# Effect Of 17β-Estrdiol On MCF-7 And MDA-MB-231 Cell Lines On EGFR Expression

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**ABSTRACT**
In comparison to non-users, OCP users had a substantially higher incidence of breast tumors classified as Luminal B, Progesterone Receptor+ (PR+), and ER+. When comparing OCP users to nonusers, the age at admission for ER+ cancer was significantly lower in the former group (45.3 years) than in the latter (52.2 years). Alternatively, compared to non-users (45.4 years), patients with basal (TNBC) cancer who were OCP users were older at the time of admission (53.1 years). Logistic analysis showed that compared to non-users, OCP users had an 18% greater risk of TNBC and an 8% lower risk of ER+, PR+, and Luminal B, respectively, with each additional year of age. The in-vitro investigation found that the MDA-MB-231 cell line, when treated with β-estradiol and then chased with Cycloheximide, had a decreased expression of EGFR. It seems that estrogen destroys EGFR via the ubiquitination route, as EGFR expression did not decrease under treatment with MG-132 and E2. There may be a correlation between OCP usage and an uptick in ER+, PR+, and Luminal B breast cancer cases. In fact, there is some evidence that OCP usage is associated with a slowed advancement of TNBC. Results from an in vitro experiment showed that estrogen ubiquitinates EGFR in MDA-MB-231 cells, destroying the protein.

**KEYWORDS:** Oral contraceptive, breast cancer, phenotype, Progesterone

## INTRODUCTION

The presence or absence of molecular tumor markers such as estrogen receptor (ER), progesterone receptor (PgR), human epidermal growth factor 2 (HER2, ERBB2), and a proliferation index (Ki67), in addition to tumor size, tumor grade, and nodal status, can show that BrCa is a diverse disease with various intrinsic tumor subtypes. A poorer prognosis and treatment responsiveness are linked to subtype differences in genomic and immunohistochemical markers, unique racial/ethnic occurrence patterns, and aggression levels. The majority of breast cancers are ER-positive (ER+). Luminal A and luminal B are subgroups that make up this category; they are present in around 60% of all malignancies. The subtype known as luminal A (ER+/PgR+/HER2− with low Ki67) accounts for about 40% of all cases. This subtype is marked by a sluggish growth rate, a lack of aggressiveness, a good survival rate, and the best response to hormone treatment. By contrast, the subtype known as luminal B, which may be defined as ER+/PgR+/HER2+ or HER2− with high Ki67, accounts for 10-20% of all cancer cases. This subtype is characterized by a greater relapse rate, histological grade, proliferative index, and a worse relapse survival rate.

The specific subtype of BrCa is ER-negative (ER−). Features such as infiltrative margin, high grade lymphoid stroma, central fibrosis/necrosis, and comedo-type necrosis are morphological hallmarks of this condition. The ER−/PgR− subgroup is linked with a lower endocrine treatment sensitivity score and the most invasive tumors (20-25%) belong to this group. Additionally, the BRCA 1 germline mutation is greater in certain malignancies. A distinct BrCa subtype with an ER−/PgR+ phenotype, accounting for around 3% of cases, is being documented more often, and this subtype is characterized by distinct molecular and clinical features. Hormone sensitivity is low, recurrence is common, and overall survival is poor in this subtype. Nevertheless, a number of studies have cast doubt on the reality of this subtype, stating that it cannot be replicated, does not make biological sense, or might be a result of a technical error in the immunohistochemistry process that led to a misclassification. positive for HER2 Of all invasive breast cancers, 10% to 34% are BrCa, which is characterized as ER−/PgR−/HER2+. Its rapid growth and dissemination patterns set it apart. It is linked to a reduced duration of illness-free status and overall survival rate. Twelve to seventeen percent of all breast cancers are triple-negative (ER−/PgR−/HER2−), and they disproportionately strike young women. There is a significant death rate and the possibility of metastases associated with this aggressive malignancy. There are numerous risk factors for breast cancer, including but not limited to: reproductive factors, genetics, lifestyle, BrCa in family history, mutation carrier, age of menarche, parity, age at first birth, breastfeeding, and exogenous hormone use. The exact causes of the various subtypes of breast cancer are still unknown. One of the first areas of study was the estrogen receptor status in breast carcinomas since estrogens play a major role in the development of BrCa by promoting the expansion and differentiation of breast ductal epithelial cells. There has also been a lot of study on the link between breast cancer risk and the use of oral contraceptives (OC).

