Estrogen fluctuation of postmenopausal Risk leading to Breast Cancer in Pakistani population

By

UFFAQ NAZ

MASTER OF SCIENCE IN BIOSCIENCES

DEPARTMENT OF BIOSCIENCES
Capital University of Science and Technology
Islamabad
2017

Estrogen fluctuation of postmenopausal Risk leading to Breast Cancer in Pakistani population

By

UFFAQ NAZ

A research thesis submitted to the Department of Biosciences,
Capital University of Science &Technology, Islamabad
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE IN BIOSCIENCES

DEPARTMENT OF BIOSCIENCES
CAPITAL UNIVERSITY OF SCIENCE & TECHNOLOGY
ISLAMABAD
2017

CAPITAL UNIVERSITY OF SCIENCE & TECHNOLOGY
ISLAMABAD
Islamabad Expressway, Kahuta Road, Zone-V, Islamabad
Phone: +92 51 111 555 666, Fax: 92 51 4486705
Email: info@cust.edu.pk, Website: http"//www.cust.edu.pk

CERTIFICATE OF APPROVAL

Estrogen Fluctuation of Postmenopausal Risk Leading to Breast Cancer in Pakistani population

by

UFFAQ NAZ
MBS153007

THESIS EXAMINING COMMITTEE

S No	Examiner	Name	Organization
(a)	External Examiner	Dr. Thair Ahmed	NUST, Islamabad
(b)	Internal Examiner	Dr. Sahar Fazal	CUST, Islamabad
(c)	Supervisor	Dr. Shaukat Iqbal Malik	CUST, Islamabad

_____

Dr. Shaukat Iqbal Malik
Thesis Supervisor
October 2017

_____

Dr. Aamer Nadeem
Head
Department of Biosciences
Dated : October 2017

_____

Dr. Muhammad Abdul Qadir
Dean
Faculty of Health and life sciences
Dated: October 2017

Certificate

This is to certify that, this thesis submitted by Miss Uffaq Naz is accepted in its present form by the Department of Bioinformatics & Biosciences, Capital University of Science & Technology, Islamabad, Pakistan; as satisfying the thesis requirements for the degree of Master of Science In Biosciences.

Forwarded for necessary action

Dr. Shaukat Iqbal Malik

(Thesis Supervisor)

.

.

Declaration

The material and information contained in this thesis is my original work. I have not previously presented any part of this work elsewhere for any other degree.

Uffaq Naz

Dedication

This thesis is dedicated to my mother, **Mrs. Shaheen Akhter**, my sister **Ms. Sumbal Kiran,** my brother, **Mr. Hamza Ali**, who is always support, encouragement, and constant love have sustained me throughout my life. I would like to dedicate this thesis to my best friend, **Mr. Muhammad Nadeem.** Thank you for always believing in me, even when I did not, and cheering me on until the end. Words cannot express how much I love you all and appreciate everything you all have done for me.

List of Contents

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

HER2	Human epidermal growth factor receptor 2
ER	Estrogen receptor
DCIS	Ductal Carcinoma In Situ
IDC	Invasive Ductal Carcinoma
ILC	Invasive Lobular Carcinoma
IBC	Inflammatory Breast Cancer
BBD	Benign Breast Disease
BRCA1	Breast CAncer gene one
BRCA2	Breast CAncer gene two
PEC	Pectoralis muscles
ELISA	Enzyme-linked immunosorbent assay

ACKNOWLEDGMENT

I bow my head before **ALLAH Almighty** in gratitude who blessed me the courage to complete this thesis. My whole life moments are devoted for his praise. All respect and regards to the holy prophet **Hazrat Muhammad (peace be upon him),** who came as guider towards the real destination.

I am very thankful to my supervisor **Dr. Shaukat Iqbal Malik**, for giving me a present supervision during this thesis work. His continuous guidance, discussion and support especially at my hard time made this project work possible.

I am highly obliged in my deepest and sincerest gratitude to my respected teacher **Dr. Sahar Aqads** for her keen interest, benevolent attention, vital and intellectual suggestion in my thesis work. I would never forget her polite and persistent support.

I am thankful to **Dr.Aamer Nadeem**, Head of Department of Bioinformatics and Biosciences, Capital University of Science & Technology, Islamabad, for Provision of all possible facilities. My deepest thanks go to **Sir Shahid Hussain**, Lecture, and Department of Bioinformatics & Biosciences. I would like to thank all the staff member of **Pakistan Institute of Medical Sciences (PIMS) Islamabad.**

Uffaq Naz

ABSTRACT

Cancer is currently a global issue. It is the vital health problem in the different regime of the world. It is an uncontrolled proliferation of cells followed by mal function of the cell cycle check points. Breast carcinoma is a heterogeneous disease and starts in the breast tissue that is the reason it is called breast cancer. Hormones are the chemical messenger that goes into the circulatory system from an endocrine organ to another organ or gathering of organs to manage an extensive variety of physiological procedures. Estrogen is a female sex hormone that is responsible for physical feature and reproduction. Most important cause of breast carcinoma is a hormonal factor which also known as reproductive and menstrual factors. Hormones are showing significant reasons for carcinoma. The hormonal imbalances such as estrogen imbalance can become one of the reasons for breast cancer. The risk factor that involves the premenopausal women have low out comes compared to the post menopausal women. This researched was design to addressed estrogen hormonal level in post menopausal breast cancer woman with reproductive factors in Pakistani population. Results were analyzed through SPSS version 19 and estrogen hormone level was linked with age and tobacco usage in post menopausal woman breast cancer in Pakistani population. Therefore this research could be used for broader perspective estrogen level in Pakistani population.

CHAPTER 1

INTRODUCTION

Cancer is currently a global issue. It is the vital health problem in the different regime of the world (Siegel, Ward, & Brawley, 2011). It is an uncontrolled proliferation of cells followed by mal function of cell cycle check points (Saeidpour & Torabizadeh, 2013). It developed from normal cells that have the ability to proliferate unexpectedly and ultimately turn malignant. These cells grow clonally into tumors and ultimately have the ability to metastasize ("The biology of cancer stem cells," 2007). Tumor is subdivided into two types such as benign and malignant. A tumor which does not spread is called a benign tumor. A tumor which does spread and is invasive in nature is called malignant (Zaman, 2010). A malignant tumor is life alarming; it has developed due to the carcinoma of the mammary gland by malignant cells (Memon, Khan, Raza, & Noor, 2014).

Tumors are named after the part of the body from which they begin such as breast cancer starts in the breast tissue that is the reason it is called breast cancer (Khuwaja & Abu-Rezq, 2004). It's often maximum going on women cancer, and major public health problem worldwide. It is uncommon in men, with an occurrence less than 1% that of female breast cancer because males do not create milk delivering breast, although it is uncommon in man's but their breast cells and tissue can still produce disease (Siegel & Naishadham, 2013). It is common malignancy in women's causing 459,000 deaths (Youlden, Cramb, Dunn, & Muller, 2012).

In order to metastasis cell need ability to migrate and invade into surrounding tissue cells. After invading they circulate into blood vessels or lymphatic system. The lymph system has an important role in extending the Carcinomas. They carry the blood away from the gland. Lymph are little ball like collection of immune responses which helps fighting against pathogens moreover they are vessels extending throughout body like other vessels (vein, artery, nerve). Besides the pathogens lymphatics also carry the waste material like pus and membrane fluids. Lymph nodes are growing because breast tumor cells can penetrate lymphatic vessels. In the

axillary region the lymph nodes and vessels are numerous. Inside the Pec muscles (pectoralis major and minor) known as the inner lymph nodes. These vessels are attached to lymph nodes, infraclavicular nodes either above or below the collarbone.

During mammary gland development different biological process occur in the mammary gland which also takes part in breast cancer development and progression (Ercan, Diest, & Vooijs, 2011). Every breast has lobes consisting of 15-20 sections. These lobes are subdivided into many smaller sections known as lobules. The lobules and lobes are joined by thin tube are known as ducts and drainage into nipples (Israyelyan, 2003).

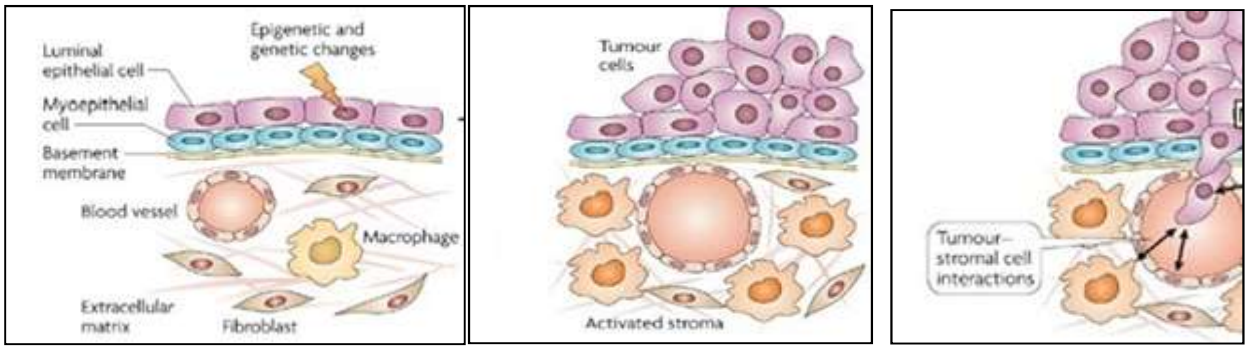

Figure 1 tumor cells progression and metastasis in normal cells.

The whole breast is lined by two types of cells epithelial and myoepithelial cells. If the mutation is occurring in the cell, tumor cell grows and develop and it has the possibility to proliferate neighboring tissue and other organ due to the genetic mutation. These cells grow faster than surrounding cells such as shown in (Figure1). The lumen of each duct is surrounded by luminal cells and formed cuboidal or columnar epithelial cells layer. Myoepithelial cells provide support and contract to move milk during breastfeeding (Polyak & Hu, 2005). The malignancy development in lines of ducts known as ductal growth and it is the most common kind of breast tumor in distinctive populations (Wasif, Maggard, Ko, & Giuliano, 2010).

Breast carcinoma is a heterogeneous disease. It is a combination of a growing number of familiar biological subtypes (Carey, Perou, Livasy, & Dressler, 2006). Carcinoma of mammary gland can be split into subtypes according to the clinical, histological and molecular classifications system. The clinical classification is based on the Classification of Malignant Tumors and includes stage, grade, size, affected lymph nodes and metastases. Histological, breast tumors are divided into

ductal and lobular carcinomas. There are four subtypes defined by tumor marker expression, luminal sub type, Basal-like, Her2-overexpressing and normal breast-like tumors (Weigelt & Baehner, 2010), the basal subgroup having the worst prognosis, HER-2 over-expressing having a poor prognosis, luminal B an intermediate prognosis and the normal breast-like and luminal A subgroups having a good prognosis. The basal and HER-2 over-expressing groups are ER-negative, whereas the Luminal subgroup is ER-positive reflecting the significant role of ER status in prognosis (Yersal & Barutca, 2014).

The non spreading carcinomas tends to remain inside the ductules and not to move outwards, but the ultimate factor that predicts the breast carcinoma is that either it will spread or not (Gomez, Quach, & Horn-Ross, 2010). If cancer cells disseminate outside the basement membrane of the milk duct or milk making glands in the lobules and grows into surrounding adjacent normal tissue the cancer is known as invasive. Invasive cancers can extend to further parts of the body and form metastases (secondary tumors) at distant sites including Lungs, Liver, Brain, and Bone which are the internal organs of the body. Tumor of the breast can initiated from breast tissue and from the internal lining about milk ducts, alternately from those lobules and supplying ducts for milk. It could additionally start in the covering regions which is particularly least common and is incorporate fat and connective tissues (Shaukat, Ismail, & Mehmood, 2013).

Histological type of breast cancer includes Ductal carcinoma in situ (DCIS), Invasive or infiltrating ductal carcinoma (IDC), Invasive (infiltrating) lobular carcinoma (ILC) and inflammatory breast cancer (IBC). DCIS is a kind of non-invasive breast cancer. It means that the tumor cells are just in the ducts. They cannot spread to the other vital organs and basically the lymphatics, and even not spread to the breast tissue through the membrane or the barrier of the ducts. At this stage of cancer all the women can be cured (Allred, 2010). The cancer which starts in the ducts overrunning the breast tissues along with them affecting the other organs, is IDC which is one of the most common type of Carcinoma among all others, ¾ of the breast cancers are due to this type (Anderson, Schairer, Chen, & Hance, 2006). ILC before spreading to the other parts the first step is the extension in the wall of lubules. This type is about 1 in 10 invasive breast cancers (Arpino, Bardou, & Clark, 2004). IBC is the type of invasive breast cancer and also it is an uncommon type of cancer and account for about 1% to 3% of all breasts.

IBC is the type of invasive breast cancer and also it is mostly unusual type of carcinoma as it accounts for only $1/10^{th}$ of the mammary gland. When IBC occur in women feels warm and the skin appear crimson. It makes the breast skin look thick and skin starts to shed along with increase of size and uncomfortable feelings (Anderson et al., 2006).

New lump or mass the most frequent symptoms of the breast cancer. When the woman feels a lump it can cause a breast cancer, but the majority of lumps are not cancerous. Breast cancer symptoms include a liquid advancing with the areola. There might be systems including pale skin, bone breaking pain, syncope and due to chronic cases the lymph buds or bubbles could get swollen and this can cause serious problem by extending the diseases (Dannhauser & Berg, 2011). Such type of symptoms are produced is inflammatory breast cancer.

Easily diagnose frequent types of breast cancer is through biopsy. The lump can be removed by a process known as lumpectomy and biopsy, through an excisional biopsy. Some of them need specialized lab exam. Physicals examination is the most frequently screening methods, and mammography for good degree of accuracy, mammography can suggest for lump of cancer or a few other lesions (Saslow, Hannan, & Osuch, 2004). In the lump get through the clear fluid is highly unlike to cancerous. Magnetic resonance imaging (MRI) and ultrasound is also done for good diagnosis (Yu, Liang, & Yuan, 2010).

Some breast cancer treatments are to obliterate the tissue inside of the breast by removing the nearby lymph nodes or perhaps can be done. In somehow whole breast is removed by the surgery process known as mastectomy. If the tumor is presided only in a particular part without affecting the whole breast the lump is only removed known as the Lumpectomy. High energy waves are used to kill the cancer cell in radiation therapy. Chemotherapy by blood is another process by using particular drug types to fight the disease but the consequences are more apparent e.g fatigue, cold sweats, early menopause, and vomiting. Other treatment is hormone therapy that is use drugs to avoid hormones, particularly estrogen, from fueling the growth of breast cancer cells.

