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Correlation between duration of estrogen exposure with grading of breast cancer

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ABSTRACT

Introduction: Estrogen is known to contribute significantly to the formation and growth of breast tumors. High levels of serum estrogen and long-term exposure to estrogen are associated with an increased risk of breast cancer. The mechanism of estrogen involvement in the process of carcinogenesis is not known for certain. Many things are risk factors for breast cancer, between early menarche, slow menopause, with families with a history of breast cancer, obesity and a high-fat diet, age of upward productivity, first pregnancy in old age, and hormones. The purpose of this study was to see whether there is a long relationship of estradiol exposure to breast cancer grading.

Methods: Study design using cross sectional model, a sample of all breast cancer patients treated in the sub-section of surgical oncology rs dr muwardi March-mei 2016 as many as 108 with the criteria of breast cancer erα positive. Analysis by using Pearson correlation to see the strength of relationship between variables and see the presence or absence of long exposure relationship with grading by using ono-way ANOVA.

Result: 108 samples with ERα positive breast cancer, age 31 - 85 years, with longest exposure >40 years. Age of menarche, history of pregnancy, first childbirth, age of menopause, family history, and grading showed a relationship with prolonged exposure to estradiol, p<0.05. The longer the estradiol exposure of 39.89 years indicates the presence of high grade (grade III), p-value <0.05.

Conclusion: The duration of estradiol exposure in breast cancer patients will affect the grading of cancer.

Keywords: estradiol, long exposure, breast cancer grading.

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INTRODUCTION

Breast carcinoma is the most common malignant tumor and the leading cause of death in women with more than 1.000.000 cases occurring worldwide each year. Many things are risk factors for breast cancer, between early menarche, slow menopause, with families with a history of breast cancer, obesity and a high-fat diet, age of upward productivity, first pregnancy in old age, and hormones. Although the results of epidemiological studies show many factors that increase the risk of developing breast cancer in women, the denominator of the most commonly used risk factors is levels and duration of endogenous and exogenous estrogen exposure.[1]

Estrogens are known to contribute significantly to the formation and growth of breast tumors. High levels of serum estrogen and long-term exposure to estrogen are associated with an increased risk of breast cancer. The mechanism of estrogen involvement in the process of carcinogenesis is not known for certain. The most accepted theory holds that estradiol (E2) binding to α (ERα) estrogen receptors will stimulate cell proliferation and mutations resulting from replication errors occurring during pre-mitotic DNA synthesis. The continuous promotional or stimulating effects of estradiol (E2) lead to cell growth in which mutations occur. Over a period, the number of mutations that accumulate will suffice to induce the occurrence of neoplastic transformation.[2]

A recent molecular study of the role of estradiol against breast carcinogenesis, found that estradiol can act independently. Estrogen levels also contribute to the risk of distant metastasis in breast cancer patients with negative estrogen receptor (ER) tumors. Estrogen promotes growth, normalization, and angiogenesis of estrogen receptor (ER) negative breast cancer cells by systemic induction of host angiogenesis and recruitment of bone marrow stromal cells.[3,4]

In this study, we observed the duration of estrogen exposure in patients with breast cancer, looking at breast cancer risk factors such as patient age, family history and genetics, menstrual and reproductive history, and exogenous and endogenous hormones by comparing with the cancer grading.estrogen exposure in patients with breast cancer, looking at breast cancer risk factors such as patient age, family history and genetics, menstrual and reproductive history, and exogenous and endogenous hormones by comparing with the cancer grading.[5]

METHODS

Study design and subject

Study design using cross sectional model, with inclusion criteria of all women diagnosed with ERα positive breast cancer treatment in Sub Division of Oncology Surgery Dr. Moewardi Hospital in March-May 2016, patients were willing to participate as a sample in the study and agreed to do an estradiol examination and history taking for the risk factors of breast cancer. The exclusion criteria are patient with recurrent breast cancer.

Diagnosis of breast cancer is established based on histopathology results, including tumor grade, and immunohistochemistry. History taking is performed on patients aged at diagnosis, age of menarche, history of pregnancy, age at first childbirth, age of menopause, history of family planning and family history of cancer. All samples were given informed consent to be willing to follow this study, taken blood samples for blood samples for estradiol examination.

Examination of Estradiol

In premenopausal women, blood sampling is performed on days 21-22 of the menstrual cycle, with an estimated 28-day regular cycle. In women with abnormal menstruation or FP users and postmenopausal women serum estradiol levels are examined at any time.

Examination of estradiol was performed using 5 ml of blood serum. Stored in room temperature (maximum 3 hours). The test procedure is by way of channeling 25 µL of each standard, control and sample with new disposable ends into the proper well. Distributing 200 µL conjugate enzymes into each well. Stirred thoroughly for 10 minutes. It is important to perform perfect stirring at this stage. Incubate for 120 minutes at room temperature (without closing the plate). Shake quickly well, rinse well 3 times with a dilute wash solution (diluted solution) (400 µL per well). Scribbles well on sharp paper on absorbent to release drip remnants.

