

ASSESSMENT OF CREATINE KINASE AND LACTATE DEHYDROGENASE ACTIVITIES OF POSTMENOPAUSAL WOMEN IN EKPOMA, EDO STATE, NIGERIA

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ABSTRACT

In a bid to investigate the influence of menopausal on coronary heart disease, plasma creatine kinase (CK) and lactate dehydrogenase (LDH) enzymes were analysed on a prospective cohort of 100 women attending Irrua Specialist Teaching Hospital (ISTH), Irrua, Edo state-Nigeria. They were divided into two groups; postmenopausal women (n = 50) which serve as the test group and the premenopausal women (n = 50) which serves as the control group. The control group were aged 25-40 with a mean age of 34 years, while the postmenopausal women were aged 45 years and above with a mean age of 58 years. Information on menopausal status and other risk factors were obtained while venous blood samples were collected for the analysis of enzyme activities herein studied using standard laboratory procedures. Results which are presented as mean \pm standard deviation showed significantly higher ($P < 0.05$) CK and LDH activities in postmenopausal women (82.88 ± 23.63 ; 174.28 ± 76.63 respectively) compared to those of premenopausal women (48.40 ± 50.76 ; 126.44 ± 50.76 respectively). These findings therefore confirmed increase incidence of coronary heart diseases associated with menopause.

keywords: Menopause, lactate dehydrogenase, creatine kinase, arteriosclerosis

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INTRODUCTION

The word “menopause” [Greek word “Pausis” meaning ‘cessation’ and the root ‘men’ meaning ‘monthly’] literally means the “end of monthly cycle”. Walker and Herndon (2008), describe it as change in human females where the end of fertility is traditionally indicated by the permanent stopping of monthly menstruation or menses. On the other hand, the term “perimenopause” which literally means “around the menopause”, refers to the menopause transition year, a span of time both before and after the date of the final episode of flow (Soules *et al.*, 2001). The menopause transition and postmenopause itself, is a natural life change, not a disease state or a disorder. The transition itself has a variable degree of effects: it can be a difficult time of life for some women, less so for others.

Although, menopause is perhaps most easily understood as the opposite process to menarche; the start of the monthly periods. According to Goff *et al* (2002), a woman has reached menopause when she has not had a menstrual period for 12 months in a

row. However, menopause in women cannot satisfactorily be defined simply as the permanent “stopping of the monthly period” because in reality what is happening to the uterus is quite secondary to the process; it is what is happening to the ovaries that is the crucial factor. As an illustration of the central role of the ovaries, it is worth pointing out that when for medical reasons the uterus has to be surgically removed (hysterectomy) in a younger woman, her period will of course cease permanently, but as long as at least one of her ovaries is still functioning, the woman will not have reached menopause. Even without the presence of the uterus, ovulation and the release of the sequence of reproductive hormone will continue to cycle on, until menopause is reached. In contrast to this, in circumstances where a woman’s ovaries are removed (Oophorectomy), even if the uterus were to be left intact, the woman will immediately be in “surgical menopause”. Surgical menopause is a menopause which is induced both suddenly and totally by removal of both ovaries prior

to the natural menopause (Minkin *et al.*, 1997; Kato *et al.*, 1998).

In younger women, during a normal menstrual cycle the ovaries produce estrogen, testosterone and progesterone in a cyclical pattern under the influence of follicle stimulating hormone (FSH) and luteinizing hormone (LH) which are both produced by the pituitary gland (Burger, 1994). However, the menopause is characterized by marked and often dramatic variation in FSH and estrogen (estradiol) level (Burger, 1994). Menopause is based on natural or surgical cessation of estradiol and progesterone production by the ovaries, which are part of the body's endocrine system of hormone production (Simpson and Davis, 2001). Menopause can be classified based on the level of testosterone, estrogen and progesterone which plays a number of critical roles in a woman's health during and after menopause. Based on this, twelve (12) types of menopause can be identified and are as described in the free encyclopedia (Wikipedia; accessed, 2011).

The signs and effect of the menopause transition can begin as early as 35 or at mid to late 40s. The duration of perimenopause with noticeable bodily effects can be as brief as a few years during which many women undergo noticeable and clinically observable physical changes resulting from hormonal fluctuations such as "hot flash" or "hot flush" sensation (Freeman *et al.*, 2007; Twiss *et al.*, 2007).

