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ANALYZING THE ROLE OF OBESITYY AND MENSTRUAL STATUS IN DEVELOPING BREAST CANCER

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ABSTRACT

Breast cancer is unrestrained growth and growth of cells that start in the breast tissues. It is most commonly diagnosed cancer in women and is chief cause of cancer death in women in the world. Prevention of breast cancer is a big challenge for the world. Best prevention of breast cancer is early diagnosis, which results in increasing survival rate of breast cancer patients. Researches in the field of breast cancer has made remarkable progress in last two decades, which results in better understanding of the disease, increased public awareness, early diagnosis with improved and modern screening methods, effective and less virulent treatments and improved survival rate. These discoveries are an important step forward in the ongoing battle against breast cancer. In this study, division of breast cancer into different types on the basis of invasiveness (invasive, non-invasive) and frequency (common and rare), stages of breast cancer on the basis of invasiveness, risk factors associated with breast cancer, how to manage breast cancer including different treatments for different categories of patients, all were discussed in detail. Statistical analysis showed that there were no significant differences in the results of patients with obesity related to their menstrual status when compared to healthy controls.

Keywords: Ductal Carcinoma In Situ, Lobular Carcinoma In Situ, Tris- Acetate EDTA.

I. INTRODUCTION

Cancer is usually termed after its appearance in the body part. So, Breast cancer infers to the uncontrolled growth & multiplication of cells which begin in the breast tissue (Khuwaja and Abu-Rezq, 2004).

Human breast has 2 kinds of tissues: glandular & stromal tissues. Glandular tissues consist of milk producing gland and ducts and stromal tissues are the fat tissues & fibrous connective tissues. Also, there are lymphatic tissues which are part of the immune system that moves lymph into circulatory system, is also found in the breast (Sharma et al., 2010).

Breast cancer is most recurrent malignant tumor in the world, responsible for 10.4% of all cancer-causing deaths in females aged between 20 and 50 (Siegel et al., 2019).

Breast cancer is the most common cancer in the world in females overall. In 140 of 195 countries in the world, breast cancer is most frequently diagnosed in women (McGuire, 2016).

Breast cancer is a progressive disease that can spread to the organs which are far apart from its origin like brain, liver, bone & lung, which make the treatment difficult almost impossible to cure. Preventive measures & early detection using modern techniques can give high survival rate. like in North America, they have 5-year comparative living rate higher than 80% because of early diagnosis of cancer (DeSantis et al., 2016).

Many variables, including gender, age, estrogen, familial history, gene alterations and mutations, and an unhealthy and poor lifestyle, might raise the chance of getting breast cancer (Majeed et al., 2014).

The majority of breast cancer cases occur in females, and the rate in females is 100 times greater than in males (Siegel et al., 2018).

Obese women are more likely to be identified at a later stage of cancer, with larger tumors and nodal involvement, but research findings have shown that obesity has an independent predictive influence on the chance of re-occurrence, free of illness surviving capability, and on the whole surviving capacity (Majed et al., 2008).

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According to the WHO website, 2.3M women were diagnosed in 2020 with breast cancer. Breast carcinoma has been detected in 7.8M females globally in the last 5 years, making it the most common cancer in the world. Breast cancer causes the most disability-adjusted life years in females across the world. Breast carcinoma affects the women of any age after adolescence in all country on the earth, with frequency increasing with age. For all of these motives, a reliable and accurate method is required to help in the early identification and diagnosis of breast cancer illnesses in order to limit the mortality rate. Machine-learning techniques may be used extensively in the field of medical analysis (Dahiwade et al., 2019).

Lots of genes are responsible for breast cancer in humans. Oncogene & anti- oncogene mutations & deviant amplification is responsible for tumour initiation or carcinogenesis (Sun et al., 2017).

Estrogens, both endogenous and exogenous, have been linked to an increased risk of breast cancer. In premenopausal (around menopause) women, the ovary produces endogenous oestrogen, and ovariectomy (removal of an ovary or ovaries) can lessen the risk (Hormones and Breast Cancer Collaborative Group, 2013).