Survival analysis

Research methodology using survival analysis, data were taken from the medical records of breast cancer patients in the Surgical Oncology Departement of Dr. Moewardi Hospital from March to May 2016. History taking includes age at diagnosis, menarche age, pregnancy history, age at first childbirth, age of menopause, history of family planning and family history with cancer. All samples are breast cancer patients who have been treated in accordance with the applicable protocols based on stadium. Possible bias may occur in a sample of patients receiving hormonal therapy (anti-estrogen). This study is a cross sectional study with the aim to see the relationship duration of exposure to estradiol with breast cancer grading. The duration of exposure to estradiol was divided into 4 groups, exposure group <20 years, exposure 20 - 29 years, exposure 30 - 39 years and exposure >40 years. Analysis to see the strength of relationship between each characteristic subjects by using Pearson correlation. Analysis to see whether or not there is a long relationship of estradiol exposure with grading using One Way ANOVA, test considered significant if $p<0.05$.

Table 1. Subject Characteristics

Characteristics		Amount	%
Age	30 – 39	16	14.8%
	40 – 49	44	40.7%
	50 – 59	26	24.1%
	60 – 69	13	12.1%
	70 – 79	8	7.4%
	80 – 89	1	0.9%
Age of menarche	≤12 y.o	7	6.5%
	> 12 y.o	101	93.5%
Pregnancy history	No children	8	7.4%
	1 – 2	60	55.5%
	≥ 3	40	37.1%
Age at delivery of first child	< 20 y.o	7	7%
	20 – 30 y.o	16	16%
	>30 y.o	77	77%
Age of menopause	< 45 y.o	11	22.9%
	45 – 49 y.o	10	20.8%
	50 – 54 y.o	20	41.7%
	55 – 59 y.o	6	12.5%
	>60 y.o	1	2.1%
Family Planning history	Without Family Planning	51	47.3%
	< 10 years	36	33.3%
	≥10 years	21	19.4%
Grading	I	10	9.3%
	II	40	37%
	III	58	53,7&
Ki67	Positive	63	58.4%
	Negative	45	41.6%

RESULTS

Subject was taken from March to May 2016 at Dr. Moewardi Hospital, 303 patients with breast cancer diagnosis, 108 patients with ERα were positive, and 195 were negative ERα. With the youngest age 31 years old and the oldest 85 years. Subject characteristics are shown in Table 1.

Based on age, the highest frequency in the 40-year age decade was 40% with the longest exposure for 30-39 years, only statistically insignificant. ($p>0.05$). Based on age at menarche, most

Table 2. Relationship Characteristics Subjects With Duration of Estradiol Exposure

Characteristics		Exposure Length				p-value
		< 20	20 – 29	30 – 39	> 40	
Age	30 – 39	1	4	8	3	0.860
	40 – 49	1	9	20	14	
	50 – 59	1	7	11	7	
	60 – 69	1	3	5	4	
	70 – 79	0	4	1	3	
	80 – 89	0	0	0	1	
Age of menarche	≤12 y.o	3	4	0	0	<0.001
	> 12 y.o	1	23	45	32	
Pregnancy history	No children	4	2	0	2	<0.001
	1 – 2	0	22	29	9	
	≥ 3	0	3	16	21	
Age at delivery of first child	< 20 y.o	4	1	1	1	0.025
	20 – 30 y.o	0	2	8	6	
	>30 y.o	0	24	36	25	
Age of menopause	< 45 y.o	2	3	5	1	<0.001
	45 – 49 y.o	0	5	2	3	
	50 – 54 y.o	0	2	3	15	
	55 – 59 y.o	0	0	2	4	
	>60 y.o	0	0	0	1	
Family Planning history	Without Family Planning	2	18	24	7	<0.001
	< 10 years	2	8	18	8	
	≥10 years	0	1	3	17	
Grading	I	3	7	0	0	<0.001
	II	1	19	20	0	
	III	0	1	25	32	
Ki67	Positive	4	15	21	23	0.685
	Negative	0	12	24	9	

Table 3. Association between duration of estradiol exposure with breast cancer grading

Grading	Frequency	Mean	Standard Deviation	95% CI
I	10	22.10	3.38	11.69 – 21.86
II	40	29.60	4.75	3.30 – 12.73
III	58	39.86	5.15	7.85 – 13.73

frequencies >12 years were 93.5% with 30-39 years of exposure, with p<0.05. Based on pregnancy history, 55.5% 1 - 2 children, with 30-39 years old exposure, with p<0.05 (Table 2).

Based on age at delivery of first child the highest frequency at age >30 years counted 77% with 30-39 years old exposure. Based on the age of menopause, the highest frequency in the decade 50 - 54 years as much as 20% with a long exposure >40 years, p<0.05. Age of menopause, most frequencies in the decade 50 - 54 years as much as 20% with a long exposure >40 years, the value of p<0.05 (Table 2).

