Levels of Estrogen, Carcinoembryonic Antigen and Cancer Antigen of Breast in Breast Cancer patients

By

Hala Ahmed Abdelhadi

B. Sc. Faculty of Science

University of Khartoum

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Supervisor

Dr. Barakat El Hussein

Department of Biochemistry

Faculty of Veterinary Medicine

University of Khartoum

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DEDICATION

I dedicate this work to my family –big and small- and to whoever heartily helped me to reach this step

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With a thankful heart I would like to acknowledge my supervisor Dr. Barakat El Hussien who guided me through and spared no effort to help and to Dr. Eltayeb Ahmed Eltayeb who gave time and expertise to make this work a success. Not forgetting my colleagues in the Sudan Atomic Energy Commission who geared the work from the very start, especially Mr. Waleed Abdalbagi Ahmed for his hands in sample collection and more.

Abstract

This study was conducted during the period from February 2004 to July 2004; with the objective of measuring the levels of estrogen (E2), carcinoembryonic Antigen (CEA) and cancer antigen of breast (CA-15.3) so as to facilitate the early diagnosis of breast cancer and to determine the involvement of these parameters as risk factors for breast cancer.

Ninety blood samples were collected from Sudanese females, divided into two groups; control group and patients groups. The patients group was sixty Sudanese females visiting the Radio Isotope Center, Khartoum (RICK) and they were confirmed as breast cancer patients by histopathology.

The levels of the above mentioned parameters were determined by using radioimmunoassay technique.

The results showed that, no significant ($p=0.05$) difference between the levels of the estrogen in patients compared to the control, on the other hand there was non-significant ($p>0.05$) elevation in CEA levels in the patients with breast cancer compared to the control. The level of CA15.3 was significantly ($p<0.0001$) higher in the breast cancer patients compared to the control.

الخلاصة

أجريت هذه الدراسة في الفتره من فبراير 2004 الى يونيو 2004 بهدف التأكد من العلاقة بين هرمون الاستروجين والمستضد الجنيني ومستضد سرطان الثدي واحتمال الإصابة بسرطان الثدي.

تم جمع عينات دم من ستين مريضة بسرطان الثدي من مركز علاج الأورام بالخرطوم (مستشفى الذرة) ، وجمعت ثلاثون عينة دم أخرى من نساء غير مصابات لاستخدامها كمجموعة تحكم.

كل العينات تم تحليلها بتقنية المناعة الإشعاعية (Radioimmunoassay) بمعمل هيئه الطاقة الذرية.

بعد إجراء التحاليل الإحصائية على النتائج أتضح أنه لا توجد فروقات معنوية فى كل من هرمون الاستروجين ($p=0.05$) والمستضد الجنيني($p>0.05$) بين النساء المصابات بسرطان الثدى ومجموعة التحكم، ومن ناحية أخرى أوضحت هذة الدراسة أن هناك زيادة مؤثرة فى مستضد سرطان الثدي فى النساء المصابات بسرطان الثدى بالمقارنة مع مجموعة التحكم ($p<0.0001$).

ومن هذا نخلص أن هذه الدراسة أعطت مؤشرا جيدا لاستخدام بعض موسمات الأورام في المساعدة في التشخيص المبكر ، ومن أقوى هذه الموسمات مستضد سرطان الثدي.

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INTRODUCTION

Breast cancer is ranked as the second common cancer around the world. It is most common in females, though males can develop breast cancer as well; it is believed to be the second cause of death (US mortality public, 2004). The relationship between developing breast cancer and some parameters has been stated; amongst them were estrogen (E2), carcinoembryonic antigen (CEA) and cancer antigen of breast (CA 15.3) (Lupulescu, 1995; Fletcher, 1996; Pamies, *et al.* 1996).

CEA is one of the first tumor markers to be identified and characterized and has been evaluated in a wide range of malignancies, including breast cancer (Sikorska, *et al.* 1988). The availability of the CA 15.3 tumor marker encouraged its use as a tumour marker for breast cancer favorably for its high sensitivity in both early and advanced diseases. Nevertheless, CEA is still a widely used test for monitoring breast cancer patients (Van Dalen, *et al*. 1996).

Estrogen is important for normal sexual development and is essential for the normal functioning of the female organs needed for childbearing such as the ovaries and uterus (Massaro, *et al.* 2004). Estrogen as risk factor is strongly suggested to have role in the development of breast cancer (Toniolo, *et al*. 1995). However, some studies reported that no relationship between the estrogen concentrations and the breast cancer (Sturgeon, *et al*. 2004).

Objectives of the study

This study was conducted to satisfy the following objectives:

1- The main objective of this study is to determine the levels of estrogen E2, carcinoembryonic antigen CEA and cancer antigen of breast CA-15.3 in breast cancer patients compared to the control.

2- The use of these parameters in the early diagnosis of breast cancer.