In developed communities, especially breast cancer incidence rates are increasing throughout the world (Bray, McCarron, & Parkin, 2004). According to the most recent worldwide cancer statistics report of Ferlay, more than 1,675,000 women are diagnosed with this disease each year

and furthermore more than 500,000 die of it ("GLOBOCAN 2012 v1. 0, cancer incidence and mortality worldwide: IARC CancerBase No. 11," 2013).

It is the commonest tumor and the second majority basic reason for disease-related deaths among females (Baloch, Khosa, & Bangulzai, 2016; McPherson, Steel, & Dixon, 2000). According to 2010 report the Prominent causes seen in women of Europe is the process of ageing because with the increase in age the risk factor of breast cancer increases mostly due to the hormonal imbalance (Strumylaitė, Mechonošina, & Tamašauskas, 2010). In United States expected breast cancer diseases are analyzed every year is 230,000. In 2017 it is also predictable that in the U.S. population recent spread of breast carcinomas, it would be; 252,710 ("Cancer screening in the United States, 2017: A review of current American Cancer Society guidelines and current issues in cancer screening," 2017). It is rare in men, but it does occur. In 2017 it is also predictable that in the U.S. population recent spread of breast carcinomas, it would be; 2,470.

There is a sudden rise in the incident of breast tumor in Pakistan. Its occurrence starts from the subcontinent where the rate of breast cancer is higher i.e. Pakistan, than the rest of the Asian countries after the Middle East (Liede, Malik, Aziz, Rios, & Kwan, 2002). Around the Asian populations, the increase scale of breast carcinoma occur in Pakistani population indicates that after nine women's, one women having risk of this diseases (Shin, Carlos, & Varghese, 2012; Sohail & Alam, 2007). In Pakistan it is expected that every year 90,000 women suffering from breast cancer each year (Yasin, Afridi, & Khan, 2014).

Carcinoma of breast is non-communicable complex and multifactorial disease that is caused by various environmental (Lichtenstein, Holm, & Verkasalo, 2000) and genetic factors. The vast majority population reveals to two inherited situations from claiming breast tumor association in abnormal genes: *BRCA1* (Breast Cancer gene one) and *BRCA2* (Breast Cancer gene two) (Yoshida & Miki, 2004) and hormonal components incorporate progesterone and estrogen. In two fundamental cellular processes BRCA1 and BRCA2 are involved. These fundamental cellular processes are transcriptional regulation and DNA damage repair (Welcsh & King, 2001). When mutations will occur in particular genes in human genome than the breast carcinoma will occur. This could transfer from one parental generation to another genotypic generation. Another gene role in it is P53, which mostly it acts the function of tumor suppresser by DNA transcription, cell cycle, and cell death. In the breast cancer the mutation of p53 is detected that

are first pointed mutation which ends the function of wild type p53 (Lacroix, Toillon, & Leclercq, 2006).

Most broad categories of risk factors are divided into established and speculated risk factors (Memon et al., 2014). Established risk factors are gender, age, genetic factors, family history/ personal history of breast cancer, ethnicity and reproductive factors (Dannhauser & Berg, 2011). Speculated risk factors or not having pregnancy history, or with ageing the lower chances of getting pregnant other than that the contraception methods for terminating pregnancy (Kluttig & Schmidt-Pokrzywniak, 2009).

Hormones are the chemical messenger that goes into the circulatory system from an endocrine organ to another organ or gathering of organs to manage an extensive variety of physiological procedures. Estrogen is a female sex hormone that is responsible for physical feature and reproduction. It is formed in adipocytes, lower pituitary gland and female reproductive part (Henderson, Ross, & Bernstein, 1988). It controls the development of the uterine coating among the initial segment and the structure of the breast changes and disturbance of the menstruation. Estrogen manages pregnancy (Simpson, 2003). Estrogen not only plays a vital role in breast and uterus but also the visceral organs, in female population estrogen wander around and in to a targeted tissue on the estrogen receptor sites (Ritchie, Hahn, Roodi, & Bailey, 2001).

Most important cause of breast carcinoma is a hormonal factor which also known as reproductive and menstrual factors (Key, Appleby, Barnes, & Reeves, 2002). Hormones are showing significant reasons for carcinoma. The hormonal imbalances such as estrogen imbalance can become one of the reasons for breast cancer. The risk factor that involves the premenopausal women have low out comes compared to the post menopausal women (Butt, Haider, Arif, & Khan, 2012). But post-menopause cause more breast cancer in women than pre-menopause (Yager & Davidson, 2006). Additional epidemiologic data suggest that the rise in the chances of carcinoma of breast is linked with enhanced estrogen exposure during a woman's lifetime (Santen, 2014).

It is proven that the major factor of breast cancer links directly with estrogens. The level of estrogen in the blood of post menopausal women is less than the level in breast tissue which is nearly 20 to 60 times higher (Landeghem, Poortman, & Nabuurs, 1985), The activity of breast is

enhanced by the cancerous tissues in which estrodial levels are higher (Jefcoate, Liehr, Santen, & Sutter, 2000).

Many risk factor studies have been done in different populations which are causing breast cancer. Some environmental risk with estrogen studied in the European population. But it is not yet studied in Pakistani population, causing postmenopausal breast cancer. This study is going to find out the reproductive factors and estrogen role in causing postmenopausal breast cancer.

Aims and Objectives

Post menopause and hormones are risk factors of causing breast cancer. It has been investigated in different population that estrogen plays a vital role in causing post menopause in woman and breast cancer. In Pakistani population comparative study of pre and post menopause has been done in 2012 in Karachi with reproductive factors. There is still need to study postmenopausal affect with estrogen causing breast cancer in Pakistani population.

Objectives

- To identify the role of estrogen hormone level either elevated in post menopausal breast cancer woman in Pakistani population or not.
- To identify role of which reproductive factor is involved in breast cancer Post menopausal woman of Pakistani population with estrogen hormone.
- To identify that either higher age is greater risk for Post menopausal breast cancer or not.

CHAPTER 2

LITERATURE REVIEW

Breast anatomy

The composition of breast includes fatty tissues and granular tissues with both epithelial and stromal elements epithelial elements comprise of milk-producing glands (lobules) and ducts. Stromal elements comprise of adipose tissue, connective tissue, blood and lymphatic vessels (Edge & Compton, 2010) as Figure 2. Glandular tissue consists of lobes and smaller subsections lobules, which connected to duct and drainage into the nipple. Ducts are formed by two cell layer (epithelial and myoepithelial) surrounded by fibroblast. Epithelial cell is responsible for milk synthesis and releases into the lumen (Gusterson & Stein, 2012). The breast is also having blood and lymphatic vessels. Nourishment and Oxygen are supplied through blood in arteries and capillaries. Breast lymphatic drainage takes place through the axillary lymph nodes (Russo & Russo, 2004). This whole network of breast helps in fight against infection.

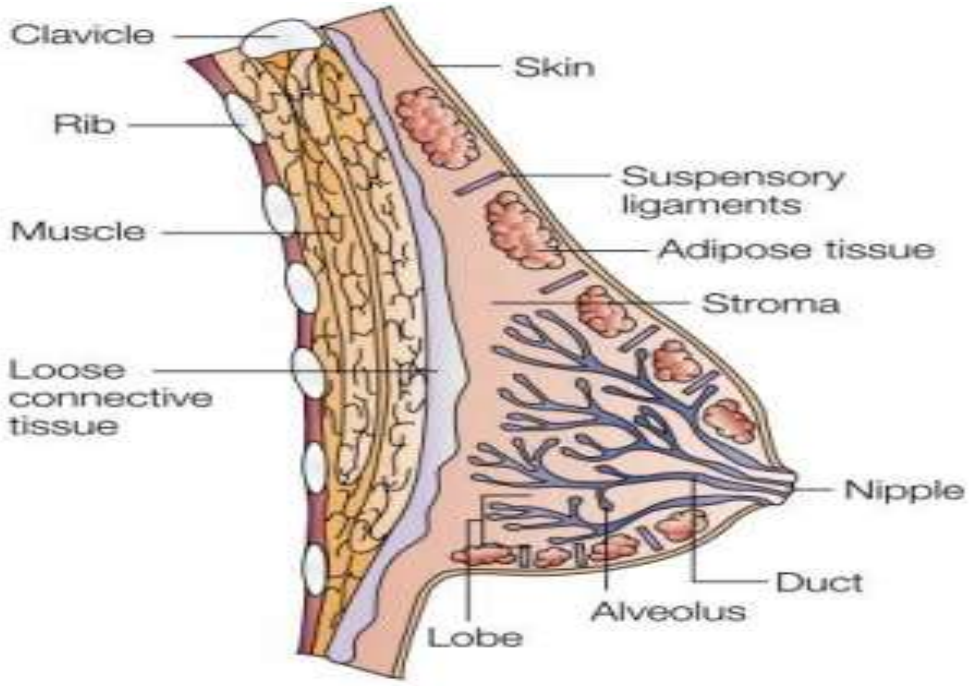

Figure 2 Anatomy of human glands (simak & coombes, 2002)

Breast cancer development

Body cells normally grow and divide in a specialized order and died after several rounds of replication. Cells of breast abnormal growth and change in abnormal tissues formation lead to breast cancer (Khuwaja & Abu-Rezq, 2004). It develops in the internal lining of the drain ducts alternately lobules (Sharma, Dave, & Sanadya, 2010). When the cells grow uncontrollably; the breast cancer develops. The cancer cell grows on an increasable high speed than the other cells and the uncountable cells when invade the adjoining organs than it is known as malignancy.

Tumor features

Tumor size has long been recognized to be one of the most important prognostic indicators. Larger breast cancer tumors are associated with poorer rates of survival compared with tumors smaller in size (Soerjomataram, Louwman, & Ribot, 2008). The absence or occurrence of metastases to the regional lymph nodes is also of prognostic importance with regard to disease free and overall survival. While regional metastasis is partially a function of time, nodal involvement is also considered to indicate a more biologically aggressive breast cancer phenotype (Nassar, Wallis, Andea, Dey, & Adsay, 2001). Staging of cancers is a method of determining the anatomic extent of a cancer based on its natural history, which is relevant to therapeutic decision-making and determining overall prognosis (Edge & Compton, 2010). TNM staging is determined by the combined score on three components, including: tumor size, lymph nodes involvements in regional area and distant metastasis (Edge & Compton, 2010).

Benign breast disease

Benign breast disease (BBD) represents small changes in normal breast tissue which could indicate a multiplied chance of invasive breast cancer, or may also behave as a non-obligate precursor lesion (Page et al., 2003). BBD is generally sub-classified into three types of lesions, based on their severity and associated risk for subsequent breast cancer, including: (i) non-multipliable disease, (ii) multipliable disease without atypical cells (iii) multipliable diseases with atypical cells (Dupont & Page, 1985). Non-proliferative breast lesions are not associated with increased risk of breast cancer (Hartmann, Sellers, & Frost, 2005).

Rapid growth of breast disease without atypia is associated with moderately increased risk of subsequent breast carcinoma (Shnitt & Connelly, 2004), while breast cancer risk is increased 3.5- to 6-fold when atypical hyperplasia (ductal or lobular) is present.

Stages of Breast Cancer

Staging of the breast cancer are based on the level of invasion of primary tumor and clinical size, the clinical deficiency or occurrence of clear axillary lymph nodes and evidence of their local invasion, collectively with the clinical and imaging evidence of distant metastases.

Stage I – in this stage the tumor size is less than 2 cm during the initial stages the tumor is limited only to the breast and will remain resided over there.

Stage II – in this next stage invading of tumor occurs by the help of lymph nodes, is now spread towards the axillary region. The tumor is either less than 2 cm or not.

Stage III – It can be seen that the tumor size increases above 1.9 inches and the axillary lymph nodes swells because of the extension of tumor due to increase in size after that the nearby area is chest and the tumor will spread to towards it where breast is located

Stage IV – in the last stage the malignant tumor has invaded to the nearby organs causing large number of cellular death (Israyelyan, 2003).

Classification of breast cancer

Breast cancer categorization is mostly based on specialized cell type which they formed. Specialized cell types include epithelial, mesenchymal and fibroepithelial. Epithelial tumors are invasive in nature that can penetrate the basement membrane, can invade adjacent tissue and regional node and can even metastasize to adjacent sites ("World Health Organisation classification of tumors of the breast," 2012) This tumor includes tubular carcinoma, ductal carcinoma in situ and tubular neoplasia. The tumor in the mesenchymal stem cell includes benign vascular lesion, lipoma, liposarcoma, angiosarcoma, and osteosarcoma.The fibroblast tumors include fibroadenoma, phyllodestumors, and hamartoma (Montserrat, 2015).

Histological type of breast cancer includes Ductal carcinoma in situ (DCIS), Invasive or infiltrating ductal carcinoma (IDC), Invasive (infiltrating) lobular carcinoma (ILC) and inflammatory breast cancer (IBC). DCIS is a kind of non-invasive breast cancer. It means that the tumor cells are just in the ducts. They cannot spread to the other vital organs and basically the lymphatics, and even not spread to the breast tissue through the membrane or the barrier of the ducts. At this stage of cancer all the women can be cured (Allred, 2010). The cancer which starts in the ducts overrunning the breast tissues along with them affecting the other organs, is IDC which is one of the most common type of Carcinoma among all others, ¾ of the breast cancers are due to this type (Anderson et al., 2006). ILC before spreading to the other parts the

first step is the extension in the wall of lubules. This type is about 1 in 10 invasive breast cancers (Arpino et al., 2004).

IBC is the type of invasive breast cancer and also it is an uncommon type of cancer and account for about 1% to 3% of all breasts. IBC is the type of invasive breast cancer and also it is mostly unusual type of carcinoma as it accounts for only $1/10^{th}$ of the mammary gland. When IBC occur in women feels warm and the skin appear crimson. It makes the breast skin look thick and skin starts to shed along with increase of size and uncomfortable feelings (Anderson et al., 2006).