The results indicate that overall, OC usage is linked to a slightly elevated incidence of breast cancer in the community. Researchers have found conflicting results when looking at the impact of oral contraceptives on the chance of developing various subtypes of breast cancer. Some studies have shown an increased risk, while others have found no such effect. We achieved this goal by systematically reviewing and analyzing case control studies that focused on this subject. According to a meta-analysis of 54 studies, the risk of breast cancer is 24% higher for women who are currently using oral contraceptives (OCs). The risk is 16% higher during the first five years after ceasing OC usage, but it almost goes away after ten years. Due to a lack of research evaluating extended periods after cessation, however, the exact duration for which risk stays raised is unknown. Further research has linked long-term OC usage to an increased risk of incident breast cancer. White women provided the bulk of the data used in these investigations.

**LITERATURE REVIEW**

Dikshit (2012) Mumbai is one of the cities in India where the number of new cases of breast cancer is on the rise. These have most likely resulted from a shift toward a way of life more typical in industrialized nations. To better develop rational cancer control programs within the nation, it is vital to analyze breast cancer trends and estimate the future burden. We analyzed changes in breast cancer incidence rates from 1976 to 2005 using data from the population-based Mumbai Cancer Registry. The data was stratified by age category, with younger cases (25–49) and older cases (50–74) being considered separately. The estimated annual percentage change (EAPC) was measured by fitting age-period-cohort models and then using the net drift. The number of breast cancer cases and age-adjusted rates were projected around 2025 using population forecasts and age-period cohort models. Incidence rates of breast cancer rose sharply among women aged 65 and above over the last three decades (EAPC = 1.6%; 95% CI 1.1-2.0), whereas younger women had an even smaller but statistically significant 1% rise (EAPC = 1.0; 95% CI 0.2- 1.8). There were non-linear effects of time and cohort; a trends-based model projected that the number of incident cases would almost double by 2025, going from 1300 per annually in 2001–2005 to more than 2500 per annum in 2021–2025. Breast cancer has been more common in Mumbai during the last 20 to 30 years, especially among women of childbearing age. The bulk of breast cancer diagnoses will afflict older women, and the number of cases is expected to increase to over 2500.

Xu (2021) The prognosis is worse for young women with breast cancer since the disease is often detected at a later stage. Over the last quarter of a century, we looked at the incidence of breast cancer in women ages 20–49 broken down by race/ethnicity, hormone receptor status, tumor stage, and the influence of period and cohort effects. Methods: We drew on information from thirteen SEER registries covering the years 1993–2002 and eighteen SEER registries covering the years 2003–2017. For 222,424 women with primary invasive breast cancer between the ages of 20 and 49, we determined age- standardized incidence rates and annual percent change (APC). We next stratified the participants by race/ethnicity, hormone receptor status (ER and PR), and tumor stage (I-IV). Using the 1948 cohort and the 1993–1997 era as reference groups, respectively, we conducted age-period-cohort analysis (IRR) to examine the impacts of age, period, and cohort on incidence trends. After being steady from 1993 to 2010, the incidence of invasive breast cancer among women aged 20 to 49 years rose between 2010 and 2017. The rate of growth was 0.67 percent (95% CI: 0.32 to 1.03). Throughout the 25-year span (1993-2017), there were disparities based on race. The incidence rates among non-Hispanic White (NHW) and non-Hispanic Asia/Pacific Islander (NHAPI) women increased significantly (APC = 0.58%, 95%CI: 0.34 to 0.82), Hispanic women (APC = 0.59%, 95%CI: 0.34 to 0.83) and non-Hispanic Black (NHB) women did not increase significantly (APC = 0.14%, 95%CI: -0.06 to 0.34). For ER+ tumors, the incidence was higher, whereas for ER- tumors, it was lower: ER+/PR+ (2.39%, 95%CI: 2.20 to 2.58), ER+/PR- (1.46%, 95%CI: 1.05 to 1.87), ER-/PR+ (-6.33%, 95%CI: -7.31 to -5.33), and ER-/PR- (- 0.70%, 95%CI: -1.09 to -0.32). Lower rates among high-net-worth women seems to be the primary driver of the decline in ER-/PR- malignancies. There was an increase in the incidence of tumors in stages I, II, and IV (APC = 0.31, 95%CI: 0.07 to 0.55) and 2.88 (95%CI: 2.37 to 3.39) respectively, but the incidence of tumors in stage III reduced (0.81%, 95%CI: -1.04 to -0.59). The cohort effect was about ten times bigger than the period effect, while both had an influence on incidence. From 1948–1958, the age-specific relative risk by birth cohort fell, but from 1958–1993, it rose gradually. Compared to women born in the 1948 cohort, those born in the 1988 and 1993 cohorts had a higher incidence of breast cancer (IRR = 1.17, 95%CI: 1.07 to 1.28) and 1.22, 95%CI: 0.99 to 1.51, respectively). Results: The rise in ER+ tumors is the primary factor behind the alarming increase in the incidence of breast cancer among young women. The emphasis of prevention efforts should be on reducing ER-tumors while simultaneously addressing the variables that are causing the growth of ER+ tumors.