CHAPTER ONE

LITERATURE REVIEW

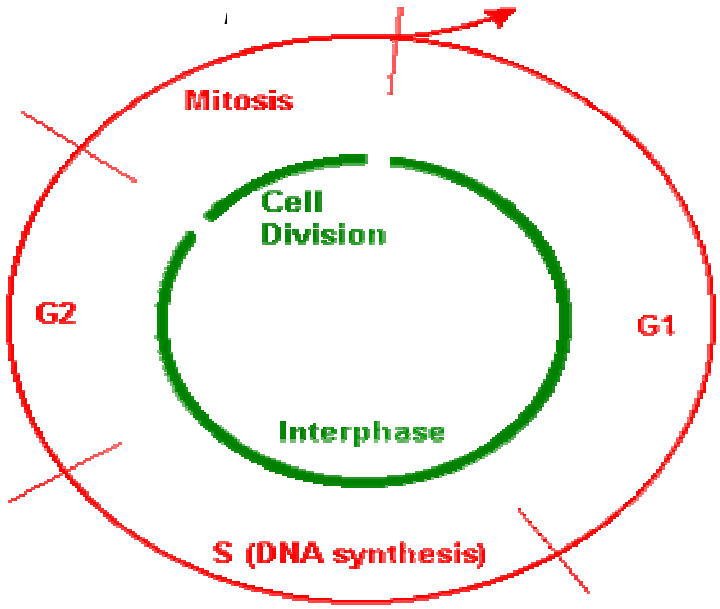
1.1 Cell cycle

Cells engaged in proliferation pass through four phases which together constitute the cell cycle (Fig. 1). In gap-1 (G_1) preparation is made to enter DNA synthesis. In S, DNA synthesis occurs. In G_2 the cell assembles the machinery for distributing the newly replicated chromosomes equally to the two daughter cells which are generated in M, mitosis. A great many cells in normal tissues, however, are in none of these phases. Some are in a non-proliferating state called G_0, or growth arrest, from which they can be activated to enter the cell cycle in G_1. Others can no longer the cycle; they are committed to terminal differentiation and death. The process whereby cells stimulated to move from one phase of the cycle to the next is called cell cycle control. Incontrast, the control of entry to the proliferative state from G_0 is called growth control (Andrew and Tim, 1993).

1.1.1 Cell growth control

Cells move from the quiescent G_0 state into G_1 in response to specific stimuli. The best known of these are locally active peptides and lipoproteins called growth factors. Some growth factors act on wide spectrum of cell types; examples include platelet derived growth factor (PDGF), the somatomedins or insulin-like growth factors (IGFs) and the acidic and basic fibroblast growth factors (a-and b-FGF) which, despite their name, stimulate proliferation in many cells in addition to fibroblasts.

Fig. 1: Schematic Drawing of the Cell Cycle



Epidermal growth factors (EGF) acts predominantly on epithelial cells. Tumor-derived growth factor alpha (TGFα) is a closely related peptide. Some growth factors have the opposite effect and inhibit proliferation of certain target cells (e.g. Transforming Growth Factor $_{\beta,}$

TGF_{β}). Many familiar hormones also exert positive or negative growth factor activity on their target cells (e.g. thyroid hormones on thyroid epithelium, progesterone on mammary epithelium). Growth factors influence their target cells by binding to the specific receptors. Sometimes they stimulate their target cells to synthesize and display receptors to other factors, so rendering the cell capable of response to proliferative stimuli to which it was previously inert (Gary and Arthur, 2004).

1.2 Tumors

Tumors are an abnormal mass of tissue, the growth of which exceeds and is uncoordinated with that of the normal tissues, and persists in the same excessive manner after cessation of the stimuli which evoked the change (Pamies, *et al.* 1996)

Tumors are divided into two classes, benign and malignant; the critical distinction between them is their mode of growth. Benign tumours grow by expansion, compressing or displacing surrounding normal tissue, but malignant tumors grow by local infiltration, destroying the tissue through which they invade. Benign tumors grow at the site of origin only, but malignant tumors may spread to distant sites, by the blood stream, in the lymphatics, or across tissue spaces such as the peritoneal or pleural cavities, or the cerebrospinal fluid space (Philip and Harris, 2001). This extremely important and dangerous property of malignant tumours called metastasis, and the secondary tumors which grow at the distant sites are metastases. Malignant tumors also tend to have a faster rate of growth than benign tumors. As a result, benign and malignant tumors have different clinical effects. By virtue of their position, benign tumors may cause symptoms by compression of adjacent structures, or in certain specific circumstances they may secrete hormones which contribute to the manifestations of disease. Incontrast, malignant tumors always have the

potential to cause death as a result of their aggressive growth behavior. Cancer is the common term in use and is defined as malignant tumor which is invasive and metastatic (Daugaard, 2001).

1.3 Breast cancer

With the possible exception of skin, breast cancer is the most common human female cancers throughout the world, although men can also develop this disease. It is more than 100 times more common in women than in men. During the mid-1980s mortality from cancer of the breast overtook that of every other female cancer to become the commonest cause of cancer death (Key, 2001). Breast cancer can develop at any age, but the risk of developing it increases as women get older. It is the second leading cause of cancer death for American women. While 5% to 10% of breast cancers are related to an inherited defect in one of two genes (BRCA1, BRCA2), the majority of cases develop for reasons we do not yet understand (Tonin, 2000). As a general rule, those at higher risk of developing breast cancer include women whose close relatives have had the disease (Colditz *et al*. 1996), women who have not had children and women who had their first child after the age of 30 (Kelsey and Horn-Ross, 1993).

Breast cancer divided into three stages, reflecting the extent to which the cancer has spread in the body.

1.3.1 Early stage

Breast cancer is usually confined to the ducts (which transport milk to the nipple during lactation or to the lobules (small areas of tissue where milk is produced in the breast) and is known as noninvasive cancer. If the cancer is confined to the ducts, it is called ductal carcinoma *in situ* (DCIS) and if it is confined to the lobules, it is called lobular carcinoma *in situ* (LCIS). At this stage, the cancer cannot be felt as a

lump in the breast, but DCIS can sometimes be detected by mammography (Singletary, 2002).

1.3.2 Invasive stage

It is characterized by a spread of the cancer beyond the ducts or lobules and into the surrounding areas of breast tissue. At this stage, the cancer may be detected through a breast self-exam, by a clinical breast examination performed by health care professional, or by mammography (Singletary, 2002).

1.3.3 Metastatic stage

There is a huge spread to other areas of the body, including nearby lymph nodes (Singletary, 2002).

1.3.4 Risk factors

A risk factor is anything that increases your chance of getting a disease, such as cancer. Different cancers have different risk factors. For example, exposing skin to strong sunlight is a risk factor for skin cancer. Smoking is a risk factor for cancers of the lung, mouth, larynx, bladder, kidney, and several other organs, but having a risk factor, or even several, does not mean that you will get the disease. Some women who have one or more breast cancer risk factors never develop the disease, while most women with breast cancer have no apparent risk factors. Even when a woman with breast cancer has a risk factor, there is no way to prove that it actually caused her cancer (Susan, 2005).