Epidemiology

According to the Cancer statistics for African Americans 2016 report, cancer is the increasing global problem and United States the most commonly diagnosed cancer is breast cancer in women approximately 29% (DeSantis, Siegel, & Sauer, 2016). In 2008 (Boyle & Levin, 2008) Boyle et al, found that it is second common cancer in the world. It is responsible for 1.4 million new cases annually. It is the common happening form of cancer in women worldwide. It is the most frequently diagnosed cancer and accounting for 23% (1.38 million) of the total new cancer cases and 14% (458,400) of the total cancer deaths (Jemal, Bray, Center, & Ferlay, 2011). In 2012 its ratio became 25 %, 1.7 million cases and 12 %, 521,900 deaths ("Global cancer statistics," 2011) as shown in Figure 3. According to ("Breast cancer: epidemiology and etiology," 2015) Tao et al, report 2015, in 2050 it has been predicted the world wide occurrence of female breast cancer will reach approximately 3.2 million new cases per year.

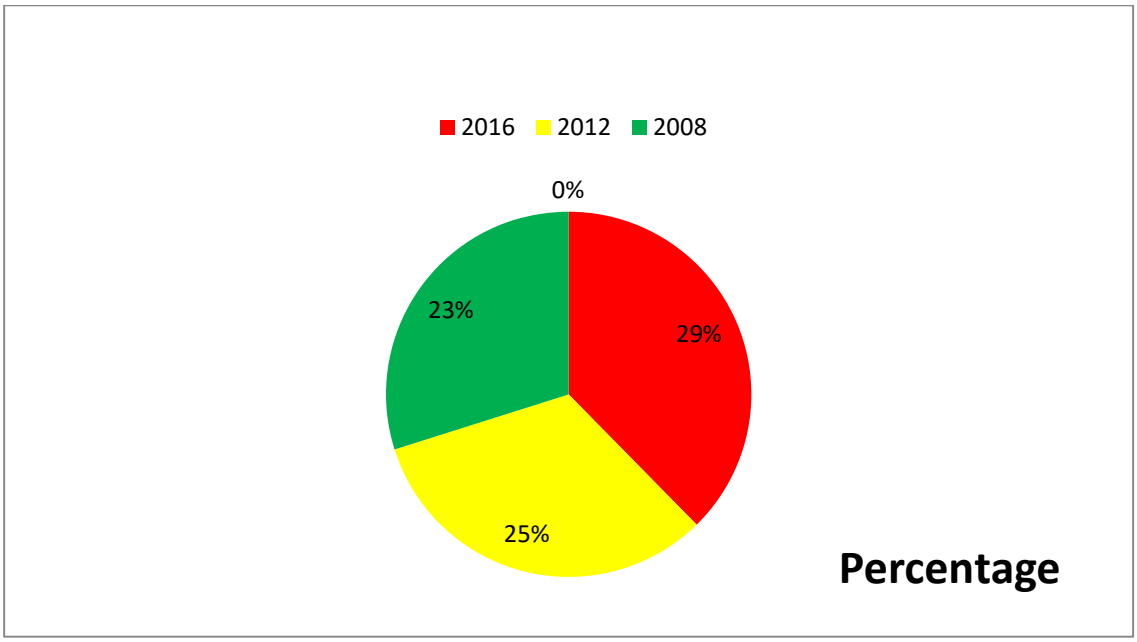

Figure 3 Percentage distribution of statistic of breast cancer 2008-2016

Literature survey of (Jemal, Bray, Center, & Ferlay, 2011) J. Ferlay et al, IS 2010 suggests that West Africa with an about 30000 new cases in 2008 and more than 16000 deaths. The occurrence is similar in Central and Eastern Europe with approximately 115000 new cases and more than 47000 deaths in 2008. Some studies suggested that Asian women were highly susceptible to breast cancer, and it was reported that the number of women with incident breast cancer in Asia was estimated at 651,000 in 2012, comprising 38.8% of all cases globally, followed by Europe (27.7% of all cases) and North America (15.3% of all cases) (Ferlay, Soerjomataram, & Dikshit, 2015). In the subcontinent the rate of breast cancer is higher in Pakistan than the rest of the Asian countries after the middle East (Liede et al., 2002). In Pakistan 98% of women are at high risk of developing breast cancer (Shin et al., 2012; Sohail & Alam, 2007). Every year 90,000 of the Pakistani women suffer from breast carcinomas (Yasin et al., 2014).

Breast cancer risk factor

There is much scientific evidence shows several risk factors participating in breast cancer risk. These risk factors can be categories in two broad terminologies such as established and speculated risk factor for breast cancer. Established risk factors include some inherited predisposition, age, family / personal history, ethnicity and reproductive factors. Speculated risk factors include alcohol consumption, smoking and breastfeeding (McPherson et al., 2000).

Speculated risk factors

ALCOHOL CONSUMPTION

Alcohol consumption is other risk factors for developing breast cancers among the addicts than the non-alcoholic. Recent studies have confirmed there is a dose-response relationship consumption of alcoholic liquids is causally related with female breast cancers. The role of heavy consuming has been long recognized, and even a moderate intake is linked with higher chances of breast carcinoma. According to a literature review of (Chou, Lerner, Harris, & Brandon, 2015), it is indicated that early age alcoholic drinking and especially ethanol consumption strongly associated with breast cancer in females.

SMOKING

In 1980 first time ("Risk of breast cancer in relation to cigarette smoking," 1988) Brownson et al., noticed that here be a positive relationship among smokers. According to (Gaudet, Gapstur, & Sun, 2013) , it is shown that women who started smoking before menarche have a 61% higher risk but those starting smoking after menarche but ≥ 11 years before the first child birth have a 45% higher risk as compared to never smokers. It has been suggested that cigarette smoking may exert a more pronounced effect on the risk of breast cancer among non-obese postmenopausal women (Luo, Horn, Ockene, & Simon, 2011). Cigarette smoking has been thought to increase breast cancer risk through the exposure to numerous well-established carcinogens of different chemical classes present in tobacco smoke, such as polycyclic aromatic hydrocarbons, N-nitrosamines, aromatic amines, and aldehydes (Trinh, 2015).

BREASTFEEDING

Breast feeding has a positive correlation with breast cancer in both pre and post menopausal women. The more the time of lactation the risk of developing breast cancer is reduces (Kwan, Kushi, & Weltzien, 2009). In 2014 (Surakasula & Nagarjunapu, 2014) found that breastfeeding plays no role in curing breast cancer which was later not approved because of the comparative studies done with western literature. A study was conducted in India in which it was describe that there is a mix relationship between breast feeding and breast cancer. The study further suggested that there is no affect of breast feeding on post menopausal women but there is significant relationship in premenopausal women (Sandhu, Sandhu, & Karwasra, 2010). The same study in Pakistan found that it is supporting to the neighboring country.

ESTABLISHED RISK FACTORS

AGE

Breast cancer risk increases significantly with growing age (MaJ et al., 2013). The disease is relatively rare among women under the age of 40, after which the incidence of breast cancer rises rapidly until menopause when the rate of increase becomes weaker (Sandhu et al., 2010). A review article showed that menarche has a positive correlation on both pre and postmenopausal breast cancer but the association was stronger for pre-menopausal women. For each additional year of menarche, the risk of female developing breast carcinoma decreased by 9% for pre-menopausal women and by 4% for post-menopausal women (Clavel-Chapelon & Gerber, 2002). Similar findings were found in a study which showed that younger age at menarche explained 70% of pre-menopausal breast cancers while it explained only 27.5% of post-menopausal breast cancers (Gao, Shu, Dai, & Potter, 2000). Recently according to the Butt, et al. in 2012 shows that younger age at first live birth decreased risk in both pre and post-menopausal women.

PERSONAL OR FAMILY HISTORY

The risk of breast cancer is advanced among women whose close blood relatives develop the disease (Haq, Haq, & Haque, 2009). Having a first-degree relative (mother, sister or daughter) with breast cancer doubles a woman's risk, and having two affected first-degree relatives increases the risk by ~3.5 times (Pharoah, Day, & Duffy, 1997). The increase in risk is also more pronounced when the relative had been diagnosed at a young age (below 50 versus ≥ 50 years of age) (Colditz, Kaphingst, & Hankinson, 2012).

RACE/ETHNICITY

A different study has shown that breast cancer survival rate differs with ethnicity. Epidemiology of cancer varies with ethnicity according to research studies a chronic stage of breast cancer was diagnosed in Asia (Jack, Davies, & Møller, 2009). The high risk factor of cancer was present excessively in European countries than in Asian migrant of England (Wild, Fischbacher, & Brock, 2006).

BREAST DENSITY

Breast density has been shown to be an issue for the development of breast cancer, with hazard growing with the extent of mammographic breast density (Boyd, 2011). Although breast density is stimulated through genetics, it's also laid low with a few different factors. In maximum women, it'll exchange over time, reducing with age. It is similarly decreased via being pregnant and menopause. Breast density is decrease amongst women with better body weight because of the better percentage of fatty tissue (Harris, Tamimi, & Willett, 2011).

RADIATION EXPOSURE

Radiation is an established hazard issue for breast cancers and excessive exposure to radiation ought to be averted. The impact of radiation on the breast is strongly associated with age at exposure, the younger the lady is exposed, the more the excess threat (Darweesh, 2009).

REPRODUCTIVE AND MENSTRUAL FACTORS

Reproductive and menstrual factor are also called hormonal factors are the most important risk factor for breast cancer. These factors include menopausal effect and hormones like estrogen, progesterone and exogenous hormones.

MENOPAUSE

Menopause does no longer purpose cancer, however the threat of growing most cancers increases a women ages. Breast cancer increases in those women who experiences menopause after age 45 and those who began menstruation after12 (Liede et al., 2002). According to one observation Women experiencing menarche before 12 years have higher risk of developing breast cancer then those who are older than 14 years of age (Clemons and Goss 2001). Likewise, not on time menopause is related to a threat elevation of 3% for every behind schedule year (Cuzick 2003)

Women reproductive menopause is of two group's pre and post menopause. Women with irregular menstruation status have been considered inside premenopausal institution till age of 41 years, and the women whose menstruation was stopped for 12 months above the age of 45 would be accepted in the post menopausal institution (Clavel-Chapelon & Gerber, 2002). Some studies found that a higher age at first live birth is protective only for postmenopausal women (Ursin, Bernstein, Wang, Lord, & Deapen, 2004).

In 2000 studies shows that premenopausal women had reduced the risk of breast cancer due to estrogen metabolic pathway (Muti, Bradlow, Micheli, & Krogh, 2000). Some research said that younger age at menarche multiplied breast cancer chance simplest in premenopausal women, while a few said improved risk most effective for postmenopausal women (Pathy, Yip, Taib, Hartman, & Saxena, 2011). In a few studies executed previously, age at menarche became determined to be related to both pre-and post-menopausal breast most cancers even as in some other observe, it had no affiliation with both pre- and post-menopausal breast most cancers (Butt et al., 2012). In 2010 studies shows that the post-menopausal group with breast cancers showed higher parity in comparison with the pre-menopausal breast cancer group. In 2014 studied suggests that early onset of menarche was found to be related to each pre- and post-menopausal patients (Surakasula & Nagarjunapu, 2014). Some research executed and was confirmed that the pre and post menopausal women who had delayed menarche less likely develop the risk factor of

breast cancer than those who had early menarche according to the research done on Indian women (Sandhu et al., 2010). It is still questionable that the post menopausal women have a lower chances of breast cancer who had different aging pattern and maternity pattern than those of pre menopausal women (Surakasula & Nagarjunapu, 2014). In some cases it was concluded that there is an early menarche but a late menopause in these cases the chances of breast cancer is high.

HORMONES

Hormones play an important role in the causing of breast cancer because women are exposed to different hormonal changes and that makes some of them postmenopausal and some pre-menopausal (Butt et al., 2012). But post menopause cause more breast cancer in women than pre-menopause (Yager & Davidson, 2006). Additional epidemiologic data suggest that an increase in the risk of breast cancer is associated with enhanced estrogen exposure during a woman's lifetime (Santen, 2014).

Estrogen is a female sex hormone that is responsible for physical feature and reproduction. It is lipid in nature and belongs to steroidal hormone group. It involves in different female diseases like uterine, lungs (Omoto et al., 2001), ovarian (Lanneret et al., 2006) and breast cancer (Schellenberger & Park, 2010). Production of estrogen is done in reproductive parts of female, lower pituitary and adipoctyes (Henderson et al., 1988). It controls development of the uterine coating amid the initial segment at puberty when menstruation starts the development of female parts starts like the formation of breasts, along with these the other body functions also enhances (Simpson, 2003). The major hormone which plays the role in release follicular cell from the ovary is estrogen, which helps release the follicle and then enhances the luteinizing hormone which regress the corpus luteum if the egg is not fertilized ("Intraovarian actions of oestrogen," 2001). Estrogen not only plays a vital role in breast and uterus but also the visceral organs, in female population estrogen wander around and in to a targeted tissue on the estrogen receptor sites (Ritchie et al., 2001).

Literature studies proven that the major factor of breast cancer links directly with estrogens. The level of estrogen in the blood of post menopausal women is less than the level in breast tissue which is nearly 20 to 60 times higher the blood (Landeghem, Poortman, & Nabuurs, 1985). The activity of breast is enhanced by the cancerous tissues in which estrodial levels are higher (Jefcoate et al., 2000). A study was conducted in which oral hormones were the made the basis, according to this study the breast cancers were shown to have a direct relationship with the hormonal therapy. It was further described that the risk factor would increase if the intake would be in regular but if discontinued the effects would pass away eventually (Kubba, 2003). A mini pill of synthetic progesterone did not have profound effects to cause the cancer hence it was prescribed the patients who were previously exposed to the risk factor. It was concluded that the risk factor is directly proportional to the length of exposure (Reeves et al., 2006).

CHAPTER 3

MATERIAL AND METHOD

Study Area

Main focused area of this research was hormonal fluctuation of postmenopausal risk leading to Breast Cancer in Pakistani population. Therefore, the participants that best fit to research were people of major hospitals of Islamabad. More specifically, these participants were taken from Pakistan Institute of Medical Sciences (PIMS) Islamabad Pakistan, for the purpose of narrowing down to research. The work presented here comprises of sample collection, blood faction separation, biochemical and statistical analysis.

Blood sample collection

In current study, the subjects with control and affected were selected that were post menopausal women above 40 year age. For participants in research, demographic factors such as menstrual and reproductive history and biochemical measurement were taken. Informed consent was signed from each participant after elucidating purpose of this research. Ethnic committee proved questionnaires were filled by each participant. Blood sample were drawn from both control and affected participants and immediately transferred to Gel heprin tube. Each tube was labeled with ID and blood sample were stored in refrigerator until before centrifugation.