Based on history of Family Planning most frequent is breast cancer patients with family planning >10 years as much as 33.3% with long exposure for >40 years, p <0.05. While based on grading, 53.7% is grade III with a long exposure >40 years with a value of p<0.05. For Ki67 58.4% was positive Ki67 with exposure time >40 years and p>0.05. Sixty samples of patients have not been menopausal with the longest exposure for 30-39 years (Table 2)

Based on One Way ANOVA analysis, it was found that the exposure time of average exposure estradiol grading III is 39.86, while average exposure time of 29.60 at grading II and exposure time 22.10 describe grading I (p<0.05) (Table 3).

DISCUSSION

In the process of estrogen metabolism, estrogen is a steroid and one of the few aromatic molecules in humans. The major estrogens in the human body are estradiol and estrone, and 16-hydroxyestradiol (estriol). Estrone is reversibly converted to estradiol by the action of 17β-hydroxysteroid dehydrogenase enzyme. Androstenedione produced from theca cells during the follicular phase of the menstrual cycle, serves as a precursor of estrone and testosterone in the ovaries and peripheral tissues. Testosterone, in turn, is converted to estradiol by the action of aromatase enzymes in peripheral tissues.[5,7]

Menarche is a critical time for the development of mammary cells. Breast tissue maturation begins at the time of menarche and progresses at a constant rate until the birth of the first child. Experiments in mice showed that the non-differentiated duct cells would increase the effects of carcinogenesis and DNA1 damage. Histologically, early menarche was significantly associated with increased lobular tumor development compared with ductus tumor. The rate at which menarche occurs is caused by the variation of genes in each that can determine when a person has menarche. Physical activity performed at the time of menarche until the first pregnancy also affects a person can be at risk for breast cancer.[6,8]

In premenopausal women, estradiol is synthesized in the ovaries whereas in postmenopausal women, estrone synthesized in peripheral tissues is aromatase. Aromatase (CYP19), encoded by the CYP19A1 gene, is the enzyme limiting the rate of androgen conversion catalase to estrogen. Endogenous levels of the hormone estrogen in menopausal women are closely related to breast cancer. The high estrogen exposure can be caused by several conditions such as never giving birth or giving birth first at age 35, not breastfeeding, menopause at age >50 y.o, long-term use of hormonal contraceptives, and menarche

at <12 years.

While this study obtained on exposure to estradiol 30-39 years found in the history of pregnancy with 1 - 2 children and age at the birth of the first child> 30 y.o. In patients with a history of Family Planning ≥10 years with exposure> 40 years.

Estrogen metabolism may also play a role in the etiology of breast cancer. Estrone and estradiol can be irreversibly dihydroxylated on the C-2, C-4, or C-16 chains of the steroid ring to produce different estrogen metabolites in its bioavailability against breast tissue and estrogen receptor activation. Also, 2- and 4-hydroxylation will form catechol estrogens which will be oxidized to form mutagenic quinones; this process is prevented by the methylation of one of the adjacent hydroxyl groups. Estrogen and estrogen metabolites can also be conjugated through sulfation or glucuronidation, each of which modifies bioavailability.

Fuhrman et al. (2012), the 2-hydroxylation estrogen metabolite ratio for estrogen and the ratio of the 4-hydroxylated catechol route to the methylationated 4-hydroxylation pathway were statistically significantly associated with breast cancer risk, even after adjustment for unconjugated estradiol. Based on studies of estrogen levels of estrogen and estrogens higher estrogen catechol is found in patients with breast cancer.[10]

The prognosis of breast cancer is best characterized by the combined expression of receptors i.e. estrogen receptor, progesterone receptor (PR) and HER2 than with single receptor status alone. Based on the research shows that at younger age the tumor more express HER2 usually ER and negative PR with aggressive tumor biology. Patients with positive ER / PR and negative HER2 are more likely to be older. Menopausal obesity was also associated with a more consistent increase in risk of breast cancer for positive ER subtypes 10,11,12,13. This is consistent with our study, where in patients with positive ER, the duration of exposure of estradiol will affect the grading of cancer, the longer the estradiol exposure will lead to higher grading.[11-13]

In this study, we were unable to calculate the true levels of estradiol in the study sample, because all of the research samples were breast cancer with ERα positive so they were still on anti-estrogen therapy, which made the estradiol levels we can not measure exactly, and we can only calculate the duration of estradiol exposure before the patient is diagnosed with cancer.

CONCLUSION

All 108 samples of patient with ERα positive breast cancer, showing age and Ki67 results did not affect the duration of estradiol exposure, while menarche, history of pregnancy, age at first childbirth, age of menopause and family history of contraceptives showed an effect on duration of estradiol exposure. The longer the exposure to estradiol will result in high grading of breast cancer. The duration of this exposure due to the accumulation of estrogen metabolites in the form of catechol estrogen that is mutagenic is instrumental in the process of carcinogenesis of breast cancer.

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