There are different kinds of risk factors. Some factors, like a person's age or race, can't be changed. Others are linked to cancer-causing factors in the environment. Still others are related to personal choices such as smoking, drinking, and diet. Some factors influence risk more than others, and your risk for breast cancer can change over time, due to

factors such as aging or lifestyle (Susan, 2005). The following are some risk factors:

Gender: simply being a woman is the main risk factor for developing breast cancer. Because women have many more breast cells than men do and perhaps because their breast cells are constantly exposed to the growth-promoting effects of female hormones, breast cancer is much more common in women. Men can develop breast cancer, but this disease is about 100 times more common among women than men (Kelsey and Horn-Ross, 1993).

Aging: Age is the most important risk factor for women, the risk of developing breast cancer increases as you get older. About 18% of breast cancer diagnoses are among women in their 40's, while about 77% of women with breast cancer are older than 50 when they are diagnosed (Kelsey and Horn-Ross, 1993).

Cancer genes: certain changes in DNA can cause normal breast cells to become cancerous. Some genes contain instructions for controlling when our cells grow, divide, and die. Certain genes that promote cell division are called oncogenes (such as BRCA1, BRCA2). Others that slow down cell division, or cause cells to die at the right time, are called tumor suppressor genes (p53). It is known that cancers can be caused by DNA mutations (changes) that "turn on" oncogenes or "turn off" tumor suppressor genes.

The (p53) gene is a tumor suppressor gene. When it is mutated, it no longer functions to suppress abnormal growth and cancer is more likely to develop. Certain inherited DNA changes can cause a high risk for developing cancer in people who carry these changes and are responsible for the cancers that run in some families.

Most DNA mutations related to breast cancer, however, occur in single breast cells during a woman's life rather than having been inherited. Acquired mutations of oncogenes and/or tumor suppressor genes may result from radiation or cancer-causing chemicals. So far, however, studies have not been able to identify any chemical in the environment or in our diets that is likely to cause these mutations, or a subsequent breast cancer. The cause of most acquired mutations remains unknown (Tonin, 2000). Recent studies have shown that about 10% of breast cancer cases are hereditary as a result of gene changes (mutations). The most common gene changes are those of the BRCA1 and BRCA2 genes (Tonin, 2000). Normally, these genes help to prevent cancer by making proteins that keep cells from growing abnormally. However, if you have inherited changed gene from either parent, you are at increased risk for breast cancer.

Family history of breast cancer: breast cancer risk is higher among women whose close blood relatives have this disease. Having one first-degree relative with breast cancer approximately doubles a woman's risk, and having two first-degree relatives increases her risk 5-fold. Although the exact risk is not known, women with a family history of breast cancer in a father or brother also have an increased risk of breast cancer (Colditz, *et al*. 1996). Altogether, about 20% to 30% of women with breast cancer have a family member with this disease.

Race: white women are slightly more likely to develop breast cancer than are African-American women. But African-American women are more likely to die of this cancer because their cancers are often diagnosed later and at an advanced stage when they are harder to treat and cure. There is also some question about whether African-American women have more

aggressive tumors. Asian, Hispanic, and Native American women have a lower risk of developing breast cancer (National Academy of Sciences, 2005).

Previous breast biopsy: women whose earlier breast biopsies detected proliferative breast disease without a typical or usual hyperplasia have a slightly higher risk of breast cancer (1.5 to 2 times greater than other women). Having a previous biopsy result of a typical hyperplasia increases a woman's breast cancer risk by 4 to 5 times. Having a biopsy specimen diagnosed as fibrocystic changes without proliferative breast disease does not affect breast cancer risk (Morris, 2003).

Previous breast radiation: women who as children or young adults have had radiation therapy to the chest area as treatment for another cancer (such as Hodgkin's disease or non-Hodgkin's lymphoma) have a significantly increased risk for breast cancer. Some reports found the risk to be 12 times normal. This varies with the age of the patient at the time of the radiation. Younger patients have a higher risk. If chemotherapy was also given, the risk is lowered because the chemotherapy often stops ovarian hormone production (John and Kelsey, 1993).

Menstrual periods: women who started menstruating at an early age (before age 12) or who went through menopause at a late age (after age 55) have a slightly higher risk of breast cancer (Colditz, *et al*. 1995).

Chemical carcinogenesis: there are some chemicals that play role in the breast cancer (Nicola, *et al*. 2003).

Diethylstilbestrol (DES) Therapy: in the 1940's through the 1960's some pregnant women were given diethylstilbestrol because it was thought to

lower their chances of losing the baby. Some studies have shown that these women have a slightly increased risk of developing breast cancer (Calle, *et al*. 1996).

Oral contraceptive: it is still not certain what part of oral contraceptives might play in breast cancer risk. Studies have suggested that women using oral contraceptives have a slightly greater risk of breast cancer than women who have never used them. Women who stopped using oral contraceptives more than 10 years ago do not appear to have any increased breast cancer risk. When considering using oral contraceptives, women should discuss their other risk factors for breast cancer with their health care team (Brinton, *et al*. 1995).

Alcohol: use of alcohol is clearly linked to a slightly increased risk of developing breast cancer. Compared with nondrinkers, women who consume 1 alcoholic drink a day have a very small increase in risk, and those who have 2 to 5 drinks daily have about 1½ times the risk of women who drink no alcohol. Alcohol is also known to increase the risk of developing cancers of the mouth, throat, and esophagus (Ferraroni, 1998).

Not having children: women who have had no children or who had their first child after age 30 have a slightly higher breast cancer risk (Kelsey and Horn-Ross, 1993).

Hormone replacement therapy: it has become clear that long-term use (several years or more) of hormone replacement therapy (HRT) after menopause, particularly estrogens and progesterone combined increase your risk of breast cancer. Moreover it may also be that they increase your chances of dying of breast cancer (Colditz, *et al*. 1995).

If the females are still have the uterus, doctors generally prescribe estrogen and progesterone (known as combined HRT). Estrogen relieves menopausal symptoms and prevents osteoporosis. But estrogen can increase the risk of developing cancer of the uterus. Progesterone is added to prevent this. If the females are no longer having the uterus, then only estrogen is prescribed, this is commonly known as estrogen replacement therapy (ERT).