Centrifugation

Centrifugation is method by which the solid practices dense down to the bottom and the light substances stay on the top. It is done under high speed in a machine known as centrifugation machine. Mostly in science it is use to separate plasma from the blood. It is a necessary procedure for obtaining high-quality blood supernatant (Zheng et al 2010). The two basic types of centrifugation are deferential and the second is density gradient centrifugation. Centrifugation was performed for each labeled sample and plasma was extracted from samples.

Blood fraction separation

Blood is a combination of ethryocytes, leucocytes and plasma cell fragments when looks like viscous red water but it contains a lot more than just water and these can be seen by the method of centrifugation to separate the substances (Armstrong et al., 2008). In increasing order, the specific gravity of blood components is plasma, platelets, leucocytes and red blood cells (RBCs). Blood fractioning is a process of separating blood into its components and generally it is done by the process of centrifugation. The plasma component of blood was extracted after centrifugation and it was transferred in eppendorf tube and then each eppendorf tube was labeled.

Biochemical Analysis

Biochemical Analysis is a technique used for finding modern drug; it is an authentic way to test the latest achievements (Glick et al., 2009). Biochemical analysis was carried out using diagnostic kit for detection of hormonal level. Enzyme-linked immunosorbent assay (ELISA) was performed according to standardized procedure and protocol.

ELISA

It is Enzyme linked immunoassay (ELISA) kit, which are primarily used for the detection of antigen and antibody. It is Enzyme linked immunoassay (ELISA) kit, which initially used for identification of protein in blood to test for antigens and antibodies. Estradiol (E2) Immunoassay test kit was used to detect the hormone level of both control and affected participants. The ELISA is used to detect the viral infections and hormones along with the body defense to a particular infection. It is a useful technique which was designed to carry out a large scale diagnostic test as it is easy to perform yet very handy.

Assay principle

ELISA is a technique in which polystyrene 96-well plates are used and incubated well. It contained a negative and a positive serum which was to be tested. It was originally to detect the attached antibodies or antigens.

Assay procedure

First of all protected, the chosen broad selected line wells within the holder, and distributed the standard solution of 25 ul with subject and control samples into suitable wells.

After samples and standard solution distribution, 100 ul of estradiol-HRP conjugate Reagent was putted into each well.

In next step 50ul of rabbit anti-Estradiol (E2) reagent were distributed in each well and microtiter plate carefully mix for 30 seconds until it mixed completely.

In next step this microtiter plate incubated at room temperature (18-25oC) for 90 minutes.

After incubation, microtiter plate washed with distilled water for 5 times.

After washing, 100 ul of TMB Reagent was distributed in each wells, and carefully mixed for 10 seconds.

In next step this microtiter plate incubated at room temperature (18-25oC) for 20 minutes.

After incubation, 100 ul stop solution was added in every well for stopping the further reaction, and then gently blend for 30 seconds.

In next step it was confirmed when blue color of wells changed into yellow color completely.

Finally 450nm absorbance of microtiter well within 15 minutes checked.

Statistical Analysis

Statistical analysis was done through SPSS 19 version. Frequency distribution of estrogen hormone level of both case and normal was carried out by descriptive analysis. The correlation between menarche age and breast cancer, cancer age and estrogen level, menopausal age and breast cancer was carried out to identify the parentage and the behavior of risk factor association. Pearson chi square test analysis for case and control risk factor association was carried out.

CHAPTER 4

RESULTS AND DISCUSSION

Blood sample collection

In current study analysis population of participant's were categories into two groups, controls and affected. In this research criteria of age above 40 and postmenopausal women were consider for the affected subjects. The subjects with genetic history were excluded in entire research. The most population belongs to Punjab of Pakistan and some limited number of population belong to Khyber Paktunkhwa, because the samples were collected Pakistan Institute of medical sciences (PIMS) Islamabad, Pakistan. Demographic factor of the participants such as age, gender, ethnicity, reproductive and menstrual history, exogenous hormone use [oral contraceptive (OC) and hormone replacement therapy (HRT) use], lifetime smoking and alcohol consumption history were gather using questionnaire. The study was proved by the ethnic committee of Capital University of Sciences and Technology and informed consent were also obtained from each participant, the Performa is attached in Annexure 1. A total of 80 Subject were included in the study. 54 belong to affected patients and 26 belong to control subjects.

After Performa filling informed consent taken all subjected participants were requested for the blood sample donation. The subjected participants were asked to remove clothes from the forearm for selection and assessment of suitable arm. The phlebotomist looks for suitable vein as they lie just below the skin and there are a few nerves ending. Participant arm was placed downward for vein puncture and the tourniquet was tight in such a way that radial pulse was still palpable. The tourniquet was not being place longer than one and half minutes, If the Veins are not clearly visible than it should be taped the surface of the skin to making veins visible.

Skin was warped with alcohol wipes and left for 30 sec to dry. Needle was injected in vein and the phlebotomist draws the blood from vein. Furthermore, these samples were kept in Green-Top Tube (Sodium Heparin) and labeled with subject ID number. Sodium Heparin tube is used mostly for collection **of** plasma or whole blood for special tests. Blood sample were immediately stored in refrigerator for further analysis. In this research criteria of age above 40 and postmenopausal

women were consider for the affected subjects. The subjects with genetic history were excluded in entire research.

Centrifugation and blood Fraction separation

Centrifugation is a method that separates solids from liquids and liquids of different densities from each other through the use of centrifugal force. It is finished inside a centrifuge. It is a necessary procedure for obtaining high-quality blood supernatant (Zheng et al 2010). Subject ID labeled blood sample were taken out from refrigerator and centrifuged within 15 minutes at 3000 rmp in centrifugation machine. After centrifugation when the supernatants are settled down, 3ml plasma was extracted from tubes through pipit and transferred to ependrof tubes. This analysis results in to blood fraction separation of all the control and affected breast cancer patient.

Biochemical analysis

In order to analysis the subjected participant estrogen hormone level, biochemical analysis was done through ELISA kit. ELISA kit was prepared with the standardize principle and procedure.

First of all protected, the chosen broad selected line wells within the holder, and distributed the standard solution of 25 ul with subject and control samples into suitable wells. After samples and standard solution distribution, 100 ul of estradiol-HRP conjugate Reagent was putted into each well. In next step 50ul of rabbit anti-Estradiol (E2) reagent were distributed in each well and microtiter plate carefully mix for 30 seconds until it mixed completely. In next step this microtiter plate incubated at room temperature (18-25oC) for 90 minutes. After incubation, microtiter plate washed with distilled water for 5 times. After washing, 100 ul of TMB Reagent was distributed in each wells, and carefully mixed for 10 seconds. In next step this microtiter plate incubated at room temperature (18-25oC) for 20 minutes. After incubation, 100 ul stop solution was added in every well for stopping the further reaction, and then gently blend for 30 seconds. In next step it was confirmed when blue color of wells changed into yellow color completely. Finally 450nm absorbance of microtiter well within 15 minutes checked. After that it was placed in ELISA machine. It result were taken such as shown in table 1.

Table 1 Estrogen Hormonal level in Control and Breast cancer Affected woman samples result

	1	2	3	4	5	6	7	8	9	10
A	21.005	26.046	9.38	53.675	14.524	29.772	20.409	9	52.122	26
B	185	17.342	10.447	27.228	5.128	9.034	9.503	12	8.66	24.517
C	22.837	33.381	10.233	23.543	24	22.335	8.282	19	12	5.769
D	209.47	5.372	8.984	16.212	28.956	1.064	20.597	9.936	26.77	7.89
E	4.719	29.351	48.899	30.157	33.979	69.167	123.033	13.274	27.537	15.859
F	5.9	94.707	20.678	5	17.089	13.746	11	17.464	18.117	17.337
G	22.615	28.918	47.26	109.677	10.929	39.829	13	17.446	6.969	16.942
H	9.987	23.095	24.869	29.905	66.26	74.986	14.191	16.674	20.26	5.9

In above table first 54 cells are showing the values of breast cancer subjects and last 24 values are control estrogen values. These values are produced after ELISA test performance.

Statistical analysis

All the statistical measures were done through the Statistical analysis tool SPSS version 19. Mean level and standard deviation of estrogen E2 hormone was measured in both normal and case groups. This analysis provides average mean and standard deviation of Estrogen hormone shown in table 2.

Table 2 Mean Estrogen Hormone (E2) Level in both Case and Normal

Group	**Number**	**Estrogen Hormone E2**	
		Mean	**Standard Deviation**
Case	54	35.41	40.84
Normal	26	16.15	7.64

This result revealed that Case mean value for estrogen is 34.95 which indicates, it's above referenced normal Estrogen level below 18 of Postmenopausal breast cancer. Such as normal postmenopausal woman mean value in table above is 16.15 which agreed to the normal referenced value.

Chi square Test

Chi square test or x^2 test is short name of Pearson's test. It determines whether there is an association between categorical variables or not. It only determines *relationship* between categorical variables, and cannot provide any inferences about reason. In SPSS it uses a possibility table and examines the information. This table (also known as a cross-tabulation, crosstab, or two-way table) is course of action on which information is arranged as stated by two categorical variables. Chi square calculates values on formula of

$$X^2 = (\text{Obesrved value} - \text{Expectedvalue})2 \Big/ \text{Expected value}$$

Correlation between Menarche age and Breast Cancer_ ER Level

Previous reported study found that postmenopausal women had reduced the risk of breast cancer due to estrogen metabolic pathway (Muti et al., 2000). Last decade review study concludes that menarche age has positive association with pre and post menopausal breast cancer. Earlier the menarche more the chances of breast cancer risk, if menarche late in occurring than female developing breast carcinoma decreased by 9% for pre-menopausal women and by 4% for post-menopausal women (Clavel-Chapelon & Gerber, 2002). Some case studies shows that age at menarche do not affecting in breast cancer development in females. Sandhu et al., 2010 shows that pre and post menopausal women who had delayed menarche less likely develop the risk factor of breast cancer than those who had early menarche in India. It is still questionable that the post menopausal women have a lower chances of breast cancer who had different aging pattern and maternity pattern than those of pre menopausal women (Surakasula & Nagarjunapu, 2014).

By assuming that, might be elevation of estrogen level in post menopausal woman due to the association with age at menarche. Breast cancer female estrogen association with age at menarche was analyzed. Early onset of menarche might be associated to breast cancer because some previous studies agreed that early menarche involved in Postmenopausal breast cancer.

Chi square association test for dependant variable breast cancer Estrogen level was performed. Results of analysis are shown in table 3.

Table 3 Case Processing Summary of Post menopausal breast cancer estrogen Level and Menarche age

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
ER Level * Menarche	54	100.0%	0	.0%	54	100.0%

Table 4 Cross tabulation table of Postmenopausal woman breast cancer and age at menarche

			Menarche		Total
			>13	<13	
ER Level	>18	Count	24	16	40
		Expected Count	23.7	16.3	40.0
		Residual	.3	-.3	
	<18	Count	8	6	14
		Expected Count	8.3	5.7	14.0
		Residual	-.3	.3	
Total		Count	32	22	54
		Expected Count	32.0	22.0	54.0

Table 5 Chi-square Test result of Postmenopausal woman breast cancer and age at menarche

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	.035[a]	1	.851		
Continuity Correction[b]	.000	1	1.000		
Likelihood Ratio	.035	1	.852		
Fisher's Exact Test				1.000	.547
Linear-by-Linear Association	.034	1	.853		
N of Valid Cases	54				

a. 0 cells (.0%) have expected count less than 5. The minimum expected count is 5.70.

b. Computed only for a 2x2 table

Although expected counts meet requirements but P-value is greater than selected confidence 0.05, so it concluded that there is not enough evidence to suggest an association between early menarche and breast cancer postmenopausal woman estrogen level elevation. Because in current study, estrogen level increases those who got late menarche as shown in bar char. These result agreed with the previous study of Butt et al., 2012.

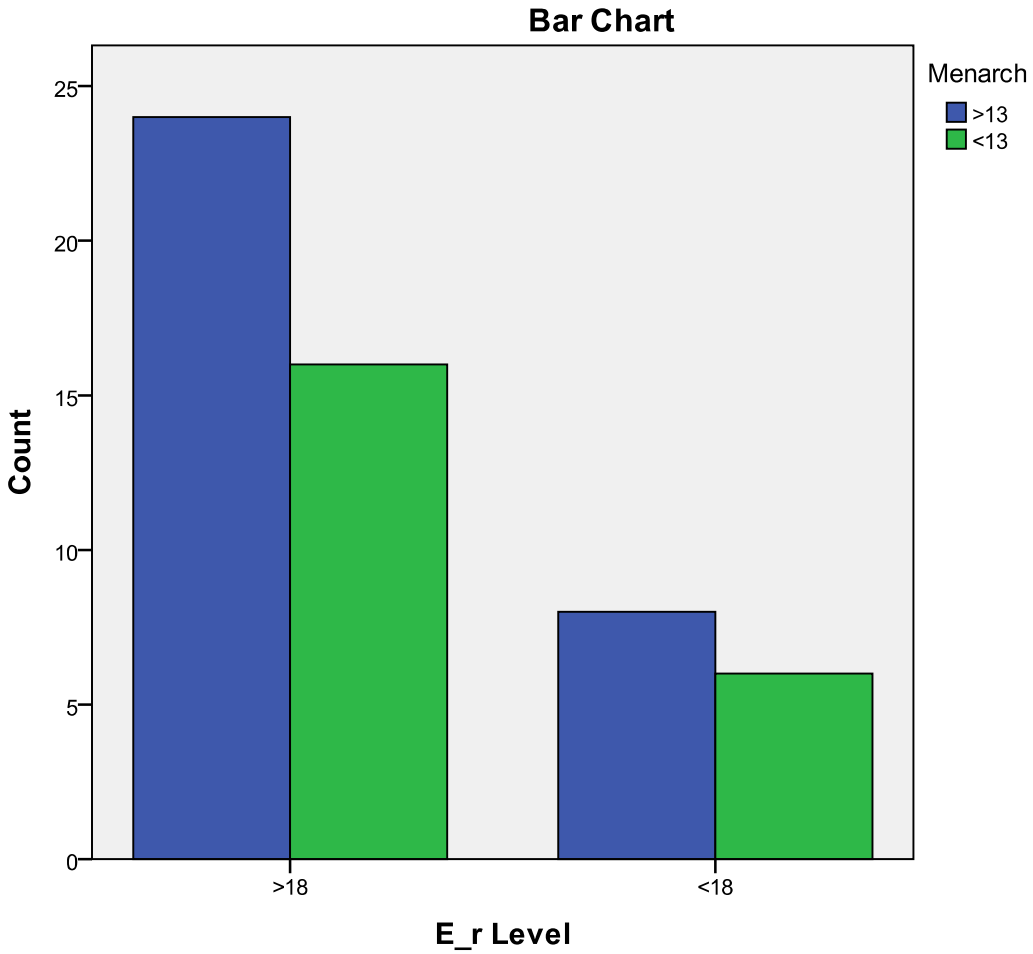

Figure 4 correlations between menarche and breast cancer_ER level

Correlation between Menarche age and Normal ER Level

Hypothesis that late menarche is associated with low level of estrogen in postmenopausal woman that is below 18. Or late menarche is not associated with low level of estrogen in postmenopausal woman. To found this relationship chi square was performed. And results are shown below.

