post-menopausal Nigerian women.

Methods

This study was a cross-sectional survey of post-menopausal women conducted at the University of Benin Teaching Hospital, Benini City, Edo State, Nigeria, between June 2015 and April 2016. Ethical clearance for the study was obtained from the ethics committee of the Edo State Ministry of Health, Benin City, while informed consent was given by the participants before the commencement of the study. A semi-structured questionnaire was used to collect the socio-demographic and medical history of the participants. Two hundred participants were randomly recruited, comprising 150 post-menopausal women, aged 50 years and above and who had not menstruated for at least one year, without any chronic disease and not on oestrogen replacement therapy. Also, 50 apparently healthy pre-menopausal women, aged ≤40 years who were pre-menopausal and not on oestrogen-based contraceptives were recruited as controls. Full blood count, red cell indices, relative plasma viscosity, whole blood viscosity, fibrinogen, Prothrombin, and PTTK were determined while neutrophil –to-lymphocyte and platelet-to-lymphocyte ratios were calculated.

Inclusion and exclusion criteria

Apparently healthy women who had natural menopause without any hormonal or surgical intervention and weighing 55–60 kg with a height of 150–160cm were enrolled. Weight- and height matched women who were having regular menstruation were included as control subjects. The age of the controls was 30–40 years. Women with lifestyle habits such as tobacco chewing and smoking known to have diabetes mellitus and hypertension, surgically induced menopause or

history of coagulopathies, thyroid diseases, and those on medications known to affect the haematological and haemorrheological values were excluded from the study.

Blood sample collection

Eight millilitres of blood were drawn from the participants in the morning hours. Three millilitres of the venous blood were dispensed in Ethylene diamine tetra-acetic acid (EDTA) containers with anticoagulant to blood ratio of 1: 99 parts. Another 3mL of the blood was emptied into 0.33mL of 3.2% sodium citrate to determine partial thromboplastin time with kaolin (PTTK) and prothrombin time (PT). Also, 2mL of the blood was dispensed into a plain container, allowed to clot and separated to obtain serum used for oestradiol level assay by Enzyme-Linked Immunosorbent Assay (ELISA) technique. The full blood count, partial thromboplastin time with kaolin and prothrombin time were determined on the same day the blood samples were collected.

Sample Analysis

Full blood count was conducted using the automated ERMA Haematology auto analyser PCE-210N (Diamond Diagnostic; Holliston, USA). The partial thromboplastin time with kaolin (PTTK) and prothrombin time (PT) were assayed manually using reagents supplied by BIOLABS Diagnostics, Maizy, France.

Partial Thromboplastin Time with Kaolin (PTTK)

A 1:9 anticoagulant to blood ratio sample was collected into a clean container and centrifuged using the bucket centrifuge for 15 minutes at 1000g to obtain platelet-poor plasma. Plasma (0.1ml) was dispensed into a clean, dry glass test tube, 0.1ml of pre-warmed kaolin/platelet substitute aliquot was added to the test tube and incubated at 37^{o}C in a water bath for two minutes. The sample was re-calcified with 0.1ml of 0.025M calcium chloride. A stopwatch started immediately while tilting the tube back and forth within the water bath while observing for clot formation. At first sight of clot formation, the watch was stopped, and the result was recorded in seconds.

Prothrombin time (PT)

Citrated plasma (0.1ml) was dispensed into a test tube and incubated at 37^{o}C for two minutes in a water bath. Pre-warmed thromboplastin/calcium chloride reagent (0.2ml) was added to the test tube using an automatic pipette. The stopwatch was started concurrently while tilting the tube in the water bath and examining for clot formation. At first sight of clot formation, the stopwatch was stopped, and the result was recorded in seconds.

Whole Blood and Plasma Viscosity Measurements [14]

Whole blood and plasma viscosity were measured using a low-cost syringe method to measure relative plasma viscosity (RPV) and relative whole blood viscosity (RBV).