Obesity and high-fat diets: obesity is associated with an increased risk of developing breast cancer, especially for women after menopause (which usually occurs at age 50). Although ovaries produce most of estrogen, fat tissue can change some other hormones into estrogen. Having more fat tissue can increase your estrogen levels and increase your likelihood of developing breast cancer (Bergstrom, *et al*. 2001)

1.3.5 Factors reducing breast cancer incidence

Physical activity: exercise and cancer is a relatively new area of research. Recent studies show that strenuous exercise in your youth might provide life-long protection against breast cancer and that moderate to strenuous physical activity as an adult can lower breast cancer risk. More research is being done to confirm these findings (Albanes, *et al*. 1989).

Breast feeding: Some studies suggest that breast feeding may slightly lower breast cancer risk, especially if breast feeding is continued for 1.5 to 2 years. Other studies found no impact on breast cancer risk (Furberg, *et al.* 1999).

The explanation of this may be that both pregnancy and breast feeding reduce a woman's total number of lifetime menstrual cycles. This

may be similar to the reduction of risk due to late menarche (start of menstrual periods) or early menopause, which also decrease the total number of menstrual cycles. One study concluded that having more children and breast feeding longer could reduce the risk of breast cancer by half (Furberg, *et al.* 1999).

1.4 Tumor markers

Tumor markers are substances that can often be detected in a higher than normal amounts in the blood, urine or body tissues in some patients with certain type of cancer. Tumor markers are produced either by the tumor itself or by the body in response to the presence of cancer or certain benign conditions (noncancerous) (Daugaard, 2001).

Tumor markers can be used for screening a healthy population or a population at high risk for the presence of cancer; making a diagnosis of cancer or a specific type of cancer; determining the prognosis in a patient; and for monitoring the course in a patient in remission or while receiving surgery, radiation, or chemotherapy (Lindblom, 2000).

1.4.1 Carcinoembryonic antigen (CEA)

Carcinoembryonic antigen (CEA), an oncofetal glycoprotein, it is a glycoprotein of approximately 180-200 k D, that is associated with the plasma membrane of tumor cells, from which it may be released into the blood, it is expressed in normal mucosal cells and over-expressed in adenocarcinoma, especially colorectal cancer. CEA elevations also occur with other malignancies such as breast and lung cancer. Non-neoplastic conditions associated with elevated CEA levels include cigarette smoking, peptic ulcer disease, inflammatory bowel disease, pancreatitis, hypothyroidism, biliary obstruction, and cirrhosis. Levels exceeding 10 ng per mL are rarely due to benign disease (Fletcher, 1996).

1.4.2 Cancer antigen of breast cancer (CA 15.3)

CA 15.3 is a mucinous antigen defined by 2 monoclonal antibodies. These have been raised against human milk fat globule membranes (115 D8) and metastatic breast cancer cells (DF3) respectively. The antigen is an epitope of Polymorphic Epithelial Mucin (PEM), a high molecular weight (300-400 K D), heavily glycosylated protein encoded by the MUC1 gene. Depending on the tissue the level of glycosylation, and therefore, the molecular weight, can vary considerably. While CA 15-3 is a normal product of breast cells, in cancer it is frequently over-expressed and also spread more widely on the cell membrane rather than restricted to the apical surface. Additionally altered glycosylation may expose additional epitopes for recognition (Pamies, *et al.* 1996).

1.4.3 Comparison between CEA and CA15.3

CEA is one of the first tumor markers to be identified and characterized (Sikorska, *et al.* 1988). CEA has been evaluated in a wide range of malignancies, including breast cancer. Several studies have reported that positive serum CEA levels at the time of primary breast cancer diagnosis may represent a negative prognostic parameter (Gaglia, *et al*. 1988) and correlate with the stage of disease (Pavesi, *et al*. 1994). Several authors have shown that an increase or a decrease in the CEA levels may reflect the status of disease progression or regression (Mughal, *et al*. 1983). CEA may be useful in the postsurgical follow-up of breast cancer patients for an early diagnosis of recurrence (Robertson, *et al*. 1999). The availability of the CA 15.3 tumor marker in the last decade has greatly reduced the value of CEA in breast cancer management, and recent studies discouraged the routine use of the CEA assay because of its low sensitivity in both early and advanced diseases compared with CA

15.3. Nevertheless, CEA is still a widely used test for monitoring breast cancer patients (Van Dalen, *et al*. 1996).

1.5 Estrogen

Estrogen (17 β – E 2) is a female sex hormone required for the growth and function of breast cells. Estrogen E2 - also called estradiol- is one of three naturally occurring estrogens (estrone E1, estradiol E2 and estriol E3) and is the most potent of the three estrogens in inducing estrogen effect. It is important for normal sexual development and is essential for the normal functioning of the female organs needed for childbearing such as the ovaries and uterus. Estrogen helps to control a woman's menstrual cycle. It is important for the normal development of the breast. It also helps to maintain healthy bones and the heart. All of these are estrogen target tissues-organs or tissues that estrogen can influence (Massaro, *et al.* 2004). During the childbearing years from puberty to menopause, organs called the ovaries produce estrogen. After menopause, when the ovaries no longer make estrogen, body fat is the primary source for estrogen that is made by the body (Lupulescu, 1995).
The main beneficial effects of estrogen include its role in:
- Programming the breast and uterus for sexual reproduction.
- Safeguarding the heart by controlling cholesterol production in ways that limit the buildup of plaque in the coronary arteries.
- Preserving bone strength by helping to maintain the proper balance between bone buildup and breakdown.

17 β – E 2 is the major estrogenic steroid with a molecular weight of approximately 272, which is secreted by the ovaries. The primary function of E2 is to prepare the uterine mucosa for the presentational stage. It also suppresses the production of FSH and stimulates preovulatory LH release from the pituitary. E2 concentration is frequently

useful in evaluating a variety of menstrual dysfunctions, estrogen producing tumors, feminization in children and cirrhosis. E2 concentration is an essential parameter in the monitoring of the induction of ovulation and ovarian hyper stimulation. In males, E2 level in serum is low. Abnormal levels may be indicative of testicular tumors (Pan, 1994).

1.5.1 Estrogen and breast Cancer

In breast tissue estrogen triggers the proliferation of cells lining the milk glands, thereby, preparing the breast to produce milk if the woman should become pregnant.