Table 6 Case Processing Summary of Post menopausal normal woman estrogen Level and Menarche age

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
Normal ER Level * Age of menarche	26	100.0%	0	.0%	26	100.0%

Table 7 Cross tabulation of postmenopausal woman Estrogen level and Age of Menarche

			Age of menarche		Total
			>13	<13	
Normal ER Level	>18	Count	1	4	5
		Expected Count	4.0	1.0	5.0
		Residual	-3.0	3.0	
	<18	Count	20	1	21
		Expected Count	17.0	4.0	21.0
		Residual	3.0	-3.0	
Total		Count	21	5	26
		Expected Count	21.0	5.0	26.0

Table 8 Chi-square Test result of Postmenopausal normal woman and age at menarche

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	14.718[a]	1	.000		
Continuity Correction[b]	10.273	1	.001		
Likelihood Ratio	12.412	1	.000		
Fisher's Exact Test				.002	.002
Linear-by-Linear Association	14.152	1	.000		
N of Valid Cases	26				

a. 3 cells (75.0%) have expected count less than 5. The minimum expected count is .96.

b. Computed only for a 2x2 table

The corresponding p-value of the test statistic is so small that it is cut off from display. Instead of writing "p = 0.000", it instead write the mathematically correct statement $p < 0.001$. The expected counts meet requirements with selected confidence 0.05, so it concluded that there is an association between late menarche and breast cancer postmenopausal woman estrogen level elevation. It is supportive to previous analysis Butt et al., 2012. Bar chart result is shown below.

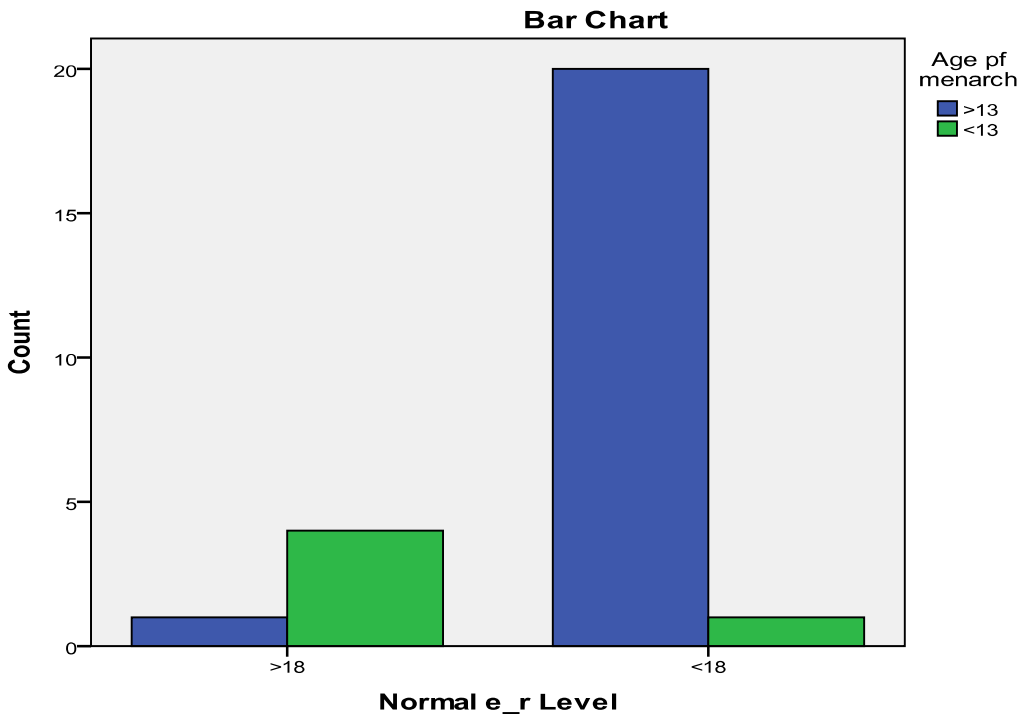

Figure 5 Normal ER level and age of menarche relationship

Correlation between Breast Cancer_ER level and menopausal Age

Menopause itself does not causing breast cancer but with the passage of time as age increases risk of breast cancer become higher. Menopause early and late both is associated with breast cancer (sandu et al., 2010). To address menopausal role in current study, it was checked through the correlation analysis. And cut off value for late menopause consider as above 47 year. Chi square performed for testing hypothesis that breast cancer postmenopausal woman menopausal age is associated with breast cancer.

Table 9 case processing summary of Post menopausal breast cancer woman estrogen Level and Menopausal age

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
Breast Cancer ER_Level * Menopause	54	100.0%	0	.0%	54	100.0%

Table 10 Cross tabulation of postmenopausal breast cancer woman Estrogen level and Age of menopause

			Menopause		Total
			<47	>47	
Breast cancer ER_Level	>18	Count	5	35	40
		Expected Count	8.9	31.1	40.0
		Residual	-3.9	3.9	
	<18	Count	7	7	14
		Expected Count	3.1	10.9	14.0
		Residual	3.9	-3.9	
Total		Count	12	42	54
		Expected Count	12.0	42.0	54.0

Table 11 Chi square test result of postmenopausal breast cancer woman estrogen level and age of menopause

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	8.438[a]	1	.004		
Continuity Correction[b]	6.407	1	.011		
Likelihood Ratio	7.659	1	.006		
Fisher's Exact Test				.007	.007
Linear-by-Linear Association	8.281	1	.004		
N of Valid Cases	54				

a. 1 cells (25.0%) have expected count less than 5. The minimum expected count is 3.11.

b. Computed only for a 2x2 table

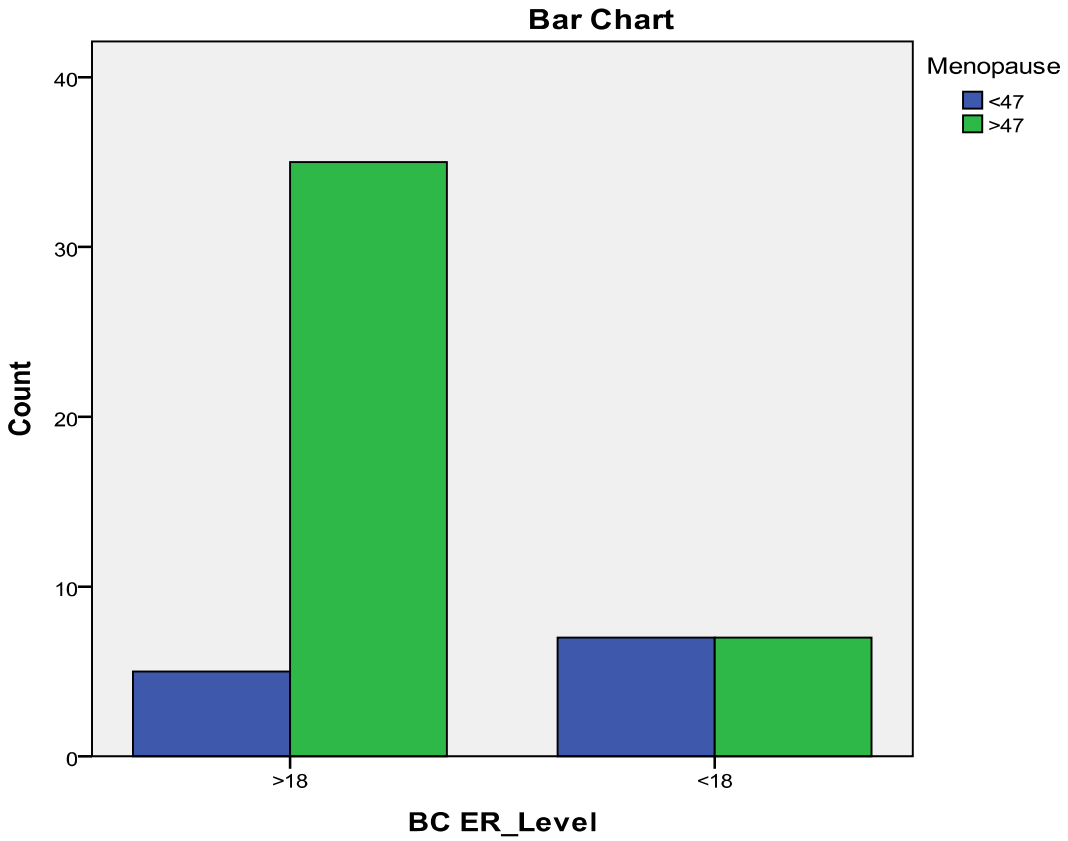

Figure 6 Menopause age and Breast cancer ER_level

Statistics relations of age at menopause and Estrogen level indicates here that Post menopausal woman breast cancer estrogen level increases with late menopause after cut off value 47 year age menopause. Although expected count is less than 5 which is 3.11 and p-value is p=0.004 and lower than 0.05 cutoff confidence interval. It is concluded that late menopausal age is associated with breast cancer postmenopausal woman and estrogen level elevation. Previous reported study of sandu et al., 2010 indicates that risk of above 45 year and late menopause increase breast cancer incidences. It indicated that if woman arrive at menopause in between 55-years instead of 45 than has 30 percent higher risk.

Correlation between Normal ER level and menopausal Age

Hypothesis that postmenopausal normal woman early menopause before cutoff value 47 is associated with low level estrogen. Or early menopausal not associated with low level of menopause. Chi square was performed and result was analyzed.

Table 12 Case Processing Summary of Post menopausal normal woman estrogen Level and Menopausal age

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
Normal E_R Level * Menopause	26	100.0%	0	.0%	26	100.0%

Table 13 Crosstabulation of Post menopausal normal woman estrogen Level and age menopause

			Menopause		Total
			<47	>47	
Normal E_R Level	>18	Count	3	2	5
		Expected Count	3.7	1.3	5.0
		Residual	-.7	.7	
	<18	Count	16	5	21
		Expected Count	15.3	5.7	21.0
		Residual	.7	-.7	
Total		Count	19	7	26
		Expected Count	19.0	7.0	26.0

Table 14 Chi-Square result of Post-menopausal normal woman estrogen Level and age of menopause

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	.538[a]	1	.463		
Continuity Correction[b]	.030	1	.863		
Likelihood Ratio	.507	1	.477		
Fisher's Exact Test				.588	.411
Linear-by-Linear Association	.517	1	.472		
N of Valid Cases	26				

a. 2 cells (50.0%) have expected count less than 5. The minimum expected count is 1.35.

b. Computed only for a 2x2 table

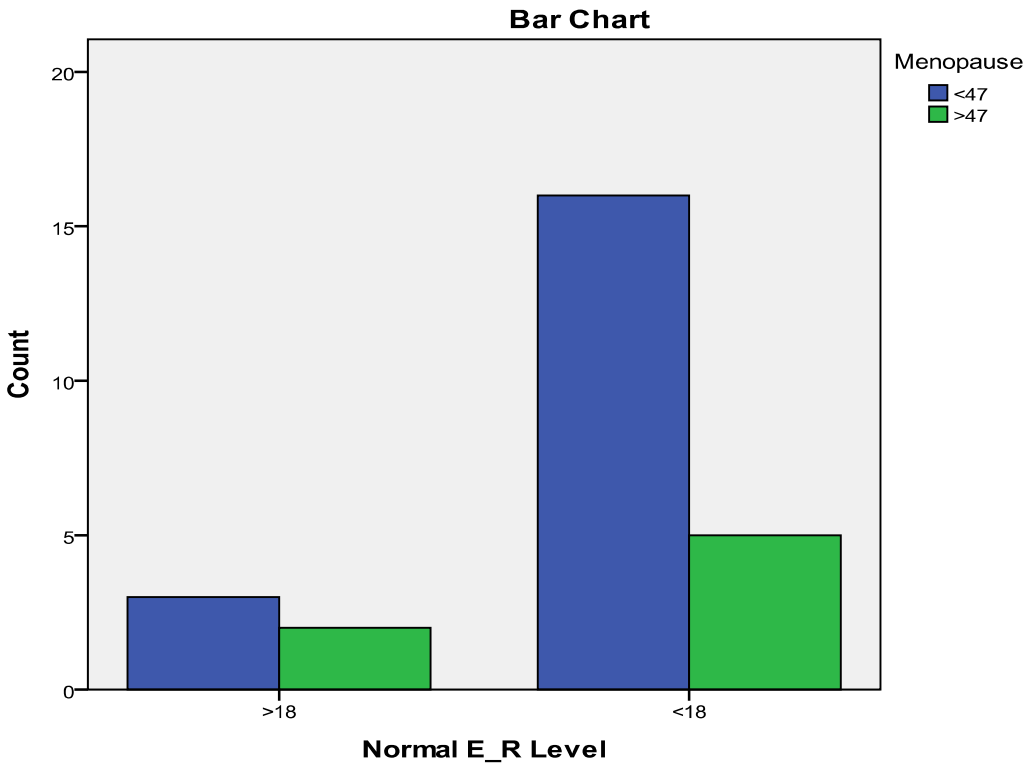

Figure 7 menopause and control ER level

Corresponding p- value is higher than the p= 0.05 confidence interval because the data set is small. And counted expected cells are also low that is 1.35. Chi square value for this test is 0.538 and p value is 0.463. according to this it concluded that early menopause does not related to low level estrogen level normal postmenopausal but if counts were consider to predict than is related. Because below cutoff 18, estrogen level postmenopausal woman belongs to group of early menopause.