Uncontrolled use of alcohol & a diet containing high amount of fat are risk factors that can raise the risk of breast carcinoma. Excessive alcohol intake can increase the estrogen-related hormones in the blood; also it can activate the oestrogen receptor pathways, which is linked to an increased risk of breast cancer. According to some studies, drinking 35 to 44 grammes of alcohol each day raise the breast cancer risk by 32% approximately, with the intake of each extra ten grammes of alcohol per day raise the risk by 7.1 percent approximately (Collaborative Group on Hormonal Factors in Breast Cancer, 2002; Jung et al., 2016). Excess fat consumption in the modern diet, particularly saturated fats, is linked to poor diagnosis in patients with breast cancer (Makarem et al., 2013). Evidence suggests that tobacco smoking, especially at a young age, increases the breast cancer risk (Catsburg et al., 2015; Kispert and McHowat, 2017).

Types of the Breast Cancer

Non-Invasive Type of the Breast cancer

Cancer that confined to the ducts and do not extend to the other fat and connective tissues of the breast. The earliest and most common kind of breast carcinoma is ductal carcinoma in situ (DCIS) (90% of cases), which is non-invasive. LCIS which is Lobular carcinoma in situ is infrequent type of the breast carcinoma that is non-invasive and considered as risk factor of breast cancer disease (Olcina et al., 2008).

Invasive Type of the Breast Cancer

Cells which infiltrate the neighbouring fat tissues & fibrous connective tissues of breast after advancing the duct and lobular walls. Cancer must not spread to the lymph nodes or else other organs to be insidious Invasive breast cancers include infiltrating ductal carcinoma, Medullary carcinoma, and infiltrating lobular carcinoma. (Olcina et al., 2008).

Signs and Symptoms

The most common sign to identify breast cancer is a bump in breast or armpit. Women should do monthly self-examination of their breast and become familiar with the texture, periodic changes, size, color, shape, and skin state. Symptoms like swelling in the breast, lump or mass, armpit swelling in lymph nodes, nipple pain, nipple skin changes, scaly or pitted skin around nipples, clear or bloody fluid discharge from nipple,a continuous tenderness of breast, breast pain or discomfort, all these can be the signs of breast cancer. Underarm lymph nodes are present in the advanced stage of illness, along with other symptoms such as bone pain, shortness of breath, lack of appetite, unintended weight loss, migraines, neurological discomfort or fatigue (Stephan, 2007).

Symptoms are classified into three major symptom categories:

- Breast lump
- Non-lump breast symptoms including pain in breast, breast shape or skin deformities abnormalities and nipple abnormalities.
- Non-breast symptoms including tiredness, breathlessness, axillary symptoms, neck lump, and pain in back (Koo et al., 2017).

Previous research shows that, non-lump breast symptoms to non-malignant factors such as hormonal fluctuations, trauma, or nursing are the causes of delayed diagnosis and delayed help seeking in women (Ramirez et al., 1999; O'Mahony et al., 2013; Khakbazan et al., 2014).

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Among all,

- The most frequent symptom is breast lump, which was reported by around 83% of all women having breast cancer.
- Nipple anomalies are at second number, with 7% reported symptom in women.
- Breast pain is at 3rd with 6%.
- Other abnormalities like breast skin, shape and texture changes are reported in 2% of women patients (Koo et al., 2017).

Majority of women belonged to one of four symptom categories:

- 'Lump only' (76%);
- 'Non-lump only' (11%);
- 'Both lump and non-lump' (6%);
- 'Non-breast symptoms' (5%) (Koo et al., 2017).

Breast cancer risk is linked with the age at first menstruation and the age at last menstruation, according to epidemiologic research (Bernstein and Ross, 1993).

Both of these factors determine the number of menstrual cycles a woman has in her lifetime and, as a result, the quantity of ovarian hormones she is exposed to during each cycle. A lower number of lifelong ovulatory cycles have been recommended as a source to lessen the chance of having breast tumours (Henderson et al., 1985).