Exactly 2ml of the whole blood sample was drawn using a syringe, and the syringe was fixed in a vertical position. The syringe's plunger was removed, and the blood was allowed to flow freely into a collection vessel. The flow of the blood was observed and timed using a stopwatch.

RBV was calculated using the following equation:

RBV$= t_{blood}/ t_{water}$

Where t_{blood} = the time of flow of 2ml of whole blood.

t_{water} = the flow time of 2ml of distilled water (standard).

RPV was measured using a plasma sample with the same procedure described for WBV above.

The following equation calculated RPV:

RPV$= t_{plasma}/ t_{water}$

Where t_{plasma} = the time of flow of 2ml of plasma

t_{water} = the flow time of 2ml of distilled water (standard).

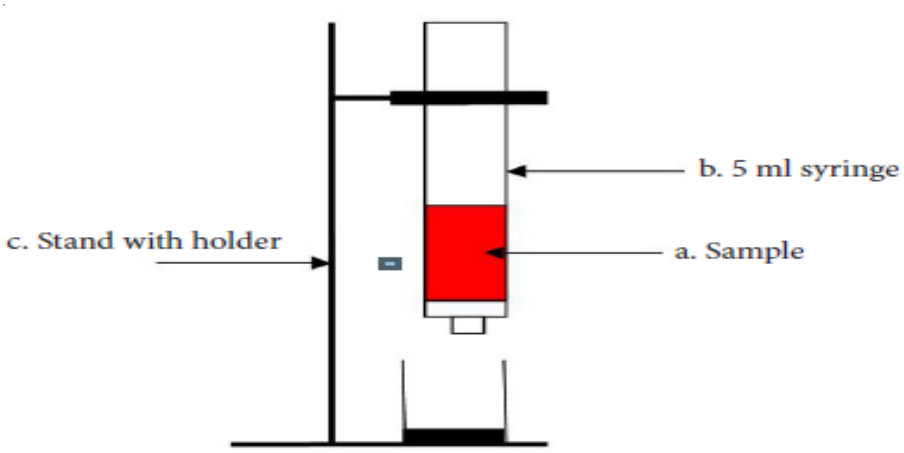

Figure 1: Schematic diagram of simple syringe method for measuring RBV and RPV

Plasma Fibrinogen Determination [15]

The plasma sample was diluted (1:10 dilution), and 200ul of each plasma dilution was dispensed into a test cuvette and incubated at 37^0C for two minutes. A hundred microlitre of the room temperature fibrinogen reagent was added rapidly, and simultaneously the timer started. The clotting time was recorded in seconds, and all the samples were processed in duplicate.

A standard curve was created by plotting the average clotting time against fibrinogen concentration on a log-log graph. The concentration (mg/dl) was on the x-axis and clotting time (sec) on the y-axis. The assigned fibrinogen value on the normal control was used to determine fibrinogen values for the dilution.

Results

Table I compares some haematological indices between post-menopausal and pre-menopausal women. The mean age (p = 0.001), platelet count (p = 0.013), neutrophil (p = 0.03), neutrophil to lymphocyte ratio (p = 0.044) were significantly higher in postmenopausal women, while the total lymphocyte count was significantly lower (p = 0.04) in postmenopausal than premenopausal women.

Table II shows that the mean serum oestradiol, plasma viscosity (PV), relative plasma viscosity (RPV), whole blood viscosity (WBV) and PTTK were significantly lower (p = 0.005) among post-menopausal women compared to pre-menopausal women. However, the difference in the mean prothrombin time (PT) and fibrinogen were not significant between post-menopausal and pre-menopausal women. Table III shows the correlation of haematological parameters with age. Only RPV correlated positively with age, while the correlation between the other measured indices and age were insignificant.

Discussion

Several changes occur in the physiological indices of post-menopausal women, and some of these are known to increase the risk of cardiovascular diseases such as stroke and ischemic heart disease. These alterations may include changes in fat distribution and metabolism and fibrinolytic activities. [16]

Table I: Comparison of selected haematological indices between post-menopausal and pre-menopausal women.