In addition to these important beneficial effects of estrogen that are mentioned above, it can also be harmful. The most serious problem arises from the ability of estrogen to promote the proliferation of cells in the breast and uterus (Lupulescu, 1995). Although this ability to stimulate cell proliferation is one of estrogen's normal roles, it can also increase a woman's chance of developing breast or uterine cancer.

During each menstrual cycle, estrogen normally triggers the proliferation of cells that form the inner lining of the milk glands in the breast. If pregnancy does not occur, estrogen levels fall dramatically at the end of each monthly menstrual cycle. In the absence of high estrogen levels, those milk gland cells that have proliferated in any given month will deteriorate and die, followed by a similar cycle of cell proliferation and cell death the following month (Elks, 1993).

When cells acquire mutations in specific genes that control proliferation, such as proto-oncogenes or tumor suppressor genes, these changes are copied with each new generation of cells. Later, more

mutations in these altered cells can lead to uncontrolled proliferation and the onset of cancer (Rosen, 2005).

Although estrogen does stimulate cell proliferation, therefore, if one or more breast cells already possesses a DNA mutation that increases the risk of developing cancer, these cells will proliferate (along with normal breast cells) in response to estrogen stimulation. The result will be an increase in the total number of mutant cells, any of which might thereafter, acquire the additional mutations that lead to uncontrolled proliferation and that lead to cancer (Murphy, 1997).

In other words, estrogen-induced cell production leads to an increase in the total number of mutant cells that exist. These cells are at increased risk of becoming cancerous, so the chances that cancer may actually develop are increased.

Even in women who do not have any mutant breast cells, estrogen-induced proliferation of normal breast cells may still increase the risk of developing cancer. A cell must duplicate its DNA molecules prior to each cell division, thereby ensuring that the two new cells resulting from the process of cell division each receive one complete set of DNA molecules. But the process of DNA duplication occasionally makes mistakes, so the resulting DNA copies may contain a mutation. If one of these spontaneous mutations occurs in a gene that controls cell growth and division, it could lead to the development of cancer (Murphy, 1997).

CHAPTER TWO

MATERIALS AND METHODS

This study was conducted during the period from February 2004 to July 2004; all samples were collected from Sudanese females visiting the Radio Isotope Center, Khartoum (RICK) with the objective of measuring some parameters associated with the development of breast cancer to derive conclusions about the feasibility of early determination and to look into the involvement of these parameters as risk factors for breast cancer.

2.1-Subjects

Ninty Sudanese females their age range from 25-70 years were included in this study. These females were divided into two groups, patients group and the control group.

The patients group included sixty Sudanese females suffering from breast cancer visiting the Radio Isotope Center, Khartoum (RICK). The patients were confirmed as CA breast by histopathology. The control group included thirty healthy Sudanese females.

2.2 Blood sampling

Five ml of venous blood from median cubital vein were obtained from all subjects, then serum was separated and stored frozen at (-20 °C) until analyzed.

2.3 Biochemical methods

The method used to measure serum estrogen (E2) is based on radioimmunoassay (RIA) technique, while carcinoembryonic antigen (CEA) and cancer antigen of breast (CA 15.3) were measured using immunoradiometric assay (IRMA).

2.3.1 Principles of radioimmunoassay (RIA)

Radioimmunoassay (RIA) is an ideal example of limited reagents method. As it uses antibodies at a critical (limited) concentration, based on the principle that Antigen (Ag) and tracer -labeled antigen- (Ag*) compete with a limited amount of antibody (Ab) to form Ag-Ab or Ag*-Ab complexes.

Ag + Ab + Ag* → Ab─Ag (complex) + Ab─Ag* (complex) +Ag + Ag*.

In this reaction the supernatant Ag and Ag* is separated from the complex Ab─Ag + Ab─Ag*, and the activity of this complex is counted using gamma counter. Quantification is made by comparison to known analyte standards set up under identical reaction conditions (Chapman, 1996).

2.3.2 Principles of Immunoradiometric assays (IRMA)

Antibody (Ab) in a solid phase reacts with (Ag); the complex that formed is washed with washing buffer. The tracer–labeled antibody (Ab*) is then added to the complex to form sandwich complex. The supernatant Ag and Ab* is separated from the complex Ab─Ag─ Ab* and then the gamma counter is used to count the activity.

Ag + Ab + Ab* → Ab─Ag─ Ab* (complex) + Ag + Ab*.

2.4 Estrogen determination

This procedure for the direct determination of serum estrogen is based on competitive binding principle of RIA. Standards and patient samples are preincubated with estrogen antibody. ^{125}I-E2 then competes with estrogen in the standards and the patient samples for a fixed and limited number of estrogen antibody sites. After incubation, separation of bound from free is achieved by the precipitate (PR) method. The antibody

bound fraction is precipitated and counted. Patient sample concentrations are read from a calibration curve (Ccuilleronc and Forestm, 1990).

2.4.1 Reagents

- ^{125}I-E2 derivative solution (10ml, red, tracer).
- E2 antiserum solution (10 ml, blue).
- Seven vials of E2 standards, the standards contain respectively 0, 10, 30, 100, 300, 1000, and 2000 (pg/ml).
- precipitating solution (PR).

2.4.2 Assay procedure

- The tubes were labeled in duplicates: T (total counts), NSB (nonspecific binding), A (maximum binding), B through G. Additional tubes were labeled for samples.
- 200 µl of the zero standards A into the NSB and A tubes were pipetted.
- 200 µl of B through G and samples were pipetted into prelabeled tubes.
- 100 µl E2 antiserum were dispensed into all tubes except the NSB and T tubes.
- All tubes were mixed using vortex mixer and incubated for 2 h at room temp. (18-28 °C).
- 100 µl^{125}I- E2 derivative were dispensed into all tubes, and mixed.
- All tubes were incubated for 1 h at room temp. (18-28 °C).
- 500 µl of precipitating solution were added to all tubes (except the T tube), and mixed.
- Then all tubes were incubated for 10 minutes at room temperature.
- Centrifugation was done for 20 minutes and the supernatant was discarded, and the activity was read in gamma counter.

