TITLE

CARDIOVASCULAR RISK FACTORS IN PREMENOPAUSAL WOMEN WITH BREAST CANCER AFTER OOPHORECTOMY

BY

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ABSTRACT

Oophorectomy is a management modality for Pre-Menopausal women with Breast Cancer (PMBC) resulting in reduced oestrogen levels and artificial menopause. Metabolic alterations occur during natural menopause while reduced levels of oestrogen (a known cardioprotective factor) may alter plasma lipids, lipoproteins and other cardiovascular risk factors. The effects of low estrogen levels, which occur in oophorectomised women are not known. This study was therefore designed to investigate the effects of oophorectomy on cardiovascular risk factors.

In this longitudinal study, 69 women were recruited from the University College Hospital, Ibadan and environs. They consisted of 25 PMBC age-matched with 25 Pre-Menopausal women without Breast Cancer-Control (PreMC) and 19 Post Menopausal women without Breast Cancer as control for oophorectomised women (PostMC). Demographic (age, ethnicity, occupation and educational status), reproductive history (parity, age at menarche and menopause, age at first live birth, stage and duration of disease), Body Mass Index (BMI) and blood pressure (systolic-SBP and diastolic-DBP) were obtained from all groups by standard methods. Blood (10 mL) was collected at the luteal phase from PreMC, PostMC, PMBC before oophorectomy and 1, 3 and 6 months after oophorectomy. Plasma obtained was used for analyses of total Cholesterol (TC), High Density Lipoprotein Cholesterol (HDLC) and triglycerides levels by spectrophotometric methods. Low Density Lipoprotein Cholesterol (LDLC) and Very Low Density Lipoprotein Cholesterol (VLDLC) concentrations were computed using Friedwald's formulae while estradiol level was assayed by EIA. Ratios of HDLC to TC, LDLC to HDLC and triglyceride to HDLC (indicative of low density lipoprotein particle size) were also evaluated. Data were analysed using ANOVA, Student's t test and Pearson correlation coefficient and differences significant at p=0.05.

Significantly lower triglycerides (134.8±10.0mg/dL, 171.22±3.0mg/dL), VLDLC (27.0±2.9mg/dL, 34.2±1.9mg/dL) levels and triglyceride to HDLC ratio (5.9±0.8, 15.0±4.3) but higher estradiol (261.0±19.0pg/mL, 102.12±29.3pg/ml), DBP (78.8±2.1mmHg, 70.1±2.5mmHg) and SBP (123±3.8mmHg, 112.4±3.2mmHg) levels were observed in PMBC before oophorectomy compared with PreMC respectively. PostMC had significantly higher SBP (125.3±4.9mmHg, 112.4±3.2mmHg) and TC levels (165.4±13.1mg/dL, 127.2±11.4mg/dl) than PreMC respectively. The BMI (kg/m^2) of PMBC, PreMC and PostMC were similar (25.0, 25.2 and 26.0 respectively). The PMBC at 3 months had increased levels of triglycerides (182.0±21.0mg/dL, 135.0±10.8mg/dL), VLDLC (36.5±1.4mg/dL, 27.0±2.0mg/dL) and TC (185.5±17.6mg/dL, 135.2±9.8mg/dL) compared with PMBC before oophorectomy respectively. When PMBC at 3 months was compared with PostMC, the HDLC (36.0±4.1mg/dL, 25.0±2.7 mg/dL) and estradiol (158.3±31.5pg/mL, 55.3±10.6pg/mL) levels were significantly higher. The HDLC level was also higher in PMBC at 6 months compared to PostMC (47.1±8.8mg/dL, 25.0±2.7mg/dL). The plasma estradiol level of PMBC before oophorectomy reduced from 261.0±69pg/mL

to 77±23.4pg/mL at 1 month, increased to 158.0 ± 31.5pg/mL at 3 months but reduced to 89.0±25pg/mL at 6 months.

Elevated levels of high density lipoprotein and estradiol were found in oophorectomised women with breast cancer. Increased estrogen from one month to six months after oophorectomy could be due to extraglandular estrogen production.
The safe effects of oophorectomy on lipids and lipoproteins in premenopausal Nigerian women with breast cancer suggest low cardiovascular risk.

Keywords: Breast Cancer, Oophorectomy, Pre-menopausal lipoproteins, Cardiovascular risk
Word count: 485

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Adigun Omolola

CERTIFICATION

This is to certify that this research work was carried out by Adigun Margaret Omolola in the Department of Chemical Pathology, Faculty of Basic Medical Sciences, College of Medicine, University of Ibadan, Nigeria.

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ATTESTATION

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DEDICATION

This study is dedicated to the Glory of the Lord God Almighty.

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ABBREVIATIONS

ANOVA	-	Analysis of Variance
ApoA 1	-	Apolipoprotein A – 1
BMD	-	Bone mineral desnity
BMI	-	Body mass index
CEA	-	Carcinoembryonic antigens
CHD	-	Coronary heart disease
CVD	-	Cardiovascular disease
DBP	-	Diastolic blood pressure
DNA	-	Deoxyribonucleic acid
E_2	-	Oestradiol (Oestrogen)
EBCTC	-	Early Breast Cancer trialists collaborative group
EDTA	-	Ethylene diamine tetra acetate
ERT	-	Estrogen replacement therapy
HDLC	-	High density lipoprotein-cholesterol
HRT	-	Hormone replacement therapy
Ht	-	Height
IBCR	-	Ibadan Cancer Registry
IDL	-	Intermediate density lipoprotein
Kg	-	Kilogram
LDLC	-	Low density lipoprotein-cholesterol
LRCP	-	Lipid Research Clinic Programme
M	-	Metre
mg/dl	-	Milligram per decilitre
OHDL	-	Oxidised High Density Lipoprotein
PEPI	-	Postmenopausal Estrogen/Progestin Interventions
p	-	Probability
pg/ml	-	Picogram per millilitre
PMBC	-	Premenopausal breast cancer
PreMC	-	Premenopausal control
PostMC	-	Postmenopausal control

Postmeno	-	Postmenopausal
Premeno	-	Premenopausal
r	-	Pearson correlation
S.EM	-	Standard Error of mean
SBP	-	Systolic blood pressure
SEER	-	Surveillance, Epidemiology and End Results
TC	-	Total Cholesterol
TG	-	Triglycerides
UCH	-	University College Hospital
VLDLC	-	Very low density lipoprotein-cholesterol
Wt	-	Body Weight
µl	-	Microlitre
ml	-	Millilitre
<	-	Less than
>	-	Greater than
+	-	Plus
-	-	Minus
i.e	-	that is

CHAPTER ONE

1.0 INTRODUCTION

Breast cancer is the commonest malignant disease among women in all developed countries (Ewertz, 1996) and the incidence is increasing in developing countries. The worldwide incidence of breast cancer as estimated in 1985 was 720,000 new cases per year, corresponding to 19% of all cancers (Parkin *et al.*, 1993). Malignacy of the breast is one of the commonest causes of death in women aged between 40 to 44 years (Wernberg *et al*., 2009). The incidence of breast cancer is increasing daily all over the world, particularly in areas that used to have low incidence. Cancer of the breast is so wide spread that it has been become a genuine problem for public health, with about one women in ten developing it in her life time throughout the world (Miller and Bulbrooke, 1986; Owiredu *et al*., 2009). It primarily affects women, however, its occasionally affects men too, female to male ratio of breast cancer prevalence being 100:1 (Wernberg *et al*., 2009).

The American nation focused concerted effort on prevention of cancer over 27 years ago, but despite the increase in cancer awareness and research in different communities, the African-American community in U.S.A continues to maintain the highest breast cancer mortality rates in the country (Figure 2.1). The possible role of genetic factors in the incidence of cancer has been speculated as a probable reason for this. Cancer of the breast is responsible for the death of millions of women world-wide every year (Owiredu *et al*., 2009).

In 1995, 180,000 cases of breast cancer were recorded in U.S.A with 45,000 deaths. Although accurate incidence data for breast cancer in the Nigerian community are scanty, a previous study indicated that the commonest cancer in Nigerian women is cancer of the breast followed closely by carcinoma of the cervix (Solanke, 1996) Table 2.1. Also a preliminary finding showed that the prevalence of cancer of the breast in Nigeria could be as high as 1.16 per 1000 per year and this suggests that about 27,840 new cases of breast cancer would have occurred in Nigeria as by the year 2000 (Adebamowo, Personal Communication). Table 2.2 shows the prevalence of cancer in Ibadan women (2001 – 2006). Breast cancer has the highest prevalence of all malignant cases recorded for the six years.

Being female is the main risk factor for the development of breast cancer. Many other factors such as family history, benign breast disorders among others affect the risk of breast cancer. However, the etiology of breast cancer remains largely unknown. In fact 75% of patients with breast cancer have no identifiable risk factor other than sex and age. The disease is commoner in women than men and it is 100 times more common in women than men (Ewertz, 1996; Wernberg *et al*., 2009). Studies have consistently shown that oestrogen is the primary stimulant for breast epithelial proliferation in women (Pike *et al.*, 1993).

The higher the number of ovulatory cycles the women in a population have, the higher the incidence of breast cancer in that population and this seems to explain most of the international variation seen in the incidence of breast cancer worldwide (Henderson *et al.*, 1988). Dietary and nutritional epidemiology is an alternative explanation for the international variation in the incidence of breast cancer but even this may be working through an effect on hormone metabolism by a yet unidentified pathway. The risk factor of most importance in breast cancer is either evidence of or the result of variations in the exposure to endogenous and exogenous estrogen. Factors associated with increased exposure to estrogen increase risk while those associated with reduced exposure reduce risk. Thus early onset of menarche, late onset of menopause, low parity, nulliparity, late first full term pregnancy, menstrual irregularity, failure to breast feed, use of hormonal contraception and hormone replacement therapy are all associated with higher risk of breast cancer. Late onset of menarche, early onset of menopause, high parity, early first full term pregnancy, regular menstruation, breast feeding, non-use of hormone contraception and hormone replacement therapy are all associated with reduced exposure to estrogen and so are protective against breast cancer. The peak age of incidence of breast cancer in Nigeria is 43 years which is at least a decade earlier than in Caucasians (Adebamowo, 1998). About 66% of breast cancer patients in Caucasians are post-menopausal while post-menopasal patients account for just 20% of Nigerian breast cancer patients (Adebamowo, 1998). A report in the Vanguard dailies (April, 2009) by Prof. Ifeoma Okoye, Chairperson of Breast Without Spot (BWS) initiative said that about 9,000 new cases of breast cancer among women aged 25 yrs – 35 yrs (premenopausals),

occurred annually in Nigeria. Over 80% of these cases were detected late when rapid deterioration has set in, and in most cases death occurred. In the same report, the Minister of Women Affairs, Mrs. Salamotu Hussaini Suleiman, suggested that the key point in drastically reducing cases of breast cancer in Nigeria remains early detection through self examination and professional attention that will lead to cure.

Breast cancer is usually treated by a combination of surgery, radiotherapy, hormonal manipulation and chemotherapy (Kennedy, 1974). The usual method of hormonal manipulation in pre-menopausal women is oophorectomy (removal of the ovaries) which may be surgical, medical; or by radiation (Kennedy, 1974, Bilimoria and Jordan, 1996). Nearly a hundred years ago, ovarian ablation was shown to be an effective treatment for advanced breast cancer in premenopausal women (Bilimoria and Jordan, 1996). Its role in the adjuvant setting was reviewed by the Early Breast Cancer Trialist Collaborative Group (EBCTCG) (1992) and it was shown to reduce mortality, and improve disease free survival. Different treatment modalities have since been advocated to improve the management of patients with cancer of the breast. The effectiveness of chemotherapy in the treatment of breast cancer may largely be the result of partial ovarian ablation produced in premenopausal women. The comparison of efficacy of combination chemotherapy with earlier trials of oophorectomy has demonstrated the superiority of oophorectomy (Bilimoria and Jordan, 1996). Surgical oophorectomy is the preferred method of hormonal manipulation in the Surgical Oncology Unit of the University College Hospital (UCH), Ibadan for premenopausal patients with cancer of the breast. The patients are then subjected to artificial (surgical) menopause which is marked by cessation of ovulation and invariably cessation or reduced production of estrogenic hormones. Surgical oophorectomy is effective and cheap and it circumvents the problem of compliance with daily oral medications. It is of benefit in premenopausal women who have not received chemotherapy irrespective of their nodal status. The onset of menopausal symptoms in patients who have had surgical oophorectomy is sudden and the symptoms can be severe but it tends to be of shorter duration than natural menopausal (Anonymous, 1996). Radiation oophorectomy creates an artificial menopause which may not be permanent but with a gentler onset. Medical oophorectomy is done with Tamoxifen in

doses of 20mg daily. It is usually used for 5 years. Medical oophorectomy is effective and widely used. It is of benefit in both pre and post menopausal, oestrogen receptor positive (ER+ve) breast cancer patients but not beneficial to patients with oestrogen receptor negative (ER-ve) tumours.

Menopause is known to be associated with symptoms of depression, sleeplessness and irritability. Hormone replacement therapy (HRT) has been part of the management strategy for these menopausal symptoms. Both natural and surgical menopause are associated with an increase in the risk of coronary heart disease (CHD) (Kannel *et al.*, 1976) probably caused by the cessation of estrogen production (Robinson *et al.*, 1959, Rosenberg *et al.*, 1981). Endogenous female sex hormones have significant effects on circulating levels of plasma lipids and apolipoproteins (Tonolo *et al.*, 1995) and have been known to have a protective effect on the heart by lowering cholesterol levels in premenopausal women (Bachman and Liu, 1998). Cross sectional studies suggest considerable influence of menopause on serum lipids and lipoproteins in women. Fukami *et al.*, (1995) have shown that serum lipids and lipoproteins are significantly altered as a consequence of menopause resulting in a more atherogenic profile in the post menopausal period. In some populations, the pattern of hormone secretion and excretion as well as lipids and lipoproteins levels are affected by geographical variations (Rhoads *et al.*, 1976; Heiss *et al.*, 1980; Woods *et al.*, 1987). In South Western Nigeria, the profiles of circulating levels of progesterone and estradiol-17β (E_2) in women have been reported to be similar to those found in other populations and races (Dada *et al.*, 1984). Type II hyperlipoproteinemia is common in post menopausal women and estrogen replacement therapy has been identified as an effective treatment for this (Tikkanen *et al.*, 1978). Hydroxylated and oxygenated Metabolites of estradiol were also found to produce hypocholesterolemic effect in oophorectomised rats without producing any estrogenic activities in the reproductive tissues of the rats (Bachman and Liu, 1998).

Data from experimental and clinical studies suggest that female sex hormones especially estrogens play a fundamental role in the etiology of breast cancer (Ewertz, 1996). A large number of epidemiological studies of estrogen replacement therapy and incidence of breast cancer have been reported (Palmer *et al.*, 1991; Yang *et al.*, 1992;

Kvale, 1992; Colditz *et al.*, 1995). Generally analysis has suggested that there may be a modest increase in breast cancer risk with increasing duration of hormone treatment (Kvale, 1992; Brinton and Schraire, 1993; Colditz *et al.*, 1995). In addition to breast cancer risk, there is an increase in other side effects during HRT, such as weight gain and resumption of menses (Judd *et al.*, 1983; Henderson *et al.*, 1993).

Several epidemiological studies have established a relationship between the use of oral contraceptives and changes in serum lipids and lipoproteins and consequently an increased incidence of cardiovascular disease (Whynn *et al.*, 1969; Beral, 1976; Knopp *et al.*, 1982). Many of these previous studies have not controlled factors such as steroid content of the oral contraceptives preparation, duration of use, time of blood sampling and other factors which influence lipids and lipoproteins.

Serum lipids and lipoproteins are also known to vary during the menstrual cycle (Nduka and Agbedana, 1993; Cullinane *et al.*, 1995). Generally, at the pre-ovulatory phase, total and LDL cholesterol are increased significantly. At the ovulatory phase, when progesterone secretion begins to increase, the HDL cholesterol as well as Apo A_1 also increases. The ovulatory phase is associated with a marked elevation of estrogen (estradiol 17β) and this probably affects the plasma HDL and total cholesterol levels at this phase. Actually, it has been previously reported that estrogen administration to pre-menopausal women caused an increase in HDL cholesterol (Shaefer *et al.*, 1983). The Luteal phase seems to be the best period for sampling for lipids (Nduka and Agbedana, 1993; Adebamowo, 1998;). Variation of serum lipids in breast cancer patients has been reported in Europe and America (Vatten and Foss, 1990; Hoyer and Engholon, 1992). However, epidemiological evidence of relationship of serum cholesterol and triglyceride and breast cancer incidence is inconsistent. Some workers reported an inverse relation between serum cholesterol and breast cancer incidence (Vatten and Foss, 1990). They found that breast cancer incidence rate was low (0.53) among women with serum cholesterol in the highest quartile of up to 329mg/100ml, compared to those in lowest quartile of about 200mg/100ml. For serum triglyceride, an association that was not statistically significant with breast cancer incidence was found. Hoyer and Engholon (1992) on the other hand reported a negative relation between serum triglyceride and breast cancer

risk. Willet *et al*. (1984) found a reduced serum cholesterol in patients with breast cancer while Tomberg *et al.* (1984) found an increased serum cholesterol in patients with breast cancer. In the Nigerian community, not much is known about the variation of serum lipids in women suffering from breast cancer. Olabinri (1997) assessed the serum cholesterol, triglyceride, Vitamin A and B-Carotene in Nigerian patients with cancer of the breast and found that the serum levels of total cholesterol as well as those of Vitamin A and B-Carotene were significantly reduced while serum TG concentration remained unchanged.

Increased plasma levels of total cholesterol and LDL cholesterol (LDL being the major carrier of cholesterol in man), are associated with increased risk of CHD (Kannel *et al.*, 1971; Gordon *et al.*, 1977). HDL cholesterol is also known as a powerful independent predictor of coronary heart disease (CHD) in middle-aged men as well as in elderly women (Gordon *et al.*, 1977; Quintao *et al.*, 1991). Oral estradiol-17β has been shown to raise the level of HDL cholesterol in menopausal women by slowing down its clearance rate (Quintao *et al.*, 1991). HDL cholesterol and LDL cholesterol are hence described as two important factors correlating with the increased risk of CHD (Newman *et al.*, 1986). Additional discriminatory power to detect CHD is provided by the concentration of Apo B (major apolipoprotein of LDL) and Apo A_1 (major apolipoprotein in HDL) (Brunzell *et al.*, 1984). Thus an increased Apo B or decreased Apo A_1 or increased ratio of Apo B to Apo A_1 are associated with coronary heart disease (CHD).

1.1 **Justification for the Study**

(1) Evidence shows that relationship of Serum Cholesterol and Triglycerides (TG) and breast cancer incidence is not consistent (Moysich *et al*., 2000).

(2) Variation of serum lipids in breast cancer has been reported in Europe and America (Vatten and Foss, 1990; Hoyer and Engholon, 1992), while in this community, not much is done on lipids in women suffering from breast cancer.

(3) Breast cancer is commoner in pre-menopausal than in post-menopausal women in this community and in fact metastatic breast cancer has been shown to be the leading cause of death in women aged 40 – 55 years (Stepley and Ackland,

1998; Wernberg *et al*., 2009). The mortality and loss caused by this disease is a serious health concern for professionals and the public.

(4) Also, the major management strategy for pre-menopausal breast cancer patients in this community is bilateral surgical oophorectomy. This leads to a reduction or cessation of oestrogen production and induces premature menopause into the patients. Natural menopause is known to be associated with increased risk of CHD development, a leading cause of death in men and women worldwide. While plasma lipids and lipoproproteins, especially LDL cholesterol are among the risk factors for the development of CHD, there is no available information on effect of oophorectomy on lipids of pre-menopausal women with breast cancer.

(5) Oestrogen is also a known cardioprotective factor and its reduction may alter plasma lipids and lipoproteins levels possibly predisposing *oophorectomised* women to increased risk of cardiovascular disease (Demirbag *et al*., 2005).

(6) There are also racial and ethnic differences in the incidence and mortality of breast cancer (National Cancer Institute, 1988 – 1992).

1.2 Aims and Objectives

The specific aim of this study was to evaluate the effect of induced menopause (by oophorectomy) on the variation in plasma lipids and lipoprotein levels, which are well established risk factors for coronary heart disease in pre-menopausal breast cancer patients. This was achieved by:

i. Measurement of the baseline plasma lipids, lipoproteins and oestradiol levels in pre-menopausal patients suffering from cancer of the breast in this community and also in controls; and,

ii. Progressive monitoring and measurement of concentrations of plasma lipids, lipoproteins and estradiol in the patients as they get induced to menopausals (after oophorectomy) at intervals of 1 month, 3 months and 6 months after oophorectomy.

iii. Assessment of the risk/benefit ratio of oophorectomy as a treatment modality for pre-menopausal patients with breast cancer by an evaluation of the plasma

lipid and lipoprotein profile and plasma estrogen status of the patients at the specified intervals during the period of study.

1.3 Significance of the Study

It is believed that the study might impart an understanding of the pathogenesis of the dyslipoproteinemia in induced menopause by surgical oophorectomy and this should help the physicians to better select and monitor therapy for pre-menopausal patients suffering from breast cancer in this community.

1.4 Research Hypothesis

Induced premature menopause by bilateral surgical oophorectomy may result into adverse atherogenic lipid profile as it occurs in natural menopause and may be doing more harm to the premenopausal breast cancer patients being treated than good. As it could lead to higher risk of CHD development in these patients.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 What is Breast Cancer?

Breast cancer is a disease condition in which the cells of the breast (mammary gland) start growing independently on their own without the body needing or instructing them to do so i.e. uncontrolled proliferation of breast cells (Amurawaiye, 2005). The shape of these abnormal cells under the microscope is bizarre and irregular. These cells also acquire the ability to leave their sites in the breast and spread to other parts of the body by various means. This "*spreading*" is called metastasis. The reason why cancers are so dangerous is this ability of cancer cells to spread.

2.2 Origin of Breast Cancer

Breast cancer was recognized as far back as 1600B.C by ancient Egyptians. It was then described as the cold bulging tumour of the breast and its lymphatic spread was associated with poor prognosis. Over the last 50 years, as many as one in every eight women has been affected during their lifetime (Ries *et al.*, 1999). Today, breast cancer is seen not just as a single disease, but rather as a general term used to describe a number of different types of cancers which occur in the breast (Leek and Harris, 2002). It is now the most frequently occurring malignant disease in women (Lynn and Ries, 1995) which has posed a significant health problem in industrialized Western World (Forbes, 1997) (Figure 2.1) and it is also gaining as much significance in developing countries (Adebamowo, 2005) (Table 2.1 and Table 2.2).

2.3 Recognition and Diagnosis of Breast Cancer

The earliest evidence of breast cancer that one can recognize is the presence in the breast of a lump. This is also variously referred to as a swelling, tumour or mass. It looks like a "boil" but it is usually painless. A breast that is habouring early breast cancer does not actually look or feel different from normal and as such all breast lumps need to be removed and tested for the diagnosis of breast cancer. Moreover,

considering the age distribution of breast cancer disease in Nigeria and the poor state of diagnostic oncology facilities, it is highly recommended that excisional biopsy for

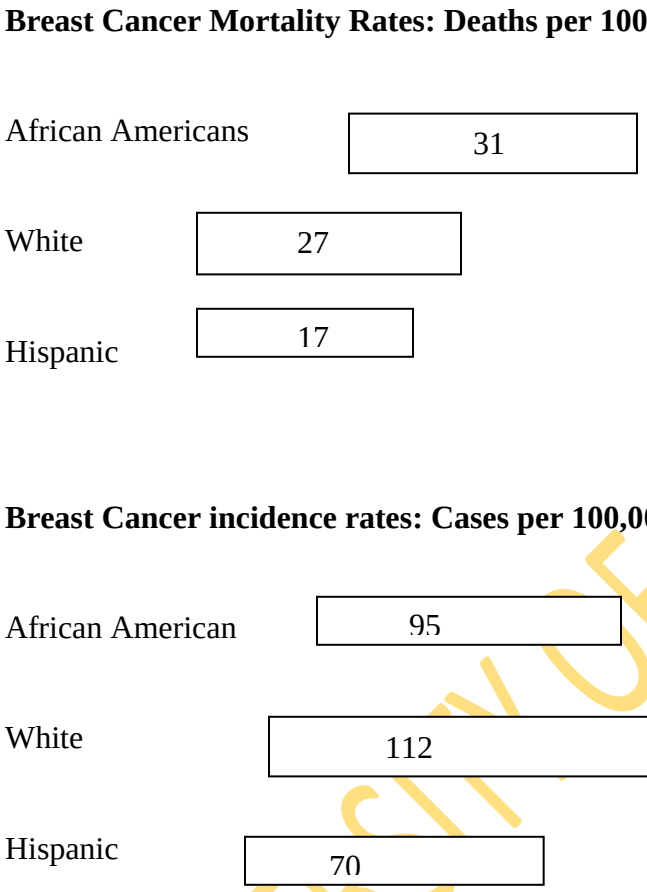


Figure 2.1: Breast Cancer: High Mortality and Low Incidence for African American women (1988 – 1992)

Source: *Miller et al., (1996). Racial/Ethnic Patterns of Cancer in the United States (1988-1992). National Cancer Institute NIH Pub. No. 96-4104. Bethesda, MD.*

Table 2.1: Common Cancers In Nigerian Females in 1991

Total Number of Cases = 818

Breast	29.7%
Cervix	23.2%
Chorion & Hydatiform	4.2%
Ovary	3.6%
Liver	2.3%
Connective & Soft tissue	2.2%
Burkitt's lymphoma	2.1%
Other Malignant Neoplasm of Lymphoid Tissue	2.1%
Other Malignant Neoplasm of Skin	2.0%
Other Malignant Neoplasm of Uterus	2.0%

Source: Ibadan Cancer Registry (IBCR) Department of Pathology, University College Hospital, Ibadan.

Table2.2: Cancer Trends in Ibadan 2001 – 2006

Commonest Malignant Cases In Women In Order of Prevalence 2001 – 2006

SITE	2001	2002	2003	2004	2005	2006
BREAST	263	323	254	396	378	438
CERVIX UTERI	216	227	176	279	349	337
CORPUS UTERI	1	7	18	27	25	21
OVARY	24	29	30	33	38	42
LIVER	32	46	40	17	59	16
NON-HODGKINS LYMPHOMA	17	14	22	19	13	12
RECTUM+COLON	27	37	41	46	45	39
THYROID	15	31	24	14	15	26
CONNECTIVE, SOFT TISSUE	18	33	21	25	14	11
BONE	18	37	20	24	25	15

Source: Ibadan Cancer Registry (IBCR) Department of Pathology, University College Hospital, Ibadan.