There are two phases to each menstrual cycle: follicular and luteal. Progesterone levels are low in the follicular phase, whereas oestrogen levels rise in expectation of ovulation. Both progesterone and oestrogen are increased during the luteal period. The luteal phase has a generally constant length, but the follicular phase has a wide range of lengths. As a result, women whose menstrual cycles are longer spend a lot of time in the follicular phase than women whose menstrual cycles are shorter (Aron et al., 2001).

The luteal phase has much higher breast cell proliferation than the follicular phase. Following that, epidemiologic research has shown that combining exogenous oestrogen with progestin elevates the chances of breast carcinogenesis above and above the effect of oestrogen alone. These studies focused on postmenopausal women, and it's unclear whether the same exogenous hormone combos affect premenopausal breast cancer risk.

Women who spend a lower proportion of their menstrual cycle in the luteal phase due to a prolonged follicular phase or anovulation might have a lower chance of having breast carcinomas since oestrogen and progestin combined enhance breast cell proliferation (Masters et al., 1977; Meyer, 1977; Colditz et al., 1995; Rossouw et al., 2002).

It is thought for a long time that estrogen performs a primary role in the genesis of breast tumors in women who are overweight. Estrogens are necessary for the development and maintenance of a healthy breast. They encourage cell proliferation and growth. Estrogen, on the other hand, has been linked to the induction and maintenance of breast cancer in studies. By boosting estrogen levels, having more fat tissue increases your risk of breast cancer (Kendall et al., 2007).

A strongly important pattern of raising chances of mortality as a consequences of breast malignancies with greater BMI (kg/m2 values) was discovered in prospective research of almost 350,000 United States females (Calle et al., 2003).

The International Breast Cancer Study Group has done seven randomized clinical trials involving 6,792 eligible patients. Body mass index had no impact on illness-free surviving capability but also had an important and crucial detrimental impact on the whole surviving rate (Berclaz et al., 2004).

Obesity was independently associated predictive parameter for illness-free surviving ability along with as a whole surviving rate in a retro-active multi-levelled assessment of 2,887 cases with breast malignancies that were node-positive and were included in the International Group of Breast, which included docetaxel addition to doxorubicin-dependent chemotherapeutics (De Azambuja et al., 2010).

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II. MATERIALS AND METHOD

In this research, about 2–3ml of blood was collected from each participant (patients and controls). Blood was collected in vials containing EDTA from each enlisted individual. Blood as a whole was used for isolating DNA. For this, standard processes (salt extraction method) were applied with some changes. Blood samples that were obtained were stored and frozen in a freezer at -20° Centigrade for later use. Working with blood can be hazardous, some of the times blood may act as biohazard, therefore proper precautions were taken when working with it. Separated deoxyribonucleic acid (DNA) was measured using an ethidium bromide gel and stored at -20 °C for later use. Relevant rules and guidelines were followed in all processes performed.

Preparation of Reagents

109.54 g of sucrose (0.32 M), 0.321 g of Tris-HCl (10 mM) and 1.016 g (5 mM) of MgCl2 diluted with deionized distilled water to form the total volume of 900 ml for Lysis buffer A. After the solution was autoclaved, 10 ml of Triton X 100 was added and shaken for 30 minutes until a uniform mixture was achieved. The final pH was 7.6 and kept at 4 °C.

5.844 g (100 mM) of NaCl, 1.488 g (4 mM) of disodium ethylene diamine tetra- acetic acid, 2.422 g (20 mM) of Tris-HCl, and 10 ml SDS diluted with autoclave distilled water in a total volume of 100 ml for Lysis buffer B. The final pH was regulated to 7.4 and the final volume was 1000 ml was made. After that, it was kept at room temperature.

DNA isolation

Total number of 38 blood samples (31+7) was obtained from the sick-people suffering from this disease and from the healthy controls, respectively. They were kept at 4 °C. In this research, DNA was obtained using the modified salt technique, which is a simple and non-toxic DNA extraction technique.