Correlation between Breast cancer Age and Estrogen Level

It is believed that, higher the age than higher would be risk of having breast cancer. Breast cancer risk increases significantly with growing age (MaJ et al., 2013). The disease is relatively rare among women under the age of 40, after which the incidence of breast cancer rises rapidly until menopause when the rate of increase becomes weaker (Sandhu et al., 2010). In order to predict either estrogen level is increases with age or not in post menopausal breast cancer women of current sample this correlation was performed. It's revealed that if age is high than greater the postmenopausal increase estrogen level risk in breast cancer. This result also matches with Lafta et al study.

Table 15 Correlation between Breast cancer Age and Estrogen Level

			BC_ER_Level				Total
			<10	<18	<30	Above 30	
Case Age	51----------60	Count	7	1	7	8	23
		% within Case Age	.3	.0	.3	.3	1.0
	61----------70	Count	1	3	5	1	10
		% within Case Age	.1	.3	.5	.1	1.0
	>70	Count	3	1	13	4	21
		% within Case Age	.1	.0	.6	.2	1.0
Total		Count	11	5	25	13	54
		% within Case Age	.2	.1	.5	.2	1.0

In Age and estrogen level association test it was observed that above 18 estrogen level 70% woman are associated and 30 % not associated. As age increases and Postmenopausal breast cancer patient estrogen hormone level increases.

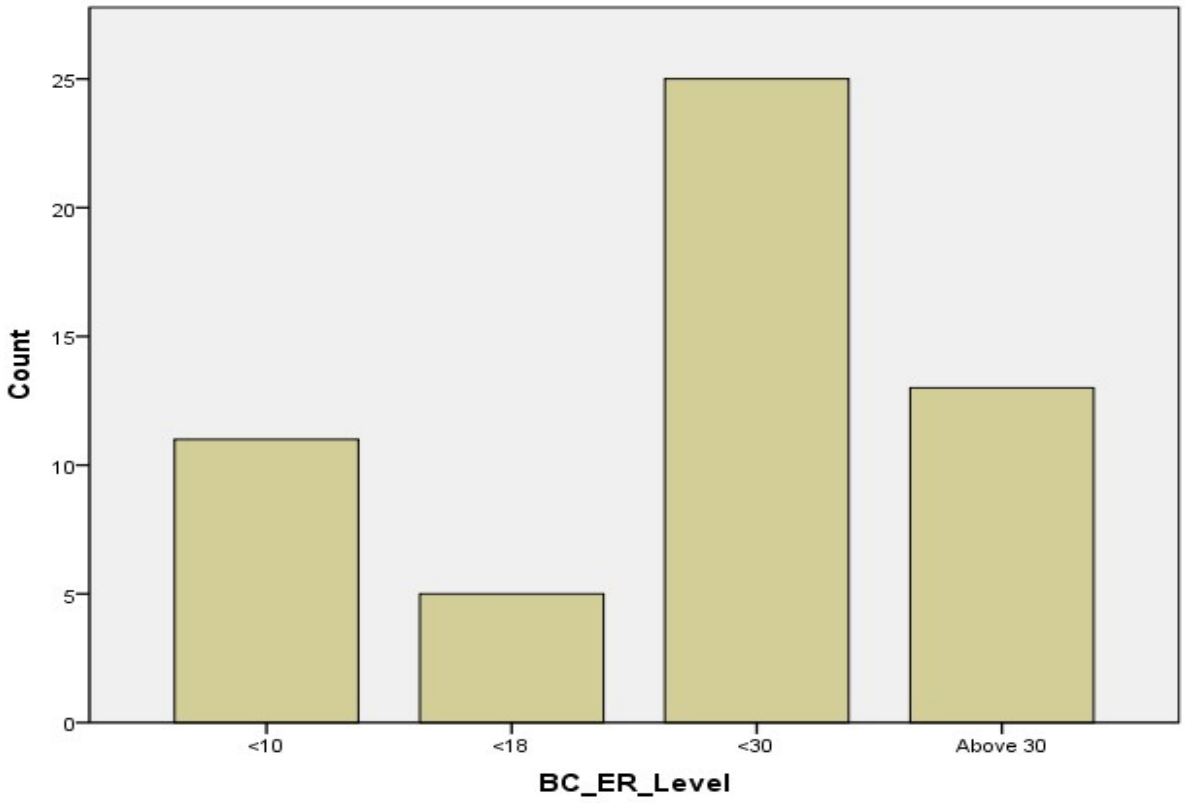

Figure 8 Estrogen level of postmenopausal breast cancer

Count distribution of estrogen level in post menopausal breast cancer is greater than the normal estrogen level. This histogram indicates that most of the post menopausal breast cancer women having greater than 18 estrogen hormone level in 70%.

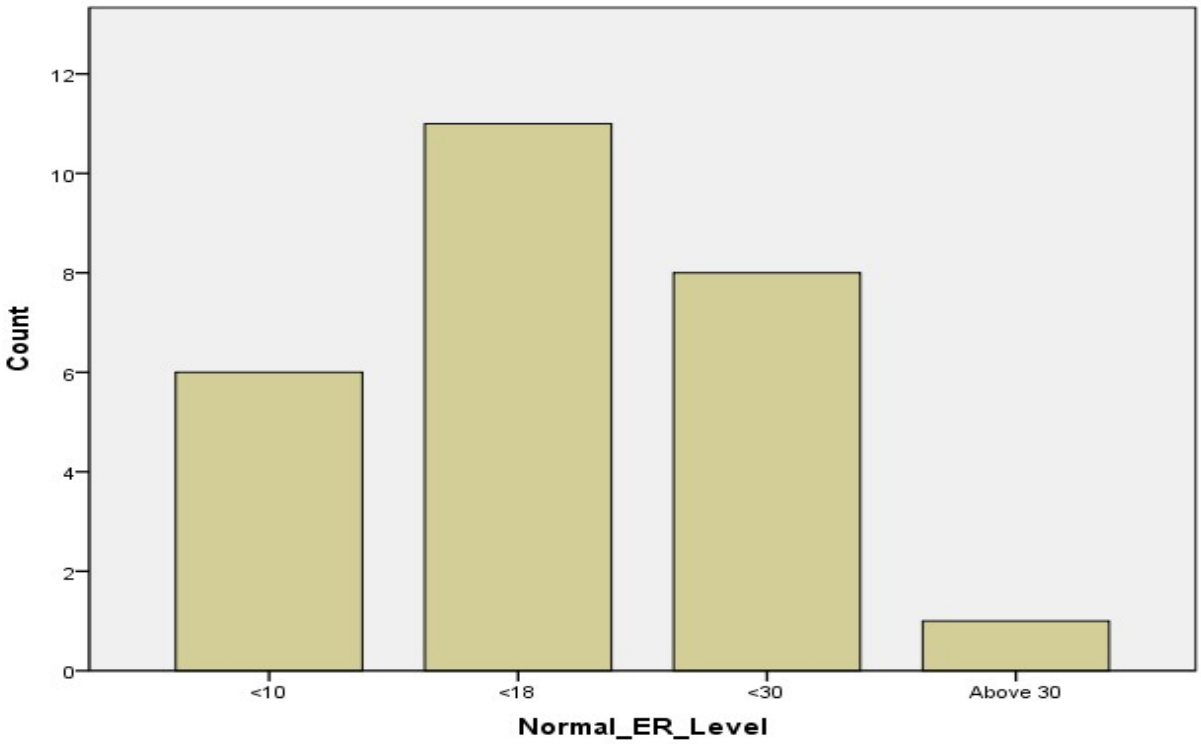

Figure 9 Estrogen Level of Normal postmenopausal woman

Count distribution of estrogen level in post menopausal normal women is less than 18 estrogen level frequencies are high. This histogram indicates that most of the post menopausal women having less than 18 estrogen hormone levels in 60%.

Pearson's Chi Square Correlation analysis on breast cancer and Tobacco usage

Smoking and tobacco use in daily life increases risk of breast cancer. But some studies founded that it is not associated actively in breast cancer. To address that, either tobacco is actively associated in breast cancer of this population size or not. Correlation analysis was done with Pearson's chi square test. It result positive association with breast cancer. This analysis was performed on large sample as compare to previous analysis. Assumption of hypothesis that tobacco using associated with breast cancer.

Table 16 Case Processing Summary of Post menopausal breast cancer woman and tobacco usage

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
Breast Cancer * Tobacco	80	98.8%	1	1.2%	81	100.0%

Table 17 cross tabulation of Post menopausal woman breast cancer and tobacco usage

			Tobacco		Total
			NO	YES	
Breast Cancer	NO	Count	17	9	26
		Expected Count	10.4	15.6	26.0
		Residual	6.6	-6.6	
	YES	Count	15	39	54
		Expected Count	21.6	32.4	54.0
		Residual	-6.6	6.6	
Total		Count	32	48	80
		Expected Count	32.0	48.0	80.0

Table 18 Chi –Square test result of Post menopausal breast cancer woman and tobacco usage

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	10.342[a]	1	.001		
Continuity Correction[b]	8.834	1	.003		
Likelihood Ratio	10.329	1	.001		
Fisher's Exact Test				.002	.002
Linear-by-Linear Association	10.213	1	.001		
N of Valid Cases	80				

a. 0 cells (.0%) have expected count less than 5. The minimum expected count is 10.40.

b. Computed only for a 2x2 table

Pearson chi square analysis of Breast cancer and tobacco usage and non usage was analyzed. Most evidences founded that show link between active tobacco usage and breast cancer. It revealed that smoking is associated with breast cancer. With significant chi square test value of 0.004 which is less than P value of 0.05 and it's met counts of all the cells greater than 5. It is concluded that tobacco usage is associated with breast cancer in this study.

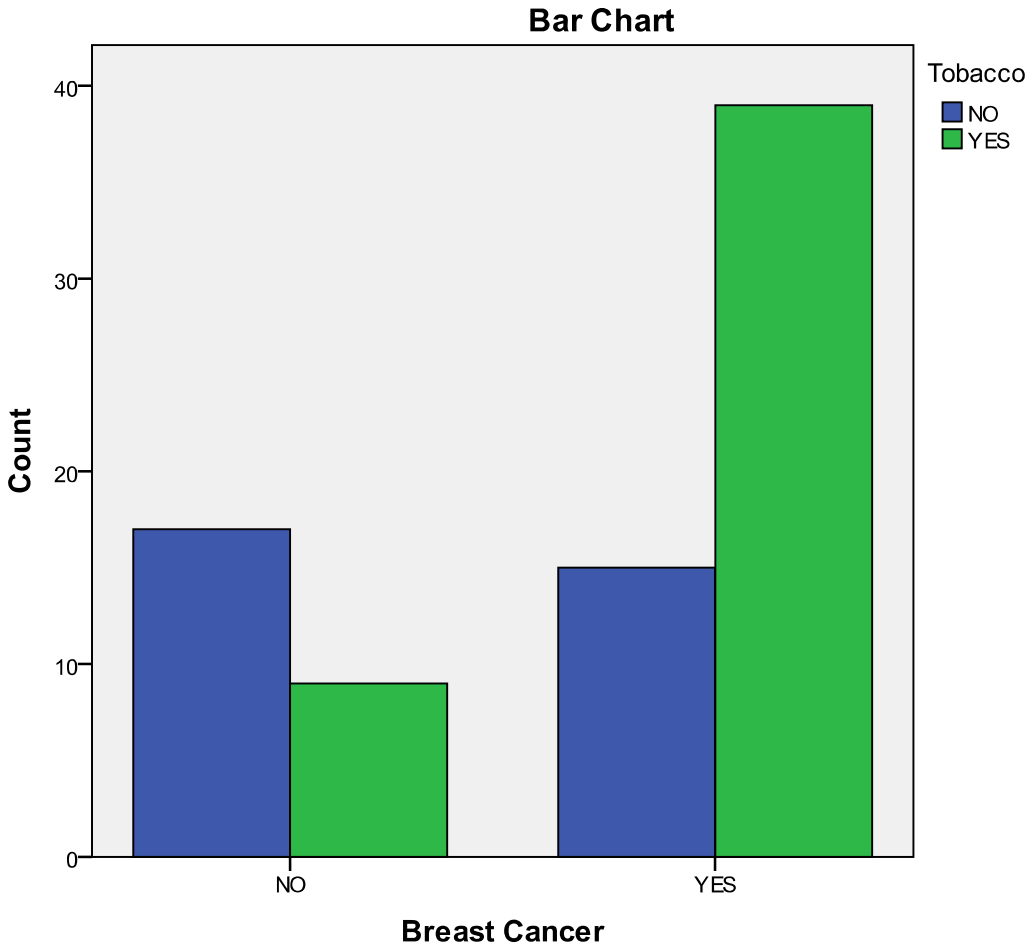

Figure 10 Breast cancer Postmenopausal woman and tobacco Concentration relationship

Pearson's Chi Square Correlation analysis on breast cancer and breast feeding

To address that, either breast feeding is actively associated in breast cancer of this population size or not. Correlation was done with Pearson's chi square test. In this analysis yield that breast feeding is not associated with breast cancer. The chi square test significant value is 0.532 which is higher than cutoff value of 0.05, although result metes the expected counts. Counts bar shown whole statistics relation.