The normal range of females' estrogen is 31- 417 pg/ml.

2.5 Carcinoembryonic Antigen (CEA) determination

Carcinoembryonic antigen (CEA) was measured using the Immunoradiometric assay (IRMA).

The CEA assay is a two-side (sandwich) assay in which two mouse monoclonal antibodies, directed against two different epitopes of the molecules, are employed. Samples or standard are incubated in tubes coated with first monoclonal antibody, in the presence of the second ^{125}I labeled monoclonal antibody. After the incubation, the liquid content of the tubes are aspirated and the excess of unbound labeled antibody is removed by washing. The bound radioactivity measured in gamma counter is proportional to the CEA concentration. Unknown values of the samples to be assayed are determined by interpolation from the standard curve (Bormer, 1991; Nap, *et al*. 1992).

2.5.1 Materials provided

- 1 vial ^{125} I-labeled CEA, red, lyophilized.
- 5 vials CEA standards, lyophilized. Nominal values are as follows:
  5, 10, 20, 40,80 ng/ml.
- 1 vial CEA antibody, blue lyophilized.
- 1 vial precipitation solution.
- 1 vial incubation solution.

2.5.2 Reagents preparation

- 11 ml incubation solution was added to ^{125} I-labeled CEA.
- 1 ml incubation solution was added to each vial of CEA standard.
- 11 ml incubation solution was added to CEA antibody.

2.5.3 Procedure

- The tubes were labeled in duplicates: T (total counts), NSB (nonspecific binding), A (maximum binding), B through F. Additional tubes was

labeled for samples.

- 100 µl of incubation solution were added into the appropriate tubes.
- 100 µl of standards and samples were added in the specific tubes.
- 100 µl of antibody were added into the appropriate tubes.
- All the tubes were mixed gently and incubated at 37 °C for two hrs.
- 100 µl of ^{125} I-CEA were added into each tube.
- All the tubes were mixed gently and incubated at 37 °C for two hrs.
- 500 µl precipitant solutions were added into each tube except T tube.
- All tubes were mixed and incubated at room temperature for half an hour.
- All tubes were centrifuged for 20min.
- The supernatant was discarded.
- The remaining activity of all tubes were counted using gamma counter.

The normal value of carcinoembryonic antigen is below 10ng/ml

2.6 Cancer Antigen of breast CA 15.3 determination

Cancer antigen of breast is measured using the Immunoradiometric assay (IRMA).

2.6 .1 Reagents preparation

- 5ml of distilled water were added to redissolve the standard from A to E.
- The concentrations of the standards were A: 11, B: 28, C: 73, D: 140,
  E: 230 U/ml.
- All the samples were diluted 10 times with dilution buffer.
- The antibody coated tubes were labeled 0 for nonspecific binding and A to E for standard in duplicates and for patients samples S1, S2, …… Sn.

- Two uncoated tubes were labeled for total counts.

2.6.2 Assay procedure

- 200 µl of incubation buffer were added into 0 tube and 150µl of incubation buffer into all other tubes except T tube.
- 50 µl of standard and diluted sample were added to the corresponding tubes.
- The tubes were mixed gently and incubated for two hours at 37°C.
- All the solutions of the tubes were aspirated completely.
- 1ml of distilled water were added to each tube and decanted. This step was repeated again.
- 200 µl of ^{125} I anti CA15.3 monoclonal antibody were added to all tubes.
- The tubes were mixed gently and incubated for three hours at room temperature.
- The solution of all tubes was aspirate completely and all the tubes were washed twice with 1 ml distilled water except T tube.
- The activity was count for 1 minute in gamma counter.

The normal value of cancer antigen of breast is below 40 U/ml.

2.7 Equipments used

1- Syringe, Germany5ml 0.7X30mm/22G X11A.

2- Storage tubes.

3- Tubes: polystyrene assay tubes code No.5001, round bottomed tubes with a capacity of 5ml and Intermediate g.mm, from allied from Amersham International Ltd. Amersham, U.K.

4- Pipettes (25-1000µl) and disposable tips from eppendorf Gmbt, Itamurage 65, Fed, Re p. Gsermany.

5- Adjustable repeating syringe (50 µl - 2ml per shot) and comb tips from eppendorf Gmbt, Itamurage 65, Fed, Re p .Germany.

6- Vortex mixture, model G560-E from scientific industries ink Bohemia N.Y11716, USA.

7- Incubator.

8-.Refrigerated bench top centrifuge model Centra-7R, from International equipment company, 300 second Avenue, need hard high TS, AM, 02194, USA

9- Multi detector gamma counter NE 1612 from nuclear enterprises Ltd. Sight hill Edinburgh, UK.

2.8 Statistical analysis

Statistical analysis was performed using the software Statistical Package for Social Sciences (SPSS) including data evaluation and tests for significance. Graph plots were done via windows excel version.

CHAPTER THREE

RESULTS AND DISCUSSION

Ninty subjects were included in this study divided into two groups, patients group (sixty) and control group (thirty) their ages range from 25-70 years. The patients group was confirmed as CA breast by histopathology.

For all subjects, the serum levels of estrogen E2, carcinoembryonic antigen CEA and cancer antigen of breast cancer CA 15.3 were measured using radioimmunoassay technique.

3.1 Estrogen concentrations

The level of estrogen in the patients group and control is presented in table (1) and Fig. (2). E2 concentrations (pg/ml) measured in this study for the control and patients group were within the normal range. Therefore, no significant ($p = 0.05$) difference in the serum levels of estrogen was detected between the control subjects and the breast cancer patients.

Estrogen plays an important role in breast cancer (Colditz, 1998). Our data showed that no difference between the control and the patients with breast cancer concerning estrogen level. These findings agree with the works of Jones, *et al*. (1987); Reed, *et al*. (1983); Helzlsouer, *et al.* (1994); Sturgeon, *et al*. (2004) who found that no convincing or consistent difference between normal women and women with breast cancer concerning estrogen level.

Although some studies agree with our results, there were some studies which found good associations between the serum levels of estrogen and breast cancer. Toniolo, *et al*. (1995); Hankinson, *et al*.