- Cold buffer A and blood with ratio (1:1) were mixed in a 15-millilitre falcon vessel, in which two volumes of cold sterile distilled water was added. (Because a large number of samples were to be treated, a master mix was created in sterile glassware).
- 6 to 8 times, the tube was inverted. It was kept on ice for 10-15 minutes. As a result, the membrane of red blood cells became weak.
- After that, a 15-minute centrifugation at 3500 rpm (4°C) was conducted.
- Just after the centrifugation was completed, two-third of the supernatant from the other side of the pellet containing red blood cells was carefully dumped into 10% bleach solution. Because red blood cells lacked a nucleus and genetic material.
- The remaining white blood cell solution was resuspended in lysis buffer A (2 ml) and cold sterile distilled water (6ml). (Because a large number of samples were to be treated, a master mix was created in sterile glassware).
- The above mixture was centrifuged for almost 15 minutes at 3500 rpm (4°Centigrade). Then supernatant was gently removed without dislodging the pellet.
- The washing process was done several times until a white creamy pellet was obtained.
- The pellet obtained in the previous stage was treated with Lysis buffer B (5ml) and SDS (500 µl). The pellet was re-suspended by aggressively vortexing for 30-60 seconds, or until totally dissolved. The white blood cell membrane and nucleus membrane were lysed using Lysis buffer B.
- The liquid containing the pellet was then inverted 2-3 times with a 25 µl solution of proteinase K (20 mg/ µl).
- In a water bath, the proteinase K containing pellet was incubated over night at 45°C or for 2 hours at 55°C.
- After the removal of samples from water bath, they were kept on ice for 10 minutes.
- After that, 4ml solution of 5.3 M NaCl was added. If a hazy solution was not detected, the samples were put back on ice. The samples were vortexed for 15 seconds.
- Then it was instantly centrifuged at 4500 rpm (4°C) for 20 minutes. The supernatant was then transferred into a 15 ml falcon tube without dislodging the pellet.
- At 4500 rpm for 20 minutes at 4°C this supernatant was centrifuged again. And then instantly transferred to the fresh 50 ml falcon tube.

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- An equal volume of cold isopropanol kept at -20°C was poured to the falcon tube and gently inverted 5-6 times to precipitate DNA or kept in the freezer over night.
- The sample was centrifuged at 4500 rpm (4°C) for 20 minutes.
- Finally, at the bottom of falcon tube, a little volume of the pellet in the form of a white cluster of DNAs was shown. After the centrifuge procedure, the supernatant was removed and 1000 µl of ethanol (70 percent) was added to the remaining pellet.
- This mixture was centrifuged at 4500 rpm (4°C) for 10 minutes, and supernatant was discarded instantly without dislodging pellet.
- The place of the DNA on the tube was marked. The DNA in the falcon tube was dried entirely at room temperature for 15-20 minutes or in an incubator for 10 minutes.
- After drying, 300-400 µl of low TE buffer/sterile distilled water was added and left overnight to dissolve the DNA.
- On the same day or a few days following DNA isolation, at 70°C the DNA was heated in a water bath for 1 hour to inactivate any residual nucleases.
- Kept the DNA solution at -20°C for further use.

DNA analysis

The obtained DNA's quality was tested using agarose gel electrophoresis and spectrophotometry. The procedure used for gel preparation is explained below:

Gel preparation

- Agarose was mixed with TAE buffer to make the solution. 0.70g agarose was measured and put to a 100 ml conical flask containing 75 ml of 1x TAE buffer.
- To dissolve the agarose, the mixture was heated for 1-2 minutes in a microwave oven or in a hot water bath. (In case of microwave oven, care should be taken, because the solution might over boil and spill out of the flask).
- The temperature of the solution was reduced to around 60°C by rotating the flask occasionally for 5 minutes to cool evenly.
- To the melted agarose, 15 µl ethidium bromide was added (ethidium bromide intercalates to the DNA and can easily be seen under UV light).
- The casting tray's ends were sealed, and the 16-well comb was properly placed.
- After pouring the agarose TAE solution into a mould, it was allowed to cool and harden and milky white color was obtained.
- The comb and sealing were carefully removed. The gel was put in a gel box that contained TAE buffer. The gel was placed so that the chamber wells were nearest to the chamber's negative electrode.
- The electrophoresis chamber was filled to roughly 2-3 mm above the gel with 1x TAE buffer (20 ml 50x TAE buffer in 980 ml distilled water).