Moreover, considering the age distribution of breast cancer disease in Nigeria and the poor state of diagnostic oncology facilities, it is highly recommended that excisional biopsy for histological evaluation be done for all cases of breast lumps (Frazee *et al.*, 1996). This will help in treating benign breast disease and provide tissue for detailed study of pathologic prognostic factors that may influence choice of treatment in malignant cases. Laboratory evaluations such as full blood count, electrolytes and urea, abdominal ultrasound, chest x-ray etc, are usually sufficient in the absence of metastatic disease. Nuclear scans, liver function tests, CT scans of the brain and the liver, serum tumour markers such as CEA and CA 15-3 are neither sensitive nor specific enough and are too expensive for routine use (Hayes *et al.*, 1986; Adebamowo and Ezeome, 1996).

Since the paradigm of "painless breast lump" as a presenting feature of breast cancer is unknown to the majority of the population in the developing world, the development of culturally appropriate health education materials is an urgent task. In order to reduce the likelihood of breast lump being ignored in the young female, there is need to emphasize the epidemiology of breast cancer as seen in this environment (Adebamowo, 1998).

2.4 Predisposing Factors of Breast Cancer

The aetiology of breast cancer still remains largely unknown but there is a clarity about the risk factors that influence the development of the disease. In majority of patients with breast cancer, the major identifiable risk factors are sex and age. The disease is 100% more common in women than men. The age incidence of breast cancer shows a steeply rising curve from early adulthood to menopause after which the gradient of the curve falls (Adebamowo, 1998). The disease is rare before the age of 20 years. In Caucasians, less than 0.5% of patients with breast cancer are younger than 30 years while in Nigeria, 12% of patients are younger than 30 years. Furthermore, in Nigeria, only 20% of patients with breast cancer are post-menopausal women while in Caucasians, over 67% of the patients are post-menopausal women (Adebamowo, 1998). This is probably due to ethnicity and lifestyle factors.

Data from experimental, epidemiological and clinical studies suggest that female sex hormones, especially oestrogens play a fundamental role in the aetiology of breast cancer. Studies have also confirmed the role of prolonged exposure to endogenous and exogenous oestrogens in the aetiology of breast cancer disease and this has been identified as the risk factor of most importance (Henderson *et al.*, 1988; Key *et al*., 2001, Russo and Russo, 2006; Chlebowski *et al*., 2007).

Having a first birth before age of 20 years reduces the lifetime risk of breast cancer by 50% (since oestrogen exposure is reduced) in comparison to nulliparity, while women with delayed first full term pregnancy have a higher risk. A relationship actually exists between pregnancy, abortion and breast cancer. First trimester abortions, whether spontaneous or induced occurring before first full-term pregnancy increase the risk of breast cancer (Henderson *et al.*, 1997). This is because the maternal serum oestradiol rises to high levels during the first trimester of the first pregnancy and to a lesser extent in the second and later pregnancies. However, completing the first pregnancy to term assures of the beneficial effects in terms of reduction of risk of breast cancer because the hormonal patterns that accompany the second and third trimesters are not as high as in the first trimester.

2.5 Role of Anthropometric Factors in Aetiology of Breast Cancer

The evidence for a role of anthropometric factors (i.e. height, weight, Body Mass Index, Waist Hip Ratio) in breast cancer aetiology is becoming clearer and stronger (Friedenreich, 2001) though uncertainties remain regarding the associations.

There is a positive association between breast cancer risk and height (Cold *et al*, 1998). In pre-menopausal women, risk of breast cancer increases with height but risk decreases with increasing body weight or Body Mass Index (i.e. obesity). There is no association with increased waist to hip ratio. In post-menopausal women, there is an increased risk with increase in all the anthropometric variables (Cold *et al.*, 1998). A possible major contributor to the rising incidence of breast cancer in the developed world is the increasing prevalence of obesity in these countries due to consumption of westernized diet (Flegal *et al.*, 1998; Popkin and Doak, 1998). Over nutrition and obesity are therefore the key determinants of post-menopausal breast cancer

worldwide (de Waard *et al.*, 1960; Friedenreich, 2001; Adebamowo *et al.*, 2003). Breast size is also a possible predictor of breast cancer risk. There is an increased risk of developing breast cancer in large-breasted-lean women (Egan *et al.*, 1999).

2.6 Other Risk Factors of Breast Cancer

i. **Age at Menarche, Menopause and First Live Birth:-** Early onset of menarche at less than 11 years of age results in 20% increased risk of breast cancer than onset at greater than 14 years. Late menopause also increases the risk. Age at first live birth of less than 20 years results into half the risk that nulliparous women and those with first live birth at greater than 35 years have (Key *et al*., 2001).

ii. **Race/Ethnicity:-** There is low incidence in African-Americans but high mortality. This is due to advanced stage presentation and socio-economic factors such as decreased access to healthcare and lower use of mammography screening because of the cost. Also, due in part to genetic factors (Olopade *et al., 2002).*

iii. **Diet and Environmental Factors:-** Dietary fat and alcohol intake in moderate or heavy level increase the risk of breast cancer while increased intake of β-carotene decreases the risk of breast cancer (Hamajima *et al.*, 2002). Studies have also shown (though not consistent) that there is decreased risk of breast cancer in pre-menopausal women who exercise (Beral *et al*., 2002; Barnard *et al*., 2006).

iv. **Breast Feeding:-** The longer women breast feed the greater reduction in risk of breast cancer (Collaborative group on Hormonal Factors in Breast Cancer, 2002). Lower incidence of cancer of the breast in developing countries like Nigeria may be largely attributed to the more frequent and longer nursing of infants.

v. **Environmental Toxins:-** E.g. organochlorine pesticides. Effects of these on breast cancer risks are being intensively investigated as there is concern that they could have oestrogenic effects on humans and so constitute a risk factor of breast cancer.

As a society gets more affluent, and living standards improve, age at menarche decreases (i.e. early onset of menstrual period) while age at menopause increases (i.e. late onset of menopause) (King *et al*, 1993). Also, the demands of education and

career may increase the number of women who delay child bearing and reduce the duration of breast feeding. Again, with better healthcare facilities and increased contraceptive use, women also tend to have fewer pregnancies. The prolonged exposure to endogenous oestrogens that result from all these factors combined may contribute to increasing incidence of breast cancer in our community (Adebamowo, 1998).

2.7 Hereditary Risk of Breast Cancer

Another important risk factor for breast cancer is positive first-degree family history. In recent years, there has been an explosion of knowledge on the role of genes in the aetiology of breast cancer. Genetic predisposition to cancer results from mutation in oncogenes, tumour suppressor genes, apoptosis genes or mismatch repair genes (McPherson *et al*., 2000). It is well known that women at increased risk of breast cancer as well as ovarian cancer generally belong to families in which other members of the family have had these diseases. It is now known that these diseases may be inherited from fathers or mothers as a gene mutation (alteration in DNA sequence) in either of two susceptibility genes called BRCA 1 on chromosome 17 and BRCA 2 on chromosome 13. It is estimated that 10% of all cases of breast cancer result from inherited factors. These two genes, BRCA1 and BRCA2 are believed to be responsible for about 85% of these cases. Every individual is born with two copies of about 100,000 different genes. One copy of each gene comes from the mother and the other copy from the father. BRCA1 gene was the first gene found to play a major role in hereditary breast cancer (BRCANALYSIS, 1998). These genes help normally to prevent cancer by making proteins that keep cells from growing abnormally. However, if a woman inherits a mutation in BRCA1 or BRCA2 from her mother or father, the breast now becomes more susceptible to the development of cancer. Many mutations have been reported in these genes and these findings lead to the possibility of primary prevention of breast cancer through the use of genetic screening (Grann *et al*., 1999). In Nigeria, effort is being made to identify the spectrum of mutations present in the population, their penetrance and expressivity (Gao *et al*., 1998).

Many other genetic mutations which possess more clinical importance but may be of lower penetrance than BRCA1 and BRCA2 as well as further BRCA genes are being investigated (Narod, 1998). It was found for example, that heterozygous carriers of an autosomal recessive gene – Ataxia-Telangiectasia, located on chromosome 11 and found in 1.4% of the general population have 5 to 8-fold increased risk of breast cancer (Waha *et al.*, 1998). The A-T mutation (ATM) is associated with late onset breast cancer in contrast to the tendency of BRCA mutation associated breast cancer to present with early onset disease (Athma *et al.*, 1996).

It is speculated that 6.6% of all breast cancers in the United States may be in women who are A-T heterozygotes. Other risk factors for breast cancer include benign breast disease, showing hyperplasia with or without atypia, complex fibroadenoma and prior diagnosis of breast, uterine, colon and or ovarian carcinoma. Among the other risk factors that have been recognized are ionizing radiation, obesity (in menopausal women only) psychosomatic factors, alcohol consumption and high fat diet (Purohit *et al*., 2002; Barnard *et al*., 2006).

2.8 Effects of Socio-Economic Status (SES), Environmental and Life Style Factors on Breast Cancer Incidence:

Reports showed that SES factors account for a substantial proportion of breast cancer outcome disparities. Breast cancer pathogenesis and epidemiology are complex and are influenced by the myriad of environmental and life-style factors that impact on lifetime hormone exposures. The genetic, SES, and cultural features associated with ethnic background complicate the picture further. These factors converge for the African-American women to yield patterns of a relatively lower breast cancer incidence, higher mortality and younger age distribution. Breast cancer incidence is lower in African-Americans than in Caucasian-American women, yet the African-American women have a 19% higher breast cancer mortality rate than white women (Gupta, 1998). This is partially explained by the more advanced stage distribution that is seen among African-American patients with breast cancer (Newman, 2005). This is attributed to African-American women's lack of education about the disease resulting in poor healthcare and health habits including eating culture and also to some other

economic barriers to health care access such as poverty rates, likelihood of lacking medical insurance and likelihood of relying on public insurance (e.g. Medicaid). These barriers are twice as high for African-Americans compared with Caucasian-Americans and they certainly contribute to delays in breast cancer diagnosis and treatment, ultimately resulting in higher mortality.

Moreover, breast cancer risk clearly increases as a function of age and there is higher prevalence of early child bearing in African-Americans compared with Caucasian-American women (Olopade *et al*., 2002). As such, there is younger age range risk of breast cancer in African-Americans and higher incidence of early-onset breast cancer than in Caucasian-Americans. African-American women under 45 years of age have greater incidence of breast cancer than Caucasian-American women in this young age range, while for women over age of 50 years, incidence rate for Caucasian-Americans is greater than that for African-Americans (Olopade *et al*., 2002; Newman, 2005).

Since breast cancer risk is a function of environmental and lifestyle exposures and genetic factors, it is appropriate to compare the patterns of the disease among African-Americans with data regarding the epidemiology of breast cancer among native African women. It is however unfortunate that there is paucity of population-based data regarding breast cancer incidence and mortality in Africa, being a large continent which comprised of many diverse nations. Nevertheless, available data revealed several provocative parallels between African-Americans and native Africans' breast cancer patients. Though breast cancer is a relatively unusual malignancy in African countries, several investigators have documented a younger age distribution and greater prevalence of high-grade (late stage) estrogen receptor negative (ER-) disease among breast cancer patients in the Ghanaian and Nigerian populations of West Africa. These are similar to the patterns of breast cancer reported among African-American women. Western African populations of course served as the source for most of the slave trade to colonial North America and so they share a common ancestry with present generation African-American (Newman, 2005).

Genetic predisposition alone, cannot account for the majority of international variations in breast cancer incidence rates. Relation between diet and cancer is also of

importance and infact there are very strong nutritional variations among many specific cancers. For instance, there are strong correlations between the total fat intake per capital and the national rate of breast cancer mortality among women (Walter *et al.*, 1984).

Several BRCA-related breast cancers in African-American women have been identified and these studies were comprehensively reviewed by Olopade *et al.* (2002). Reports demonstrated that African-American women tend to under utilize genetic counseling services. There is also racial/ethnicity related variation in levels of endogenous hormones (Randolph *et al.*, 2004; Lasley *et al.*, 2002).

Poola *et al*. (2002) also reported that the beta (β) isoform in the estrogen receptor (which may be protective against abnormal proliferative changes in the mammary tissue) may be disproportionately low in African-American women.

Tumours of African-American breast cancer patients are significantly more likely to be hormone-receptor negative (ER), aneuploid and node-negative. As biomedical research and genotyping pools develop further, contemporary studies are identifying other provocative characteristics that appear to be specific to African-American breast cancer patients. Such studies examined variations in cytochrome P450 polymorphisms, estrogen receptor β, p53 and other markers. Polymorphisms in cyto p450/Cyp enzymes are known to have variable prevalence rates among different ethnically defined subsets of the population and could potentially alter effectiveness of chemotherapy (Newman, 2005).

2.9 The Nodal Status and ER-Status of Breast Cancer

When axillary lymph nodes are affected, the cancer is described as node-positive and when there are no axillary lymph nodes affected, it is described as node-negative. When the breast tumour is oestrogen responsive, it is termed oestrogen receptor positive (i.e. ER + ve) and when the tumour is not oestrogen responsive, it is oestrogen receptor negative (i.e. ER –ve). Treatment selection for breast cancer is based primarily on assessment of endocrine-responsive or endocrine non-responsive disease according to the presence of oestrogen and progesterone receptors in the primary tumour. Absence of detectable steroid hormone receptors indicates an

endocrine non-responsive disease (i.e. ER-ve). Tumours containing as few as 1% of cells staining for steroid hormone receptors are regarded as potentially endocrine-responsive disease (i.e. ER+ve) (Cheung, 2007).

Patients with this type of disease might benefit from the addition of endocrine therapies to the treatment programme i.e. adjunctive programme. Infact, patients with Node-ve and strongly ER+ve tumours (i.e. with 90% of tumour cells stained) might be treated with endocrine therapy alone. Patients with Node+ve and a low ER disease (i.e. 9% of tumour cells stained) would be candidates for combined chemoendocrine therapy while those with endocrine-non responsive disease (i.e. with absence of detectable steroid hormone receptors, (ER-ve), should be offered adjuvant chemotherapy alone and not be given endocrine therapy (Adebamowo, 1998; Cheung, 2007).

2.10 Staging of Breast Cancer

Staging provides a tool for documenting the extent of disease, choosing treatment options, comparing result of treatment and prognosticating. Breast tumour is staged with respect to its local morphology, auxiliary lymph node involvement and presence of distant metastasis (Odelola, 2005). Several systems of staging have been recommended.

American Joint Committee on Cancer recommends that Stage 1 describes an invasive breast cancer in which the tumour measures up to 2cm and no lymph nodes involved. Stage II describes invasive breast cancer in which tumour measures more than 2cm but not more than 5cm with or without movable auxillary nodes and without distant spread. Stage III is divided into III_a and III_b. Stage III_a describes invasive breast cancer in which tumour measures larger than 5cm or there is significant involvement of lymph nodes. Stage III_b describes invasive breast cancer with tumour of any size and with homolateral metastatic supraclavicular or infraclavicular nodes or oedema of the arm but without distant metastasis. This includes inflammatory breast cancer, an aggressive type of breast cancer with redness involving part or the entire breast. Stage IV describes invasive breast cancer with tumour of any size with or without regional spread but with evidence of distant metastasis (American Cancer Society, 1983).

The TNM staging of breast cancer is commonly used in this community (Table 2.3). This staging system could yield an extensively large number of possible combinations (about 108). However, this system may have outlived usefulness in most developed countries in the face of era of mammographically detected lesions. This system has a low discriminating value and does not incorporate the new prognostic indications identified by molecular biology techniques.

The extent of spread of cancer disease in a patient could also be described by Manchester "staging" "(I-IV)"

- **Stage 1:** When there is a lump in the breast and it is usually small, less than 5cm in diameter
- **Stage II:** When there is lump in the breast and small movable lumps in the armpit of the same side too
- **Stage III:** When there is a lump in the breast and the armpit but the lumps in the armpit are bigger and joined together
- **Stage IV:** When there is evidence of disease outside the breast and the armpit

Stages I and II are regarded as early breast cancer while stages III and IV are advanced breast cancer. In the absence of a screening program, the staging of breast cancer at diagnosis is a reflection of the degree of awareness of the disease in the population.

In a review in Nigeria, there were no T1 tumours found and 72.8% of the patients had Manchester Stage III or IV breast cancer with axillary nodal involvement at diagnosis in 95.6% (Adebamowo and Adekunle, 1999). The common sites of metastatic disease presenting clinically are pleura with respiratory distress; bones with pain, pathological fracture and cord compression when the spine is involved; the brain and the liver.

Table 2.3 TNM staging of breast cancer

Primary Tumour (T)	Description
TX	Primary tumour cannot be assessed
TU	No evidence of primary tumour
Tis	Carcinoma in situ: Intraductal carcinoma, lobular carcinoma in situ, or Paget's disease of the nipple with no tumour
TI	Tumour 2cm or less in greatest dimension
TI mic:	Microinvasion 0.1cm or less in greatest dimension
Tia	Tumour more than 0.5cm but not more than 0.5cm in greatest dimension
TIb	Tumour more than 0.5cm but not more than 1cm in greatest dimension
Tic	Tumour more than 1cm but not more than 2cm in greatest dimension
T2	Tumour more than 2cm but not more than 5cm in greatest dimension
T3	Tumour more than 5cm in greatest dimension
T4	Tumour of any size with direct extension to (a) chest wall or (b) skin only as described below
T4a	Extension to chest wall
T4b	Oedema (including peau d'orange) or ulceration of the skin of the breast or satellite skin nodules confined to the same breast
T4c	Both (T4a and T4b)
T4d	Inflammatory carcinoma

Regional Lymph Nodes (N)	Description
NX	Regional lymph nodes cannot be assessed (e.g. previously removed)
NO	No regional lymph node metastasis
N1	Metastasis to movable ipsilateral axillary lymph node(s)
N2	Metastasis to ipsilateral axillary lymph node(s) fixed to one another or to other structures.
N3	Metastasis to ipsilateral internal mammary lymph node(s)
Pathologic Classification pN)	**Description**
pNX	Regional lymph nodes cannot be assessed (e.g. previously removed, or not removed for pathologic study).
pNO	No regional lymph node metastasis
pN1	Metastasis to movable ipsilateral axillary lymph node(s)
pN1a	Only micrometastasis (none larger than 0.2cm)
pN1b	Metastasis to lymph node(s), any larger than 0.2cm
pN1bi	Metastasis in 1 to 3 lymph nodes, any more than 0.2cm and al less than 2cm in greatest dimension
pN1bii	Metastasis to 4 or more lymph nodes, any more than 0.2cm and all less than 2cm in greatest dimension
pN1biii	Extension of tumour beyond the capsule of a lymph node metastasis less than 2cm in greatest dimension
pN1biv	Metastasis to a lymph node 2cm or more in greatest dimension
pN2	Metastasis to ipsilateral axillary lymph nodes that are fixed to one another or to other structures.

pN3	Metastasis to ipsilateral internal mammary lymph node(s)
Distant Metastasis (M)	**Description**
MX **MO** **MI**	Distant metastasis cannot be assessed No distant metastasis Distant metastasis (included metastasis to ipsilateral upraclavicular lymph node(s)

2.11 Treatment of Breast Cancer

Breast cancer is believed to be frequently a systemic disease from the onset involving complex tumour-host interactions. This suggests that breast cancer is already metastatic at the time of diagnosis. As such, over half of women with localized breast cancer will ultimately develop metastasis.

Early breast cancer is treated by surgery i.e. removal of the entire breast (mascectomy) or a part of it with or without radiotherapy which is the cornerstone of management of the primary cancer. Micrometastasis may however be present even in the very early stages and surgery almost invariably leaves behind some malignant cells. Consequently, drug treatment has to be considered for all women and in all stages of breast cancer. (Adebamowo, 1998).

The medical treatments available for breast cancer include: use of endocrine agents (hormone therapy) which alters the rate of growth of cancer cells by adjusting the hormonal composition of the body. The anti-oestrogens such as Tamoxifen, megestrol acetate etc, are the drugs of first choice for endocrine treatment especially of menopausal breast cancer patients. Tamoxifen has partial oestrogenic activity and this accounts for a mixture of beneficial and adverse effects. Also available is, cytotoxic chemotherapy which involves the use of dugs to destroy cancer cells that may have spread to other parts of the body. These drugs are known to attack all rapidly dividing cells in the body, normal and cancer cells alike. They are therefore relatively crude disease-fighting weapons that have limited effectiveness and leaves patients weak and nauseated. However, new cancer drugs before cytotoxic chemotherapy are coming up on the scene that gives a renewed hope to patients with cancer. An experimental pill called Gleevec is one of such drugs. This was discovered by Lemonick and Park and has been found to target cancer cells with uncanny precision according to a Vanguard dailies report (August, 2001).

2.11.1 Endocrine Aspects of the Clinical Management of Breast Cancer

Endocrine therapy has been used as an effective treatment for breast cancer over 100 years. Infact, it is the first successful systemic treatment for cancer (Wyld *et al.*, 1998). The initial acceptance of this therapy followed George Beaton's

observations in 1896 that in a proportion of pre-menopausal women with advanced breast cancer, bilateral oophorectomy results in disease regression. This subsequently led the interest and enthusiasm of physicians to be focused on the use of cytotoxic chemotherapy regimens and so in the 1970s came Tamoxifen and a range of other new endocrine agents such as Aromatase inhibitors. Tamoxifen is now the most widely prescribed of the anticancer agents. The current availability of measurements of hormone receptor levels allows better selection of patient's whose tumours might be hormonally responsive (Adebamowo, 1998).

However, there is a risk of second malignancies associated with Tamoxifen use. In the mid 1980s, a number of authors reported the occurrence of endometrial cancers in patients with breast cancer receiving Tamoxifen (Barakat, 1996). Tamoxifen has been shown to be carcinogenic in rats (Greaves *et al.*, 1993; Hard *et al.*, 1993). High doses of Tamoxifen have been shown to produce liver tumours. In humans too, an association between Tamoxifen use and an increased risk of endometrial cancer has been noted (Jordan, 1995; Barakat, 1996).

It is known that oestrogen use increases the incidence of endometrial cancer in post-menopausal women (Grady *et al.*, 1995) and Tamoxifen therapy increases the likelihood of finding oestrogenic effects in the uterus. Therefore, it is likely that oestrogenic effects of Tamoxifen on the uterus contribute to its carcinogenic potential (EBCTCG, 1992).

Tamoxifen has a predominantly antioestrogenic role in the breast, but in most other tissues, its properties are mostly agonist oestrogenic effects. As in the case of estrogenic hormone replacement therapy (HRT), Tamoxifen has been shown to have both beneficial and detrimental effects on general health in post-menopausal women via its agonist effects in non-breast tissues.

On cardiovascular disease risk, the potential mechanism for a protective role of Tamoxifen are suggested by studies of Love *et al.*, (1990) and Powles (1996) which showed alterations in cholesterol metabolism with Tamoxifen therapy that produced a reduction in LDL/HDL ratio. When potential benefits of the oestrogenic agonist properties of Tamoxifen are considered, such as improved lipid profile and increased

bone mineral density, the benefits of Tamoxifen therapy far outweigh any risks associated with its use (Love *et al.*, 1990; Powles, 1996).

Other new anti estrogens other than Tamoxifen have emerged and also the aromatase inhibitors. However, none of these new antioestrogens (including Toremifene and Falsodex) has shown greater efficacy than Tamoxifen or any better potential to advocate any of them as a replacement for Tamoxifen as first line therapy (Howel *et al.*, 1996; Gradishar and Jordan, 1997).

2.11.2 **Adjunctive Treatment or Supportive Agents**

In general, about one third of an unselected group of patients are likely to respond to hormonal manipulation while women with oestrogen receptor positive (ER+) tumours have about 50 – 60% chance of response. Patients who are elderly and with disease confined to soft tissue or bones or with a long disease free interval before metastases developed are more likely to have endocrine therapy-responsive tumours. Those who respond to one endocrine therapy are likely to respond to subsequent hormonal manipulations. Conversely, younger patients or those with oestrogen receptor negative (ER-) tumours or with metastases in viscera or with a short disease-free interval are best treated with combination chemotherapy. In general, combinations chemotherapy are more effective than single drugs. Antracyclines (i.e. doxorubicin or epirubicin) are the major components of first line combination chemotherapy (Stephen, 1998).

2.11.3 **Aromatase Inhibitors**

These also offer a greater breath of endocrine therapy with lower toxicity. These are a class of compounds which are already beginning to replace progestogens as second-line endocrine therapy following Tamoxifen therapy failure in post-menopausal patients with advanced breast cancer disease. These are currently undergoing trials as alternatives to Tamoxifen in first-line adjuvant therapy (Wyld *et al.*, 1998). The biosynthesis of oestrogen occurs in a process in which the aromatase enzyme system converts the male sex hormones, androgens to female sex hormones, estrogens. In pre-menopausal women, this occurs principally in the ovaries. In post-

menopausal women, the aromatases present in liver fat, muscle, fatty tissues of the breast and breast tumours and brain carry out the peripheral conversion of circulating androstenedione to estrone and subsequently to estradiol (Figure. 2.2).

The inhibition of this aromatase enzyme complex in post-menopausal women can cause a marked decrease in tissue oestrogen levels.

The first aromatase inhibitor to be used therapeutically in the management of patients with breast cancer was aminoglutethimide (Wyld *et al*, 1998). This was introduced as a medical alternative to surgical adrenalectomy in post-menopausal women. Others are letrozole, exethestane and anastrozole.

2.11.4 **Radiotherapy**

This is the use of high-energy radiation to destroy the cancer cells. Rapidly dividing cells are particularly sensitive to damage by radiation and for this reason, some cancerous growths can be controlled or eliminated by irradiating the area containing the growth.

Externally, irradiation can be carried out using a gamma beam from a radioactive Cobalt 60 source. In developed countries however, the much more versatile linear accelerators are now being utilized as a high-energy x-ray source (gamma beam and x-ray being much the same (Camphausen and Lawrence, 2008).

Internal irradiation is by administering or planting a small radiation source, usually a gamma or beta emitter in the target area. Iodine 131 is usually used to treat thyroid cancer. It is also used to treat non-malignant thyroid disorders. Iridium – 192 implants are used especially in the head and breast. These are produced in wire form and introduced through a catheter to the target area. After administering the correct dose, the implant wire is removed to shielded storage. This short-range procedure (i.e. branchy therapy) gives less overall radiation to the body and is more localized to the target tumour and cost effective (Frank *et al*., 2007). Many of these therapeutic procedures are palliative, usually to relieve pain.