Studies have shown that after menopause, women experience an increase risk of heart disease, osteoporosis and bone fracture (Nelson *et al.*, 1994; Simkin-silverman *et al.*, 1995). Researchers have also connected this pattern to decreasing levels of the female hormone estrogen during menopause (Twiss *et al.*, 2007). In this regards, estrogen has been reported to be associated with higher level of high-density lipoprotein cholesterol (HDL-C) and lower-density lipoprotein cholesterol (LDL-C) (Demissie *et al.*, 2006). As such, Demissie *et al.*, (2006), reported that withdrawal of the natural estrogen that occurs in menopause leads to lower HDL and higher LDL and thus increasing the risk of heart disease. Of interest is the activities of plasma creatine kinase (CK) and lactate dehydrogenase (LDH) enzymes which are known to be distributed in a variety of tissues and their serum levels elevated in a variety of clinical conditions such as myocardial infarction, haemolysis, and disorders of the liver, Kidney, lungs, brain damage and Muscle, which serves as markers for the state of tissue or organ (Schumann *et al.*, 1998).

The aim of the present study therefore is to investigate the influence of menses cessation (menopause) on markers of coronary heart diseases via assessing the level of plasma creatine kinase (CK) and lactate dehydrogenase (LDH) enzymes activities.

MATERIALS AND METHODS

Study area: This cross-sectional study was carried out in Ekpoma, Esan West Local Government Area of Edo State. Ekpoma is a rural area located at latitude 6.75°N and longitude 6.13°E of the Greenwich Meridian with an estimated population size of 59,618 people (CSSR, 2008).

Study population: The study population comprised a total of one hundred (100) women made up of fifty (50) apparently healthy postmenopausal women aged 45 and above, and fifty (50) apparently healthy reproductive women between the ages of 25 and 40 years as control group. Subjects were randomly recruited in the study area.

Inclusion criteria: This includes apparently healthy postmenopausal women who are indigene of the study area.

Exclusion criteria: Include pregnant women, smokers, athletic and adolescent females.

Sample collection: After an informed consent was obtained from the subjects, five milliliters of blood samples were collected by venipuncture technique from subjects (both test and control) into lithium heparinized vacuum tubes, mixed gently and spun at 1000 rpm for 10 minutes at room temperature to obtain plasma which was extracted into plain tubes and frozen at -4°C until required for further analysis.

Sample analysis/assay: Determination of plasma Creatine kinase (CK) activity was carried out using the method described in the kit assay system (RANDOX United Kingdom) while plasma lactate dehydrogenase (LDH) activity was estimated by spectrophotometric method of Societal (1989) and Scientific Committee (1982) with kit assay system (BioSystem, Spain).

Statistical analysis: The data obtained were analysed statistically, the Mean±standard deviation values were calculated in each case. The Students t- test statistical method was employed for comparison using SPSS software package version 16.0. A P-value ($p \leq 0.05$) was considered statistically significant at 95% confidence level.

RESULTS

Table 1 shows the results obtained in the investigation in their SI unit (U/L) and reveals the

mean \pm standard deviation of plasma CK and LDH activities of postmenopausal women and control group as 82.88 ± 23.65 ; 48.40 ± 23.00 and 174.28 ± 76.63 ; 126.44 ± 50.76 respectively.

Table 1: Comparison of creatine kinase (CK) and lactate dehydrogenase (LDH) activities in plasma of test and control subjects

Parameters	Control (n=50)	Test (n=50)	t – value	p – value
CK (μ /L)	48.40 ± 23.00^a	82.88 ± 23.65^b	7.39	< 0.05
LDH (μ /L)	126.44 ± 50.76^a	174.28 ± 76.63^b	3.68	< 0.05

Results are mean \pm standard deviation; values in a row having different superscript are statistically different at $p \leq 0.05$; CK = Creatine kinase; LDH = Lactate dehydrogenase