Sample preparation

2µl loading buffer was added to each of the 5 µl samples of DNA Loading buffer served two needs:

- It was helpful in gel loading due to the colored dye and allowed to visualized how far the DNA had moved;
- It contained glycerol, which increased the thickness of the DNA sample and caused it to settle at the bottom of the gel well rather than diffusing in the buffer.
- The DNA samples were carefully loaded into the gel's wells. Each sample's order was recorded.

Agarose Gel Electrophoresis

- After adding samples, the gel box was closed with a lid and the electrodes were attached.
- The electrode wires were linked to the power source, and the electrodes: the black negative end (cathode) and the red positive end (anode) were properly attached. The negatively charged DNA samples began to move across the gel towards the anode (red). The negative electrode was positioned on the side where the DNA samples were deposited.
- The power source was set at 80-100 volts. The most severe allowable voltage varies with the size of the electrophoresis chamber. It should not exceed 5 volts/cm between cathode and anode.

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- The gel was run until the samples had travelled two-thirds of the way.
- Then the electricity to the chamber was turned off. The electrode wires were disconnected from the power supply. The cover of the electrophoresis chamber was lifted.
- The tray of gel was carefully removed from the gel box and put on the UV trans-illuminator for DNA analysis. The photograph was taken to show DNA fragments of various sizes.

Spectrophotometric analysis of DNA

Extracted DNA was subject to spectrophotometric analysis. Absorbance at 260 (A260) showed the quantity of DNA and the ratio of absorbance at 260 and 280 nm (A260/A280) showed the quality of DNA.

After calculation of concentration of DNA for each DNA sample. The DNA aliquots having concentration (50ng/µl) were prepared and kept at -20°C.

Statistical analysis

Questionnaires were studied and data was analyzed using SPSS software. Chi- square test was applied on the risk factors (obesity & menstrual status) found in breast carcinoma patients and compared with healthy controls.

III. RESULTS AND DISCUSSION

The study population comprised of total subjects (n=38), consisted of 31 cases and 7 controls. Parameters studied were Obesity and Menstrual Status including pre- menopausal and post-menopausal females.

Results for Obesity

In the current study, total 23(100 %) were cases and 7(100 %) were controls. Out of 23 cases, 6(26.1%) were obese and 17(73.9%) were not obese, whereas out of 7 controls 0(0.0%) were obese and 7(100.0%) were not obese. The statistical analysis showed no significant difference between two groups (X2=2.283, p=0.131) (Figure 07).

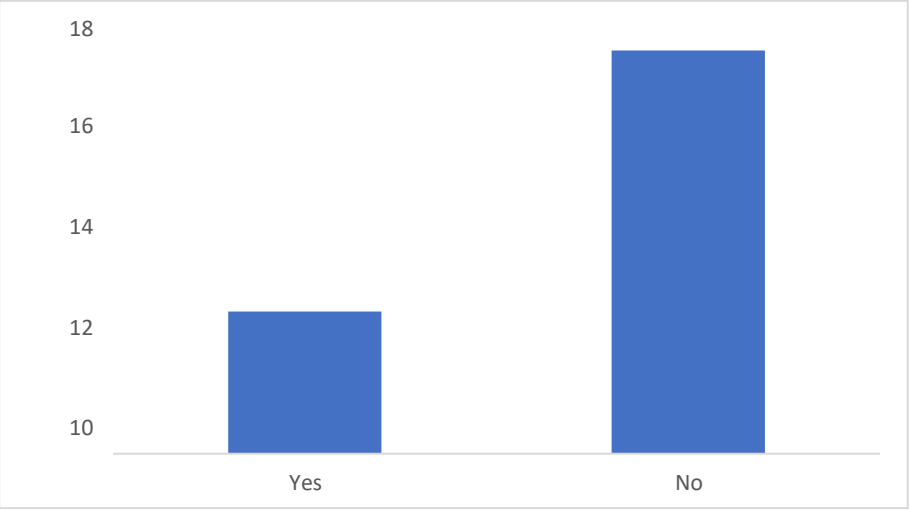

Figure 1: Obesity status

Results for Menstrual status

In the current study, total 16 (100%) were cases and 7 (100%) were controls. Out of 16 cases, 6(37.5%) were pre-menopausal females and 10(62.5%) were post- menopausal females, whereas out of 7 controls, 5(71.4%) were pre-menopausal females and 2(28.6%) were post-menopausal females. The statistical analysis showed no significant difference between two groups (X2=2.246, p=0.134) (Table I).