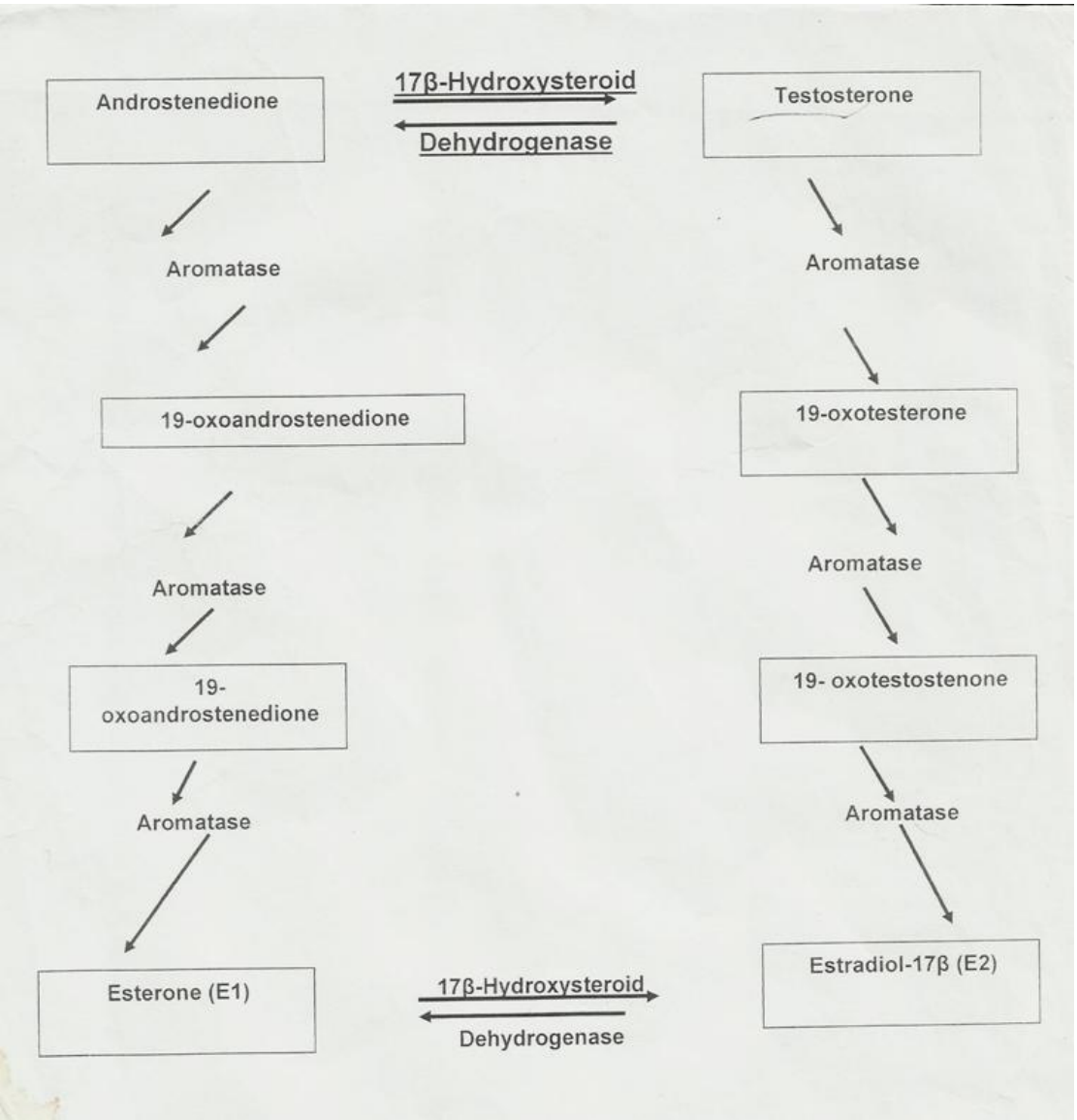


Figure 2.2: Biosynthesis of Oestrogens by the Aromatase Enzyme System (Nimrod and Ryan, 1975).

2.11.5 Ovarian Ablation:

In pre-menopausal patients; oophorectomy should be considered before any other endocrine manipulation. This is by surgery or radiation. Ablation could also be caused by chemotherapy or hormonal treatment. The agonists of gonadotropin releasing hormone (i.e. buserelin, goserelin, leuprolide and triptorelin) suppress pituitary release of Follicle Stimulating Hormone (FSH) and Leutenising Hormone (LH). This results in lack of gonadal endocrine stimulation and castration levels of circulating oestradiol (Stephen, 1998). These drugs may be given intramuscularly or as in-plants. Tamoxifen a non-steroidal antioestrogen at 20mg daily for five years is also effective in castration and it is widely used. It is of benefit in both pre and post-menopausal ER+ve early breast cancer while it does not benefit patients with ER-ve tumours. Responses similar to surgical castration (oophorectomy) have been observed, though of slower onset. The choice of breast cancer treatment should be made jointly by the clinician and patient.

2.12 Outcome of Treatment (Prognosis)

This refers to average result from treating large numbers of patients. The result in any one individual may differ. Generally, prognosis depends on many factors and these include:

- Stage at diagnosis:- The earlier the stage the better the outcome
- Nature of the cancer in the individual i.e. ER status: - This is discerned from laboratory tests that are done on the tumour after its removal from the patient.
- Compliance with an adequate and well formulated treatment plan

Patients with papillary, tubular and mucinous tumours have better prognosis than others while Blacks have worse prognosis than Whites. The younger patients do not benefit from hormonal manipulation as much as the older ones.

When there are no auxiliary nodes (armpit swelling) and the cancer is 3cm or less in diameter, more than 90% of such patients are alive in five years while 60% of patients with 2-4 armpit nodes involved will be free of disease in five years compared to 40% of those with 6-10 nodes involved that will be free of disease in five years. In

overall consideration, it is advantageous to come to the hospital early and start treatment (Adebamowo, personal communication).

2.13 Implications of Surgical Oophorectomy

After surgical oophorectomy, there is cessation or reduced production of oestrogens. This implies that menopause sets in i.e. surgical menopause or artificial menopause. The toxic effects of this includes hot flushes and other symptoms of natural menopause. Menopausal symptoms onset is sudden in patients who had surgical oophorectomy and the symptoms could be severe but of shorter duration than natural menopause (Adebamowo, 1998).

2.13.1 Menopause

Menopause marks the end of a woman's reproductive life and it is a period of rapid change in hormonal balance. The primary deficit at menopause is ovarian. The change is popularly associated with a number of signs and symptoms; such as hot flushes, menstrual irregularity and atrophic changes in the vagina and the urinary tract, and with some changes such as loss of bone mineral density (BMD) that may precipitate serious longer-term concern. The reduction in oestrogen level at menopause is often associated with accelerated development of coronary atherosclerosis and osteoporosis as these two diseases increase in incidence and severity after menopause (Hammond, 1997).

The hot flush is the classic sign of menopause (Bungay *et al*., 1980). It is the major clinical symptom of American women during the transition interval. This may cause mild inconvenience to the extent of being incapacitating in rare circumstances. The hot flush is a sudden transient sensation that ranges from warmth to intense heat, spreading over the face, scalp and anterior thorax. It is usually accompanied by erythema and perspiration and often followed by a chill (Kronenberg *et al*., 1984). In some women, palpitation and anxiety accompany the flush. Each episode usually lasts from 30 seconds to several minutes. The frequency and severity of flushes are quite variable. Women who have undergone surgical menopause such as oophorectomized women tend to have more hot flushes. It is highest in the first two post-menopausal

years. Episodes usually diminish in frequency and severity over time. Some patients however may continue to have the severe episodes for decades (Mckinlay and Jefferys, 1974). Severe hot flushes may disrupt lifestyle and function and often increase in frequency and severity in hot and humid weather, in confined spaces or with ingestion of caffeine, alcohol or spicy foods. The flushes are also often increased by stress and tension and may be less frequent or less severe in obese women.

2.13.2 Cardiovascular Disease Risk

The male versus female risk of cardiovascular disease increases after adolescence until the forties (40s) when it begins to increase more steeply in women than in men. The risk becomes essentially the same in both sexes after 70 years of age. In women who lack oestrogen earlier in life, as in surgical menopause, the risk of cardiovascular disease also increases earlier.

Epidemiological studies have indicated that pre-menopausal women have a decreased risk for the development of artheriosclerosis when compared to that of age-matched males but the decreased risk is annulled following menopause (Stampfer and Colditz *et al.*, 1991).

Important investigations into cardiovascular disease in women have been focused on obesity, plasma lipid levels, hypertension, diabetes mellitus, cigarette smoking, sedentary lifestyle and low estrogen levels (Hu *et al*., 1999). Aberrations in these factors accelerate cardiovascular status risk, while improvement on these factors reduce coronary risk.

2.13.3 Osteoporosis

This is also a problem of significant importance in post-menopausal state. Skeletal mass increases steadily throughout childhood, peaking at the median age of 25 years, after which a period of relative stability follows (Recker *et al.*, 1992). Peak bone density tends to be higher in African-Americans than in Whites and Asians. After a period of relative stability, bone loss begins in both sexes with more rapid acceleration occurring in women when they are oestrogen deficient (as in menopause) regardless of age or cause i.e. natural or surgical (Hammond, 1997).

The health protection effect of the reproductive life of a woman is lost at menopause and there are increased mortality risks of these two major problems of Oesteoporosis and Cardiovascular diseases. Practice of preventive measures for these diseases is much more effective than therapy that is begun once the disease has shown symptoms.

The probability that menopause has occurred in a woman depends on age and duration of amenorrhea (Burger, 1996). The probability of being menopausal after six months of amenorrhea is 46% in women that are 45-52 years old and 75% for women over the age of 52 years (Wallace *et al.*, 1979). This implies that no clear endocrine change occurs at the time of the last menstrual cycle at early menopause.

2.14 Steroid Hormones

The precursor for steroid synthesis is cholesterol. The main sites of production of steroid hormones are the adrenal glands, the gonads and the placenta. All these steroid-producing tissues except the placenta can make cholesterol from acetate. However, the main source of cholesterol for steroidogenesis is plasma cholesterol carried in the blood mainly by low-density lipoprotein (LDL). High affinity LDL receptors are present in steroid producing cells which facilitate entry of cholesterol into the cells (Brown *et al.*, 1979).

Steroid Nomenclature: Steroid hormones have a eryhydrocyclopentanephenanthrene nucleus with three six-carbon and five-carbon rings. Steroid nomenclature is based on the system of numbering shown in Figure 2.3. Progestogens and corticosteroids contain the 21-carbon atom (C_{21}) pregnane nucleus, androgens contain the C_{19} androstrane nucleus and oestrogens contain the C_{18} oestrane nucleus. Steroidogenesis can only proceed from $C_{21} \rightarrow C_{19} \rightarrow C_{18}$ and not in the reverse direction.

By convention, the chemical names of steroids are based on the carbon nucleus which they contain. The abbreviated name of the basic nucleus may be preceded by numbers indicating the position and number of hydroxyl groups (e.g. 3β, 17α ------ diol). The site(s) and number of double bonds may be indicated after the abbreviated basic name (e.g. Pregn-4-ene). Finally, the site(s) and number of ketone groups are indicated (e.g. 3,20-dione). Thus, the chemical name of 17α –hydroxyprogesterone is

17α – hydroxyl-pregn-4-ene-3, 20-dione. The order of the prefixes and suffixes varies with different systems of nomenclature. Almost all naturally occurring active steroids are nearly flat molecules and substituents below and above the plane of the molecule are designated α (-----) and β ( ) respectively. The terms Δ_4 (delta 4) and Δ_5 indicate the position of a double bond in the 4 – 5 and 5 – 6 positions respectively. Dehydro implies elimination of a hydrogen atom, and deoxy elimination of an oxygen atom.

2.14.1 **Oestrogen and Breast Cancer Risk: The Relationship**

Oestrogen is a hormone that is a chemical messenger in the body. It is important for normal sexual development and it is essential for the normal functioning of the female organs needed for child bearing such as the ovaries and uterus. Oestrogen helps to control a woman's menstrual cycle. It is important for the normal development of the breast. It also helps maintain healthy bones and the heart. All of these are therefore estrogen target tissues and organs that estrogen can influence. During the childbearing years (i.e. from puberty to menopause) the ovaries produce oestrogen. After menopause, when the ovaries no longer make estrogen, body fat in the extra glandular areas is the main source of oestrogen made by the body.

The effect of ovarian hormones, such as oestrogen, on breast cancer risk was first shown over 100 years ago when researchers found that removing the ovaries of women with breast cancer improved their chances of survival. Recent studies have also shown that women who had their ovaries removed early in life have a very low incidence of breast cancer (Hulka, 1997; Kauf and Barakat, 2007). Oophorectomy thus reduces risk of breast cancer in pre-menopausal women. Similarly, rats and mice whose ovaries have been removed develop few if any breast tumours (Kauf and Barakat, 2007). Also, men who do not have ovaries normally and so have low blood levels of estrogen, have low breast cancer rates compared to women (Wernberg *et al*., 2009).

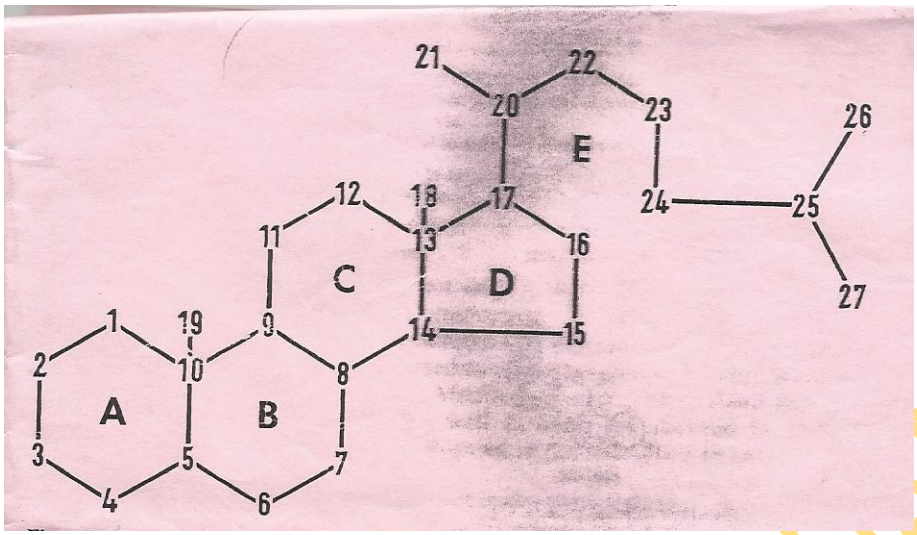


Figure 2.3 Steroid nucleus and system of numbering (Baird *et al*., 1969).

Evidence from many studies suggests that life-long exposure to oestrogen and perhaps to other ovarian hormones plays an important role in determining breast cancer risk (Key *et al.*, 2001; Chlebowski *et al.*, 2007). Studies that have identified risk factors for breast cancer have found that women who experience menarche at an early age or menopause at a later age have a higher risk of breast cancer. This supports the theory that the number of menstrual cycles a woman has and hence the length of exposure to endogenous estrogen during her lifetime increases her risk for breast cancer.

2.14.2 **Mechanism of Action of Estrogen**

During each menstrual cycle, estrogen as a chemical messenger together with other ovarian hormones signal cells in the breast to divide and multiply. Oestrogen also signals the cells of the uterus to divide. It is the other hormones that actually signal the ovaries to make oestrogen and then the ovaries secrete oestrogen into the blood stream. Oestrogen travels through the blood but only the cells in estrogen target tissues, like the breast and uterus can recognize and use oestrogen because they have oestrogen receptors. Oestrogen also has a complementary shape that allows it to fit into an oestrogen receptor in a "lock and key" manner. The oestrogen and the oestrogen receptor therefore bind to form a unit that enters the nucleus of the cell. The oestrogen-receptor unit binds to specific regulatory sites on the cells DNA and this begins a series of events that turns on oestrogen-responsive genes. These specialized genes instruct the cell to carry out important activities. Some of these signaling proteins can tell the cell to divide.

Oestrogen is also present in the body in different forms and the oestrogen-receptor can bind with these different forms of oestrogen. Some forms of oestrogen are stronger than others. Stronger forms are more likely to initiate cell division than weaker ones. In addition, some forms of oestrogen stay in the body longer than others. (Olopade, 2005).

2.14.3 Cell Division and Cancer Process

One characteristic of a cancer cell is that it multiplies out of control. The progression from a normal cell to a cancer cell is a multistep process that includes the build-up of damage to the DNA in key genes that control cell division. Damage to a gene in DNA is called **mutation**. This damage may happen in several different ways. Rarely a child may inherit a mutated gene from a parent. For example, the breast cancer genes – $BRCA_1$ and $BRCA_2$, may have mutations that parents can pass on to children. More commonly, a chemical or radiation that damages the DNA may cause mutation.

Another cause of DNA damage is when a mutation arises by chance. The chance mutation is a result of the cell making a mistake in copying its DNA during cell division.

Since oestrogen stimulates cell division, it can increase the chance of making a DNA copying error in a dividing breast cell. Oestrogen can also have the effect of making a spontaneous or chemically-induced mutation permanent, since it influences the rate of cell division. Also, because mistake in DNA becomes permanent if the cell divides and passes on the mutation, so oestrogen-stimulated cell division can increase the chance of making a mutation permanent.

Oestrogen can also indirectly stimulate cell division by instructing a target cell to make receptors for other hormones that stimulate breast cells to divide. (Brown *et al*., 1979).

2.14.4 Plasma Sex Hormone at Menopause

The oestrogens are a family of female sex hormones synthesized in a variety of tissues. They include Oestrone (E_1) 17β-Oestradiol (E_2), and Oestriol (E_3) (Figure 2.4). 17β-Oestradiol is the primary oestrogen of ovarian origin. Cauley *et al* (1989), found a lack of relation between age, years since menopause and serum oestrogens. Infact, majority of previous studies with a few exceptions, have not reported a significant decline in estrogens with age or time since menopause in post-menopausal women (Chakravart *et al.*, 1976; Judd *et al.*, 1976; and Cauley *et al.*, 1989). A major change is also known to occur post-menopausally in the source and nature of

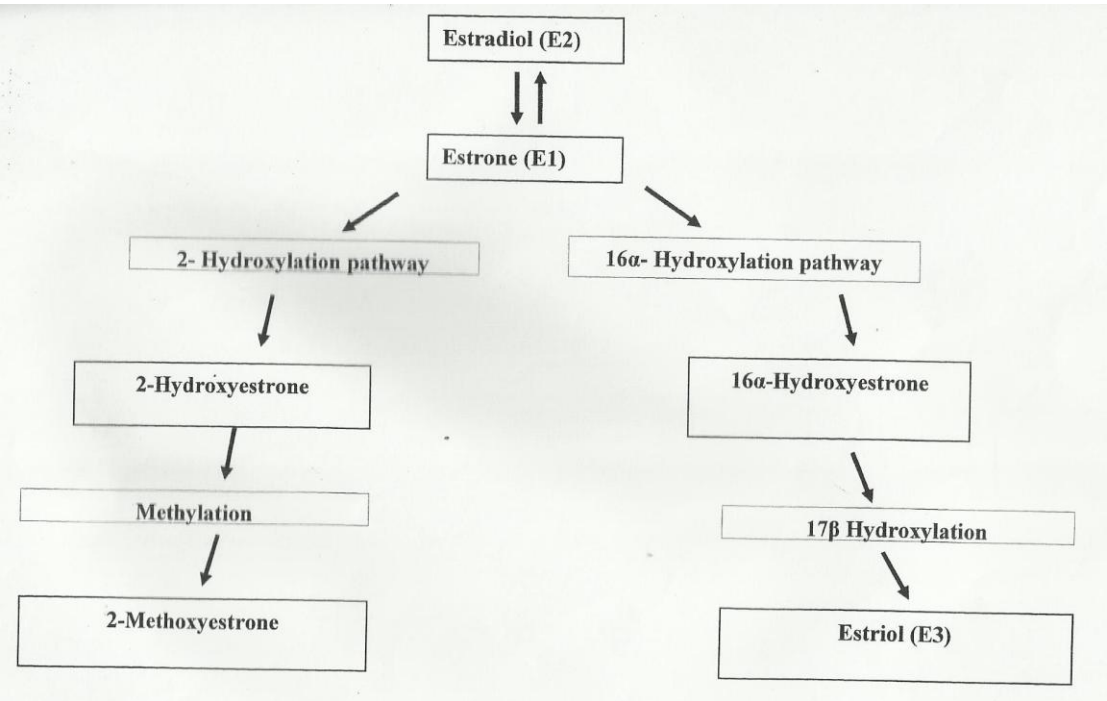


Figure 2.4 Main pathways of Oestradiol Metabolism in Humans

circulating oestrogens. Oestradiol is a more biologically potent oestrogen, but it is not the predominant oestrogen in post-menopausal women and so its concentrations are extremely low in post-menopause. Oestrone on the other hand, is the primary circulating oestrogen hormone quantitatively in post-menopausal women, and it is not specifically bound in both pre and post-menopausal women (Sitteri *et al.*, 1982). Oestrone is principally produced in post-menopausal women by the peripheral conversion of adrenal androstenedione a precursor of androgens (Sitteri *et al.*, 1982; Marshall *et al*., 1977). Oestradiol is also formed from aromatisation of plasma testosterone which is derived from the conversion of androepiandrostenedione and by the reduction of oestrone (Figure 2.2) (Nimrod and Ryan, 1975).

The major drop in oestrogen appears to occur in the peri-menopausal and immediate post-menopausal period with little subsequent change with increasing age (Chakravart *et al.*, 1976; Longcope *et al*, 1990).

The ovarian stroma cells still continue to secrete androgens after natural menopause and thus women who have undergone oophorectomy tend to have lower plasma androgen values (Cauley *et al.*, 1989).

The basal level of oestrone in post-menopausals averages approximately 100pm/1 compared with 40pm/1 for oestradiol (Burger, 1996). The degree of obesity was found to be a major determinant of oestrone and oestradiol but there is a weak association between obesity and androgens (Cauley *et al.*, 1989). The degree of extraglandular conversion of adrenal androgen precursors is the major determinant of plasma oestrone and the degree of conversion which is also a function of excess body fat is greatly increased after menopause (Marshall *et al*., 1977). Obese women therefore have higher oestrone than their normal weight counterparts (Kwekkeboom *et al.*, 1990). Cauley and others (1989) found a 40% higher oestrone levels in obese women than levels in non-obese women.

The measurement of aromatase activity in breast tumour and peripheral tissues showed a higher activity of the enzyme in breast tumours (Singh *et al.*, 1999). It has been shown that tumours were capable of producing factors that stimulate aromatase enzyme activity in adjacent tissues (Miller and O'neil, 1987). In an attempt to identify potential aromatase stimulators in breast tumours, Reed *et al.* (1992) detected

Interlukin 6 (IL-6) a proinflammatory cytokine as the most potent factor. Infact, advances in breast cancer research have the emerging trend of cytokines being important regulators of oestrogen synthesis in breast cancer. This suggests an association between breast cancer risk and immune-suppression (Reed and Purohit, 1997).

2.15 Effect of Menopause on Serum Lipids and Lipoproteins

Studies have shown that menopause is associated with an increase in serum cholesterol especially total and LDL cholesterol (Hallberg and Svanborg, 1967; Kannel *et al.*, 1976). Some studies have also indicated that plasma triglyceride (TG) concentrations may increase and plasma HDL cholesterol levels decrease around the time of menopause (Mathews *et al.*, 1989; Jensen et al., 1990; Farish *et al.*, 1991). Infact a time-course study has revealed that the levels of LDL cholesterol started to increase gradually already two years before natural menopause while concomitantly a gradual decrease in HDL cholesterol occurred (Jensen *et al.*, 1990).

A qualitative analysis of low-density lipoprotein (LDL) subfraction by Campos *et al.* (1988) demonstrated that LDL particles may be smaller in size in post-menopausal women in comparison to pre-menopausal women. Probably, this is related to "small, dense LDL" which is regarded as a risk factor for coronary heart disease (CHD). Treatment of post-menopausal women with equine oestrogens was found to lower the proportion of large LDL particles but did not increase the fraction which corresponds to "small, dense LDL" (Campos *et al.*, 1993). Another report by Van der Mooren *et al* (1994), described the changes in LDL subfraction profile towards a smaller particle size during hormone replacement therapy (HRT) with oestradiol and dihydrogesterone. There is some evidence that "small dense LDL" has an increased susceptibility for oxidative modification (De Graaf *et al.*, 1971) and report suggested that estradiol has an antioxidant capacity which could protect LDL against oxidation (Sack *et al.*, 1994).

2.16 Menopause and the Risk of Atherosclerotic Disease Development

The role of menopause in the development of atherosclerotic disease remains controversial (Bonithon Kopp *et al.,* 1990). While several studies have shown an association between precocious or artificial menopause and increased risk of coronary heart disease (CHD) (Parish *et al.,* 1967; Bengtsson *et al.,* 1973; Gordon *et al.,* 1978; Rosenberg *et al.,* 1981; Svanberg, 1982; Colditz *et al.,* 1987), the influence of natural menopause on the cardiovascular disease risk is not clear. Two of the most important prospective studies on menopause have given conflicting results. Natural post-menopausal women as well as oophorectomized women had higher incidence rates of CHD than pre-menopausal women of the same age in the Framingham study by Gordon *et al.* (1987), while on the other hand, natural menopause was not significantly associated with an increased risk of CHD in contrast to bilateral oophorectomy in the Nurses Health Study by Colditz *et al.,* (1987).

To examine whether menopause may result in higher risk of CHD, the approach consists in exploring the relationship between menopausal status and CHD risk factors among which are lipids (especially Triglycerides and Cholesterol and its LDL fractions) blood pressure, obesity, blood glucose, and fibrinogen. There are some evidence that post-menopausal women have higher cholesterol levels than pre-menopausal women but no consistent associations have been found between menopause and blood pressure, triglycerides, fibrinogen, blood glucose or obesity (Weiss, 1972; Kannel *et al.,1976*; Hjortland *et al.,* 1976; Lindguist, 1982; Baird *et al.,* 1985; Shibata *et al.,* 1987, Bengtsson and Lindguist, 1979).

However, two studies undertaken on Indian and black American women failed to find any difference in total cholesterol between post-menopausal and pre-menopausal women (Hamman *et al.,* 1975; Baird et al., 1985). This suggests an influence of ethnic and racial factors in the effects of menopause on the atherogenic process.