Variables	*Post-menopausal Mean ± SEM (n= 100)*	*Pre-menopausal Mean ± SEM (n= 50)*	*P-value*
Age (Years)	54.70±0.30	29.60±0.50	0.000
PCV (%)	39.90±0.50	40.22±0.60	0.494
Hb conc. (g/dl)	12.27±0.20	12.79±0.20	0.144
MCV (fl)	78.89±0.60	81.10±1.00	0.601
MCH (pg)	23.56±0.40	26.11±0.40	0.966
MCHC (g/dl)	29.58±0.20	30.22±0.20	0.271
PLT ($x10^9$/l)	187.15±5.00	159.84±7.00	0.013
TWBC ($x10^9$/l)	4.26±0.10	4.68±0.20	0.668
Lymphocytes (%)	41.45±2.00	49.94±2.00	0.043
Neutrophils (%)	44.59±2.00	34.70±2.00	0.035
Monocytes (%)	13.74±0.50	15.04±0.80	0.115
NLR	1.07 ±0.05	0.83 ±0.01	0.045
PLR	4.51 ±0.10	3.20 ±0.21	0.044

PCV - Packed Cell Volume; Hb conc. – Haemoglobin concentration; MCV - Mean Cell Volume; MCHC - Mean Cell Haemoglobin Concentration; PLT - Platelet count; TWBC - Total White Blood Cell count; NLR - Neutrophil-to-lymphocyte ratio; PLR - Platelet-to-lymphocyte ratio.

Table II: Comparison of some serum oestradiol, some coagulation and haemorheological parameters between post-menopausal women and pre-menopausal women

Variables	*Post-menopausal Mean ± SEM (n= 100)*	*Pre-menopausal Mean± SEM (n= 50)*	*P-value*
Oestradiol (pg/ml)	41±2.16	265.2±4.6	0.001
PV (ml/min)	5.89±0.10	7.35±0.30	0.031
RPV	2.72±0.07	3.53±0.20	0.032
WBV (ml/min)	13.63±0.20	41.14±2.00	0.039
PT (sec)	14.52±0.20	15.72±1.00	0.726
PTTK (sec)	34.82±0.70	37.32±2.00	0.045
Fibrinogen (g/l)	4.36±0.10	4.29±0.20	0.411

PV – Plasma viscosity; RPV – Relative plasma viscosity; WBV – Whole blood viscosity; PT – Prothrombin Time; PTTK – Partial Thromboplastin Time with Kaolin.

Any condition that causes an imbalance between thrombogenic and anti-thrombogenic mechanisms predisposes humans to either an increased risk of bleeding or a hypercoagulable state. When blood clots develop within blood vessels, an individual is at increased risk of developing thromboembolic events such as deep vein thrombosis or pulmonary embolism. Parts of these venous blood clots can break off and migrate to the lungs, causing pulmonary embolism. Also, arterial clots can travel to other organs, such as the brain, heart, liver, and kidneys, cutting off blood flow to those organs and causing infarction. [7,8] The higher risk of cardiovascular disease among post-menopausal women due to possible coexisting lifestyle factors such as physical inactivity, high calorie/high-fat diet, and other stress conditions necessitated this study. Oestrogen treatment in post-menopausal women can influence blood clotting by increasing plasma fibrinogen and activity of some coagulation factors. Some have reported that

oestrogen influences coagulation by increasing gene transcription of blood clotting proteins. [16, 17] Early identification of haemorheological and coagulation abnormalities may be helpful in the prevention of cardiovascular complications and the choice of a treatment regimen.