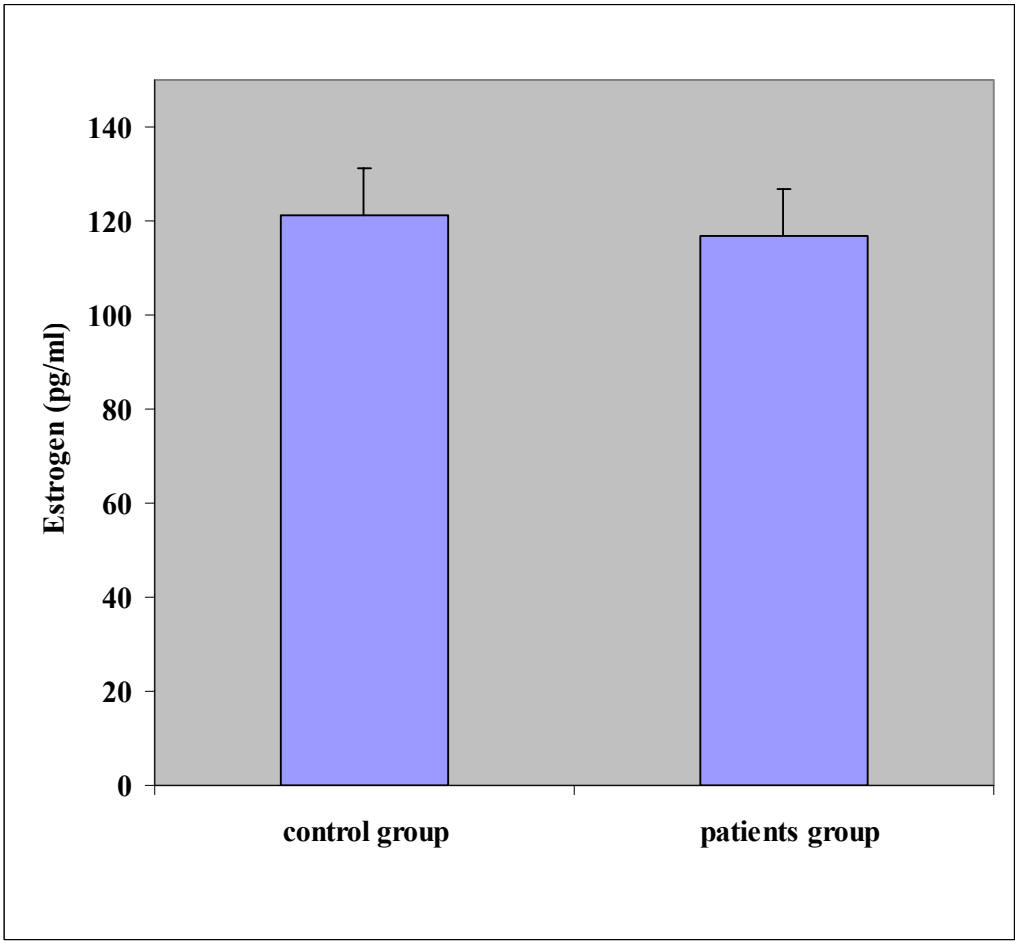
(1998) and Missmer, *et al.* (2004) reported that a positive association between breast cancer and the level of estrogen.

Table (1): The levels of estrogen, carcinoembryonic antigen and cancer antigen of breast cancer in breast cancer patients and control subjects

E2 (pg/ml) Mean ± SD		CEA (ng/ml) Mean ± SD		CA 15.3 (U/ml) Mean ± SD	
control	Breast caner patients	control	Breast caner patients	control	Breast cancer patients
121.10±84.15402[a]	116.65±127.1558[a]	28.4±41.22914[a]	44.50±76.59674[a]	16.30±6.78349[a]	59.16±78.4194[b]

Means within the same raw having different superscript letters are significantly different at ($p<0.0001$).

Fig. (2): Estrogen levels in patients with breast cancer and control subjects



3.2 CEA concentrations

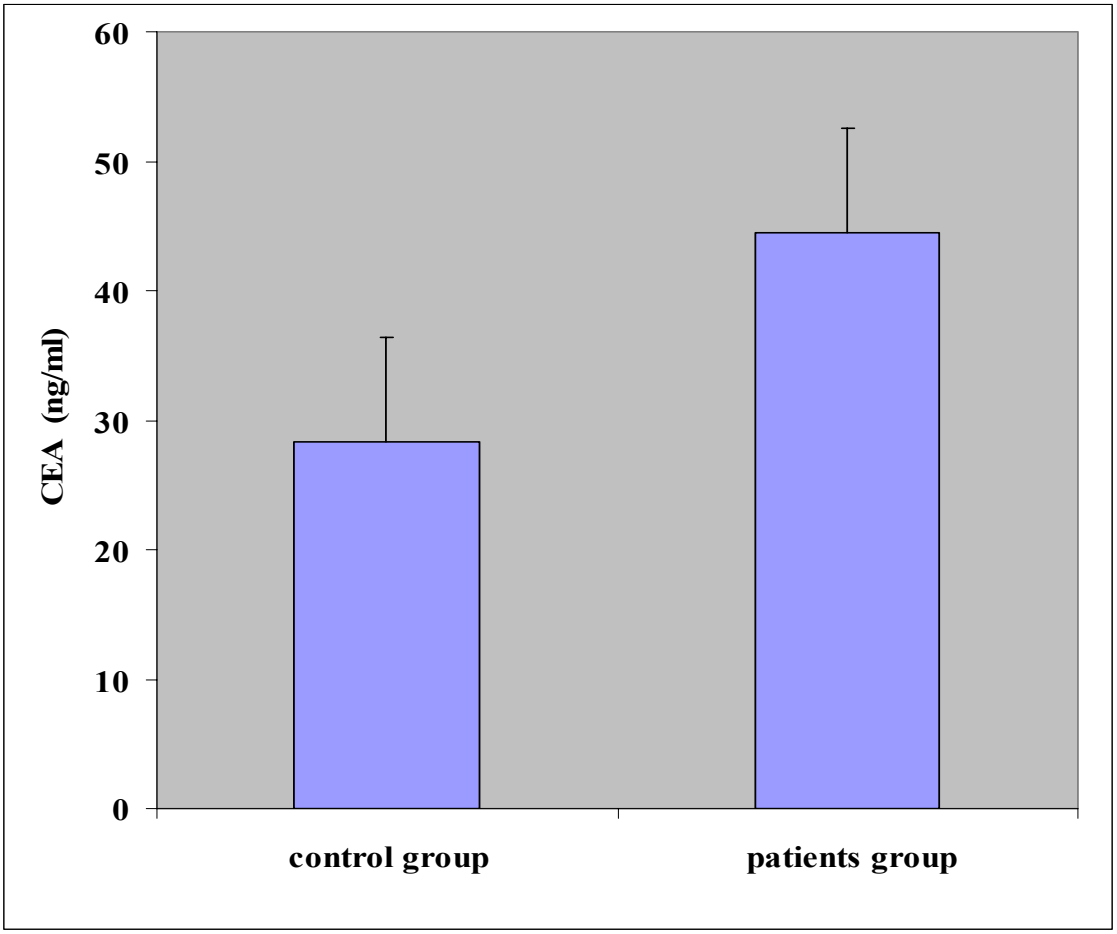
As shown in table (1) and Fig. (3), the CEA mean values were non-significantly ($p>0.05$) higher in patients group compared to the control group.