2.17 Sex Differences in Plasma Lipoproteins

Studies have confirmed that the plasma lipoprotein profile of women confers an advantage for coronary heart disease risk (Seed, 1991). The first scientists to

observe gender differences in lipoprotein were Barr and his group (1951). They demonstrated that women had consistently higher levels of alpha lipoproteins (i.e. HDL) and lower levels of beta lipoproteins (i.e. LDL) than men. Data from Lipid Research Clinic Programs – LRCP (1979) also showed that women have a lower LDL than men until around the age of 60 years, a lower very low density lipoprotein (VLDL) level throughout life and a higher HDL level during post puberty life. The male HDL fell at puberty and this suggests a dominant suppressive effect of androgen on HDL in the male (LRCP, 1979). A study of plasma HDL cholesterol in two groups of Nigerians of different socio-economic status also showed that the women in both groups had higher levels of plasma HDL cholesterol than in men (Taylor and Agbedana, 1983). In an earlier observation, female adults in the low income group in Nigeria were shown to have higher mean serum total cholesterol and serum phospholipids but lower mean serum TG than male adults (Taylor, 1971).

Studies have elucidated the mechanisms of these sex differences in plasma lipoproteins. The lower VLDL and plasma TG levels found in pre-menopausal women were suggested to be due to a more efficient clearance of VLDL in women (Nikkila and Kekki, 1971). For the higher HDL levels seen in women, some studies have shown a higher rate of synthesis of Apo A_1 in women (Schaefer *et al.*, 1983) while others found a reduced clearance of Apo A_1 (Brinton *et al.*, 1989). Apo lipoprotein A_1 ($ApoA_1$) is the chief protein to which cholesterol and phospholipids are bound in HDL. Also, there is higher hepatic lipase activity in men than women and this justifies the lower HDL concentration found in men (Godsland *et al.*, 1987). For the lower LDL levels found in women before the age of the menopause, studies have shown that the activity of LDL receptors on the cell membrane which binds to Apo B100, the binding protein of LDL correlates with plasma oestrogen levels (Chao *et al.*, 1979; Nanjee *et al.*, 1990; Seed *et al.*, 1990).

An earlier work by Furman *et al.* (1967) and Furman (1969) also demonstrated that the age trends and gender differences in lipids and lipoproteins could be explained by the known effects of androgens and oestrogens on lipoproteins. Estrogen was reported to increase HDL and VLDL plasma levels and decrease LDL plasma levels in men and estrogen-deficient women (Furman, 1969). These reciprocal changes lead to

a variable and rather small effect on total cholesterol levels while total triglyceride and phospholipids levels are constantly elevated due to the rise in VLDL and HDL.

Qualitative differences exist between certain oestrogens in respect of their influence on serum triglyceride levels (Bolton *et al.*, 1975). Bolton *et al.* (1975) found that a "natural" preparation of conjugated equine oestrogen (0.625 or 1.25mg/day) did not alter triglyceride concentrations in oophorectomized women, while ethinyl oestradiol (2000.5mg/day) raised levels in average of about (40mg/day). In the rat, there is clear evidence that triglyceride secretion by the isolated perfused liver is higher in samples from female than male animals (Watkins *et al.*, 1972); the rate is decreased in livers of female animals ovarietomized several weeks before (though not down to male values) and is largely restored by estradiol (Watkins *et al*., 1972). The incorporation of labeled amino acid into VLDL secreted by the perfused rat liver is increased by estrogen (Afolabi, 1974). It was observed that estrogen causes beneficial changes in lipoproteins. In practice therefore, to reduce the risk of development of CHD, Hormone Replacement Therapy (HRT) is advocated. During a 5-year trial of the ability of oestrogen treatment to prevent relapse in patients with myocardial infarction, serum cholesterol levels were considerably reduced (Oliver *et al*., 1961).

It has been assumed that the lower incidence of coronary heart disease (CHD) in pre-menopausal women than in men of the same age is due to the effect of estrogen on lipoprotein metabolism. The death rate from ischaemic heart disease increases with age, but there is no acceleration in the increase in death rates after the menopause (Heller and Jacobs, 1978). There appears, however, to be an increased incidence of myocardial infarction in women who undergo an early menopause following bilateral oophorectomy (Rosenberg *et al.*, 1981).

Epidemiological studies have shown that there is a statistical association between increased levels of low density lipoproteins (LDL) and coronary heart disease and also between reduced levels of high density lipoproteins (HDL) particularly HDL_2 and coronary heart disease (Gordon *et al.*, 1977; Miller *et al.*, 1981; Betteridge, 1989; Pocock *et al.*, 1989). HDL levels in women are higher than in men and show little change from before puberty, through reproductive life and after the menopause,

whereas LDL levels are lower in pre-menopausal women than in men and rise fairly steadily throughout life.

The effect of exogenous administration of oestrogens on plasma lipoproteins depends on their chemical structure and route of administration. Thus, the effects of orally administered oestrogens are more profound than those administered parenterally. The reason why oestradiol is less likely to produce thrombosis and hypertension is that it has a shorter duration of action as it is metabolized more quickly than synthetic oestrogens, such as ethinyl oestradiol and also more quickly than the equine oestrogens present in premarin. It is currently thought that HRT with natural oestrogens is beneficial in reducing the incidence of ischaemic heart disease (Ross *et al.*, 1981) especially in younger women. In general, HRT is only given to those without significant medical disorders such as hypertension and diabetes mellitus.

2.18 Effect of Unopposed Oestrogen Hormone Replacement Therapy (HRT) on Serum/Plasma Lipids and Lipoproteins

The study of the effects of gonadal steroids on lipoproteins is of importance as it gives some insight into the complexity of lipoprotein metabolism and also because of the number of healthy women taking these compounds either as oral contraceptives or as hormone replacement therapy (HRT). In the UK alone, the number of women taking gonadal steroids as oral contraceptives amount to three million while the number taking them as HRT amount to 800,000 (Seed, 1991). There is increasing demand for HRT both for amelioration of menopausal symptoms and also for prophylactic action of oestrogen against osteoporosis. Awareness of the reduction in cardiovascular mortality in HRT users is also becoming widespread (Seed, 1991). Oestrogen is usually prescribed with addition of progestin because of the risk of endometrical cancer (Tikkanen, 1996). Unopposed oestrogen HRT is recommended only for hysterectomized women. The most commonly used oestrogens are conjugated equine oestrogens and oestradiol (in micronized form or as oestradiol valerate). In terms of biological activity 1.5-2mg of oestradiol corresponds roughly to 0.625mg of oestrogens (Tikkanen, 1996). A lot of information has accumulated regarding oestrogen effects on serum or plasma lipids and lipoproteins from studies employing

unopposed oestrogens. The oral administration of these oestrogens results in significant decreases in total and LDL cholesterol as well as Apolipoprotein B (Apo B) concentrations with a concomitant increase in HDL cholesterol (mainly caused by an increase in the HDL_2 subfraction because of reduced hepatic lipase activity) (Bush and Miller, 1987; Lobo, 1991) (Figure 2.4). Serum TG and VLDL levels are influenced differently by various oestrogen preparations. Studies indicated a dose-dependent increase in serum TG levels during conjugated equine oestrogen use (Bush and Miller, 1987). When commonly used doses of oestradiol are employed, they do not give an increase in TG levels but when higher doses (4mg or more) of oestradiol are used, increase in TG levels reportedly occurs (Fahrareus, 1988). Generally, the TG content in both LDL and HDL particles increases during oestrogen administration resulting in an increased proportion of TG in the major circulating lipoprotein species (Walsh *et al.*, 1991).

On the average, oestrogen HRT results in a 10 – 15% decrease in LDL cholesterol (PEPI writing group, 1995). The effect on LDL however depends on the initial LDL cholesterol concentration. When the initial LDL cholesterol levels are low, there is little or no effect of oestrogen HRT on these, while markedly elevated initial LDL cholesterol levels are reduced effectively (Tikkanen *et al.*, 1978). Also qualitative changes occur in the circulating LDL particles. Commonly used oestrogen preparations produce LDL particles with reduced cholesterol/Apo B ratios and enrichment in TG concentration (Moorjani *et al.*, 1991). Tikkanen (1992) proposed that the observed increase in serum/plasma HDL cholesterol levels during post-menopausal oestrogen treatment is due to a significant increase in the sex-steroid sensitive HDL_2 subfraction. Studies have indicated that HDL_2 particles (containing Apo A_1 as the sole Apo protein component) are selectively increased by oestrogen therapy (Walsh *et al*., 1991). Two different mechanisms appear to contribute to these changes. A suppression of hepatic lipase activity by oestrogen can result in reduced degradation of HDL_2 particles (Tikkanen *et al.*, 1982). Schaefer *et al*. (1982) also suggested that overall production rate of HDL protein could increase by a large dose of ethinyl oestradiol. The study of Walsh *et al*. (1994) demonstrated that 2mg of oral

oestradiol per day increased the production rate of HDL_2 Apo A_1 and to a smaller extent that of HDL_3 Apo A_1.

2.19 Effect of Combination Hormone Replacement Therapy (HRT) on Serum/Plasma Lipids and Lipoproteins

The most commonly used combination hormone replacement therapy (HRT) regimens consist of a relatively low oestrogen dose given for 21-28 days of the cycle with cyclical addition of progestin or progestestogen during the 10 – 14 last days. Some of the androgenic progestins exhibit pronounced HDL cholesterol-lowering activity. For instance, addition of levonorgestrol, a 19-nortestosterone derivative with androgenic properties in a daily dose of 125-250ug may decrease HDL cholesterol levels below the baseline level (Tikkanen *et al*., 1986).

When a daily dose of 5mg norethisterone is added to the oestrogen regimen, a similar reduction in HDL cholesterol results (Krauss, 1983). However, when commonly used doses of 17-hydroxyprogesterone-derived progestins or smaller doses of androgenic progestins are used, these usually reduce HDL cholesterol only moderately with the levels during treatment remaining close to pre-treatment levels (Hirvonen *et al*., 1981; Rijpkema *et al.,* 1990; PEPI writing group, 1995). These are thus preferable. The changes in lipoproteins provoked by HRT are concentration dependent (Seed, 1991). Progestogens (progestins) increase hepatic lipase activity and this in turn reduces HDL_2 in HRT users. Progestogens differ in their metabolic effect because they vary in androgenicity. Levornogestrol and norethisterone which are the widely used HRT in the UK are androgenic and therefore affect HDL levels but medroxyprogesterone acetate (widely prescribed in the U.S.A) and natural progesterone are not androgenic and do not affect HDL (Hirvonen *et al.,* 1987). Other synthetic progestins (such as desogestrel used in oral contraceptives) with much less androgenic activity has no effect on HDL or its chief apoprotein, Apo A1 (Kuusi *et al.*, 1985). However, newer progestins (such as gestodene and norgestimate) which do not react with androgen receptors show no androgenic activity on lipoproteins.

Androgenic progestins of the 19-nortestosterone series tend to lower serum TG levels (Rijpkema *et al*., 1990), while 17-hydroxyprogesterone-derived progestins have

little or no effect. The TG-reducing effect of some of these progestins may be considered as an advantage over conjugated equine oestrogens regimes which have TG-elevating effect. The route of administration of these gonadal steroids is also important. Transdermal, as opposed to oral administration of oestrogen results in far less alteration in lipoprotein levels with no significant increase in HDL or TG and little reduction in LDL (Fahrareus *et al*., 1982; Whitehead *et al.*, 1990).

Continuous combined therapy consisting of daily dose of both oestrogen and progestin have been introduced in some countries (Tikkanen, 1996). These combinations employ, commonly, 0.625mg conjugated equine-oestrogen or 2mg of oestradiol in conjunction with a small dose of progestin (2.5mg/day medroxyprogesterone acetate or 1mg/day norethisterone). These regimes have a benefit of avoiding withdrawal bleeding as opposed to cyclic regimens but long-term effects on lipids and lipoproteins are not yet clear. Some preliminary data indicated that compared to cyclical regimens, qualitatively similar but somewhat smaller LDL cholesterol reduction and HDL cholesterol increases could be achieved (Lobo, 1991; Newnhan, 1993). A large study of PEPI writing group (1995) however indicated equally good efficacy of combination regimens in LDL reductions and HDL increases.

With these aforementioned changes in lipoproteins during HRT, it is evident that cardiovascular disease risk is altered by the use of HRT. The Framingham study has established that post-menopausal women have an increased risk of cardiovascular disease (CVD) as compared to pre-menopausal women (Gordon *et al*, 1978). Furthermore, both cross-sectional and prospective studies have shown reduced risk from CVD in women taking HRT (Ross *et al.*, 1981; Stampfer *et al.*, 1985; Pettiti *et al.*, 1986; Hazzard, 1989). The Lipid Research Clinic Program (LRCP) (1995) also showed a two-thirds reduction in total mortality, attributable to a reduction in cardiovascular mortality in women taking oestrogens after menopause. In women at highest cardiovascular risk (i.e. post oophorectomy women), the mortality was reduced by a factor of 10 only (Bush *et al.*, 1987). These reductions in mortality were found not to be entirely due to the changes in lipoproteins (i.e. reduction in LDL and increase in HDL) but other effects of estrogen particularly on the arterial wall are suggested to be important (Seed, 1991). There is an evidence from several studies for

an effect of oestrogen on the arterial wall which may be separate from the effects on lipoproteins. For instance, Clarkson *et al.* (1987) found that in monkeys, the elevation of hormone levels caused by pregnancy (endogenous oestrogen) provides greater protection against induced atheroma (by atherogenic diet) than can be explained by changes in lipoprotein levels. Thus suggesting that there is a reduced LDL uptake by the arterial wall exposed to oestrogen. Similarly, Adams *et al.* (1990) found that oestrogen treatment in oophorectomized monkeys prevents the development of aortic and coronary atheroma.

2.20 The Roles of Interaction Between Equine Oestrogens, Low Density Lipoprotein Cholesterol (LDLC) and High Density Lipoprotein Cholesterol (HDLC) in the Prevention of Coronary Heart Disease (CHD) and Nuerodegenerative Diseases

An inverse relationship is known to exist between the level of plasma HDLC and Coronary Heart Disease (CHD) (Stampfer *et al.,* 1991). This is in part due to HDL's involvement in reverse cholesterol transport. Oxidized low-density lipoprotein (OLDL) has been implicated in the pathogenesis of atherosclerosis and neurodegenerative diseases (Steinberg *et al.,* 1989; Chung *et al.*, 1989; Sugawa *et al.,* 1997; Dracsywka-Lusiat *et al.,* 1998; Keller *et al.,* 1999). The peroxidation of the LDL molecule renders it immunogenic and causes monocyte recruitment, foam cell formation and cytotoxicity of various cells, including neurons.

Recent studies have shown that HDL can prevent the oxidation of LDL (Bonnefront-Roussellot *et al.,* 1999). The inhibition of LDL oxidation by HDL leads to a decrease in the formation of lipid peroxides, foam cell formation and cytotoxicity caused by oxidized LDL (Chung *et al.,* 1989; Ohta *et al.*, 1989; Parthasarthy *et al.*, 1990). This contributes to the existence of a strong inverse relationship between HDL and coronary heart disease (Stampfer, 1991). Evidence also suggests that HDL associated enzymes such as paroxonase may play a critical role in the protective effect of HDL on LDL (Mackness *et al.,* 1993; Sangvanich *et al.,* 2003). However, HDL itself can get oxidized and the ability of oxidized HDL (OHDL) in reverse cholesterol transport is impaired (Nagano *et al.,* 1991). Thus, oxidized HDL, in contrast to HDL

has been shown to be neurotoxic as it induces neuronal death and may play an important role in the pathogenesis of CHD (Ohmura *et al.*, 1999; Berco and Bharnoni, 2001).

The potential role of interaction between equine oestrogens; LDL cholesterol and HDL cholesterol in the prevention of CHD and neurodegenerative diseases in post-menopausal women was investigated by Perella *et al.*,(2003); Conjugate equine oestrogens including 17β-oestradiol were found to protect HDL oxidation but in a concentration dependent manner. Protection of LDL oxidation by HDL was thus enhanced by addition of oestrogen. This stresses the importance of LDL/HDL ratio and the cardioprotective effect of oestrogen was thought to be by a mechanism involving the modification of the plasma LDL/HDL ratio. A 10-fold higher concentration of HDL over LDL was required for inhibition of LDL oxidation by HDL. Lower concentrations of HDL did not provide any protection (Perrella *et al.*, 2003). This suggested that protection of LDL from oxidation by HDL is concentration dependent (Huang *et al*, 1998; Perrella *et al.*, 2003). HDL_3 was also found to be more effective than HDL_2 in protection of LDL from oxidation. Studies are however desired to determine the role of each HDL subfraction in the presence of oestrogen on the inhibition of LDL oxidation (Yoshikawa *et al*., 1997).

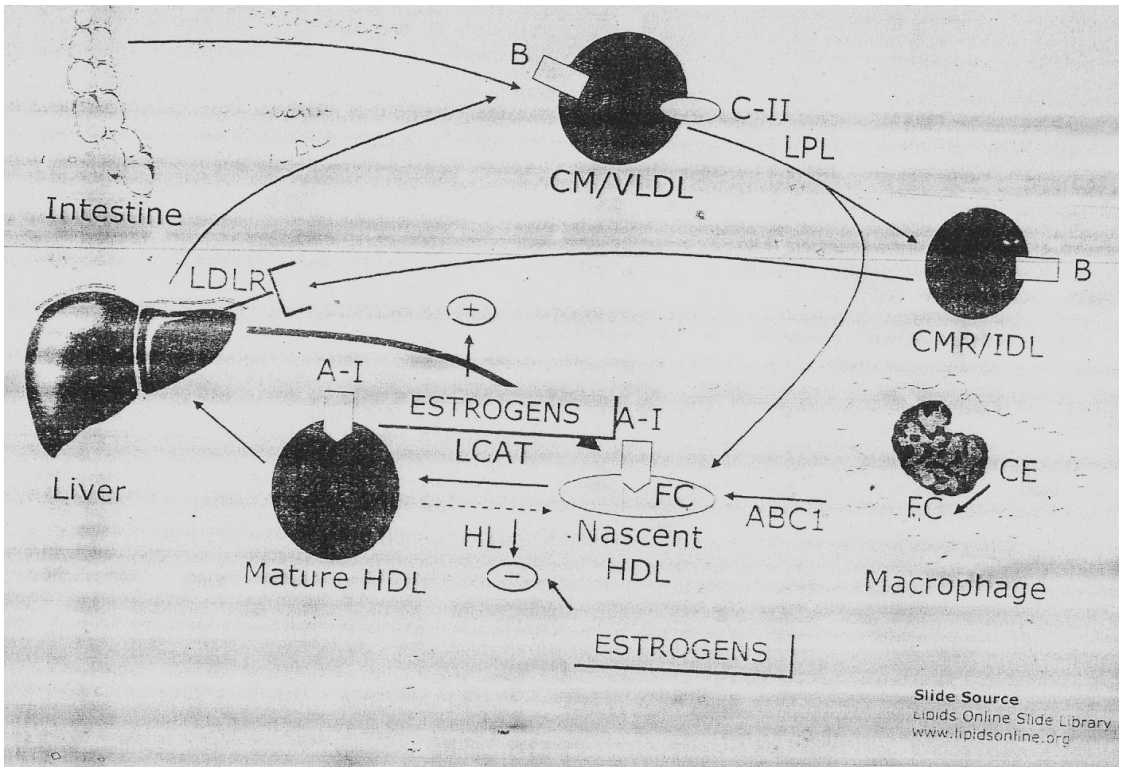
The mechanism involved in the antioxidant effects of oestrogens on HDL are not fully defined. However, different hypotheses have suggested that oestrogens may be acting as free radicals scavengers and thus able to break the free radical chain formation produced from membrane oxidation processes and hence inhibit lipid peroxidation. Oestrogens may also be able to sequester metal ions or donate a proton to reduce peroxy-free radicals (Lacort *et al.*, 1995).

The stimulation of Apo A_1 by oestrogen (Figure 2.5) may also be of importance (Zhang *et al.*, 2001). So acting as antioxidant directly and by up-regulation of antioxidant factors may be additional mechanisms that are involved in the cardioprotective and neutroprotective effects of oestrogens. Oestrogens therefore reduce the risk of CHD and neurodegenerative diseases in healthy post-menopausal women by inhibition of HDL oxidation and also by an enhancement of LDL oxidation by HDL (Perrella *et al*., 2003).

2.21 Plasma/Serum Lipids and Breast Cancer

Several clinical studies suggest the prognostic significance of serum/plasma lipid levels in breast cancer (Abu-Bedair *et al.,* 2003). There are several reports of elevated plasma lipid levels such as triglycerides (TG), total cholesterol (T.C) and low density lipoprotein cholesterol (LDL-C) in both pre and post-menopausal breast cancer patients (Bani *et al.*, 1986; Knapp *et al*., 1991; Kumark and Sacdanandam, 1991; Hover and Engholon, 1992; Abu-Bedair *et al.*, 2003; Hasija and Bagga, 2005).

Abu-Bedair *et al.,* (2003) suggested an association between high serum total lipids and total cholesterol levels and an increased risk of breast cancer in Egyptian pre-menopausal women. They found increased serum total cholesterol in pre-menopausal patients with breast cancer but not in post-menopausals. They also found increased triglycerides in the post-menopausal but not in pre-menopausal patients. Hasija and Bagga (2005) found increased serum total cholesterol and LDL-cholesterol in both pre and post-menopausal patients with breast cancer in India. Vatten and Foss (1990) in Europe reported an inverse relation between serum cholesterol and breast cancer incidence. They found that breast cancer incidence rate was low (0.53) among women with serum cholesterol in the highest quartile of up to 329mg/100ml compared to those in lowest quartile of about 200mg/100ml. For serum triglyceride, an association that was not statistically significant with breast cancer incidence was found. Hoyer and Engholon (1992) on the other hand reported a negative relation between serum triglyceride and breast cancer risk. Others found no association between the total serum cholesterol levels and breast cancer risk in pre-menopausal women (Gaard *et al.,* 1994). These reports have not been consistent.



Key: CMR = Chylomicron Remnants

CE = Cholesterol Ester

FC = Free Cholesterol

HL = Hepatic Lipase

A-I = Apolipoprotein A-I

Figure 2.5 Drug Effects on HDL: Estrogens

CHAPTER THREE

3.0 METHODOLOGY

3.1 Study Design and Subjects

3.1.1 Study Design

All subjects signed the consent forms and filled questionnaires that were endorsed by the Ethical Committee of the University of Ibadan and University College Hospital, Ibadan, (see Appendix)

The study was designed in such a way that after signing and filling the consent forms and questionnaires, patients as well as pre-menopausal controls were given regular follow up appointment visits that were consistent with the date/time of bleeding i.e. at luteal phase of their menstrual cycles (about one week to expected date of monthly flow). All subjects were also instructed to eat their last meal on the night before their appointments at around 8.00pm and not to eat anything in the morning of their visit before seeing the Doctor by around 8,00am – 10.00am. This was to ensure collection of overnight fasting (12 - 14 hours) blood samples.

3.1.2 Subjects

A total of sixty-nine (69) adult women were recruited by convenience sampling technique and used for this study. These consisted of twenty-five (25) pre-menopausal patients with breast cancer who had oophorectomy done and forty-four (44) women without breast cancer who served as controls.

The patients group included all eligible consecutive pre-menopausal women with breast cancer who were being seen at the Surgical Oncology Unit, Surgery Department of the University College Hospital (U.C.H) Ibadan and who consented for surgical oophorectomy and had it done. These were recruited under the supervision of a Consultant Surgeon.

Fifteen (15) of the patients reported at Manchester Stage III of the disease, seven (7) patients reported at Stage IV, two (2) patients at Stage II while only one patient reported at Stage I. The control group included twenty-five (25) age-matched pre-menopausal women and nineteen (19) post-menopausal women who did not present with endocrine or oncological disorders. These were recruited from Aba-Alamu

Community, Apata Ibadan and also from patients attending the Surgical Unit of U.C.H for other reasons that are not endocrine or oncological.

The attending Surgeon identified and established the menopausal status of the patients and control subjects. Women who had their menstrual flow in the last 3 months were classified as pre-menopausal, while women who had stopped menstrual flow for at least 12 months were classified as post-menopausal. This study was approved by the Ethical Committee of the U.I/U.C.H Ibadan.

3.1.2.1 **Sample Size Calculation**

The minimum sample size for this study was calculated from the formular below:

$$N = \frac{2\,[Z_\alpha\ \sqrt{2\Pi(1-\Pi)}\ + Z\beta\sqrt{P_1(1-P_1) + P_2(1-P_2)}]^2}{(P_1-P_2)^2}$$

Where:

N = Number of Subjects

Zα = Standard normal deviate of α i.e type I error at level of significance of 0.05 (95% confidence interval) = 1.96.

Zβ = Standard normal deviate of β i.e type II error at power of 80% = 0.84.

P_1 = Prevalence of Breast cancer in Nigeria = 1.16 in 1,000 per year = 0.16% (Adebamowo, 1998).

P_2 = Estimated prevalene of premenopausal breast cancer patients that consent for oophorectomy, (about eight (8) premenopausal breast cancer patients consent for oophorectomy out of about 120 to 150 breast cancer patients who report in a year at the oncology unit of U.C.H Ibadan). = 0.01%.

$$\Pi = \frac{P_1 + P_2}{2} = \frac{0.16 + 0.01}{2} = 0.085$$

$$\therefore \quad N = \frac{2\,[1.96\ \sqrt{2x0.085(0.015)} + 0.84\sqrt{0.16x0.84+0.01x\ 0.99}]^2}{(0.15)^2}$$

$$= \frac{2[1.96\sqrt{0.00255} + 0.84\sqrt{0.1344+0.0099}]^2}{0.0225}$$

$$= \frac{2\,[0.0989604 + 0.3190867]^2}{0.0225}$$

$$= \frac{2x[0.41804955]^2}{0.0225}$$

$$= 15.5347$$

$$N = 16.$$

Therefore, the minimum sample size for this study was 16 patients.

3.1.2.2 **Sample Collection**

Ten millilitre (10ml) fasting (12 - 14 hours) blood samples were then collected at luteal phase of the patients menstrual cycle (before oophorectomy) by venipuncture into EDTA (1mg/ml) bottles pre-chilled and kept in ice bath to prevent auto-oxidation of the lipids. Blood samples were again collected from the patients at 1 month (4 weeks), 3 months (12 weeks) and six months (24 weeks) after oophorectomy.