Table III: Correlation analysis between haematological variables and age of participants

Variables	*r*	*p-value*
Packed Cell Volume	-0.155	0.059
Haemoglobin concentration	-0.020	0.807
Mean Corpuscular Volume	0.122	0.138
Mean Corpuscular Haemoglobin	0.015	0.484
Mean Corpuscular Haemoglobin Concentration	0.015	0.853
White Blood Cell count	-0.008	0.925
Lymphocytes	-0.005	0.953
Neutrophils	-0.008	0.919
Monocytes	-0.023	0.780
Platelets	-0.010	0.908
Plasma viscosity	-0.073	0.372
Relative plasma viscosity	0.162	0.048
Whole blood viscosity	0.081	0.322
Prothrombin time	-0.059	0.422
PTTK	0.066	0.422
Fibrinogen	-0.051	0.536

Plasma Thromboplastin Time with Kaolin

In the present study, no significant changes were observed in PCV, Hb, MCV and MCH among post-menopausal women compared with pre-menopausal women. This finding is not consistent with previous reports. [18] Menopausal women were reported to have higher red cell counts, haemoglobin concentrations, haematocrits and increased MCV. The author reported a progressive increase in haemoglobin concentration from 40 years to 65 years of age. That was attributed to the effects of the hormonal environment at menopause and the cessation of menstruation. A study reported that the administration of oestrogen to post-menopausal women caused an increased proliferation of haematopoietic stem cells (HSCs), which explained the higher blood counts in women during their reproductive years. [19] No significant alteration in red cell indices were observed in the present study. This is presumably due to differences in the nutritional status of the subjects evaluated.

In the present study, significantly higher levels of lymphocytes, neutrophils, platelet count, NLR and PLR were observed among the post-menopausal women than pre-menopausal women. This finding aligns with a previous

study that reported that the increase in leucocyte count might be due to a rise in infections due to the changes associated with decreased levels of the hormone oestrogen, which include mucosal dryness and the change in the vaginal pH. [20] There was, however, no significant difference between the total WBC count, monocyte count and basophil count in the comparison groups.

A study reported an increase in plasma fibrinogen concentration and whole blood viscosity [21], but in the present study, the plasma fibrinogen concentration was not significantly different from that of the pre-menopausal women. The increase in blood viscosity may be attributed to a rise in the concentration of other plasma proteins other than fibrinogen and not due to PCV rise since the packed cell volume was not significantly altered between the study groups. The increase in blood viscosity coupled with changes in the vascular system previously reported may predispose post-menopausal women to cardiovascular diseases. The significantly lower PTTK without a concurrent change in PT or plasma fibrinogen concentration implies that the coagulation factor(s) of coagulation responsible for this belongs to the intrinsic coagulation pathway. [22] Previous authors reported significantly higher haematocrit and lower platelet count among post-menopausal women, a significantly lower APTT, PT and International Normalized Ratio (INR) in post-menopausal women than in the control group. This observation is not consistent in some ways with the findings from the present study. Whereas PTTK was significantly lower in this study, the PT was not significantly different between the study groups. The authors suggested that the higher values for PCV may enhance red blood cell aggregation, and the raised viscosity might aggravate the atherosclerotic risk. This study's significantly higher platelet count aligns with a previous study. [23] Elevated platelet count may increase the adhesiveness of platelets to the sub-endothelium tissues, and higher leakage of proteins through the vessel wall may increase atherosclerotic risk among post-menopausal women. Conversely, the observed higher platelet count is not consistent with the findings of other authors. [24, 25] Previous workers had reported that the significantly lower platelet count might be due to the low concentration of oestrogen in post-menopausal women.

The red cell indices, including PCV, haemoglobin concentration, MCV, MCH and MCHC, had no significant correlation with age. The total WBC count, lymphocytes count, neutrophil count, monocyte
count also showed no significant correlation with age, whereas the relative plasma viscosity showed a significant correlation with age.
This study showed no significant difference between PCV, haemoglobin concentration, MCV, MCH and MCHC of post-menopausal women and pre-menopausal women. This is in agreement with reports from a previous study. [22] However, a significantly higher level of PCV among post-menopausal women compared to pre-menopausal women had also been reported. [22]

Conclusion

Significant changes observed in some haemorheological and clotting profiles of post-menopausal women when compared to pre-menopausal women might be due to age and oestrogen deficiency. Identifying such changes at the right time may help to prevent vascular-related complications.

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