These results agree with Saccani, *et al.* (1988) and Helier, *et al.* (1990) who found that no significant difference between the patients with breast cancer and the control.

Incontrast to our results, Hayes, *et al.* (1986) and Norum, *et al*. (2001) found a significant elevation in the serum levels of CEA in patients with breast cancer than in the control.

CEA as a tumor marker is not a very precise tool for breast cancer diagnosis when it is used alone, especially for early breast cancer. Therefore, by combining it with another tumor marker such as CA 15.3, the efficiency of CEA can be improved (Omar, *et al.* 1988).

Fig. (3): Carcinoembryonic antigen levels in patients with breast cancer and control subjects



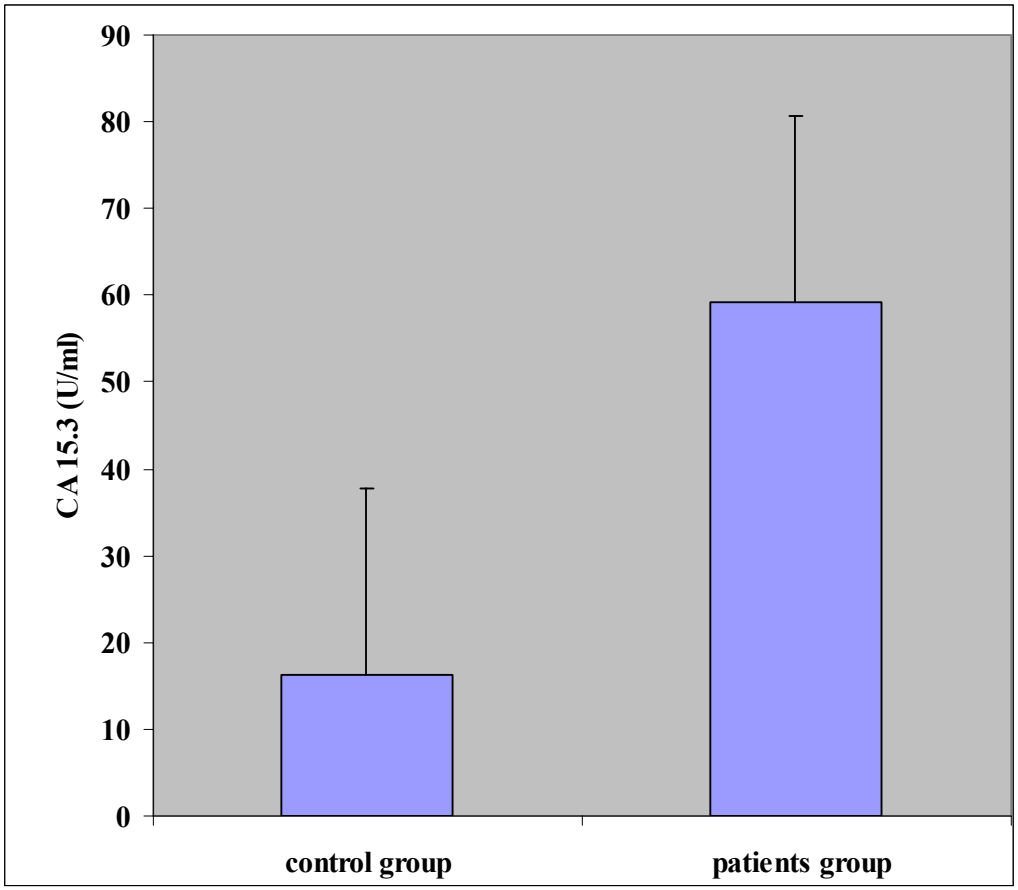
3.3 CA 15.3 concentrations

CA 15.3 concentrations were presented in table (1) and Fig. (4). The level of CA 15.3 is highly significant ($p < 0.0001$) in the patients with breast caner compared to the control.

This result is in agreement with the Hayes, *et al*. (1986) and Gion, *et al*. (1991) who reported a highly significant difference between the patients suffering from breast cancer and the control.

The result obtained in our study confirmed that CA 15.3 is a sensitive tumor marker for the evaluation and monitoring of patients with breast cancer (Hayes, *et al*. 1986).

Fig. (4): Cancer antigen of breast levels in patients with breast cancer and control subjects



3.4 Conclusions and Recommendations

It is concluded from this study that the level of CA 15.3 could be used for the early detection and diagnosis of breast cancer. Since it was significantly increased in the patients of breast cancer compared to the control.

More investigation is needed in this area, such as the relation between breast cancer and free estradiol, some hormones such as prolactin and progesterone. It is preferable that the measurement of the serum levels of hormones is supported by the measurement of the receptors so that the finding will be more reliable.

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