Fasting (12-14 hours) blood samples (10ml) were similarly collected from the control subjects (at luteal phase for the pre-menopausal group) into EDTA bottles in ice bath.

The samples were immediately centrifuged and the plasma separated and kept at -20^{o}C pending the determinations of the plasma levels of lipids, lipoproteins and oestrogen hormone i.e. Oestradiol.

The age at recruitment, weight, height, age of menarche and at first childbirth and parity of the patients and controls were recorded in the questionnaire at recruitment. The systolic blood pressure (SDP) and diastolic pressure (DBF) were similarly measured and recorded. The body mass index (BMI) values were calculated (Kg/m^2) for patients and control subjects accordingly and recorded.

3.1.2.3 Anthropometric measurements: Subjects were weighted on a bathroom scale while barefooted. They were also made to remove all other extra outer clothes e.g. , coats or headgears and asked to stand straight for the weight to be read on the bathroom scale. Measurement was done to the nearest 0.5kg. The height was measured with a wall-mounted ruler. After removing footwear, the subject was made to stand up as staright as

possible with feet together and with heels, buttocks and shoulders and back of the head touching the upright. The head was positioned such that the subject views horizontally. A direct height reading was taken through the bar window after the metal piece was gently lowered to make contact with the head. When necessary, thick hairs were compressed so as to get contact with the top of the head. Measurement was done to the nearest 0.5 cm. BMI was calculated by dividing weight (kg) by height (m) squared (m^2).

3.1.2.4 **Exclusion Criteria**

Hypertensives and those with hyperlipideamic syndromes or on drugs that affect plasma lipid levels were excluded from the study.

3.2 **Lipid and Lipoprotein Determinations**

Assays kits by HUMAN were used for these determinations and absorbances were measured by spectrophotometer (SpectroScan 60DV).

3.2.1 Total Cholesterol

The plasma total cholesterol determination of the patients and control samples were done by use of Human Cholesterol Liquicolor Kit (Richmond, 1973). This employs an enzymatic colorimetric test for cholesterol with Lipid Clearing Factor (L.C.F)

3.2.1.1 **Principle of Reaction**

Cholesterol is determined after an enzymatic hydrolysis and oxidation by cholesterol esterase and cholesterol oxidase respectively. These yield hydrogen peroxide (H_2O_2). The hydrogen peroxide reacts with 4-aminophenazene in the reagent kit in the presence of phenol and peroxidase (also in the kit) to form an indicator called quinoneimine (pink colour).

$$\text{i.e. Cholesterolester} + H_2O \xrightarrow{\text{CHE}} \text{Cholesterol} + \text{Fatty acid}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{CHO}} \text{Cholesterol 3-one} + H_2O_2$$

$$2H_2O_2 + \text{4-aminophenazone} + \text{Phenol} \xrightarrow{\text{POD}} \text{Quinoneimine} + 4H_2O$$

(pink)

Note: A special feature of benefit of this kit is the fact that it contains LCF which clears up totally any turbidity by lipeamic specimens. Lipeamic specimens usually generate turbidity of the sample-reagent mixture which leads to falsely elevated results. The Cholesterol Liquicolor test avoids such falsely elevated results through its built in lipid clearing factor (LCF). The reagent and standard are ready for use as supplied in the kit and the kit could be used for serum as well as heparinized or EDTA plasma.

3.2.1.2 Procedure

10µl of each sample, standard and control were added to 100µl of reagent. The contents of the cuvettes were vortex mixed and the tubes were incubated for 10mins at 20-25^{o}C The absorbance of the sample, control and standard were measured against reagent blank at 500nm wavelength within 60 minutes.

RGT= Reagent; STD = Standard, Humatrol quality control was used.

3.2.1.3 **Calculation**: The total cholesterol (T-Chol) concentration of the various samples (test and control) were calculated using the measured absorbance and value of the standard (supplied in the kit) according to the formular below:

$$\text{T-Chol sample} = 200 \times \frac{\Delta A\text{sample}}{\Delta A\text{STD}} \text{ (mg/dl)}$$

Note: Concentration of cholesterol Standard = 200mg/dl

Δ A = Absorbance or O.D.

3.2.2 High Density Lipoprotein Cholesterol (HDLC)

This was determined by use of HUMAN HDL Cholesterol precipitant and standard kit together with HUMAN Cholesterol liquicolor test kit (Richmond, 1973).

3.2.2.1 Principle

The chylomicrons, very low density lipoproteins (VLDL) and low density lipoproteins (LDL) in the plasma samples were precipitated quantitatively by addition of the HDL reagent (precipitant) which contains phosphotungstic acid and magnesium chloride. After centrifugation, the supernatant which contains the HDL fraction was assayed for HDL cholesterol using the HUMAN Cholesterol liquicolor test kit.

The precipitant was used undiluted (as for macro assays). The precipitant needs to be diluted 1 part to 4 parts of distilled water for semi micro assays.

3.2.2.2 Procedure for Precipitation

500µl aliquots of plasma samples (patients, control and quality control) were pipetted into respective labeled centrifuges tubes and l000µl portions of precipitant were added into each tube. These were mixed well and incubated for 10 minutes at room temperature after which the content were centrifuged for 10 minutes at 4000rpm. After centrifugation, the clear supernatant of each tube was separated into labeled tubes within one hour and cholesterol concentration was determined using the HUMAN cholesterol liquicolor reagent (as earlier described). The humatrol quality control was used to ensure precision and accuracy.

3.2.2.3 Calculation

$$\text{HDL Cholesterol} = \frac{\Delta A \text{ sample}}{\Delta A \text{Standard}} \times \frac{150}{1} \text{ mg/dl}$$

Note: Concentration of HDL Standard = 150mg/dl

Δ A = Absorbance

3.2.3 **Plasma Triglyceride Estimation**

The triglyceride concentrations of the samples of this study were estimated by use of HUMAN Triglycrides liquicolor GPO-PAP method kit (Trinder, 1969). This is an enzymatic colorimetric test with lipid clearing factor (LCF).

3.2.3.1 Principle of Reaction Method

The triglycerides are determined after an enzymatic hydrolysis with lipases. An indicator is formed from hydrogen peroxide (H2O2), 4-arnino antipyrine and 4-chlorophenol under the catalytic influence of peroxidase i.e.

$$\text{Triglycerides} \xrightarrow{\text{Lipases}} \text{Glycerol + Fatty acids}$$

$$\textit{Glycerol} + \text{ATP} \xrightarrow{\text{Glycerol kinase}} \text{Glycerol -3-phosphate} + \text{ADP}$$

$$\textit{Glycerol-3-phosphate} + O_2 \xrightarrow{\text{GPO}} \text{dihydroxyacetone phosphate} + H_2O_2$$

peroxidase

H_2O_2 + 4-aminoantipyrine +4-chlorophenol $\longrightarrow$ *quinonei*mine + *HCL* + $2H_2O$

(pink)

HUMAN Triglycerides GPO also has a built in LCF which clears up totally all turbidity caused by lipemic specimens (serum/plasma).

3.2.3.2 Procedure

10µl of each sample, standard and control were pipette into cuvettes and 1000µl of the working reagent was added to each tube. The tubes were mixed well respectively and the contents were incubated for 5 minutes at 37°C.

The absorbances of the samples, standard and quality control were measured at 500nm against the reagent blank within 60 minutes.

3.2.3.3 Calculation

$$\text{TG concentration} = \left\{ \frac{\Delta \text{ A sample}}{\Delta \text{ AStandard}} \times \frac{200}{1} \right\} \text{mg/dl}$$

TG = Triglyceride

3.2.4 Low Density Lipoproteins (LDL) and very Low Density Lipoprotein (VLDL) Cholesterol Determination using Calculation (Friedewald *et al*., 1972).

The LDL Cholesterol and VLDL Cholesterol concentrations were calculated from the total cholesterol concentration (T.C), the HDL Cholesterol concentration and (TG) according to Friedwald *et al* (1972).

i.e $[LDLC] = [T.C] - \{[HDLC] + [TG]/5\}$ mg/dl

and $[VLDLC] = TG/5$ mg/dl

3.2.5 Oestradiol [E_2] Determination

This was done by use of enzyme immunoassay (ElA) kit by Immunometric's (UK) Pugeat, *et al*., 1981. This EIA system was developed for the quantitative measurement of Oestradiol [E2] in human serum or plasma without extraction i.e. direct measurement.

3.2.5.1 Principle of Oestradiol EIA

Oestradiol in serum occurs largely bound to Sex Hormone Binding Globulin (SHBG). This protein - bound Oestradiol is unavailable for antibody binding as

antibodies can only bind to unbound or "free" Oestradiol. In direct (i.e. non-extraction) Oestradiol assays a displacing agent-which binds to the serum binding proteins but not to the antibody is used to displace oestradiol from serum binding proteins and make it available for antibody binding. Thus, total oestradiol in plasma or serum is measured.

The oestradiol EIA is a direct assay of limited type because of its *"Competitive nature"*. A specific agent is used to displace Oestradiol from binding proteins thus making it available for antibody binding.

The oestradiol in sample, controls or standards reacts with fluorescent-labelled polyclonal anti-oestradiol antibody and then equilibrates with a fixed amount of alkaline phosphatase labeled oestradiol in binding to a limited amount of the antibody. An anti fluoresin serum bound to magnetic particles is used to separate the Oestradiol/oestradiol/labeled antibody complex from unbound components by magnetic sedimentation and wash step.

The magnetic particles are incubated with enzyme substrate solution for a fixed time and the reaction is ended by addition of stop buffer. The intensity of colour produced is inversely proportional to the amount of oestradiol present in the sample.

3.2.5.2 Oestradiol EIA Assay Procedure

Approximately four hours assay the reagent were prepared as directed in the kit manual and the tubes were labeled respectively. The reconstituted standard as well as the quality control (QC) and test samples were included in duplicates in each assay batch. The set up of an assay (run) are of 100 tubes. Tubes 1 to 14 contain standards, tubes 15-100 contain test samples and QCsamples while tube 101 contains substrate blank (used at colour development stage containing 0.5ml substrate solution and 1ml. Stop buffer only). The volume of plasma needed was just 300µl

The assay procedure has four (4) main stages;

(i) **Reaction of antibody with serum/plasma**

Oestradiol antibody and displacing agent were incubated with sample for 20 minutes and at 37°C.

(ii) **Reaction of antibody with enzyme**

Labeled oestradiol: Alkaline phosphatase labeled oestradiol is added and the incubation continued for a further 20 minutes at 37°C.

(iii) **Magnetic solid phase separation step**

Anti fluorescein antibody coupled to a magnetic solid phase was added and incubated for 5 minutes at 37°C.

Fluorescein labeled anti-oestradiol antibody was then isolated by means of a magnetic separation and two wash-steps.

(iv) **Colour development step**

The magnetic parties were incubated with a coloured enzyme substrate for 60 minutes at 37 C. The presence of alkaline phosphate causes a colour change from yellow to pink. The intensity of colour produced is inversely proportional to the amount of oestradiol present in the sample.

The reaction was terminated by addition of stop buffer and the optical density of all tubes was measured at 550nm wavelength using Serozyme Spectrophotometer.

3.2.5.3 Calculation

The oestradiol concentrations of test samples were interpolated from a calibration curve.

3.3 **Statistical Analysis**

All data were analysed using statistical package of social science, SPSS 15.0.

For quantitative variables:

1. Paired Student's t-test was used to test the significance of difference between mean values.
2. Analysis of variance (ANOVA) was used to test significance of variations within and among group means and Post Hoc test was used for comparison of multiple variables.
3. Multiple regression analysis was employed to determine interrelationship between variables.

For non-quantitative variable:

4. Chi-square test was used for comparison of means for non-quantitative variable.

Probability value, $P < 0.05$ was considered statistically significant.

CHAPTER FOUR

4.0 RESULTS

Majority of the breast cancer patients studied reported at advanced stages of the disease. The percentage that reported at Manchester stage IV was 58.3 and 29.2 percent reported at Manchester stage III while 8.3 and 4.2 were the percentages that reported at stages II and I respectively. The invasive ductal and intraductal were the types of breast carcinoma presented by the patients.

Sixty four percent (64%) of the premenopausal breast cancer patients studied reported for check-up planned visits at one month after oophorectomy while fifty two percent (52%) and forty-eight percent (48%) respectively reported at three months and six months after oophorectomy.

On the whole, thirty-six percent (36%) of the patients reported for all the visits consecutively i.e at 0 month (before oophorectomy/baseline) 1 month, 3 months and 6 months after oophorectomy.

4.1 Demographic, Reproductive and Anthropometric Parameters of the Patients and Controls

Tables 4.1 and 4.2 show the comparison of the demographic, reproductive and anthropometric parameters of premenopausal and postmenopausal control subjects and premenopausal breast cancer patients using ANOVA. There were significant variations in the mean ages and parity among the groups ($P<0.05$) and Post Hoc tests (Table 4.3 and Table 4.4) showed that the significant variation in mean ages was between postmenopausal control subjects and the premenopausal breast cancer patients and also between premenopausal controls and postmenopausal controls. The patients, with a mean age of 39.0 ± 1.2 years were statistically significantly younger than the postmenopausal control subjects with a mean age of 53.0 ± 1.6 years ($P<0.01$). The mean age of the patients was however not significantly different from that of the premenopausal control subjects of 38.6 ± 1.4 years ($P=0.703$). Similarly, the postmenopausal control subjects were significantly older than the premenopausal control subjects ($P<0.01$). The significant variation in parity was between the premenopausal controls and postmenopausal controls ($P<0.01$). There were no significant variations in the parities of the breast cancer patients and the control subjects.

Table 4.1: Comparison of Age, Demographic and Reproductive Characteristics in Pre-menopausal and Postmenopausal Controls and Pre-menopausal Breast Cancer Patients using ANOVA.

Parameters	Pre-Menopausal Controls n = 25	Post Menopausal Controls n = 19	Pre-Menopausal Patients n = 25	F	p
Age (yrs)	38.6 ±1.4	53.0 ±1.6	39.0 ± 1.2	31.86	0.000*
Age at menarche (yrs)	16.0 ± 0.4	16.1 ± 0.5	15.8 ± 0.5	0.216	0.81
Parity	3.4 ±0.4	5.1 ±0.5	4.4 ±0.4	4.034	0.02 *
Age at 1st childbirth (yrs)	23.7±0.9	21.9 ±0.8	22.9 ±0.8	0.579	0.56

values are mean ±SEM

n = number of subjects

SEM = standard error of mean

p = probability

yrs = years

* = significant at $p<0.05$

ANOVA = Analysis of Variance

Table 4.2: Comparison of Anthropometric Characteristics of Pre-menopausal and Postmenopausal Controls and Pre-menopausal Breast Cancer Patients using ANOVA.

Parameters	Pre-Menopausal Controls n = 25	Post Menopausal Controls n = 19	Pre-Menopausal Patients n = 25	F	p
Weight (kg)	66.5 ±3	64.7 ±3	65.9 ±3	0.177	0.834
Height (m)	1.60 ±0.0	1.58 ±0.1	1.61 ±0.0	2.155	0.12
BMI (kg/m^2)	25.0 ±0.9	26.0 ±1.0	25.0 ± 0.9	0.302	0.74

Values are mean ±SEM

n = number of subjects

SEM = standard error of mean

p = probability

ANOVA = Analysis of Variance

Table 4.3: Post Hoc Tests of the Demographic and Reproductive Measurements in Premenopausal and Post Menopausal Controls and Premenopausal Breast Cancer Patients, (p-Values)

Parameters	p-Values Premeno Patients (n=25) Vs Premeno Controls (n=25)	p-Values Premeno Patients (n=25) Vs Postmeno Controls (n=19)	p-Values Premeno Controls (n=25) Vs Postmeno Controls (n=19)
Age (yrs)	0.53	0.000*	0.00*
Age at menarche (yrs)	0.52	0.72	0.79
Age at 1st child birth (yrs)	0.79	0.42	0.30
Parity	0.9	0.22	0.01*

n = number of subjects
p = probability
* = significant at $p<0.05$
premeno = premenopausal
postmeno = postmenopausal
yrs = years

Table 4.4: Post Hoc Tests of the Anthropometric Characteristics of Measurements in Pre-menopausal and Post Menopausal Controls and Premenopausal Breast Cancer Patients, (p-Values)

Parameters	p-Values Premeno Patients (n=25) Vs Premeno Controls (n=25)	p-Values Premeno Patients (n=25) Vs Postmeno Controls (n=19)	p-Values Premeno Controls (n=25) VS Post Meno Controls (n=19)
Weight (kg)	0.56	0.75	0.81
Height (m)	0.20	0.05	0.42
BMI (kg/m^2)	0.87	0.46	0.56

n = number of subjects
p = probability
premeno = premenopausal
postmeno = postmenopausal

The ages at menarche and ages at first childbirth in all the subjects were the same across the groups statistically (P>0.05) (Table 4.3). The mean weight, height and body mass index (BMI) of the premenopausal patients with breast cancer were 65.9+3.0kg, 1.6+0.0m and 25.0+0.9kg/m^2 respectively. These were not significantly different statistically to corresponding values of 65.5+3.0kg, 1.60+0.01m and 25.0+0.9kg/m^2 respectively for the premenopausal controls and 64.7+3.0kg, 1.58+0.05m and 26.0+1.0kg/m^2 respectively for the postmenopausal controls (Table 4.4). None of the subjects (patients and controls) were obese but were all of normal weights i.e BMI<27.0 according to Bray's cut off for obesity (Bray, 1978).

Table 4.5 shows the Pearson Chi-Squared tests of association between demographic, reproductive and anthropometric measurements of all groups. These did not show any significant relationship between any of the parameters tested except diastolilc pressure (DBP) and age of menanche in pre-menopausal control subjects (χ^2 = 5.00, P = 0.001)

The assessment of the socioeconomic status (SES) of all the subjects (patients and controls) was done by assessing their educational level and this is shown in table 4.6. All the premenopausal breast cancer patients had a formal education. Majority (46%) of the patients had secondary education while 25% had tertiary education. The premenopausal control subjects had the highest percentage with tertiary education while majority of the postmenopausal control subjects had elementary education and 21% of them had no formal education.

4.2 The cardiovascular risk factors and Oestradiol Levels (Mean ±S.E.M) in the Patient Group (before Oophorectomy) and control groups:

Table 4.7 shows the comparison of the mean plasma lipids, lipoproteins and oestradiol (E_2) concentrations in the patient and control groups using ANOVA. There were significant changes in the TC, TG, VLDLC and E_2 concentrations among the groups.

Post Hoc tests (Table 4.8) show that the mean plasma E_2 concentration in the premenopausal Breast cancer patients was significantly higher than the mean values in the premenopausal controls (P=0.001) and postmenopausal controls (P=0.00) respectively. The mean plasma TG and VLDLC levels in the patients were

significantly lower than the corresponding mean values in the premenopausal and postmenopausal control subjects (P=0.03,0.04 and P=0.01,0.004 respectively).

The mean plasma total cholesterol concentration of the postmenopausal control subjects was significantly higher than that of the premenopausal control subjects (P=0.02). The other lipid parameters were not significantly different in the two groups of control and the patients too.

The mean plasma E_2 level decreased in the post-menopausal controls compared to the mean level in premenopausal controls but the decrease was not significant (p=0.66).

The 4.9 shows the pairwise comparison of the oestradiol and other cardiovascular risk factors in the premenopausal breast cancer patients and in the premenopausal control subjects. There were significant reductions in the plasma triglycerides (TG) and VLDL cholesterol levels of the patients as well as in the triglycrides to HDLC ratio compared to the corresponding levels in the premenopausal control subjects (P=0.003). There was also a significant increase in the mean plasma oestradiol (E_2) level in the premenopausal breast cancer patients in comparison to that in the premenopausal controls (P=0.004). The mean plasma levels of total cholesterol (TC), high density lipoproteins cholesterol (HDLC) and low density lipoprotein cholesterol (LDLC) all tended to increase in the patients compared to the levels in the premenopausal controls. The ratio of HDLC to total cholesterol increased by about 27% in the patients group and the ratio of LDLC to HDLC reduced by about 4% in the patients when compared to the premenopausal control subjects. The differences were, however, not significant. Though the systolic blood pressure (SBP) and diastolic blood pressure (DBP) measurement in all the subjects were within normal ranges. ANOVA showed significant changes in SBP and DBP across the groups. The systolic blood pressure (SBP) and diastolic pressure (DBP) both increased significantly in the patient group in comparison to the premenopausal control values (p = 0.03 and p = 0.01 respectively). The mean systolic blood pressure of the postmenopausal controls was also significantly higher (P<0.02) than that of the premenopausal controls but there was no significant difference in the mean diastolic blood pressure of the two groups of control subjects.

Table 4.5: Pearson Chi-Squared Test of Demographic, Reproductive and Anthropometric Characteristics in Pre-menopausal Breast Cancer Patients and Pre-menopausal and Post Menopausal Controls

Parameters	Premeno patient n = 25	Premeno control n = 25	Post meno control n = 19
BMI (k/m^2) and DBP (mm/Hg)	$\chi^2 = 0.00$ p = 0.71	$\chi^2 = 0.490$ p = 0.68	$\chi^2 = 0.380$ p =0.73
DBP (mmHg) and Age at Menarche (yrs)	$\chi^2 = 0.616$ p = 0.74	$\chi^2 = 5.000$ p = 0.001*	$\chi^2 = 3.958$ p = 0.875
DBP (mmHg) and Parity	$\chi^2 = 1.07$ p = 0.78	$\chi^2 = 3.30$ p = 0.19	$\chi^2 = 2.11$ p = 0.55
Level of Education and Parity	$\chi^2 = 6.00$ p = 0.74	$\chi^2 = 2.60$ p = 0.86	$\chi^2 = 7.85$ p = 0.55
Education and age at 1st live birth	$\chi^2 = 3.85$ p = 0.70	$\chi^2 = 6.91$ p = 0.65	$\chi^2 = 8.35$ p = 0.50

n	=	number of subjects
χ^2	=	Chi-Squared
p	=	Significant level
*	=	significant at $p<0.05$
premeno	=	pre-menopausal
postmeno	=	postmenopausal
BMI	=	Body Mass Index
SBP	=	Systolic Blood Pressure
DBP	=	Diastolic Blood Pressure

Table 4.6: Assessment of the Socioeconomic Status (SES) of the Patients and Control Subjects

SES by Level of Education	Premenopausal Breast Cancer Patients	Premenopausal Controls	Post Menopausal Controls
	n=25	n=25	n=19
% With no Formal Education (0)	-	8.7	21.1
% With Elementary (E Education	29	26.1	36.8
% With Secondary Education (S)	46	30.4	21.1
% With Tertiary Education (T)	25	34.8	21.1

n = number of subjects

Table 4.7: ANOVA of Cardiovascular Risk Factors and Oestradiol in the Patient and Control Groups

Parameters	Pre-Menopausal Controls (n=25)	Post Menopausal Control (n=19)	Pre-menopausal Patient (n=25)	F	P
TC (mg/dl)	127.2 ± 1.41	165.4 ± 13.1	135.2 ±9.8	3.123	0.05
TG(mg/dl)	171.2 ±9.3	206.5 ±30.8	134.8 ±10.6	4.468	0.02*
HDLC(mg/dl)	24.1 ±3.0	25.0 ±2.7	31.9 ±4.1	1.830	0.169
LDLC(mg/dl)	77.2 ±12.2	105.5 ±13.7	78.7 ±11.9	1.582	0.213
VLDLC(mg/dl)	34.2 ±1.9	41.3 ±5.8	27.0 ±2.0	4.654	0.01*
HDLC/TC	0.207 ±0.0	0.169 ±0.0	0.279 ±0.1	2.523	0.09
TG/HDLC	15.01 ±4.3	9.5 ±1.4	5.90 ±0.8	4.560	0.03*
LDLC/HDLC	5.29±1.5	4.89±1.5	5.63±1.1	2.83	0.08
E2(pg/ml)	102.12±29.3	55.3 ±16.6	261.0 ±67.5	8.787	0.001*
SystolicBP(mmHg)	112.4 ±3.2	125.3 ±4.9	123.0 ±3.8	3.69	0.03*
DiastolicBP(mmHg)	70.4 ±2.5	73.2 ± 2.8	78.8 ± 2.1	3.794	0.023*

values are mean ±SEM

n = number of subjects

SEM = standard error of mean

p = probability

* = significant at $p<0.05$

E_2 = oestradiol

TC = Total Cholesterol

TG = Triglyceride

HDLC = High Density lipoprotein cholesterol

LDLC = Low Density lipoprotein cholesterol

VLDLC= VeryLow Density lipoprotein cholesterol

BP = blood pressure

ANOVA = Analysis of Variance

Table 4.8: Post Hoc Tests of the Cardiovascular Risk Factors and Oestradiol levels in the Patient Group at Baseline and in the Control Groups (p-Values)

Parameters	p-Values Premeno Patients (n=25) vs Premeno Controls (n=25)	p-Values Premeno Patients (n=25) vs Postmeno Controls (n=19)	p-Values Premeno Controls (n=25) vs Postmeno Controls (n=19)
TC (mg/dl)	0.52	0.07	0.02*
TG (mg/dl)	0.03*	0.01*	0.25
HDLC (mg/dl)	0.08	0.17	0.75
LDLC (mg/dl)	0.84	0.14	0.11
VLDLC (mg/dl)	0.04*	0.004*	0.23
HDLC/TC	0.13	0.04*	0.49
TG/HDL	0.04*	0.02*	0.28
E2 (pg/ml)	0.001*	0.00*	0.66
SBP (mmHg)	0.04*	0.70	0.02*
DBP (mmHg)	0.01*	0.13	0.26

n = number of subjects
p = probability
* = significant at p<0.05
E_2 = Oestradiol
SBP = systolic blood pressure
DBP = diastolic blood pressure
Premeno = pre-menopausal
Postmeno = postmenopausal
TC = Total Cholesterol
TG = Triglyceride
HDLC = High Density lipoprotein cholesterol
LDLC = Low Density lipoprotein cholesterol
VLDLC= VeryLow Density lipoprotein cholesterol

Table 4.9: Oestradiol and Other Cardiovascular Risk Factors in Pre-menopausal Breast Cancer Patients before Oophorectomy (i.e. Baseline) and in Pre-menopausal Controls.