Table 19 Cross Processing Summary of Post menopausal breast cancer woman and breast feeding

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
Breast Cancer * Breast Feeding	80	98.8%	1	1.2%	81	100.0%

Table 20 Cross tabulation of Post menopausal breast cancer woman and breast feeding

			Breast Feeding		Total
			NO	YES	
Breast Cancer	NO	Count	9	17	26
		Expected Count	7.8	18.2	26.0
		Residual	1.2	-1.2	
	YES	Count	15	39	54
		Expected Count	16.2	37.8	54.0
		Residual	-1.2	1.2	
Total		Count	24	56	80
		Expected Count	24.0	56.0	80.0

Table 21 Chi-Square test Result of Post menopausal breast cancer woman and breast feeding

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	.391[a]	1	.532		
Continuity Correction[b]	.133	1	.715		
Likelihood Ratio	.386	1	.535		
Fisher's Exact Test				.606	.354
Linear-by-Linear Association	.386	1	.534		
N of Valid Cases	80				

a. 0 cells (.0%) have expected count less than 5. The minimum expected count is 7.80.

b. Computed only for a 2x2 table

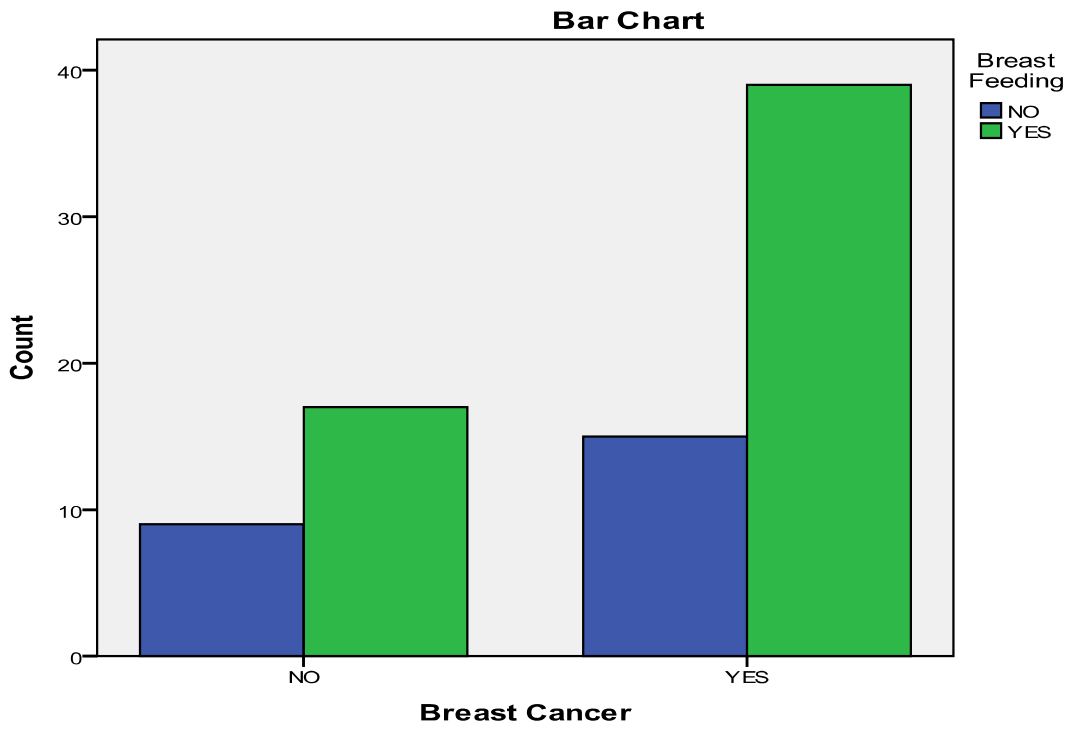

Figure 11 Post menopausal Breast cancer Woman and Breast feeding

Pearson's Chi Square Correlation analysis on breast cancer and marital status

Hypothesis that marital status is associated with breast cancer or not this analysis was performed and results are gathered.

Table 22 Cross Processing Summary of Post menopausal breast cancer woman and marital satatus

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
Breast Cancer * Marital Status	80	98.8%	1	1.2%	81	100.0%

Table 23 cross tabulation of Post menopausal breast cancer woman and marital status

			Marital Status		
			NO	YES	Total
Breast Cancer	NO	Count	8	18	26
		Expected Count	7.2	18.9	26.0
		Residual	.8	-.9	
	YES	Count	14	40	54
		Expected Count	14.9	39.2	54.0
		Residual	-.9	.8	
Total		Count	22	58	80
		Expected Count	22.0	58.0	80.0

Table 24 Chi-Square Tests of Post menopausal breast cancer woman and breast feeding

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	.206[a]	1	.650		
Continuity Correction[b]	.035	1	.852		
Likelihood Ratio	.204	1	.651		
Fisher's Exact Test				.790	.421
Linear-by-Linear Association	.204	1	.652		
N of Valid Cases	80				

a. 0 cells (.0%) have expected count less than 5. The minimum expected count is 7.15.

b. Computed only for a 2x2 table

Relationship between marital status and breast cancer was analyzed by chi square test. In this test the marital status is not significantly associated in breast cancer reduction. There is no significant relationship in postmenopausal women. Because p values to much higher than p=0.05 confidence interval.

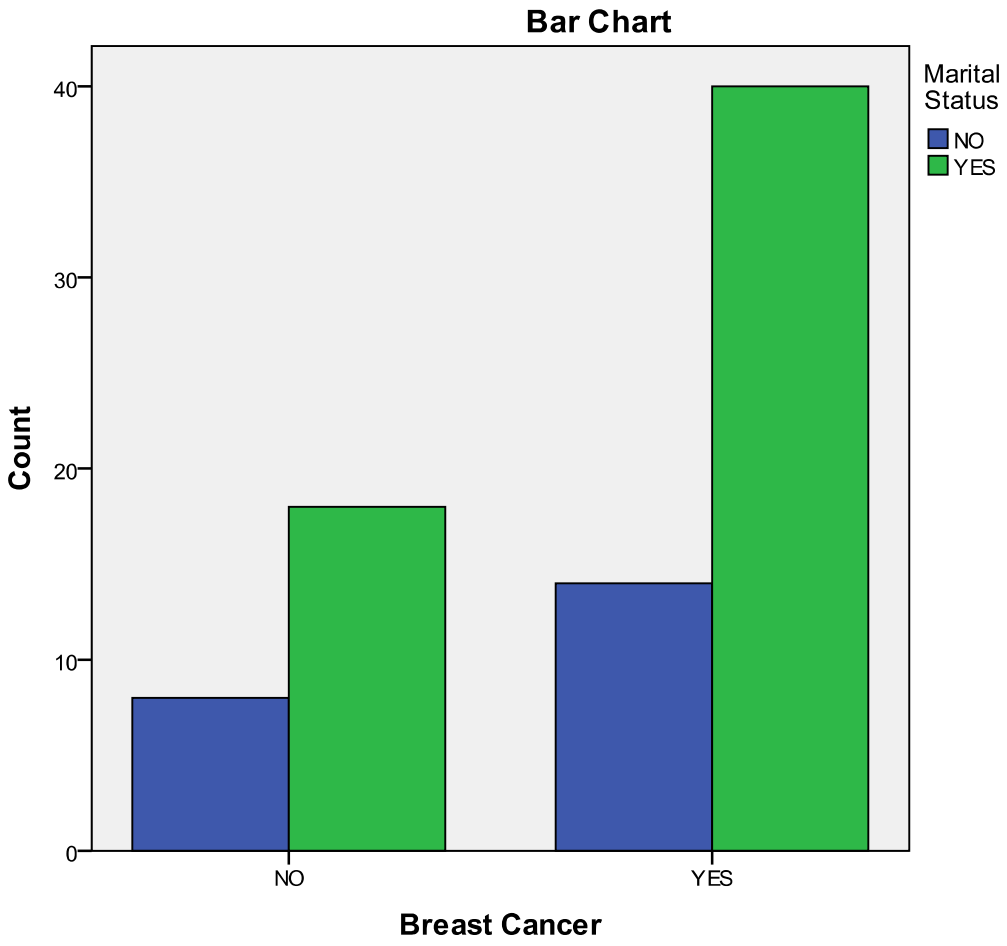

Figure 12 Postmenopausal woman breast cancer and marital status risk

CONCLUSION

Estrogen level is elevated in Postmenopausal breast cancer woman with increase of age. Tobacco used is strongly associated with breast cancer post menopausal woman and age at menarche not associated with breast cancer. Breast feeding does not reduce the risk of breast cancer in Pakistani Population of this study. Late the menopause is related to increase of age and this is the case in which postmenopausal estrogen level also increased in post menopausal breast cancer woman.

FUTURE DIRECTION

Pakistani population breast cancer post menopausal risk is increasing with age and estrogen level fluctuation also playing role in it. Study needed to done on wider scale to find more association findings of risk and Post menopausal breast cancer in Pakistani population, because this study was done one smaller data sample

REFRENCES

Allred, D. (2010). Ductal carcinoma in situ: terminology, classification, and natural history. *Journal of the National Cancer Institute Monographs*. Retrieved from https://academic.oup.com/jncimono/article-abstract/2010/41/134/890446

Anderson, W., Schairer, C., Chen, B., & Hance, K. (2006). Epidemiology of inflammatory breast cancer (IBC) 1. *Breast*. Retrieved from http://content.iospress.com/articles/breast-disease/bd000190

Arpino, G., Bardou, V., & Clark, G. (2004). Infiltrating lobular carcinoma of the breast: tumor characteristics and clinical outcome. *Breast Cancer*. Retrieved from https://breast-cancer-research.biomedcentral.com/articles/10.1186/bcr767

Baloch, A., Khosa, A., & Bangulzai, N. (2016). Novel Nonsense Variants c. 58C> T (p. Q20X) and c. 256G> T (p. E85X) in the CHEK2 Gene Identified dentified in Breast Cancer Patients from Balochistan. *Asian Pacific Journal*. Retrieved from https://www.researchgate.net/profile/Abdul_Baloch2/publication/301553197_Novel_Nonsense_Variants_c58CT_pQ20X_and_c256GT_pE85X_in_the_CHEK2_Gene_Identified_dentified_in_Breast_Cancer_Patients_from_Balochistan_Abdul_Hameed_Baloch1_Ahmad_Nawaz_Khosa1_Nasrullah_Bangulzai1_Jami/links/5719303208ae30c3f9f2bff7/Novel-Nonsense-Variants-c58CT-pQ20X-and-c256GT-pE85X-in-the-CHEK2-Gene-Identified-dentified-in-Breast-Cancer-Patients-from-Balochistan-Abdul-Hameed-Baloch1-Ahmad-Nawaz-Khosa1-Nasrullah-Bangulzai1-J.pdf

Boyd, N. (2011). Tamoxifen, mammographic density, and breast cancer prevention. Retrieved from https://academic.oup.com/jnci/article-abstract/103/9/704/919079

Boyle, P., & Levin, B. (2008). *World cancer report 2008.* Retrieved from https://www.cabdirect.org/cabdirect/abstract/20103010665

Bray, F., McCarron, P., & Parkin, D. (2004). The changing global patterns of female breast cancer incidence and mortality. *Breast Cancer*. Retrieved from https://breast-cancer-

research.biomedcentral.com/articles/10.1186/bcr932

Breast cancer: epidemiology and etiology. (2015). *Cell Biochemistry and*. Retrieved from http://link.springer.com/article/10.1007/s12013-014-0459-6

Butt, Z., Haider, S., Arif, S., & Khan, M. (2012). Breast cancer risk factors: a comparison between pre-menopausal and post-menopausal women. *JPMA-Journal of the*. Retrieved from https://www.researchgate.net/profile/Furqan_Syed/publication/228111122_Breast_cancer_risk_factors_A_comparison_between_pre-menopausal_and_post-menopausal_women/links/54bd509e0cf218d4a16a25d8.pdf

Cancer screening in the United States, 2017: A review of current American Cancer Society guidelines and current issues in cancer screening. (2017). *CA: A Cancer Journal*. Retrieved from http://onlinelibrary.wiley.com/doi/10.3322/caac.21392/full

Carey, L., Perou, C., Livasy, C., & Dressler, L. (2006). Race, breast cancer subtypes, and survival in the Carolina Breast Cancer Study. *Jama*. Retrieved from http://jamanetwork.com/journals/jama/fullarticle/202952

Chou, L., Lerner, L., Harris, A., & Brandon, A. (2015). Cancer prevalence among a cross-sectional survey of female orthopedic, urology, and plastic surgeons in the United States. *Women's Health*. Retrieved from http://www.sciencedirect.com/science/article/pii/S1049386715000699

Clavel-Chapelon, F., & Gerber, M. (2002). Reproductive factors and breast cancer risk. Do they differ according to age at diagnosis? *Breast Cancer Research and Treatment*. Retrieved from http://link.springer.com/article/10.1023/A:1014891216621

Colditz, G., Kaphingst, K., & Hankinson, S. (2012). Family history and risk of breast cancer: nurses' health study. *Breast Cancer Research*. Retrieved from http://link.springer.com/article/10.1007/s10549-012-1985-9

Dannhauser, A., & Berg, V. Van den. (2011). Prevalence of the known risk factors in women diagnosed with breast cancer at Queen II Hospital, Maseru. Retrieved from

http://scholar.ufs.ac.za:8080/xmlui/handle/11660/1226

Darweesh, A. (2009). Risk factors of breast cancer among Palestinian women in North West Bank. *Public Health Department. Faculty of Graduate*. Retrieved from https://scholar.najah.edu/sites/default/files/all-thesis/risk_factors_of_breast_cancer_among_palestinian_women_in_north_west_bank.pdf

DeSantis, C., Siegel, R., & Sauer, A. (2016). Cancer statistics for African Americans, 2016: progress and opportunities in reducing racial disparities. *CA: A Cancer Journal*. Retrieved from http://onlinelibrary.wiley.com/doi/10.3322/caac.21340/full

Edge, S., & Compton, C. (2010). The American Joint Committee on Cancer: the 7th edition of the AJCC cancer staging manual and the future of TNM. *Annals of Surgical Oncology*. Retrieved from http://link.springer.com/article/10.1245/s10434-010-0985-4

Ercan, C., Diest, P. van, & Vooijs, M. (2011). Mammary development and breast cancer: the role of stem cells. *Current Molecular Medicine*. Retrieved from http://www.ingentaconnect.com/content/ben/cmm/2011/00000011/00000004/art00002

Ferlay, J., Soerjomataram, I., & Dikshit, R. (2015). Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. *Journal of Cancer*. Retrieved from http://onlinelibrary.wiley.com/doi/10.1002/ijc.29210/full

Gao, Y., Shu, X., Dai, Q., & Potter, J. (2000). Association of menstrual and reproductive factors with breast cancer risk: results from the Shanghai Breast Cancer Study. *Journal of Cancer*. Retrieved from http://onlinelibrary.wiley.com/doi/10.1002/1097-0215(20000715)87:2%3C295::AID-IJC23%3E3.0.CO;2-7/full

Gaudet, M., Gapstur, S., & Sun, J. (2013). Active smoking and breast cancer risk: original cohort data and meta-analysis. *Journal of the*. Retrieved from https://academic.oup.com/jnci/article-abstract/105/8/515/912199

Global cancer statistics. (2011). *CA: A Cancer Journal*. Retrieved from http://onlinelibrary.wiley.com/doi/10.3322/caac.20107/full

GLOBOCAN 2012 v1. 0, cancer incidence and mortality worldwide: IARC CancerBase No. 11.

(2013). *For Research on Cancer*.