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Table 1: The pre-menopausal and post-menopausal females shown in number (%).

Parameters	Controls (n=7)	Cases (n=16)	Chi square value	p-value
Menstrual Status				
Pre-menopausal	5(71.4%)	6(37.5%)		
Post-menopausal	2(28.6%)	10(62.5%)	2.246	0.134

IV. DISCUSSION

In this study a comparison between menstrual status and obesity with the probability of having breast carcinomas have been evaluated. The purpose of this study is to analyze the link of menstrual status (premenopausal & postmenopausal) and obesity of different patients and how it affects the risk of breast cancer.

According to the results of this study, obesity is not much related with the risk of breast carcinoma in premenopausal and postmenopausal females as there were only fewer women in the data of this research who were obese in the pre-menopausal and post-menopausal stage. On the other hand, in the findings of different researcher's adult obesity is related with higher risk of having breast cancer in postmenopausal females, for every 5 kg per m2 there is an increase in 12 to 13 percent risk of breast cancer (Wiseman, 2008; Renehan et al., 2008), while childhood obesity work as a preventive measure against the chance of having breast cancer in premenopausal females. Furthermore, a negative and worst effect of obesity has been reported by many researchers in their previous findings which is highly related with the chance of having breast cancer (Liu et al., 2018).

The relationship of obesity and menstrual status with the risk of breast cancer could be affected by adding height in the data analysis, as most researchers used body mass index instead of only obesity, which is adversely linked with the chances of having breast carcinomas in older aged females (Liu et al., 2018).

In spite of the fact that many past studies have demonstrated that having a higher body mass index increases the risk of postmenopausal breast cancer, some studies have revealed that there is no relation between obesity and breast carcinoma risk (Hunter and Willett, 1993; Huang et al., 1997; van den Brandt et al., 1997).

Studies have shown that smaller tumors are identified more frequently in thinner women (Kusano et al., 2006), but no research has demonstrated that current weight, or weight gain, is related to invasiveness of the disease, also the larger breast volume will not increase the risk of breast cancer regardless of weight (Egan et al., 1999).

Although most of the studies have shown that there is an inverse relation between obesity and risk of having breast cancer in premenopausal women, but there can be no medical procedure that consider weight gain as a treatment, there are lots of complications associated with excess fat, having more fat tissues will increase the risk of breast cancer by increasing the estrogen level also obese women tend to have higher level of other hormones like insulin which is associated with higher risk of recurrence and mortality rate in women with early stage breast cancer (Bergström et al., 2001).

The effect of obesity and menstrual status on breast cancer risk is still controversial, some studies have been confirmed but most are still hypothesized because the results are inconsistent. Accurate mechanism in which the obesity shows reduced risk of breast cancer in premenopausal women and shows higher risk after menopause is still elusive (Vona-Davis and Rose, 2012).

V. CONCLUSION

Breast cancer is the uncontrolled expansion and expansion of cells that begin in the breast tissues. It is the kind of cancer that affects women the most often and is the leading global cause of cancer mortality in females. Breast cancer prevention is a major global concern. Early detection is the best defence against breast cancer, and it increases patients' chances of survival. The division of breast cancer into various types according to its invasiveness (invasive, non-invasive), frequency (common and rare), stages according to its invasiveness, risk factors connected to breast cancer, and how to manage breast cancer, including various treatments for various patient categories, were all covered in detail in this study. When obese individuals were compared to healthy

controls, statistical analysis revealed no significant variations in the outcomes connected to their menstrual status.

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