Parameters	Premenopausal controls (n=25)	Premenopausal breast cancer patients (n = 25)	% Change	t	p
TC (mg/dl)	127.17±1.41	135.18±9.8	+7.9	0.661	0.52
TG (mg/dl)	171.22 ±3.0	134.82±10.0	-23.9	3.09	0.003*
HDLC (mg/dl)	24.08 ±2.8	31.92 ±4.1	+36.6	1.65	0.08
LDLC (mg/dl)	77.24 ±11.4	78.66 ±12	+21.0	0.20	0.84
VLDLC (mg/dl)	34.2 ±1.90	26.96 ±2.0	-31.5	3.09	0.003*
HDLC/T C	0.207 ±0.0	0.279 ±0.0	+26.5	1.36	0.13
LDL C/ HDIC	5.29 ±1.5	4.89 ±1.5	-3.9	0.28	0.783
TG/HDLC	15.01 ±4.3	5.9 ±0.8	171	2.183	0.04*
E_2(pg/ml)	102.12 ±29.3	260.5 ±67.5	+70.9	3.06	0.004*
SBP (mmHg)	112.40 ±3.2	123.00 ±3.8	+10.5	2.327	0.03*
DBP (mmHg)	70.40 ±2.5	78.8±2.1	+14.0	2.825	0.01*

values are mean ±SEM

n = number of subjects
SEM = standard error of mean
p = probability
* = significant at p<0.05
% = percentage
t = Student's t-test
E_2 = Oestradiol
SBP = systolic blood pressure
DBP = diastolic blood pressure
TC = Total Cholesterol
TG = Triglyceride
HDLC = High Density lipoprotein cholesterol
LDLC = Low Density lipoprotein cholesterol
VLDLC= VeryLow Density lipoprotein cholesterol

Table 4.10 shows the pairwise comparisons of the cardiovascular risk factors, oestradiol and other cardiovascular risk factors in the pre menopausal and postmenopausal control subjects. There was a significant increase in total cholesterol (TCHO) in postmenopausal subjects compared to the pre menopausal subjects (P=0.03). The levels of TG, LDL and VLDL cholesterol increased by 17.0%, 40% and 17% respectively in the postmenopausal subjects compared to the premenopausal subjects but not significantly. The oestradiol level of the postmenopausal subjects decreased by 27% but not significantly in comparison to the premenopausal subjects. The LDLC: HDLC ratio and SBP increased by 15% and 20% respectively, while HDLC: TCHO ratio decreased by 18% in the post menopausal subjects when compared to the values in the pre menopausal subjects but not significantly. There was no significant difference in the HDL cholesterol levels of the subjects.

4.3 Plasma Lipids, Lipoproteins and Oestradiol Levels of the Patients at Baseline and at different Intervals after Oophorectomy.

Table 4.11 shows the comparison of the cardiovascular risk factors and oestradiol levels of the patients at baseline (before oophorectomy) and at different intervals of 1, 3, and 6 months after oophorectomy using ANOVA.

It was shown by ANOVA that there was a significant difference in the mean plasma oestradiol levels of the patients at different intervals after surgical oophorectomy and at baseline (0 month) but there was no significant variation in the mean values of plasma lipids and lipoproteins. The mean values of plasma total cholesterol, TG, LDL cholesterol and VLDL cholesterol of 135.2mg/dl, 134.8mg/dl, 78.7mg/dl and 27.0mg/dl respectively, at baseline increased to a peak of 184.7mg/dl, 182.5mgldl, 112.3mg/dl and 36.5mg/dl respectively, at 3 months, after oophorectomy. However, the mean value of plasma HDL cholesterol of the patients increased from 31.9mg/dl at baseline to a peak of 41.1mg/dl at 6 months after surgical oophorectomy.

Table 4.10: Comparison of Oestradiol and Other Cardiovascular Risk Factors in Women According to their Menopausal Status using Post-Hoc Test.

Parameters	Premenopausal n = 25	Postmenopausal n = 19	% Differences	p
TC (mg/dl)	125.27±11.4	165.38±13.1	32.0	0.03*
TG (mg/dl)	177.24 ±9.3	206.49 ±29.2	16.5	0.31
HDLC (mg/dl)	23.36 ±3.0	25.02 ±2.7	7.1	0.69
LDLC (mg/dl)	77.2 ± 12.2	105.5± 13.2	22.2	0.11
VLDLC (mg/dl)	35.4 ±1.8	41.3 ±5.8	16.7	0.31
HDLC/TC.	0.205±0.0	0.169 ±0.0	17.56	0.32
LDLC/HDLC	4.907±1.5	5.63 ±1.1	14.73	0.71
TG/HDLC	15.01 ±4.3	9.5±1.4	40.4	0.28
E_2(pg/ml)	75.91(±23.5)	55.26 ±16.5	27.2	0.5 0
SBP (mmHg)	112.3 ±3.2	125.26 ±4.9	20.1	0.002*
DBP (mmHg)	70.40 ±2.50	73.16 ±2.8	8.2	0.30

Values are mean ±SEM

n	=	number of subjects	TC	=	Total Cholesterol
SEM	=	standard error of mean	TG	=	Triglyceride
p	=	probability	HDLC	=	High Density lipoprotein cholesterol
*	=	significant at p<0.05	LDLC	=	Low Density lipoprotein cholesterol
%	=	percentage	VLDLC	=	VeryLow Density lipoprotein cholesterol
t	=	Student's t-test			
E_2	=	Oestradiol			
SBP	=	systolic blood pressure			
DBP	=	diastolic blood pressure			

Table 4.11 Comparison of the Cardiovascular Risk Factors in Pre-menopausal Patients with Breast Cancer before Oophorectomy (Baseline) and after Oophorectomy (1 month, 3 months and 6 months) using ANOVA

Parameters	Baseline (Before Oophorectomy) n = 25	1 Month After n=16	3 Months After n=13	6 Months After n=12	F	p
TC (mg/dl)	135.2±9.8	139.2±19.3	184.7±12.9	154.8±12.9	2.214	0.095
TG(mg/dl)	134.8±10.00	164.3±22.5	182.5±21.0	163.6±20.7	1.491	0.226
HDLC(mg/dl)	31.9±4.1	33.4±4.8	36.0±4.1	41.1±8.8	0.522	0.669
LDLC(mg/dl)	78.7±12.0	81.2±18.4	112.3±20.9	81.1±13.6	0.876	0.459
VLDLC(mg/dl)	27.0±2.0	32.7±4.4	36.5±4.2	32.7±4.1	1.497	0.244
HDLC/TC	0.279±0.0	0.325±0.1	0.235±0.0	0.245±0.01	0.489	0.691
TG/HDLC	5.9±0.8	7.7 ±2.4	6.4±1.2	6.6±1.7	0.781	0.469
E_2 (pq/ml)	261±67.5	76.6±28.4	158.3±31.5	89.0±24.9	2.931	0.05*

Values are mean ±SEM

n = number of subjects

E_2 = Oestradiol

p = probability

* = significant at $p<0.05$

ANOVA = Analysis of Varaince

E_2 = Oestradiol

TC = Total Cholesterol

TG = Triglyceride

HDLC = High Density lipoprotein cholesterol

LDLC = Low Density lipoprotein cholesterol

VLDLC= VeryLow Density lipoprotein cholesterol

Table 4.12 shows the Wilcoxon sign test of the plasma lipids, lipoproteins and oestradiol levels of the patients who reported for all visits consecutively during the study i.e at 0, 1, 3, and 6 months after surgical oophorectomy. The values of all the plasma lipid parameters increased from baseline to a peak at 3 months after oophorectomy except plasma HDL cholesterol value which increased to a peak of 47.9mg/dl at 6 months after oophorectomy. The plasma total cholesterol value at 3 months after oophorectomy was significantly higher than the baseline value while the reduction in plasma oestradiol level of the patients was significant only at 1 month after oophorectomy.

Figures 4.1 and 4.2 also show the graphical illustration of the variations of the plasma concentrations of the lipid parameters and oestradiol levels of the breast cancer patients at baseline and at the different intervals after oophorectomy. An early decrease in lipids and lipoproteins levels and subsequent increase to a peak at 3 months after Oophorectomy and a little decrease again at 6 months was observed. The bar chart illustrations are shown in figure 4.3 and figure 4.4 respectively.

Tables 4.13, 4.14 and 4.15 show the pairwise comparisons of the lipids and lipoproteins parameters and oestradiol levels at 1,3 and 6 months respectively with the corresponding baseline values. There were significant increases in the values of the plasma total cholesterol, triglycerides and VLDL-cholesterol at 3 months after oophorectomy only. There was a drastic drop in plasma oestradiol level of the patients by 70.5% at 1 month after oophorectomy in comparison to baseline mean values. The oestradiol concentration then started to increase rapidly afterwards and reached a peak at 3 months after oophorectomy as the lipids and lipoproteins also start to increase to a peak at 3 months after oophorectomy.

Comparison of the mean plasma lipid levels and oestradiol levels of the patients at different intervals after surgical oophorectomy with the corresponding mean values of the postmenopausal control subjects using ANOVA, revealed a significant difference in the oestradiol levels only (Table 4.16).

Table 4.12: Wilcoxon Sign Test of the Cardiovascular Risk Factors and Estradiol Levels of Patients who Reported Consecutively for all Visits (0, 1, 3, 6 Months) after Oophorectomy.

Parameters	Baseline Before Oophorectomy	1 Month After Oophorectomy	P	3 Months After Oophorectomy	P	6 Months After Oophorectomy	P
	n=9	n=9		n=9		n=9	
TC (mg/dl)	121.6 ±8.7	130.8 ±26.7	0.708	179.7±23.0	0.041*	151.6 ±16.1	0.095
TG (mg/dl)	146.9 ±21.6	145.9 ±28.9	0.955	175.6 ±29.6	0.238	159.6 ±26.5	0.626
HDLC (mg/dl)	41.9 ±8.5	35.7 ±7.1	0.656	37.4 ±4.7	0.677	54.6 ±9.5	0.436
LDLC (mg/dl)	56.4 ±11.9	75.3 ±27.1	0.522	107.2 ±25.8	0.096	71.9 ±760	0.482
VLDLC(mg/dl)	29.4±4.3	29.0 ±5.4	0.899	35.1 ±5.9	0.238	31.9 ±2.6	0.628
HDLC/TC (mg/dl)	0.385±0.1	0.453 ±0.1	0.431	0.253 ±1.06	0.318	0.283 ±0.07	0.516
E_2 (pq/ml)	251.7 ±93.2	76.6 ±28.4	0.028*	158.3 ±31.5	0.401	89.0 ±25.0	0.173

p values in comparison to baseline values.

values are mean ±SEM

n = number of subjects

SEM = standard error of mean

p = probability

* = significant at p<0.05

E_2 = Oestradiol

TC = Total Cholesterol

TG = Triglyceride

HDLC = High Density lipoprotein cholesterol

LDLC = Low Density lipoprotein cholesterol

VLDLC= VeryLow Density lipoprotein cholesterol

However, pairwise comparisons of the mean values of the parameters at 3 months and at 6 months after oophorectomy respectively with the corresponding values of the natural post menopausal controls (Table 4.16) showed a significant increase in HDL cholesterol of the patients at 3 months and 6 months after oophorectomy. There was also a highly significant increase in plasma oestradiol level of the patients at 3 months after surgical oophorectomy in comparison to natural postmenopausal corresponding values.

Tables 4.17, 4.18 and 4.19 show the analysis of variances (ANOVA) of the lipids and lipoproteins levels and oestradiol levels of the premenopausal breast cancer patients in relation to parity and according to stages of disease and duration of disease respectively. There were no significant variations in relation to parity nor stages of disease nor duration of disease. A trend of increased plasma levels of lipids and lipoproteins with increase in parity of the premenopausal patients with breast cancer was however observed in the result (Table 4.17).

4.4 Multiple Regression Analysis of all Parameters in Patients and Control Groups: Tables 4.20, 4.21 and 4.22 show the multiple regression analyses of all parameters in the pre-menopausal and post-menopausal controls, and the pre-menopausal breast cancer patients. Table 4.20 shows that in pre-menopausal controls height can significantly predict, SBP (R^2=0.62, df=6, P = 0.01) and DBP (R^2=0.73, df=9, P = 0.00) and body weight can predict TG to HDLC ratio (R^2=0.37, df=6, P = 0.04). Parity is also shown to predict SBP (R^2=0.60, df=6, P = 0.02) and Age of menarche is shown to predict DBP (R^2=0.73, df=9, P = 0.00). There are no significant predictions observed in the multiple regression analysis of the parameters in post-menopausal controls and pre-menopausal patients.

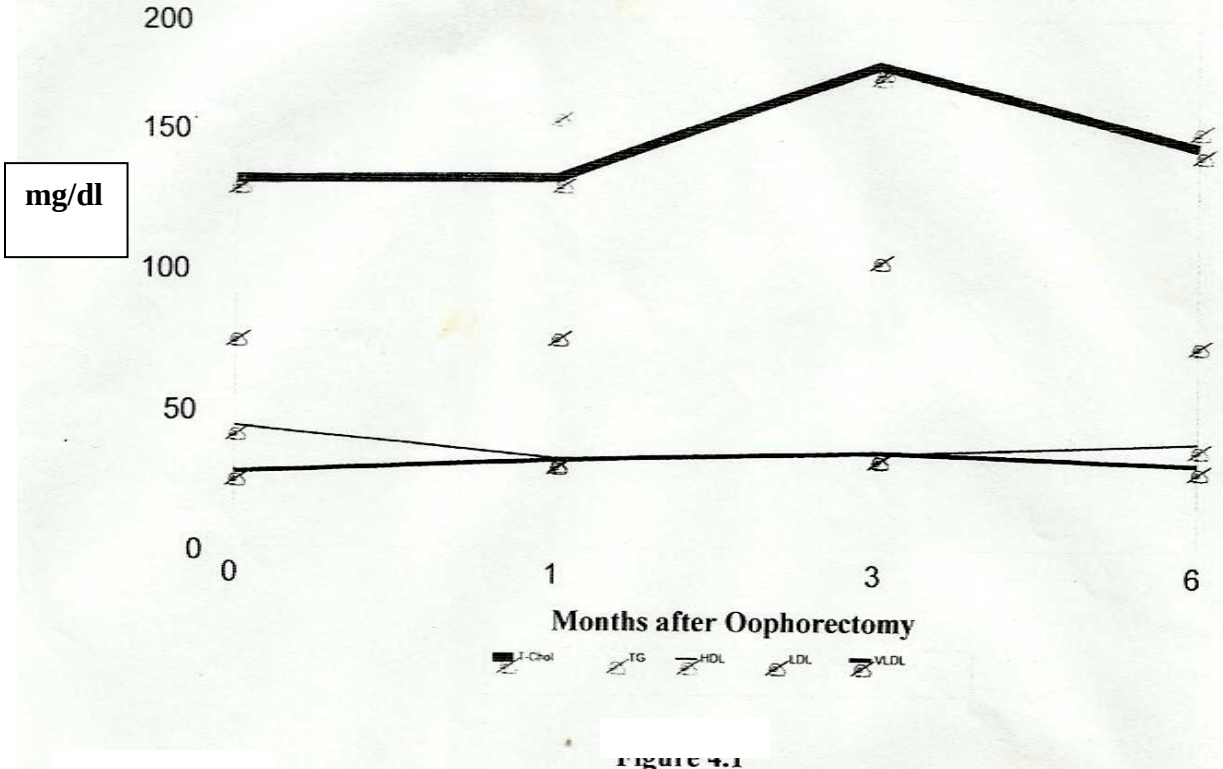


Figure 4.1: Graph showing mean values of lipids and lipoproteins profile of the patients with breast cancer after Oophorectomy

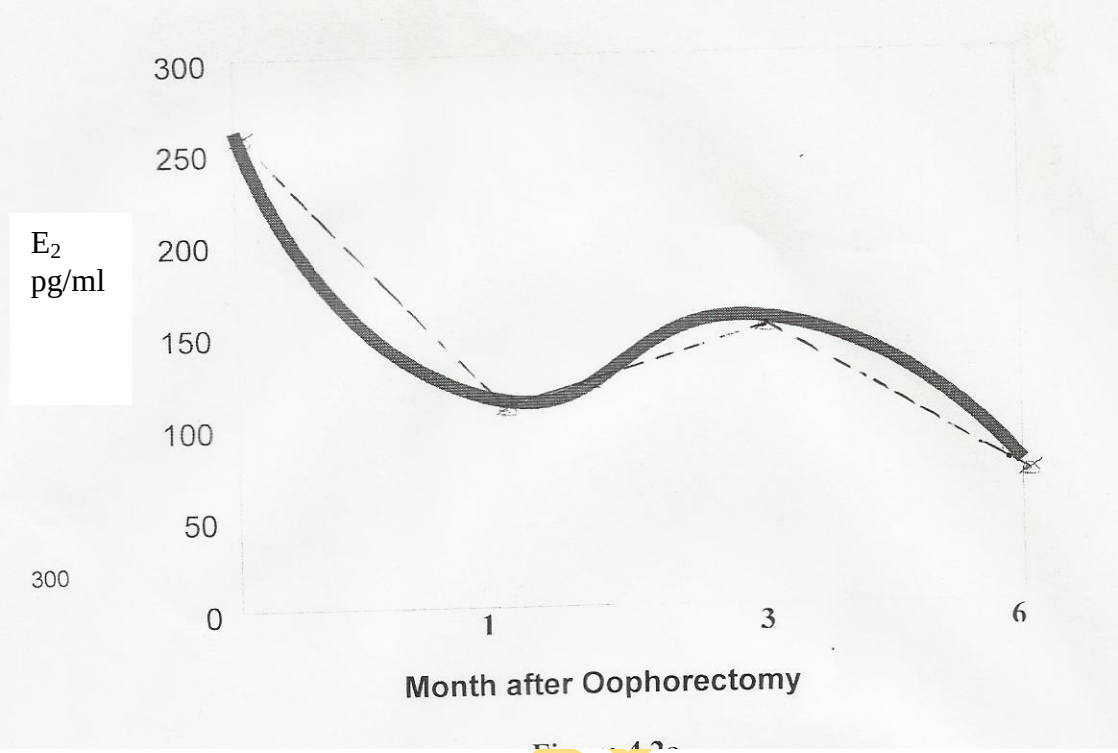


Figure 4.2: Graph showing mean values of plasma estradiol (E_2) of the patients with breast cancer after oophorectomy.

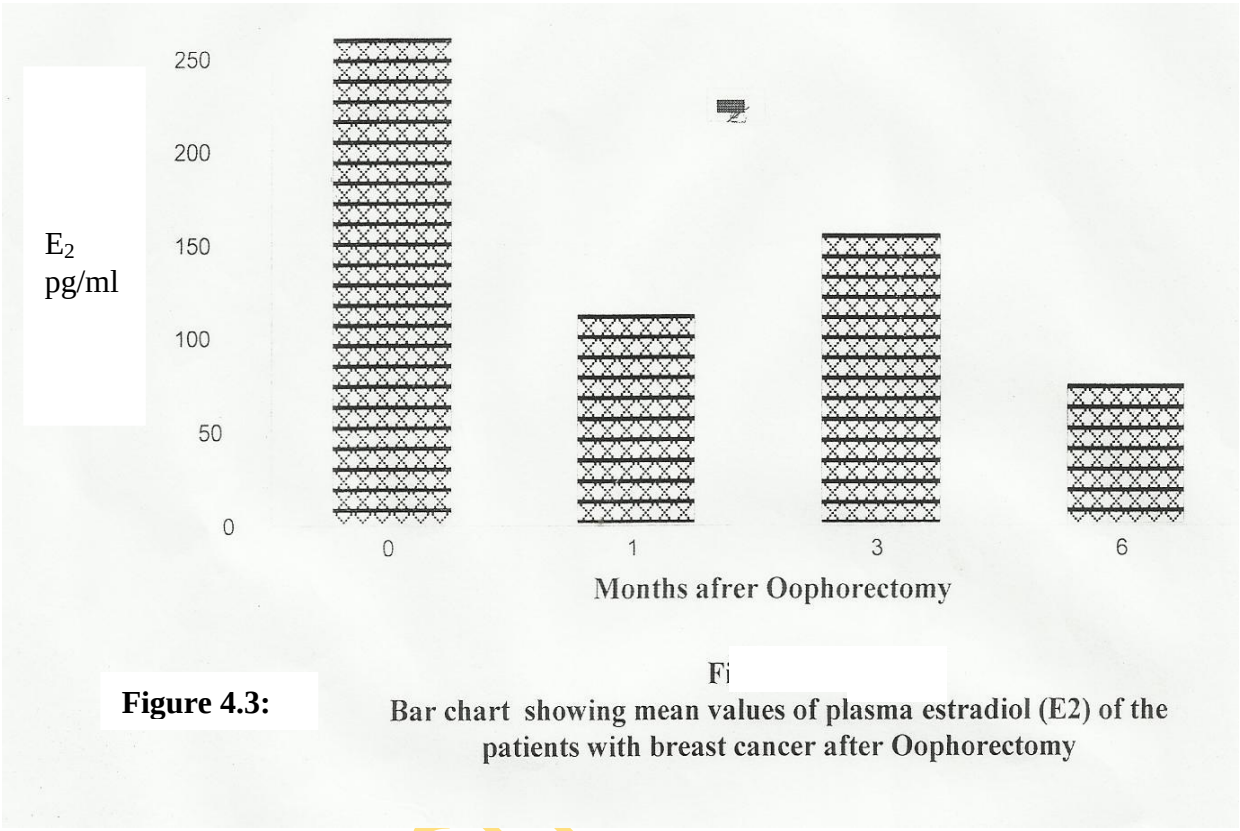
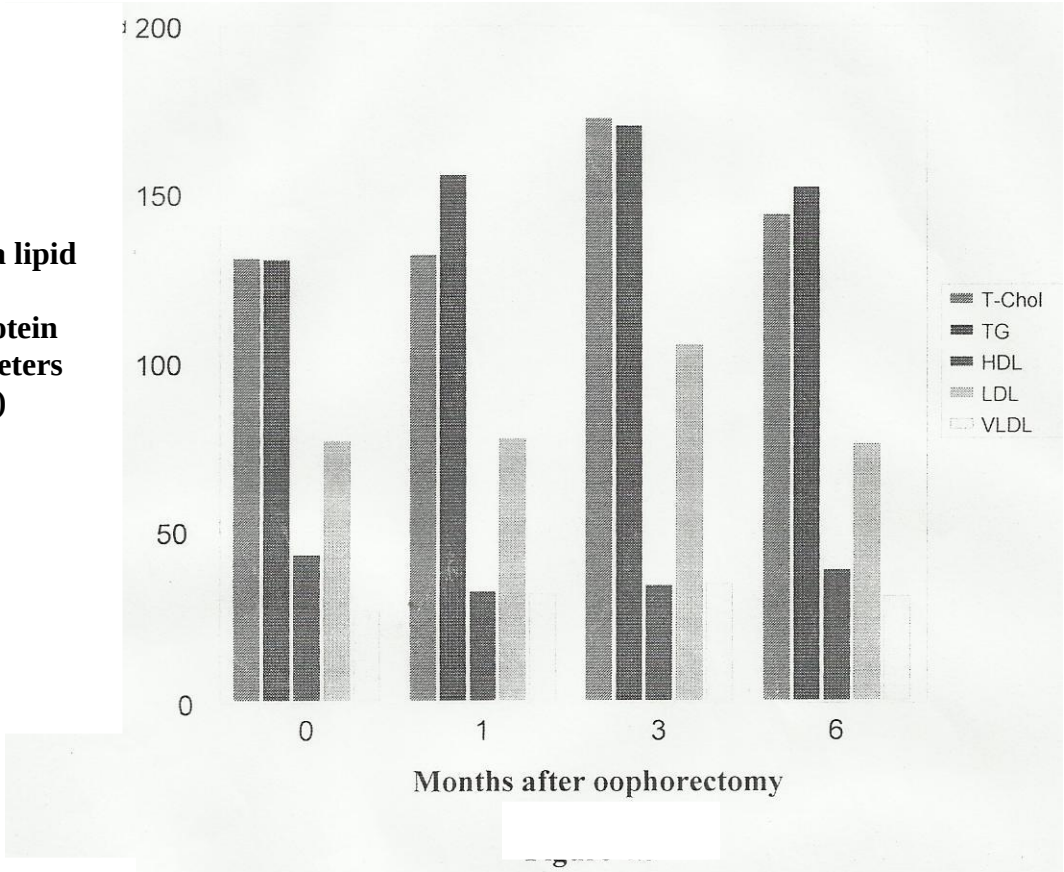


Figure 4.3: Bar chart showing mean values of plasma estradiol (E2) of the patients with breast cancer after Oophorectomy



Plasma lipid and lipoprotein parameters (mg/dl)

Figure 4.4: Bar chart showing mean values of plasma lipids and lipoproteins profile of the patients with breast cancer after oophorectomy

Table 4.13: Changes in Cardiovascular Risk Factors and Estradiol Levels of Patients at 1 Month after Oophorectomy

Parameters	Baseline Before Oophorectomy	1 Month After Oophorectomy n=16	% Change	t	P
TC(mg/dl)	135.2±9.8	139±19.3	13.0	0.206	0.838
TG (mg/l)	135±10.00	164±22.5	22.0	1.350	0.185
HDLC(mg/l)	32±4.1	33.1±4.8	4.7	0.0236	0.815
LDLC(mg/l)	79±12.0	81±18.4	3.3	0.125	>0.05
VLDLC(mg/l)	27.0±2.0	32±4.4	21.1	1.335	0.190
HDLC/HDLC	0.279±0.0	0.320±0.1	14.7	0.566	0.575
LDLC/HDLC	50±1.5	3.8±1.2	28.9	0.547	0.588
TG/HDL	5.9±0.8	7.7±2.4	32.2	1.249	0.230
E_2(pq/ml)	261±67.5	77±28.4	70.5	1.983	0.061

p-value when compared to baseline values

values are mean ±SEM

n = number of subjects

p = significance level

% = percentage

t = Student's t-test

E_2 = Oestradiol

SEM = Standard error of mean

TC = Total Cholesterol

TG = Triglyceride

HDLC = High Density lipoprotein cholesterol

LDLC = Low Density lipoprotein cholesterol

VLDLC= VeryLow Density lipoprotein cholesterol

Table 4.14: Changes in Cardiovascular Risk Factors and Estradiol Levels of Patients at 3 Months after Oophorectomy

Parameters	Baseline Before Oophorectomy	3 Month After Oophorectomy n=13	% change	T	P
TC (mg/dl)	135.2±9.8	185±17.6	36.6	2.666	0.011*
TG (mg/l)	135±10.00	182±21.0	35.4	2.331	0.025*
HDLC (mg/l)	32±4.1	36±4.1	12.9	0.625	0.536
LDLC (mg/l)	79±12.0	112±20.9	42.7	1.506	0.362
VLDLC (mg/l)	27.0±2.0	36.5±14.2	35.2	2.329	0.026*
HDLC/HDLC	0.279±0.0	0.235±0.0	15.8	0.615	0.543
LDLC/HDLC	50±1.5	56±2.2	14.3	0.296	0.777
TG/HDL	5.9±0.8	6.4±1.2	8.5	1.044	0.32
E_2(pq/ml)	261±67.5	158.1±31.5	39.2	1.154	0.261