Su (2023) The components of PM2.5 may raise the risk of breast cancer and death. The purpose of this research is to examine the relationships between breast cancer survivors in Inner Mongolia, China, and their exposure to PM2.5 over an extended period of time and various causes of death. Using information from the Inner Mongolia Regional Health Information Platform, we were able to create a cohort of 33,952 individuals diagnosed with breast cancer in Inner Mongolia between 2012 and 2021. The Tracking Air Pollution in China database was used to determine the exposure levels of each patient to PM2.5 components. Accrual hazard ratios (aHRs) and 95% CIs were estimated using Cox regression models. There was a total of 3,295. The all-cause mortality rate was 5% for black carbon, 4% for sulphate (SO42-), and 7% for nitrate (NO3-), with a 5% increase for each IQR increase in concentration in the 5 years prior to diagnosis (HR:1.05, 95%CI: 1.00 to 1.10). Additionally, there was a correlation between organic matter and an elevated

risk of death from any cause. There were similar findings when looking for correlations with the risk of mortality from respiratory, cardio-cerebrovascular illness, and breast cancer-specific causes. Older age groups and Han Chinese patients had stronger relationships. Exposure to organic matter, SO42-, black carbon, and other pollutants for an extended period of time was more strongly associated with an elevated risk of mortality from any cause, breast cancer in particular, cardiovascular illness, and respiratory diseases. As a result, there is an immediate need for better controls over pollutants from coal burning in Inner Mongolia. Potentially vulnerable groups include the elderly and Han Chinese.

Das (2024) One of the most frequent cancers in women worldwide is breast cancer. Several studies have linked menstruation and reproductive variables in women to an increased risk of breast cancer. Consequently, the purpose of this research was to investigate whether or not Indian women's risk of breast cancer screening is related to reproductive and menstrual variables. Methods The 724,115 female respondents ranging in age from 15 to 49 who participated in the 2019–2020 National Family Health Survey (NFHS-V) were the subjects of the current research. The primary measure of success in this research was the number of breast cancer screenings reported by women in the age range of 30–49. The odds ratios and 95% confidence intervals for breast cancer by menstrual and reproductive variables were estimated using logistic regression models, which controlled for possible confounders. Final Product Breast cancer screening rates were found to be significantly higher in women who experienced late menarche (OR = 2.20, 95% CI: 1.48-3.28), irregular menstrual cycles (OR = 1.29, 95% CI: 1.08-3.53), a delay in the age at first birth (OR = 1.93, 95% CI: 1.11-3.04), and the use of contraceptives pills (OR = 1.11, 95% CI: 0.74-2.10). Breast cancer screening participation was lower among women with a longer history of breastfeeding (OR=0.75, 95% CI:0.63- 0.91) and a larger number of births (OR =0.52, 95% CI: 0.10-1.03). In summary the study's findings corroborate the hypothesis that reproductive and menstrual variables contribute to breast cancer in Indian women. Consequently, our results are critical for improving readiness and creating breast cancer preventive programs. One possible strategy to reduce the occurrence of breast cancer among women of childbearing age in India is to increase public understanding of the disease and its symptoms.