DISCUSSION

The results of the present study showed that plasma CK and LDH activities in postmenopausal women were significantly elevated when compared to that of the control subjects (table 1). It has previously been reported that it is partly a lack of estrogen that increases a woman's risk for developing various acute and chronic diseases such as myocardial infarction, coronary heart disease, Duchenne's muscular dystrophy, stroke and endometrial cancer (Grady *et al.*, 1992). Hence, the results of the present study agrees with several studies which reported that increase in plasma activities of these enzymes may be due to hormonal (estrogen) influence on the cardiovascular system, which play a vital role in lipid metabolism mediated by estrogen receptor alpha and Beta ($ER\alpha$ and $ER\beta$) (Sweetin and Thomson, 1973; Gebara *et al.*, 1995; Demissie *et al.*, 2006). This is evident by the fact that at a younger age and independently of difference in life style, women are at lower risk for coronary heart diseases than men and that this disparity tends to disappear after menopause (Barrett-connor, 2003). On this evidence, the hypothesis as to the protective effect of estrogen against atherosclerosis has been based.

Also in the report of Demissie *et al.* (2006), they found out that at the transcriptional level, estrogen increases hepatic expression of apoprotein gene and the low density lipoprotein (LDL) receptor and decrease the transcription of the lipoprotein lipase (LPL) gene through $ER\alpha$. Thus, when estrogen level decrease after menopause, an increase in LPL activity is observed and this probably contributes to the increase free fatty acids (FFA) and accumulation of

abdominal fat, a factor that causes increased risk for cardiovascular disease in postmenopausal women. On the other hand, Gebara *et al.* (1995) reported that estrogen is involved in proliferation of adipocytes, whereas their deprivation at menopause increases central obesity which is associated with a more atherogenic profile, plasminogen activator inhibitor-1 (PAI-1), interleukin-6 (IL-6) CRP level, insulin resistance and several other components of the metabolic disorders, all of which contributes to the increased incidence of cardiovascular morbidity in postmenopausal women and hence increase serum activities of these enzymes which can serve as markers for cardiac disease. By implication, the results of the present study are further justified.

In the study conducted by Mendelsohn and Karas, (1999), it was observed that; estrogen possesses vasodilating effect in premenopausal women by mediating increased production of vasodilating molecules such as nitric oxide (NO) and prostracycline, as well as decreasing vasoconstricting factors such as endothelin-1. The NO in these premenopausal women attenuates the atherogenic process by decreasing the proliferation of vascular muscle cell. It also plays a central role in the production of cytokines, and decreases adhesion and accumulation of monocytes and platelets on the walls of the affected vessels, thus decreasing cardiovascular disease risk in premenopausal women and hence reduced plasma CK and LDH activities (Mendelsohn and Karas, 1999).

Jialal *et al.*, (2004) found that in postmenopausal women where estrogen is absent or highly deficient, there is increased production of reactive oxygen

species (ROS), which participate in the oxidation of LDL molecules resulting in the formation of foam cells on the vessel walls, production of proinflammatory cytokines (TNF α and IL-6) and increased NO catabolism, thus leading to atherosclerotic lesion in postmenopausal women. The loss of antioxidant activity of estrogen in postmenopausal women could also increase vulnerability to muscle damage (Cardiac and Skeletal) (Barrett- Connor and Bush, 1991; Davis *et al.*, 1994; Liu *et al.*, 2001) and hence increased serum activities of these enzymes here in study. Stamler *et al.* (1978) also reported that obesity, which is also observed in postmenopausal women were seen as indirect coronary risk due to its involvement in elevated blood pressure (hypertension) and hypercholesterolaemia leading to an increased risk of ischaemic heart disease in postmenopausal women.

From the results of this work, significant differences of plasma activities of creatine kinase (CK) and lactate dehydrogenase (LDH) were observed in postmenopausal women compared to premenopausal women. Conclusively, menopause can be said to be associated with a constellation of physical changes, some of which are directly attributed to the loss of estrogen, and hence, increased incidence of cardiovascular diseases (which is the largest health threat to women after menopause). It is important to stress however, that there are a variety of treatments available to protect postmenopausal women from developing serious health problems and most importantly, healthy lifestyle is the best preventive medicine.

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AUTHORS' CONTRIBUTIONS

Dada FL, supervised this study with assistance from Festus OO, Iweka FK, Eyaufe AO, Osagie RN, Imhanrobhor EA. and Akiyang EE.