P-Value when compared to baseline values

values are mean ±SEM

n = number of subjects

p = probability

* = significant at p<0.05

% = percentage

t = Student's t-test

E_2 = Oestradiol

SEM = Standard error of mean

TC = Total Cholesterol

TG = Triglyceride

HDLC = High Density lipoprotein cholesterol

LDLC = Low Density lipoprotein cholesterol

LDLC = VeryLow Density lipoprotein cholesterol

Table 4.15: Changes in Cardiovascular Risk Factors and Estradiol Levels of the Patients at 6 Months after Oophorectomy

Parameters	Baseline Before Oophorectomy	6 Month After Oophorectomy n=12	% Change	t	p
TC (mg/dl)	135.2±9.8	155±12.9	14.5	1.171	0.250
TG (mg/l)	135±10.00	164±20.7	21.4	1.419	0.165
HDLC (mg/l)	32±4.1	41±8.8	38.2	1.073	0.290
LDLC (mg/l)	79±12.0	81±13.6	2.8	0.124	0.902
VLDLC (mg/l)	27.0±2.0	33±4.1	21.1	1.415	0.166
HDLC/HDLC	0.279±0.0	0.245±0.0	13.9	0.444	0.659
LDLC/HDLC	50±1.5	4.0±1.0	24.5	0.521	0.606
TG/HDL	5.9±0.8	6.6±1.7	11.8	0.812	0.43
E_2(pq/ml)	261±67.5	89±25.0	65.8	1.966	0.063

p-value when compared to baseline values

values are mean ±SEM

n = number of subjects

p = probability

% = percentage

t = Student's t-test

E_2 = Oestradiol

SEM = Standard error of mean

TC = Total Cholesterol

TG = Triglyceride

HDLC = High Density lipoprotein cholesterol

LDLC = Low Density lipoprotein cholesterol

LDLC = VeryLow Density lipoprotein cholesterol

Table 4.16: ANOVA of the Cardiovascular Risk Factors and Estradiol levels of Patients at different times after Oophorectomy and of Postmenopausal Controls

Parameters	Post Menopausal Control	Patients (induced Menopausals)				
		1 Month After Oophorectomy	3 Months After Oopho	6 Months After Oopho	F	p
	n=19	n=16	n=13	n=12		
TC (mg/dl)	165.4±13.1	139.2±19.3	184.7±12.9	154.8±12.9	1.346	0.27
TG (mg/dl)	206.5±30.8	164.3±22.5	182.5±21.0	163.6±20.7	0.676	0.57
HDLC (mg/dl)	25.0±2.7	33.4±4.8	36.0±4.1	41.1±8.8	1.884	0.14
LDLC (mg/dl)	105.5±13.7	81.3±18.4	112.3±20.9	81.1±13.6	0.886	0.45
VLDLC(mg/dl)	41.3±5.8	32.7±4.4	36.5±4.2	32.7±4.1	0.729	0.54
HDLC/TC	0.169±0.02	0.320±0.06	0.235±0.04	0.245±0.05	2.280	0.09
TG/HDLC	9.5±1.4	7.7±2.4	6.4±1.2	6.6±1.7	1.143	0.37
E_2 (pq/ml)	55.3±16.6	76.6±28.4	158.3±31.5	89.0±24.9	3.522	0.02*

values are mean ±SEM

n = number of subjects

p = probability

* = significant at p<0.05

E_2 = Oestradiol

SEM = Standard error of mean

Oopho = Oophorectomy

TC = Total Cholesterol

TG = Triglyceride

HDLC = High Density lipoprotein cholesterol

LDLC = Low Density lipoprotein cholesterol

V LDLC = VeryLow Density lipoprotein cholesterol

ANOVA = Analysis of Variance

Table 4.17: Cardiovascular Risk Factors and Estradiol in Oophorectomised (i.e Induced Menopausal) Breast Cancer Patients and Natural Postmenopausal Controls

Induced Menopausal Breast Cancer Patients

Parameters	**Natural Postmenopausal Controls (n=19)**	**3 Months After Oophorectomy (n=13)**	**% Change**	**t**	**p**	**6 Months After Oophorectomy (n=12)**	**% Change**	**t**	**p**
TC (mg/dl)	165.4±13.1	185±17.6	11.7	0.900	0.330	154.8±12.9	6.4	0.545	0.601
TG (mg/dl)	207±29.2	182±21	11.6	0.591	0.542	1636(±20.7)	20.8	1.032	0.251
HDLC (mg/dl)	25±2.7	36.0±4.1	44	2.336	0.026*	41.1±8.8	64.4	2.076	0.05
LDLC (mg/dl)	106±13.7	112±20.9	6.4	0.287	0.776	81.1±13.6	30.0	1.192	0.24
VLDLC (mg/dl)	41.3±5.8	37±4.2	11.6	0.610	0.547	32.7±4.2	20.8	1.065	0.30
HDLC/TC	0.169±0.0	0.235±0.0	39.0	1.448	0.158	0.245±0.1	45.0	1.500	0.14
LDLC/HDLC	5.6±1.1	5.6±2.2	0.0	0.002	0.998	37±1.0	33.9	1.185	0.25
TG/HDLC	9.5±1.4	6.4±1.2	33.2	1.732	0.11	6.6±1.7	31.1	1.458	0.18
E_2(pq/ml)	55.3±16.6	158.3±31.5	186.3	3.194	0.004*	89.0±24.9	60.9	1.141	0. 26

p-values in comparison to Natural Postmenopausal Controls

values are mean ±SEM

n = number of subjects

SEM = standard error of mean

p = probability

* = significant at p<0.05

% = percentage

t = Student's t-test

E_2 = Oestradiol

TC = Total Cholesterol

TG = Triglyceride

HDLC = High Density lipoprotein cholesterol

LDLC = Low Density lipoprotein cholesterol

VLDLC = VeryLow Density lipoprotein cholesterol

Table 4.18: Analysis of Variance (ANOVA) of the Cardiovascular Risk Factors According to Parity of the Premenopausal Breast Cancer Patients

Parameters	Parity 0-1 (n=2)	Parity3-4 (n=14)	Parity 5-7 (n=8)	F	P
TC (mg/dl)	95±28.4	131=13.5	147±17.1	0.926	>0.05
Triglyceride (mg/dl)	127±57.4	133±14.2	137=17.1	0.034	>0.05
HDLC (mg/dl)	25±2.7	31±5.9	31±53	0.112	>0.05
LDLC (mg/l)	45.5±37.2	76±16.4	85±22.4	0.339	>0.05
VLDLC (mg/l)	25±1.5	27±2.8	27.4±3.4	0.035	>0.05
HDLC/TC	0.273±0.1	0.28±0.1	0.31±0.1	0.043	>0.05
LDLC/TC	0.273±0.1	0.28±0.1	0.31±0.1	0.043	>0.05
LDLC/HDLC	1.7±1.3	4.1±1.4	4.6±2.3	0.212	>0.05

Values are mean ±SEM

n = number of subjects

SEM = standard error of mean

p = probability

ANOVA= Analysis of Variance

TC = Total Cholesterol

TG = Triglyceride

HDLC = High Density lipoprotein cholesterol

LDLC = Low Density lipoprotein cholesterol

VLDLC = VeryLow Density lipoprotein cholesterol

Table 4.19: ANOVA of the Cardiovascular Risk Factors and Estradiol Levels (Mean±SEM) in the Premenopausal Breast Cancer Patients According to Stages of Disease

Parameters	Stages 1 & 2 n=3	Stage 3 n=14	Stage 4 n=7	F	p
TC(mg/dl)	147.9±4.4	131.9±14.2	130.0±20.9	0.142	0.868
TG (mg/dl)	93.7±15.2	144.5±15.4	129.9±14.1	1.293	0.295
HDLC (mg/dl)	37.0±7.9	29.0±5.2	38.2±9.8	0.515	0.605
LDLC (mg/dl)	92.1±4.7	76.4±16.4	69.3±27.7	0.142	0.869
VLDLC (mg/dl)	18.7±3.0	28.9±3.0	26.5±3.0	1.291	0.296
HDLC/TC	0.248±0.1	0.261±0.1	0.367±0.1	0.553	0.583
E_2 (pq/ml)	233.5±2.3	217.0±86.8	409.0±128	0.624	0.554

values are mean ±SEM

n = number of subjects

SEM = standard error of mean

p = probability

ANOVA = Analysis of Variance

TC = Total Cholesterol

TG = Triglyceride

HDLC = High Density lipoprotein cholesterol

LDLC = Low Density lipoprotein cholesterol

Table 4.20: ANOVA Table for Baseline Parameters according to Duration of Disease

Parameters	<7 months	7-15months	16 months & above	F	p
	n=8	n=9	n =7		
TC (mg/dl)	136.9±14.5	126.5 ±20.3	137.9±18.0	0.126	0.88
TG (mg/dl)	121.1 ±19.5	135.7 ±8.8	146.3±27.0	0.444	0.65
HDLC (mg/dl)	38.8 ±8.4	21.7 ±2.9	31.7 ±6.7	1.847	0.18
LDLC (mg/l)	77.7 ±19.7	74.0±23.6	77.6 ±22.2	0.009	0.99
VLDLC (mg/dl)	24.2 ±3.9	27.1 ±1.8	29.3 ±5.4	0.443	0.64
HDLC/TC	0.329 ±0.1	0.281 ±0.1	0.257 ±0.1	0.193	0.83
LDL/HDL	4.1 ±2.3	4.4 ±2.0	3.6 ±1.3	0.036	0.97

values are mean ±SEM

n = number of subjects

SEM = standard error of mean

p = probability

ANOVA= Analysis of Variance

TC = Total Cholesterol

TG = Triglyceride

HDLC = High Density lipoprotein cholesterol

LDLC = Low Density lipoprotein cholesterol

VLDLC = VeryLow Density lipoprotein cholesterol

Table 4.21: **Multiple Regression Analysis of all Parameters in Pre-menopausal Control Subjects**

	Dependent Variables										
Independent parameters (predictors)	TC mg/dl	TG mg/dl	HDLC mg/dl	LDLC mg/dl	VLDLC mg/dl	E_2 pg/ml	HDLC/ TC	LDLC/ HDLC	TG/ HDLC	SBP mmHg	DBP mmHg
	$R_2 = 0.27$	$R_2 = 0.26$	$R_2 = 0.29$	$R_2 = 0.29$	$R_2 = 0.27$	$R_2 = 0.24$	$R_2 = 0.40$	$R_2 = 0.27$	$R_2 = 0.37$	$R_2 = 0.62$	$R_2 = 0.73$
	df = 7	df = 7	df = 7	df = 7	df = 7	df = 7	df = 7	df = 7	df = 6	df = 6	df = 9
Age (yrs)	p = 0.11	p = 0.97	p = 0.54	p = 0.98	p = 0.96	p = 0.59	p = 0.07	p = 0.96	p = 0.63	p = 0.42	p = 0.10
Parity	p = 0.70	p= 0.89	p = 0.73	p = 1.00	p = 0.89	p = 0.72	p = 0.90	p = 0.89	p = 0.67	p = 0.02*	p = 11.51
Age of menarche (yrs)	p = 0.23	p = 0.06	p = 0.11	p = 0.15	p = 0.06	p= 0.60	p = 0.03*	p = 0.06	p = 0.05	p = 0.08	p = 0.00*
Age at 1st live birth (yrs)	p = 0.48	p = 0.12	p = 0.37	p = 0.45	p = 0.12	p = 0.97	p = 0.16	p = 0.12	p = 0.12	p = 0.40	p = 0.12
Weight (kg)	p = 0.12	p = 0.68	p = 0.48	p = 0.07	p = 0.66	p = 0.72	p = 0.91	p = 0.66	p = 0.04*	p = 0.40	p = 0.05
Height (m)	p = 0.09	p = 0.82	p = 0.78	p = 0.12	p = 0.83	p = 0.72	p = 0.26	p = 0.88	p = 0.012	p = 0.01*	p = 0.00*
BMI (kg/m^2)	p = 0.13	p = 0.89	p = 0.77	p = 0.06	p = 0.88	p = 0.93	p = 0.65	p = 0.88	p = 0.27	p = 0.14	p = 0.15

R^2 = regression

df = degree of freedom

p = probability

* = Significant at $p<0.05$

BMI = Body mass index

SBP = systolic blood pressure

DBP = diastolic blood pressure

TC = Total Cholesterol

TG = Triglyceride

HDLC = High Density lipoprotein cholesterol

LDLC = Low Density lipoprotein cholesterol

VLDLC = VeryLow Density lipoprotein cholesterol

Table 4.22: **Multiple Regression Analysis of all Parameters in Post-menopausal Control Subjects**

	Dependent Variables										
Independent parameters (predictors)	TC mg/dl	TG mg/dl	HDLC mg/dl	LDLC mg/dl	VLDLC mg/dl	E_2 pg/ml	HDLC/ TC	LDLC/ HDLC	TG/ HDLC	SBP mmHg	DBP mmHg
	$R_2 = 0.27$	$R_2 = 0.58$	$R_2 = 0.42$	$R_2 = 0.20$	$R_2 = 0.63$	$R_2 = 0.21$	$R_2 = 0.42$	$R_2 = 0.15$	$R_2 = 0.55$	$R_2 = 0.28$	$R_2 = 0.74$
	df = 8	df = 8	df = 8	df = 8	df = 8	df = 8	df = 8	df = 8	df = 8	df = 8	df = 8
Age(yrs)	p = 0.59	p = 0.44	p = 0.64	p = 0.61	p = 0.26	p = 0.99	p = 0.87	p = 0.89	p = 0.36	p = 1.00	p = 0.08
Parity	p = 0.71	p = 0.95	p = 0.61	p = 1.84	p = 0.80	p = 0.68	p = 0.81	p = 0.89	p = 0.83	p = 0.68	p = 0.42
Age of menarche (yrs)	p = 0.72	p = 0.85	p = 0.49	p = 0.81	p = 0.94	p = 0.45	p = 0.63	p = 1.00	p = 0.40	p = 0.22	p = 0.82
Age at 1st live birth (yrs)	p = 0.79	p = 0.84	p = 0.60	p = 0.77	p = 0.82	p = 0.73	p = 0.54	p = 0.66	p = 0.69	p = 0.96	p = 0.43
Weight (kg)	p = 0.82	p = 0.12	p = 0.90	p = 0.86	p = 0.06	p = 0.97	p = 0.94	p = 0.99	p = 0.19	p = 0.57	p = 0.76
Height (m)	p = 0.79	p = 0.11	p = 0.89	p = 0.85	p = 0.34	p = 0.94	p = 0.93	p = 1.00	p = 0.18	p = 0.53	p = 0.79
BMI (kg/m^2)	p = 0.084	p = 0.12	p = 0.87	p = 0.87	p = 0.06	p = 0.98	p = 0.97	p = 0.97	p = 0.20	p = 0.57	p = 0.78

R^2 = regression
df = degree of freedom
p = probability

TC = Total Cholesterol
TG = Triglyceride
HDLC = High Density lipoprotein cholesterol
LDLC = Low Density lipoprotein cholesterol
VLDLC = VeryLow Density lipoprotein cholesterol

BMI = Body mass index

SBP = systolic blood pressure

DBP = diastolic blood pressure

Table 4.23: Multiple Regression Analysis of all Parameters in Pre-menopausal Breast Cancer Patients

	Dependent Variables										
Independent parameters (predictors)	TC mg/dl	TG mg/dl	HDLC mg/dl	LDLC mg/dl	VLDLC mg/dl	E_2 pg/ml	HDLC/ TC	LDLC/ HDLC	TG/ HDLC	SBP mmHg	DBP mmHg
	df = 10	df = 10	df = 10	df = 10	df = 10	df = 10	df = 10	df = 10	df = 10	df = 10	df = 10
	R_2 = 0.504	R_2 = 0.48	R_2 = 0.615	R_2 = 0.48	R_2 = 0.48	R_2 = 0.673	R_2 = 0.29	R_2 = 0.591	R_2 = 0.314	R_2 = 0.43	R_2 = 0.62
Age of patient (yrs)	p = 0.358	p = 0.61	p = 0.71	p = 0.43	p = 0.61	p = 0.32	p = 0.60	p = 0.63	p = 0.53	p = 0.62	p = 0.50
Stage of disease	p = 0.383	p = 0.29	p = 0.69	p = 0.35	p = 0.29	p = 0.60	p = 0.63	p = 0.27	p = 0.63	p = 0.97	p = 0.41
Parity	p = 0.50	p = 0.95	p = 0.29	p = 0.71	p = 0.95	p = 0.12	p = 0.69	p = 0.62	p = 0.50	p = 0.63	p = 0.11
Age of menarche (yrs)	p = 0.77	p = 0.33	p = 0.36	p = 0.80	p = 0.33	p = 0.69	p = 1.0	p = 0.98	p = 0.41	p = 0.56	p = 0.44
Age at 1st live birth (yrs)	p = 0.94	p = 0.90	p = 0.96	p = 0.93	p = 0.90	p = 0.83	p = 0.96	p = 0.76	p = 0.81	p = 0.99	p = 0.79
Weight (kg)	p = 0.30	p = 0.13	p = 0.93	p = 0.29	p = 0.13	p = 0.31	p = 0.93	p = 0.57	p = 0.83	p = 0.90	p = 0.14
Height (m)	p = 0.83	p = 0.59	p = 0.24	p = 0.56	p = 0.59	p = 0.05	p = 0.85	p = 0.42	p = 0.46	p = 0.69	p = 0.59
BMI (kg/m^2)	p = 0.24	p = 0.23	p = 0.15	p = 0.16	p = 0.23	p = 0.55	p = 0.97	p = 0.48	p = 0.70	p = 0.70	p = 0.35

R^2 = regression

df = degree of freedom

p = probability

TC = Total Cholesterol

TG = Triglyceride

HDLC = High Density lipoprotein cholesterol

LDLC = Low Density lipoprotein cholesterol

VLDLC = Very Low Density lipoprotein cholesterol

BMI = Body mass index

SBP = systolic blood pressure

DBP = diastolic blood pressure

4.5 Summary of Correlations

Table 4.23 shows the square matric correlations between the mean plasma lipids and lipoproteins concentrations, age and parity of the patients, blood pressure (SBP and DBP) and plasma oestradiol values of the premenopausal breast cancer patients. There were positive significant correlations between the mean triglycerides and VLDL cholesterol values ($r = 1.00$; $p = < 0.001$) as well as between plasma total cholesterol and LDL cholesterol ($r = 0.959$; $p < 0.01$) and also LDLC/HDLC ratio ($r = 0.834$; $p < 0.001$). There was also significant correlation between age and parity of the patients ($r = 0.417$; $p < 0.05$). The plasma total cholesterol correlated negatively but not significantly with HDL/TCHO ($r = 0.584$; $p < 0.05$). These correlations were reflected in the results. The plasma triglycerides correlated negatively but not significantly with the plasma Oestradiol levels ($r = - 0.241$; $p > 0.05$), total cholesterol ($r = - 0.207$; $p > 0.05$), HDL ($r = - 0.006$; $p > 0.05$) and LDL cholesterol ($r = - 0.344$; $p > 0.05$) as well as with LDLC/HDLC ($r = - 0.230$; $p > 0.05$). The plasma oestradiol values also correlated negatively but non-significantly with the SBP and DBP ($r = - 0.521$; $p > 0.05$ and $r = - 0.222$; $p > 0.05$) respectively.

Table 4.24: Square Matrix Correlations among the Cardiovascular Risk Factors Values, Age and Parity in Premenopausal Breast Cancer Patients.

	AGE yrs	PAR	TC mg/dl	TG mg/dl	HDLC mg/dl	LDLC mg/dl	VLDLC mg/dl	HDLC/TC	LDLC/ HDLC	E_2 pg/ml	SBP mmHg	DBP mmHg
AGE(yrs)	1.000	0.471 (0.043)**	0.299 (0.146)	-0.052 (0.806)	0.058 (0.787)	0.231 (0.266)	-0.052 (0.806)	-0.193 (0.356)	0.156 (0.458)	0.436 (0.119)	-0.222 (0.338)	-0.332 (0.153)
PAR	0.417 (0.043)**	1.000	0.149 (0.487)	0.213 (0.318)	0.232 (0.286)	0.004 (0.986)	0.213 (0.318)	0.069 (0.748)	-0.083 (0.700)	-0.263 (0.363)	0.125 (0.599)	-0.089 (0.708)
TC(mg/dl)	0.299 (0.146)	0.149 (0.487)	1.000	-0.207 (0.320)	-0.363 (0.081)	0.959* (0.000)	-0.207 (0.321)	-0.584** (0.002)	0.834** (0.000)	0.241 (0.406)	0.024 (0.921)	0.071 (0.767)
TG (mg/dl)	-0.052 (0.806)	0.213 (0.318)	-0.207 (0.320)	1.000	-0.006 (0.976)	-0.344 (0.093)	1.00** (0.000)	0.034 (0.871)	-0.230 (0.269)	-0.241 (0.406)	0.034 (0.886)	-0.320 (0.169)
HDLC (mg/dl)	0.058 (0.787)	0.232 (0.286)	-0.363 (0.081)	-0.006 (0.976)	1.000	-0.542** (0.006)	-0.007 (0.975)	0.923** (0.000)	-0.596** (0.002)	-0.304 (0.312)	-0.101 (0.682)	0.032 (0.895)
LDLC (mg/dl)	0.231 (0.266)	0.004 (0.986)	0.959* (0.000)	-0.344 (0.093)	-0.542** (0.006)	1.000	-0.343 (0.093)	-0.707** (0.000)	0.897** (0.000)	0.336 (0.240)	0.031 (0.898)	0.081 (0.735)
VLDLC(mg/dl	-0.052 (0.806)	0.213 (0.318)	-0.207 (0.321)	1.00** (0.000)	-0.007 (0.975)	-0.343 (0.093)	1.000	0.034 (0.872)	-0.229 (0.270)	-0.241 (0.407)	0.034 (0.886)	-0.320 (0.169)
HDLC/ TC	-0.193 (0.356)	0.069 (0.748)	-0.584** (0.002)	0.034 (0.871)	0.923** (0.000)	0.707** (0.000)	0.034 (0.872)	1.000	-0.623** (0.001)	-0.152 (0.605)	0.037 (0.879)	0.136 (0.567)
LDLC/ HDLC	0.156 (0.458)	-0.083 (0.700)	0.834** (0.000)	-0.230 (0.269)	-0.896** (0.002)	0.897** (0.000)	-0.229 (0.270)	-0.623** (0.001)	1.000	0.429 (0.126)	0.042 (0.859)	0.114 (0.632)
E_2 (pg/ml)	0.436 (0.119)	-0.263 (0.363)	0.241 (0.406)	-0.241 (0.406)	-0.304 (0.312)	0.336 (0.240)	-0.241 (0.407)	-0.152 (0.605)	0.429 (0.126)	1.000	-0.521 (0.083)	-0.222 (0.489)
SBP (mmHg)	-0.222 (0.338)	0.125 (0.599)	0.024 (0.921)	0.034 (0.886)	-0.101 (0.682)	0.031 (0.898)	0.034 (0.886)	0.037 (0.879)	0.042 (0.859)	-0.521 (0.083)	1.000	0.734** 0.000
DBP (mmHg)	-0.332 (0.153)	-0.089 (0.708)	0.071 (0.767)	-0.320 (0.169)	0.032 (0.895)	0.081 (0.735)	-0.320 (0.169)	0.136 (0.567)	0.114 (0.632)	-0.222 (0.489)	0.734** 0.000	1.000

Values are correlations r with p in parenthesis

r = correlations

p = probability

** = significance at $p<0.05$

\- = negative correlation

PAR = parity

SBP = systolic blood pressure

DBP = diastolic blood pressure

TC = Total Cholesterol

TG = Triglyceride

HDLC = High Density lipoprotein cholesterol

LDLC = Low Density lipoprotein cholesterol

VLDLC = VeryLow Density lipoprotein cholesterol

CHAPTER FIVE

5.0 DISCUSSION AND CONCLUSION

5.1 Discussion

An advanced stage presentation of breast cancer was observed in the majority of the patients in the present study, as 88% of the patients reported at stages 3 and 4. This is consistent with what was previously observed in the African-American women patients with breast cancer in the USA. It is of course an expected outcome because of the shared ancestry of Sub-Saharan African (which includes Nigeria) with African-American women (Fregene and Newman, 2005).

Significant reduction in plasma TG was found in the Nigerian premenopausal breast cancer patients studied. Cancer patients in general are known to demonstrate significantly lower total cholesterol and LDL cholesterol in comparison to non-cancer patients but breast cancer patients as a group however, demonstrate an exception in being associated with increased total cholesterol, LDL cholesterol and triglycerides in some cases (Alexopoulus *et al*., 1987).

Florenza *et al*. (2000) also reported that association of cancer with low serum total cholesterol is well established, but the possible relationship of cancer with the cholesterol distribution among the different lipoprotein classes is less clear and inconsistent. Significantly lower values for plasma total cholesterol, HDL cholesterol and LDL cholesterol and an increased plasma triglycerides were found in cancer patients compared to non-cancer groups by Florenza *et al*. (2000) but they found increased values for breast cancer patients.

Epidemiological evidence has also shown that the relationship of serum cholesterol, TG, and breast cancer incidence is not consistent. Hoyer and Engholon (1992) reported a negative association between TG and breast cancer incidence and this is consistent with the finding in the present study of a significant reduction in TG in premenopausal breast cancer patients. In contrast, a negative association with total cholesterol alone and breast cancer was found by Vatten and Foss (1990). Olabinri (1997) also found no change in plasma TG and significantly increased plasma total cholesterol in the Nigerian breast cancer women studied. The menopausal status

of the women was however not specified. Increased plasma total cholesterol (hypercholesteroleamia) was also noted in the present study but this was not statistically significant.