Mullaguri (2024) In 2020, there were 22,61,419 new instances of breast cancer, making up 11.7% of all cancers diagnosed. Among cancer-related fatalities, 6,84,996 were attributed to the disease, placing it seventh globally in terms of incidence and 6.9% of total cancer deaths. With 10,26,171 new cases and 3,46,009 deaths, Asia accounted for 45.4% of breast cancer incidence and 50.5% of breast cancer mortality, according to GLOBOCAN. Out of all the breast cancer cases and fatalities in the world, 23.5% (5,31,086 new cases) and 20.7% (1,41,765 deaths) occurred in Europe. Estimates for new cases of breast cancer in 2022 were 2,90,560, with 43,780 fatalities, according to the SEER database. According to the National Program of Cancer Registries of the United States and the SEER database, there were 51,400 new cases of ductal carcinoma in situ and 2,87,850 new cases of invasive breast cancer in 2022. Patients with localized cancer had the best 5-year survival rate (99%), while those with metastatic cancer had the worst (29%) and clinical subtype and tumor size were the main determinants of this survival rate variable. Black women had a 5-year survival rate of 96% for localized metastatic stages, 78% for regional metastatic stages, and 20% for distant metastatic stages; white women had a rate of >99%, 87%, and 30%. In Europe, the incidence rates for the 0-44, 45-64, and 65+ age categories were 34.8%, 34.3%, and 22.5%, respectively, with fatality rates of 25.1%, 19.3%, and 15.1%. The death rates differed more among countries than the incidence did among women aged 0–39 across 185 nations. In 2020, the age categories of 15–19, 20–29, and 30-39 had cancer incidence rates of 0.1%, 5.7%, and 46.6%, respectively, with 0%, 0.4%, and 4.8% fatality rates.

Kamath (2013) Among India's top 10 killers, cancer has just emerged. While cervical cancer is the second most frequent kind of cancer in India, breast cancer is the most common type overall. Multiple national cancer registry projects have shown an upward trend in incidence, and India is currently one of the countries with the highest estimated number of breast cancer fatalities globally. In order to investigate the causes of breast cancer. The goals of this research are to (1) identify breast cancer risk factors and (2) examine the relationship between breast cancer and certain exposure variables. In Manipal, Udupi District, researchers from the Shirdi Sai Baba Cancer Hospital and Research Center performed a case-control study. The research comprised a total of 188 people, with 94 serving as cases and 94 as controls. The age range of the study's participants was 25 to 69. The age range of the cases and controls was ± 2 years. A major risk factor was a non-vegetarian diet (OR 2.80, CI 1.15-6.81). Compared to women who were illiterate, those with 7–12 years of education had a 4.84-fold increased risk of breast cancer (OR 4.84 CI 1.51– 15.46). Breast cancer is more common in women with higher levels of education compared to those with lower levels of education, and the research indicates that a non-vegetarian diet is a major risk factor for breast cancer. Because this is hospital-based research, its results may not apply outside of that specific setting.

**Oral contraceptive**

Oral contraceptives that include both estrogen and progestin are referred to as combination birth control pills, or the pill. To avoid becoming pregnant, some people choose to use oral contraceptives. Additional advantages may also be theirs.

To prevent ovulation, you should take a combination of birth control tablets. A woman's ovaries are prevented from releasing an egg by the medications. Additionally, they alter the endometrium (the lining of the uterus) and the cervix (the mucous that lines the entrance of the uterus). Due to these alterations, sperm are unable to fuse with eggs.
The amounts of estrogen and progestin in various combination birth control tablets might vary. You may decrease the frequency of your periods annually with the use of continuous-dosing or extended-cycle tablets.

**Phenotypic expression**
The phenotype of an organism includes both its outward look (physical features) and the proteins it expresses within. It is possible for one cat to have white fur and another to have black hair. Since
their fur is different colors, we say that these cats do not share a fur phenotype. In some cases, a Punnett square may be used to infer the phenotype of an organism from its genotype.
Phenotypic expression is the process by which an organism's genes manifest as its outward features. The way something looks physically and structurally may vary as a result of changes in gene expression, which in turn cause different proteins to be made. An organism's phenotypic expression may also be impacted by mutations inside its DNA. A sickle cell anemia patient is a good example of this.