Gomez, S., Quach, T., & Horn-Ross, P. (2010). Hidden breast cancer disparities in Asian women: disaggregating incidence rates by ethnicity and migrant status. *Journal of Public …*. Retrieved from http://ajph.aphapublications.org/doi/abs/10.2105/AJPH.2009.163931

Gusterson, B., & Stein, T. (2012). Human breast development. *Seminars in Cell & Developmental Biology*. Retrieved from http://www.sciencedirect.com/science/article/pii/S1084952112000663

Haq, N., Haq, M., & Haque, A. (2009). Breast Cancer Incidence and Risk Reduction with Special Reference to Muslim Countries. *Journal of the Islamic Medical*. Retrieved from http://scholarlyexchange.org/ojs/index.php/JIMA/article/view/4424

Harris, H., Tamimi, R., & Willett, W. (2011). Body size across the life course, mammographic density, and risk of breast cancer. *American Journal of*. Retrieved from https://academic.oup.com/aje/article-abstract/174/8/909/156760

Hartmann, L., Sellers, T., & Frost, M. (2005). Benign breast disease and the risk of breast cancer. *England Journal of …*. Retrieved from http://www.nejm.org/doi/full/10.1056/nejmoa044383

Henderson, B., Ross, R., & Bernstein, L. (1988). Estrogens as a cause of human cancer: the Richard and Hinda Rosenthal Foundation award lecture. *Cancer Research*. Retrieved from http://cancerres.aacrjournals.org/content/canres/48/2/246.full.pdf

Intraovarian actions of oestrogen. (2001). Retrieved from http://www.reproduction-online.org/content/122/2/215.short

Israyelyan, A. (2003). The development of molecular diagnostics for breast cancer. Retrieved from http://etd.lsu.edu/docs/available/etd-0626103-132651/

Jack, R., Davies, E., & Møller, H. (2009). Breast cancer incidence, stage, treatment and survival in ethnic groups in South East England. *British Journal of Cancer*. Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2658548/

Jefcoate, C., Liehr, J., Santen, R., & Sutter, T. (2000). Chapter 5: Tissue-specific synthesis and oxidative metabolism of estrogens. *JNCI*. Retrieved from https://academic.oup.com/jncimono/article-abstract/2000/27/95/934425

Jemal, A., Bray, F., Center, M., & Ferlay, J. (2011). Global cancer statistics. *CA: A Cancer Journal*. Retrieved from http://onlinelibrary.wiley.com/doi/10.3322/caac.20107/full

Key, T., Appleby, P., Barnes, I., & Reeves, G. (2002). Endogenous sex hormones and breast cancer in postmenopausal women: reanalysis of nine prospective studies. *Journal of the National*. Retrieved from http://europepmc.org/abstract/med/11959894

Khuwaja, G., & Abu-Rezq, A. (2004). Bi-modal breast cancer classification system. *Pattern Analysis and Applications*. Retrieved from http://link.springer.com/article/10.1007/s10044-004-0220-7

Kluttig, A., & Schmidt-Pokrzywniak, A. (2009). Established and suspected risk factors in breast cancer aetiology. *Breast Care*. Retrieved from http://www.karger.com/Article/Abstract/211368

Kubba, A. (2003). Breast cancer and the pill. *Journal of the Royal Society of Medicine*. Retrieved from http://jrs.sagepub.com/content/96/6/280.short

Kwan, M., Kushi, L., & Weltzien, E. (2009). Epidemiology of breast cancer subtypes in two prospective cohort studies of breast cancer survivors. *Breast Cancer*. Retrieved from https://breast-cancer-research.biomedcentral.com/articles/10.1186/bcr2261

Lacroix, M., Toillon, R., & Leclercq, G. (2006). p53 and breast cancer, an update. *Endocrine-Related Cancer*. Retrieved from http://erc.endocrinology-journals.org/content/13/2/293.short

Landeghem, A. Van, Poortman, J., & Nabuurs, M. (1985). Endogenous concentration and subcellular distribution of estrogens in normal and malignant human breast tissue. *Cancer Research*. Retrieved from http://cancerres.aacrjournals.org/content/45/6/2900.short

Lichtenstein, P., Holm, N., & Verkasalo, P. (2000). Environmental and heritable factors in the causation of cancer—analyses of cohorts of twins from Sweden, Denmark, and Finland.

New England Journal. Retrieved from http://www.nejm.org/doi/full/10.1056/nejm200007133430201

Liede, A., Malik, I., Aziz, Z., Rios, P. D. los, & Kwan, E. (2002). Contribution of BRCA1 and BRCA2 mutations to breast and ovarian cancer in Pakistan. *The American Journal of*. Retrieved from http://www.sciencedirect.com/science/article/pii/S0002929707603395

Luo, J., Horn, K., Ockene, J., & Simon, M. (2011). Interaction between smoking and obesity and the risk of developing breast cancer among postmenopausal women: the Women's Health Initiative Observational Study. *American Journal of*. Retrieved from https://academic.oup.com/aje/article-abstract/174/8/919/155743

McPherson, K., Steel, C., & Dixon, J. (2000). ABC of breast diseases: breast cancer—epidemiology, risk factors, and genetics. *BMJ: British Medical Journal*. Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1118507/

Memon, Z., Khan, R., Raza, N., & Noor, T. (2014). Clinical Presentation and Frequency of Risk Factors in Patients with Breast Carcinoma in Pakistan. *Asian Pacific Journal of*. Retrieved from http://europepmc.org/abstract/med/26625746

Montserrat, P. (2015). Breast cancer epidemiology: mammographic screening and molecular subtypes. Retrieved from http://dugi-doc.udg.edu/handle/10256/10301

Muti, P., Bradlow, H., Micheli, A., & Krogh, V. (2000). Estrogen metabolism and risk of breast cancer: a prospective study of the 2: 16α-hydroxyestrone ratio in premenopausal and postmenopausal women. Retrieved from http://journals.lww.com/epidem/Abstract/2000/11000/Estrogen_Metabolism_and_Risk_of_Breast_Cancer__A.4.aspx

Nassar, H., Wallis, T., Andea, A., Dey, J., & Adsay, V. (2001). Clinicopathologic analysis of invasive micropapillary differentiation in breast carcinoma. *Modern*. Retrieved from http://search.proquest.com/openview/e156c24bf65c4efb16adcd7de0420157/1?pq-origsite=gscholar&cbl=33743

Pathy, N., Yip, C., Taib, N., Hartman, M., & Saxena, N. (2011). Breast cancer in a multi-ethnic

Asian setting: results from the Singapore–Malaysia hospital-based breast cancer registry. *The Breast*. Retrieved from http://www.sciencedirect.com/science/article/pii/S0960977611000324

Pharoah, P., Day, N., & Duffy, S. (1997). Family history and the risk of breast cancer: a systematic review and meta-analysis. *Journal of Cancer*. Retrieved from http://onlinelibrary.wiley.com/doi/10.1002/(SICI)1097-0215(19970529)71:5%3C800::AID-IJC18%3E3.0.CO;2-B/full

Polyak, K., & Hu, M. (2005). Do myoepithelial cells hold the key for breast tumor progression? *Journal of Mammary Gland Biology and Neoplasia*. Retrieved from http://www.springerlink.com/index/A571552612587478.pdf

Risk of breast cancer in relation to cigarette smoking. (1988). *Internal Medicine*. Retrieved from http://archinte.jamanetwork.com/article.aspx?articleid=609303

Ritchie, M., Hahn, L., Roodi, N., & Bailey, L. (2001). Multifactor-dimensionality reduction reveals high-order interactions among estrogen-metabolism genes in sporadic breast cancer. *The American Journal of*. Retrieved from http://www.sciencedirect.com/science/article/pii/S0002929707614530

Russo, J., & Russo, I. (2004). Development of the human breast. *Maturitas*. Retrieved from http://www.sciencedirect.com/science/article/pii/S037851220400204X

Saeidpour, S., & Torabizadeh, M. (2013). Reviw on cancer stem cells. *Int J LifeSc Bt Pharm Res*. Retrieved from https://pdfs.semanticscholar.org/cbd6/2b6d5368d929dcce5ee3689a9dafc7147192.pdf

Sandhu, D., Sandhu, S., & Karwasra, R. (2010). Profile of breast cancer patients at a tertiary care hospital in north India. *Indian Journal of*. Retrieved from http://www.indianjcancer.com/article.asp?issn=0019-509X;year=2010;volume=47;issue=1;spage=16;epage=22;aulast=Sandhu

Santen, R. (2014). Menopausal hormone therapy and breast cancer. *The Journal of Steroid Biochemistry and Molecular*. Retrieved from

http://www.sciencedirect.com/science/article/pii/S0960076013001258

Saslow, D., Hannan, J., & Osuch, J. (2004). Clinical breast examination: practical recommendations for optimizing performance and reporting. *CA: A Cancer Journal*. Retrieved from http://onlinelibrary.wiley.com/doi/10.3322/canjclin.54.6.327/full

Schellenberger, J., & Park, J. (2010). BiGG: a Biochemical Genetic and Genomic knowledgebase of large scale metabolic reconstructions. *BMC*. Retrieved from https://bmcbioinformatics.biomedcentral.com/articles/10.1186/1471-2105-11-213

Sharma, G., Dave, R., & Sanadya, J. (2010). Various types and management of breast cancer: An overview. *Journal of Advanced*. Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3255438/

Shaukat, U., Ismail, M., & Mehmood, N. (2013). Epidemiology, major risk factors and genetic predisposition for breast cancer in the Pakistani population. *Asian Pacific Journal of*. Retrieved from http://www.koreascience.or.kr/article/ArticleFullRecord.jsp?cn=POCPA9_2013_v14n10_5625

Shin, H., Carlos, M., & Varghese, C. (2012). Cancer control in the Asia Pacific region: current status and concerns. *Japanese Journal of Clinical*. Retrieved from https://academic.oup.com/jjco/article-abstract/42/10/867/811767

Siegel, R., & Naishadham, D. (2013). Cancer statistics, 2013. *CA: A Cancer Journal for*. Retrieved from http://onlinelibrary.wiley.com/doi/10.3322/caac.21166/full

Siegel, R., Ward, E., & Brawley, O. (2011). Cancer statistics, 2011. *CA: A Cancer Journal for*. Retrieved from http://onlinelibrary.wiley.com/doi/10.3322/caac.20121/full

Simpson, E. (2003). Sources of estrogen and their importance. *Journal of Steroid Biochemistry and Molecular Biology*. Retrieved from http://www.sciencedirect.com/science/article/pii/S0960076003003601

Soerjomataram, I., Louwman, M., & Ribot, J. (2008). An overview of prognostic factors for long-term survivors of breast cancer. *Breast Cancer Research*. Retrieved from

http://link.springer.com/article/10.1007/s10549-007-9556-1

Sohail, S., & Alam, S. (2007). Breast cancer in pakistan-awareness and early detection. Retrieved from http://europepmc.org/abstract/med/18182132

Strumylaitė, L., Mechonošina, K., & Tamašauskas, Š. (2010). Environmental factors and breast cancer. *Medicina*. Retrieved from https://publications.lsmuni.lt/object/elaba:5367091/

Surakasula, A., & Nagarjunapu, G. (2014). A comparative study of pre-and post-menopausal breast cancer: Risk factors, presentation, characteristics and management. *Journal of Research in*. Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4078652/

The biology of cancer stem cells. (2007). *Annu. Rev. Cell Dev.* Retrieved from http://www.annualreviews.org/doi/abs/10.1146/annurev.cellbio.22.010305.104154

Trinh, T. (2015). *Determinants of breast cancer risk: focusing on mammographic density*. Retrieved from http://openarchive.ki.se/xmlui/handle/10616/44927

Ursin, G., Bernstein, L., Wang, Y., Lord, S., & Deapen, D. (2004). Reproductive factors and risk of breast carcinoma in a study of white and African-American women. *Cancer*. Retrieved from http://onlinelibrary.wiley.com/doi/10.1002/cncr.20373/full

Wasif, N., Maggard, M., Ko, C., & Giuliano, A. (2010). Invasive lobular vs. ductal breast cancer: a stage-matched comparison of outcomes. *Annals of Surgical Oncology*. Retrieved from http://link.springer.com/article/10.1245/s10434-010-0953-z

Weigelt, B., & Baehner, F. (2010). The contribution of gene expression profiling to breast cancer classification, prognostication and prediction: a retrospective of the last decade. *The Journal of Pathology*. Retrieved from http://onlinelibrary.wiley.com/doi/10.1002/path.2648/full

Welcsh, P., & King, M. (2001). BRCA1 and BRCA2 and the genetics of breast and ovarian cancer. *Human Molecular Genetics*. Retrieved from https://academic.oup.com/hmg/article-abstract/10/7/705/558549

Wild, S., Fischbacher, C., & Brock, A. (2006). Mortality from all cancers and lung, colorectal, breast and prostate cancer by country of birth in England and Wales, 2001–2003. *British*

Journal of. Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2361230/

World Health Organisation classification of tumors of the breast. (2012). *Lyon: IARC*.

Yager, J., & Davidson, N. (2006). Estrogen carcinogenesis in breast cancer. *New England Journal of Medicine*. Retrieved from http://www.nejm.org/doi/full/10.1056/nejmra050776

Yasin, F., Afridi, Z., & Khan, R. (2014). A Comparative Study of Two Medication Groups of Infants with Moderate Bronchiolitis. *IRISH JOURNAL OF MEDICAL SCIENCE*. Retrieved from http://archive.imj.ie/Archive/IPA 2014 Oral and Poster Abstracts.doc

Yersal, O., & Barutca, S. (2014). Biological subtypes of breast cancer: Prognostic and therapeutic implications. *World Journal of Clinical Oncology*. Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4127612/

Yoshida, K., & Miki, Y. (2004). Role of BRCA1 and BRCA2 as regulators of DNA repair, transcription, and cell cycle in response to DNA damage. *Cancer Science*. Retrieved from http://onlinelibrary.wiley.com/doi/10.1111/j.1349-7006.2004.tb02195.x/full

Youlden, D., Cramb, S., Dunn, N., & Muller, J. (2012). The descriptive epidemiology of female breast cancer: an international comparison of screening, incidence, survival and mortality. *Cancer*. Retrieved from http://www.sciencedirect.com/science/article/pii/S187778211200029X

Yu, Y., Liang, C., & Yuan, X. (2010). Diagnostic value of vacuum-assisted breast biopsy for breast carcinoma: a meta-analysis and systematic review. *Breast Cancer Research and Treatment*. Retrieved from http://link.springer.com/article/10.1007/s10549-010-0750-1

Zaman, S. (2010). Economics of Mammography in Norway: A Descriptive study of Mammography Screening fron Societal perspective. Retrieved from https://www.duo.uio.no/handle/10852/30391

APPENDIX

Annexure 1