Abu-Bedair *et al.* (2003) in their study on Egyptian women with breast cancer found significantly increased plasma total cholesterol in premenomenopausal breast cancer women but not in postmenopausal breast cancer patients. They also found significantly increased plasma TG in postmenopausal breast cancer patients but not in premenopausals. Their study is in agreement with that of Bani *et al.* (1986) who also found increased total cholesterol in premenopausal breast cancer patients. Willet *et al.* (1984) actually found reduced serum total cholesterol in breast cancer patients while others found no association between total serum cholesterol levels and breast cancer risk in premenopausal women (Gaard *et al.,* 1994)

One plausible explanation for the significant reduction in plasma TG observed in premenopausal breast cancer in the present study could be associated to the role of cytochrome p450 -1A2 (i.e. CYP-lA2); an enzyme that is principally responsible for the metabolism of 17β oestradiol and its activity is known to correlate with hormone levels and blood lipids and life style factors associated with breast cancer risks (Hong *et al.*, 2004}. Though CYP-1A2 activity was not measured in the present study but adequate extrapolations about it's reported effects may offer some explanations. CYP-1A2 activity varies negatively with female sex hormones especially free oestradiol as the later has inhibitory effect on the activity (Tantcheva *et al.,* 1999).

Low CYP-1A2 activity is postulated to be associated with increased breast cancer risk as it is in the case of the premenopausal breast cancer patients in this present study, while a higher CYP-1A2 activity is postulated to be associated with reduced risk (Hong *et al.,* 2004). The activity of CYP-1 A2 is also known to be lower during the late luteal phase of menstrual cycle when oestradiol is at its peak (Lane *et al.,* 1992). Moreover, in premenopausal women CYP-1A2 activity was reported to positively correlate with plasma TG, insulin levels and age but negatively correlate with total cholesterol levels and BM1 (Hong et al., 2004).

These observations, from extrapolation, could be the reason why a significantly reduced plasma TG and increased plasma total cholesterol (though not significant) together with significantly increased oestradiol level were observed in the premenopausal breast cancer patients in this present study.

Furthermore, 2 hydroxy oestrogen, a product of CYP-1A2 activity has been shown in rats to have a serum TG-reducing effect (Higa, 1990). CYP-1A2 is most active in catalysing 2-hydroxylations (Yamazaki *et al*., 1998). Infact in humans, half of the estrogens are hydroxylated at the C-2 position (Xu *et al*., 1999).

The results of the present study also indicate that HDL cholesterol was significantly increased at three to four months after surgical oophorectomy when compared with the presurgical values as well as the values in natural postmenopausal control women. After menopause, the increase in the risk of CHD is promoted by a number of metabolic and vascular changes which may in part be related to estrogen deficiency.

Alterations in plasma lipopropteins become evident at the beginning of the natural menopause, with progressive increase in total cholesterol and LDL cholesterol concentrations, while effects on HDL cholesterol levels are controversial (Cassidy and Griffin, 1999). Studies suggest that levels of HDL-cholesterol do not change significantly as a consequence of the menopause (Stevenson *et al*., 1993, Bergmann *et al*., 1997). However, it was reported in a study by Saranyaratana *et al*., (2006) that in healthy women with average age of 50$\pm$5years, the surgically menopause group had greater HDL cholesterol than the naturally menopause group. Some studies have found serum oestradiol levels to be positively associated with HDL – cholesterol levels in post menopausal women (Kuller *et al*., 1990).

Consequently, the plasma oestrogen level in the oophorectomised patients though lower than the presurgical values, was still significantly higher at three months than the corresponding values in natural postmenopausal control women. The reason for increased estrogen at three to four months after bilateral surgical Oophorectomy could be deduced from the fact that after removal of the ovaries from the patients and the attendant drastic reduction or cessation of estrogen production, then the rate of androstenedione production from extraglandular sources increased aggressively probably as a compensatory measure. Moreso that the stroma cells of the ovaries were no more available to produce androgens. The degree of conversion to oestrone and consequently to oestradiol via the aromatase enzyme complex also increased rapidly. Cholesterol being a precursor of steroid hormone and so involved in steroidogenesis also increased rapidly as well as other lipids. Hence there was an upsurge in oestradiol level at three months after oophorectomy as illustrated in figures 4.1 and 4.2. The fatty breast tumors of the patients also produce androstendione which ultimately augments the level of oestrogen after oophorectomy.

Moreover, the study by Agarwal *et al*., (1996) suggested that breast cancer patients may have an inherently higher aromatase expression in breast adipose tissue when compared with healthy women. This in turn suggests that there was probably a higher than normal aromatase activity in the fatty breast tumours of the oophorectomised breast cancer patients studied. Thus an increased estrogen production was observed in these patients at three to six months after oophorectomy.

The significantly higher oestrogen concentration may be important in the increased level of circulating HDL in the surgically induced menopause caused by Oophorectomy. In support of this, the decrease in HDL during natural menopause has been shown to increase significantly during estrogen replacement therapy (ERT) and hormone replacement therapy (HRT) i.e oestrogen plus progestin (Pickar *et al*., 1998; Tikkanen *et al*., 1999). The prevention of CHD with estrogen by HRT is aimed both at correction of the traditional set of

cardiovascular risk factors and at the direct control of vessel structure and function. (Cassidy and Griffin, 1999).

Observational prospective studies have consistently shown that exogenous estrogen therapy lowers risk of CHD (Stampfer *et al*., 1991, Meade and Berra, 1992). Estrogens have also been shown to exert acute and positive effects on the regulation of the arterial vasoreactivity in ovariectomised Rhezus Monkeys (Williams *et al*., 1992). Also Perrella *et al*. (2003) have also reported in invitro studies that HDL oxidation can be differentially inhibited by equine oestrogens and that protection of LDL oxidation by HDL is enhanced in the presence of 17β-estradiol (E_2).

The biological mechanisms by which estrogens (endogenous and exogenous) and phytoestrogens may confer cardioprotection are multifactorial, acting through serum lipoprotein homeostasis and vascular function.

The possible mechanisms for this cardioprotective effects of oestrogen include either a modification of the plasma LDL: HDL ratio (Subbiah *et al*., 1993) or estrogens acting as free radical scavengers and so are able to break free radical chain formation produced from membrane oxidation process hence inhibiting lipid peroxidation by sequestering metal ions or donating a proton to reduce peroxy free radicals (Lacort *et al*., 1995). Another mechanism is through the stimulation of Apo A_1 synthesis by estrogen which inhibits the activity of hepatic lipase and leads to increased production of mature HDL as its catabolism (clearance) is slowed down (Miller and Lane, 1984; Walsh *et al*., 1991; Quintao *et al*., 1991).

In effect, the increased HDL and the associated high level of estrogen observed in the premenopausal breast cancer women studied, at three to six months after oophorectomy may be cardioprotective in these patients.

Thus, the possible inhibition of HDL oxidation may be important mechanism by which raised estrogen concentration reduced the risk of CHD in premenopausal breast cancer patients being managed by bilateral surgical oophorectomy.

5.2 Conclusion

Premenopausal breast cancer in Nigerian women is associated with significant reduction in plasma triglycerides (TG) and non-significant increase in plasma total cholesterol.

Implication suggests that oophorectomy reduced the risk of CHD in the premenopausal patients with breast cancer because of the increase in HDL cholesterol levels at three and six months after surgical oophorectomy in comparison to natural postmenopausal status.

5.3 Recommendations

However, to further ascertain the sustenance of the cardioprotective effect, it will be of interest to have a study in which the patients could be followed up for longer period of like two years or more. Measurement of CYP – 1A2 activity could also help in affirming the cause of TG – reduction in pre-menopausal breast cancer patients.

Furthermore, although the DNA content of breast tumours of patients was not assessed in the present study, it has been postulated that DNA content appears to gradually increase in proportion with the malignancy staging of the carcinomas. Thus, the findings in the current study, showing that TG level in grade III breast cancer patients was higher than that of grades I and II by about 50% may suggest a relationship between lipid alterations and DNA synthesis in breast cancer.

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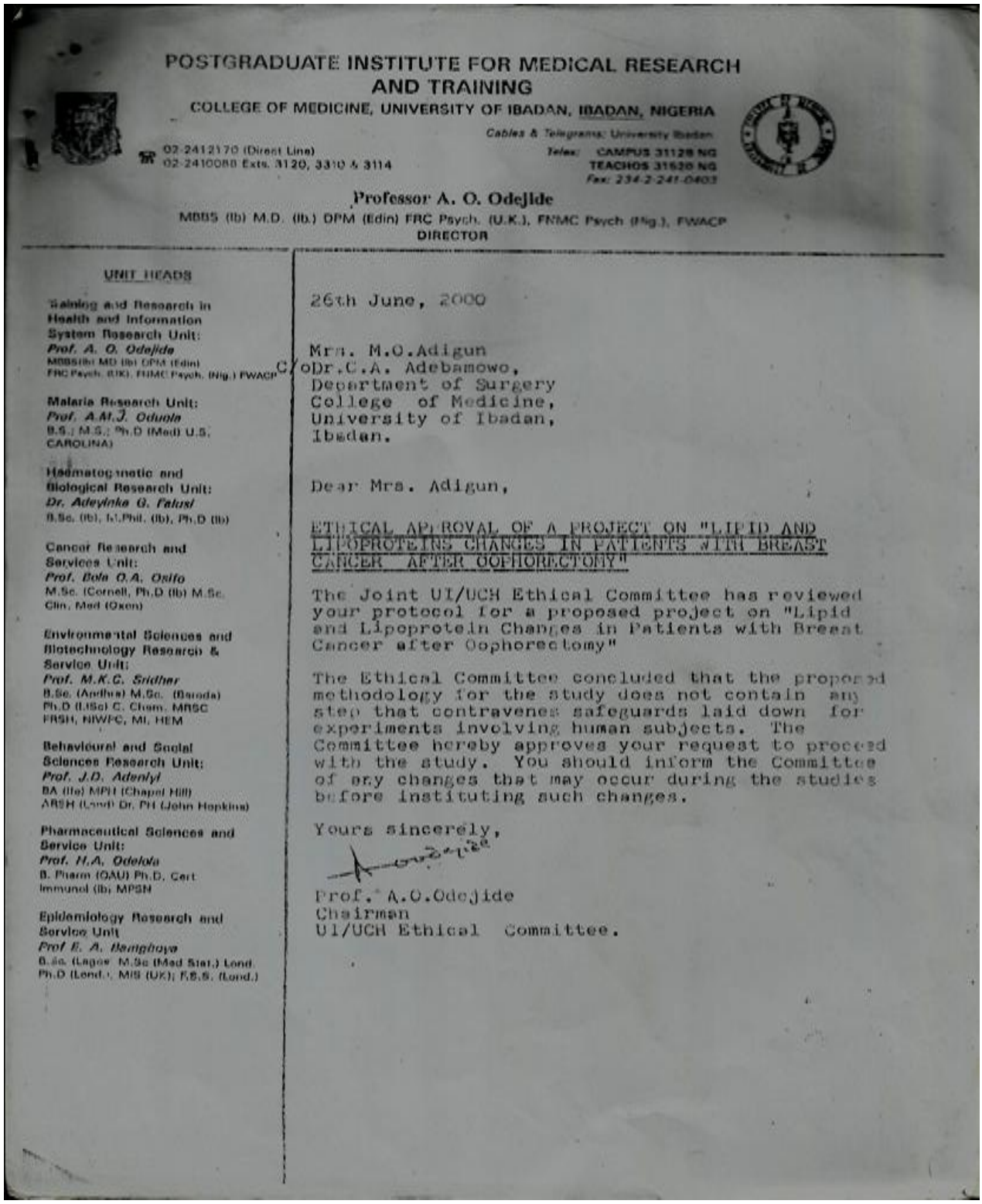
APPENDICES

Appendix I



POSTGRADUATE INSTITUTE FOR MEDICAL RESEARCH AND TRAINING

COLLEGE OF MEDICINE, UNIVERSITY OF IBADAN, IBADAN, NIGERIA

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DIRECTOR

UNIT HEADS

Training and Research in Health and Information System Research Unit:
Prof. A. O. Odejide
MBBS(Ib) MD (Ib) DPM (Edin) FRC Psych. (UK), FNMC Psych. (Nig.) FWACP

Malaria Research Unit:
Prof. A.M.J. Oduola
B.S.; M.S.; Ph.D (Med) U.S. CAROLINA)

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B.Sc. (Ib), M.Phil. (Ib), Ph.D (Ib)

Cancer Research and Services Unit:
Prof. Bola O.A. Osifo
M.Sc. (Cornell), Ph.D (Ib) M.Sc. Clin. Med (Oxon)

Environmental Sciences and Biotechnology Research & Service Unit:
Prof. M.K.C. Sridhar
B.Sc. (Andhra) M.Sc. (Baroda) Ph.D (IISc) C. Chem. MRSC FRSH, NIWPC, MI, HEM

Behavioural and Social Sciences Research Unit:
Prof. J.D. Adeniyi
BA (Ife) MPH (Chapel Hill) ARSH (Lond) Dr. PH (John Hopkins)

Pharmaceutical Sciences and Service Unit:
Prof. H.A. Odeleke
B. Pharm (OAU) Ph.D. Cert Immunol (Ib) MPSN

Epidemiology Research and Service Unit
Prof E. A. Bamgboye
B.Sc. (Lagos) M.Sc (Med Stat.) Lond. Ph.D (Lond.), MIS (UK); F.S.S. (Lond.)

C/o

26th June, 2000

Mrs. M.O.Adigun
C/oDr.C.A. Adebamowo,
Department of Surgery
College of Medicine,
University of Ibadan,
Ibadan.

Dear Mrs. Adigun,

ETHICAL APPROVAL OF A PROJECT ON "LIPID AND LIPOPROTEINS CHANGES IN PATIENTS WITH BREAST CANCER AFTER OOPHORECTOMY"

The Joint UI/UCH Ethical Committee has reviewed your protocol for a proposed project on "Lipid and Lipoprotein Changes in Patients with Breast Cancer after Oophorectomy"

The Ethical Committee concluded that the proposed methodology for the study does not contain any step that contravenes safeguards laid down for experiments involving human subjects. The Committee hereby approves your request to proceed with the study. You should inform the Committee of any changes that may occur during the studies before instituting such changes.

Yours sincerely,

Prof. A.O.Odejide
Chairman
UI/UCH Ethical Committee.

Appendix II

Department of Chemical Pathology
University College Hospital,
Ibadan.
Nigeria
Telephone: 234-2-2410088 ext 3196

Lipids Research Unit

CONSENT FOR PARTICIPATION IN RESEARCH PROTOCOL

Please indicate whether you want to read this yourself or you want it to be read to you. You may also wish to take it away and return to sign it.

Title of Protocol: **Changes in Serum Lipids and Lipoprotein Levels in Breast Cancer patients after Oophorectomy.**

Principal Investigator(s): Dr. C.A. Adebamowo, Mrs. M.O. Adigun and Prof. E.O. Agbedana.

Dear Patient:

This is to request you to participate in a research that is aimed at finding our whether oophorectomy by Surgery, Tamoxifen or radiotherapy on Breast Cancer patients can cause adverse changes in plasma lipids and lipoproteins which are known risk factors for premature coronary heart disease. The research will shed more light on how best clinicians could help in managing the breast cancer patients.

You are being involved in this study, because you have breast cancer and recieving treatment in the surgery department of University College Hospital, (UCH), Ibadan.

Procedure: The principal investigator and a trained nurse counsellor will see you. The later will spend about 15 0r 20 minutes with you asking you a variety of questions which you must answer as truthfully as possible. After this, she will request you to give about two tablespoons of blood into a tube (This will be repeated 2 or 3 times after oophorectomy for the patient group only). The blood samples will be processed in the laboratory here in U.C.H.

Risks and Benefits: The risks associated with this study are that you may be asked rather personal questions that you may find uncomfortable. Also the process of venipuncture is associated with some discomfort in the arm such as transient pain and/or local bruising and slight bleeding may occur afterwards at the venipuncture site. You will however enjoy the benefit of having helped to further the understanding of clinicians in breast cancer management. Occassionally, technical problems may warrant a repitition of the venipunture.

Confidentiality: There are multiple safeguards in place to protect you so that the results of this test cannot become public knowlege or be linked in any way with you. All information obtained during this research remain confidential. If you do desire to know the result of your test this can be made available to you.

Voluntary: Participation in this research does not have any influence on your tratment. Neither will you be denied appropriate treatment should you decide not to participate in the research.

If you have any questions regarding your rights as a study participant, please contact the principal investigator(s) on 2410088 ext. 4709 and 3196. You may also wish to contact the Ethical committee of the University College Hospital, Ibadan, Oyo state, Nigeria through the Office of the Chief Medical Director. If you decide that you no longer wish to participate in the research, you may withdraw your cconsent at any time.

Agreement: Your signature on this form indicates that you fully understand this research and have decided to participate.

Participant _____ *Date* _____

Witness _____ *Date* _____

Principal Investigator _____ *Date* _____

Appendix III

Department of Chemical Pathology
University College Hospital,
Ibadan.
Nigeria
Telephone: 234-2-2410088 ext 3196

Lipids Research Unit

CONSENT FOR PARTICIPATION IN RESEARCH PROTOCOL - CONTROL GROUP

Please indicate whether you want to read this yourself or you want it to be read to you. You may also wish to take it away and return to sign it.

Title of Protocol: **Changes in Serum Lipids and Lipoprotein Levels in Breast Cancer patients after Oophorectomy.**

Principal Investigator(s): Dr. C.A. Adebamowo, Mrs. M.O. Adigun and Prof. E.O. Agbedana.

Dear Patient:

This is to request you to participate in a research that is aimed at finding our whether oophorectomy by Surgery, Tamoxifen or radiotherapy on Breast Cancer patients can cause adverse changes in plasma lipids and lipoproteins which are known risk factors for premature coronary heart disease. The research will shed more light on how best clinicians could help in managing the breast cancer patients.

You are being involved in this study because you are a woman and you do not have breast cancer or any other oncological and endocrine problems and we wish to establish the normal levels of lipids and lipoproteins in breast cancer free women such as you. This is to enable us to find out whether there is any adverse changes caused by oophorectomy on women with breast cancer. Your participation will go a long way in helping clinicans to make quality decision on their management strategies of breast cancer patients.

Procedure: The principal investigator and a trained nurse counsellor will see you. The later will spend about 15 0r 20 minutes with you asking you a variety of questions which you must answer as truthfully as possible. After this, she will request you to give about two tablespoons of blood into a tube. The blood samples will be processed in the laboratory here in U.C.H.

Risks and Benefits: The risks associated with this study are that you may be asked rather personal questions that you may find uncomfortable. Also the process of venipuncture is associated with some discomfort in the arm such as transient pain and/or local bruising and slight bleeding may occur afterwards at the venipuncture site. You will however enjoy the benefit of having helped to further the understanding of clinicians in breast cancer management. Occassionally, technical problems may warrant a repitition of the venipunture.

Confidentiality: There are multiple safeguards in place to protect you so that the results of this test cannot become public knowlege or be linked in any way with you. All information obtained during this research remain confidential. If you do desire to know the result of your test this can be made available to you.

Voluntary: Participation in this research does not have any influence on your tratment. Neither will you be denied appropriate treatment should you decide not to participate in the research.

If you have any questions regarding your rights as a study participant, please contact the principal investigator(s) on 2410088 ext. 4709 and 3196. You may also wish to contact the Ethical committee of the University College Hospital, Ibadan, Oyo state, Nigeria through the Office of the Chief Medical Director. If you decide that you no longer wish to participate in the research, you may withdraw your cconsent at any time.

Agreement: Your signature on this form indicates that you fully understand this research and have decided to participate.

Participant _____ *Date* _____

Witness _____ *Date* _____

Principal Investigator _____ *Date* _____

Appendix IV

QUESTIONAIRE ON THE STUDY OF SERUM LIPIDS AND LIPOPROTEINS CHANGES, IN BREAST CANCER PATIENTS AFTER OOPHORECTOMY.

This study is designed to investigate the changes in the level of serum lipids and lipoproteins, after Oophorectomy in Nigerian women with Breast Cancer. The form should be filled as completely as possible. All the information provided are strictly confidential and no third party will have access to it without your express permission. If you have any questions contact any of the following - Prof. Agbedana of chemical patnology Dept U.C.H. Ibadan phone No 234-2-2410088 ext. 3196.

Dr. C. A. Adebamowo of Surgery Dept. U.C.H. Ibadan Phone No 234-2-2410088 ext. 4709.

Mrs. M. O. Adigun of Chemical Pathology Dep. (Lipids Research Room) U.C.H. Ibadan, Phone No. 234-2-2410088 ext. 2324.

DATE: ☐☐/☐☐/☐☐

1. Name (Surname first) ☐ ☐
2. Contact Address (for records only) ☐
3. Serial Number: ☐☐☐☐☐☐
4. Hospital Number: ☐☐☐☐☐☐
5. Age (in years): ☐☐
6. Ethnic Group: ☐
7. Marital Status: 1. Married ☐, 2. Single ☐, 3. Divorced ☐, 4. Widow ☐
8. Occupation: 1. None ☐, 2. Housewife ☐, 3. Trader ☐, 4. Farmer ☐, 5. Artisan ☐, 6. Professional ☐, Please specify:____________________
9. Highest level of Schooling: 1. None ☐, 2. Elementary ☐, 3. Secondary ☐ 4. Tertiary ☐ 5. Others ☐
10. Religion: 1. Christian ☐, 2. Moslem ☐, 3. Others ☐
11. Weight (in kg): ☐ Height (in cm): ☐
12. Blood Pressure: ☐
13. Duration of Disease (in months) ☐☐

14. What is the stage of Disease?

Manchester's stage ☐☐☐

TNM stage? T ☐☐☐

N ☐☐☐

M ☐☐☐

MENSTRUAL CYCLE HISTORY

15. Date of LMP (last menstrual period): ☐☐/☐☐/☐☐

16. How old was the patient when she started her period (in years): ☐☐

17. Has it been - 1. Regular ☐ or 2. Irregular ☐

18. Menopausal Status:

1. Pre Menopause ☐ 2. Natural/menopause ☐ 3. Oophorectomy. ☐

19. Age at onset of menopause (in years): ☐☐
If by Oophorectomy

20. What type of Oophorectomy:

1. Surgical ☐ 2. Radiotherapy ☐ 3. Tamoxifen ☐

21. What is the date of Oophorectomy:? ☐☐/☐☐/☐☐

22. Has the patient being on any form of hormone supplementation since onset of menopause?
1. Yes ☐ 2. No ☐

23. If yes which drug? ☐

PAST HISTORY OF PILLS

24. Has the patient used contraceptives before? 1. Yes ☐ 2. No ☐

25. If yes, is it. 1. The Pill 2. The injection or 3. Other types

26. If she used the pill, what is the name of the pill? ☐

27. At what age did she strt using it? (in years) ☐☐

28. How long did she use it for? (in years) ☐☐
Regualrly ☐ or irregularly ☐

29. If she used the injection at what age did she start using it (in years) ☐☐

30. How long did she use it (years) ☐☐

31. Is she suffering from any other systemic disease 1. Yes ☐ 2. No ☐

32. What disease? 1. Diabetes ☐ 2. Hypertension ☐ 3. Renal disease ☐ 4. Others ☐
specify ☐

33. Does the patient use any drug (of abuse) 1. Yes ☐ 2. No ☐

34. If yes: which one ☐

Appendix V

The contents of the cholesterol kit are enzyme reagent, containing:-

Phosphate buffer (PHbS)

4-aminophenazone

Phenol

Peroxidase (POD)

Cholesterolesterase (CHE)

Cholesteroloxidase (CHO)

Sodium azide (0.05%, as preservative) and

Standard cholesterol (200mg/dl)

The contents of the Triglyceride kit are:-

1. Buffer solution: containing
   - -PIPES buffer
   - 4-chlorophenol
   - Magnesium ions
   - Sodium azide
2. Enzyme reagent: containing
   - 4-animoantipyrine Glycerol-3-phosphate (GPO)
   - Lipases
   - Peroxidase (POD)
   - Glycerol kinase (GK)
3. Triglycerides Standard - 200mg/dl

The working reagent was prepared by reconstituting the contents of vial 2 (enzyme reagent) with 15ml of vial 1 (i.e. the buffer solution). The standard is supplied ready for use.

MATERIALS PROVIDED IN THE ESTRADIOL EIA KIT

These are as listed below:

E2 EIA Standards	E2 EIA standards (1.0ml each) are provided liquid in processed human serum with 0.2% (w/v) sodium azide. Ready to use
E2 EIA Antiserum	A fluorescent labeled rabbit anti-E2 antibody (20ml) is provided liquid in phosphate buffer with bovine serum azide. Ready to use. Avoid exposure to direct sunlight.
E2 EIA Enzyme E2 Label	Estradiol linked to alkaline phosphatese (20ml) is provided liquid in phosphate buffer with bovine serum proteins, and 0,2% (w/v) sodium azide. Ready to use.
EIA Separation Reagent	Provided as a suspension (20ml of sheep anti-florescein coupled to magnetic particles. (Do not freeze).
E2 EIA Serum Diluent	Serum Diluent is provided liquid (1 1ml) in processed human serum with 0.2% (w/v) sodium azide. Ready to use.
EIA wash Concentrate	Provided as a liquid concentrate (15ml) of a Tris buffer containing a surfactant and a preservative to be diluted
EIA Substrate Reagent	Provided in 1 glass bottle containing 400mg phenolphthalein monophosphate.
EIA Substrate Buffer	Provided as a liquid (55ml) read to use
EIA Stop Buffer	Provided liquid (120ml) as 2 bottles containing 60ml each of Glycine buffer pH 10.4 containing sodium hydroxide and a chelating agent. Ready to use CAUTION: CAUSTIC
E2 EIA QC Sample	Provided as 1ml lyophilized serum. An approximate target range for the E2 EIA internal QC sample supplied is given on the bottle label and on the back cover of the protocol.

Other Materials Used for the EIA

Other materials required and used for the estradiol EIA are listed below:

De-ionized or Distilled water	Good quality pure water is essential. Impurities can affect enzyme activity and hence test results.
Colorimeter/ Spectrophotometer	Any region (550nm) colorimeter/spectrophotometer may be used. Ability to measure at two wavelengths (550nm and 492nm) will extend the working range of the assay to higher doses.
Pipettes	Pipette may be manual semi-automatic or automatic. We suggest that manual 150ul pipette is used for pipetting samples and standards at the beginning of the assay, and that repeating multidose pipettes (capable of delivering 200µl, 500µl and 1ml are used for subsequent reagent additions.
Test-tubes	Round bottomed disposable 12 x 75mm clear plastic test-tubes are recommended for use with this assay.
Magnetic racks and separators	Any magnetic separator compatible with 12 x 75mm test-tubes may be used.
Vortex mixer	Rapid and preferably simultaneous, mixing of all tubes is important. A multivortex is desirable.

37°**C water-bath, refrigerator, reagent bottles, timer, measuring cylinders and beakers**