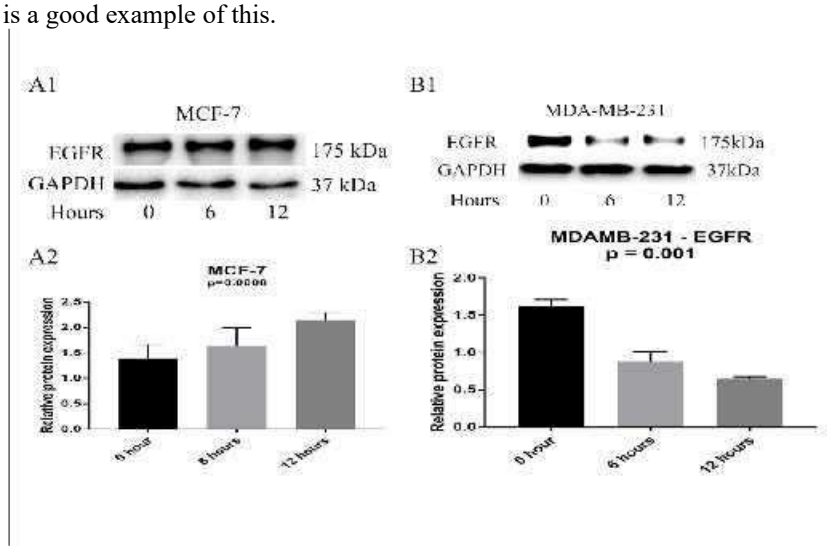


**Figure 1:** A1. Effect of 100 nM 17β-estradiol on MCF-7 cell line at 0, 6 and 12hours.

Representational blot B2. Impact of 100 nM 17β-estradiol on the MDA-MB-231 cell line at 0, 6, and 12 hours. All three replicates of the experiment werecarried out. Analysis of variance was conducted (p=0.001). In MCF-7 cell lines treated with 17β-estradiol, EGFR expression was higher at 0-, 6-, and 12-hours intervals. With a p-value of only 0.0008, After 6 hours and 12 hours, there was a 1.12%- and 1.4-fold rise in EGFR expression, respectively, that was statistically significant. The MDA-MB-231 cell lines treated with 17β-estradiol showed decreased EGFR expression at 0-, 6-, and 12-hours intervals. (Random p-value=0.0001) After 6 hours and 12 hours, there was a notable drop of 0.41% and 0.38% in EGFR expression, respectively.
The expression of EGFR was significantly decreased in cells treated with 17β-estradiol and cycloheximide at 1, 2, 3, and 4 hours. Significant (P= 0.001)From zero to four hours, EGFR expression dropped 1.52-fold. At 1, 2, 3, and4 hours, cells treated with 17β-estradiol, Cycloheximide, and MG-132 did notshow a significant decrease in EGFR expression. (P=0.05) From zero to fourhours, the expression of EGFR dropped by a pitiful 0.7-fold.

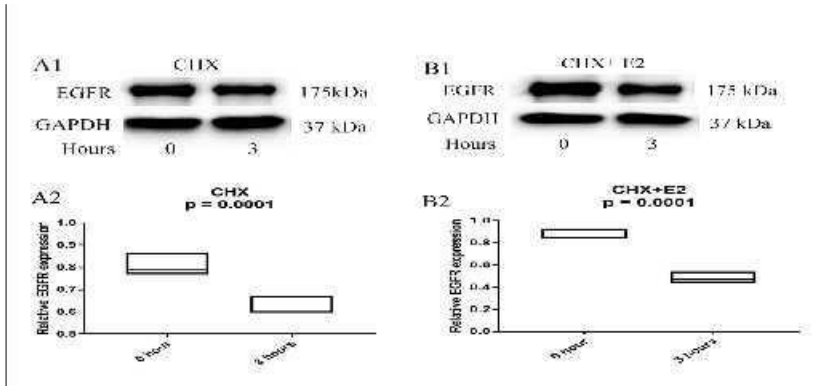


**Figure 2:** A1. Expression of EGFR in MDA-MB-231 cell line with 50 mg. Cycloheximide at 0 and 3 hours. (Representative blot) A2.

## CONCLUSION

Extensive investigation into the effects of OCP on various breast cancer subtypes has yielded more questions than solutions. We found that among ER+, PR+, and Luminal B breast cancer patients, past OCP usage was associated with an increased prevalence, relative risk, and disease progression. Contrary to popular belief, the relative risk of TNBC does not rise with past OCP usage. The usage of OCP, however, is linked to a later age at hospital admission. Consequently, it slows down the development of TNBC.

Molecular subtypes ER+, PR+, and Luminal B breast tumors were more common in OCP users compared to non-users, according to observations from human studies. When comparing OCP users to non-users, the age at admission for ER+ cancer was significantly lower in the former group (45.3 years) than in the latter (52.2 years). Alternatively, compared to non-users (45.4 years), patients with basal (TNBC) cancer who were OCP users were older at the time of admission (53.1 years). Logistic analysis showed that compared to nonusers, OCP users had an 18% greater risk of TNBC and an 8% lower risk of ER+, PR+, and Luminal B, respectively, with each additional year of age. The expression of EGFR was shown to be decreased in an in-vitro investigation using the MDA-MB-231 cell line treated with β-estradiol and a cycloheximide chase. It seems that estrogen destroys EGFR via the ubiquitination route, as EGFR expression did not decrease under treatment with MG-132 and E2.

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