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The Influence of Estradiol and HDL-Cholesterol levels on Breast Cancer in Pre-menopause and Menopause Women and its Correlation with BRCA1 Gene C61G Mutation

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Abstract

Estradiol hormones will increase the risk for breast cancer. About 5% to 10% of breast cancer patients bring with them germ line mutation. The aim of this study is to evaluate the estradiol and HDL-cholesterol levels and the correlation with BRCA1 gene C61G mutation. This research included 120 blood samples from normal females and 45 tissue samples. The results were that subjects with breast cancer in premenopause group indicating higher estradiol levels compared with menopause group. Highest HDL-cholesterol levels were found in 30-39 year group, and the lowest levels were found in the 50-59 years old group. Of all subject, only 32,5% samples with ER+/PR+, while ER-/PR- were found in 37,5%. HER-2/neu(+3) was found in 55,5% samples. For ER+/PR+ increased in the age group of 40-49 years, while ER-/PR- increased in the age group of 40-49 years (pre-menopause > menopause). HER2/neu (+3) was higher in pre-menopause than in menopause group. DNA sequencing results with no mutation or polymorphism in this SNP. There was significant difference between pre-menopause group and menopause group in estradiol and HDL-cholesterol content, ER+/PR+ was highest in pre-menopause.

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The highest ER-/PR- was found in in pre-menopause group, and the highest Her2/neu (+) was found in pre-menopause**.** BRCA1 gene C61G mutation was not found, it was suggested that polymorphism is occured in another different location.

Keywords: Estradiol; HDL cholesterol; ER/PR; HER2/neu; BRCA1 mutation C61G.

1. Background

Some risk factors are related to the increase in the case of breast cancer, including genetics, and history of reproduction [1]. Seventy five percents of all women with breast cancer do not know about its risk factors, and not all of them who are affected by breast cancer have the same risk factors. Some of these risk factors are not reversible . The increase in the exposure to estrogenic hormone will cause the increase in the risk for the incidence of breast cancer [2,3].

Germ line of BRCA1 gene mutation is about 40%-45% of the number of breast cancers which can be decreased, and every family member has high incidence in breast cancer. In this research, the evaluation on BRCA1 Gene mutation to C61G and the influence of another hormonal risk factors of breast cancer were performed.

2. Method

This research was conducted in Hospital Prima Medan, north sumatra, Indonesia.

HDL-cholesterol levels was determined by using method [3].

DNA sequensing was performed at laboratory of Biomedical Science, Medical Faculty, Andalas University, Padang, Indonesia in the period of 2016-2017.

Age Group of < 50 years and > 50 years old. Group I, 30 – 39 years Group II, 40 – 49 years Group III, 50 – 59 years Group IV,> 60 years

3. Devices and Material

The material used for isolating DNA from the blood was blood DNA isolating kits (PureLink Genomic DNA Mini Kit Invitrogen #Cat:K1820-01).

Polymerase Chain Reaction (PCR)

DNA genome was obtained by isolation using PCR technique. The quality of DNA as the result of amplification with PCR technique was seen by using agarose gel electrophoresis technique [4,5].

Detecting C61G Polymorphism with PCR Sequencing Method

BRCA1 gene polymophism in C61G allele was determined by analyzing PCR sequencing. The data of the

sequencing result was then processed by using Genious software (contig. alignment, and multiple alignments) in order to obtain SNP maturation of C61G allele.

Procedure

Blood samples were taken through antecubital vein function of 2 ml which was put into a tube containing ethylene diamine tetra acid (EDTA) anticoagulants in which DNA and PCR were extracted.

Blood DNA isolation procedure performed as the instructions of the protocol of using DNA Invitrogen isolation kit.

Specific Primer Design

Table 1: Primer Pair Used to Identify C61G Allele Polymorphism

Primer	**Sequence**
Forward	5'- CTC TTA AGG GCA GTT GTG AG -3'
Reverse	5'- TTC CTA CTG TGG TTG CTT CC -3'

The sequence of BRCA1 Gene Nucleotide Base in determining C61G mutation can be downloaded in the National Center for Biotechnology (NCBI) Gene Bank and accessed by using code through http://www.ncbi.nlm.nih.gov website. The primer can be selected by considering the requirement of a primer and paying attention to the location of C61G allele polymorphism which can be known with Avaff restriction enzyme. The selective primer pair has the sequence of 5'- CTC TTA AGG GCA GTT GTG AG -3'for C61G primer (forward) and 5'- TTC CTA CTG TGG TTG CTT CC -3 for C61G primer (reverse).

4. Research result & discussion

Table 2: The differences of laboratory indices in the age group < 50 years and > 50 years old of normal subjects.

Variable levels	concentration based on age	
	< 50 yr	> 50 yr
Estradiol (pg / dl)	106.9±95.8	25.6±34.8
Cholesterol (mg/dl)	151.1±84,6	140.0±89.0
Triglyceride (mg/dl)	108.65±93,48	92.4±74.0
HDL-cholesterol (mg/dl)	30.15±15,1	30.9±20.1
LDL-cholesterol (mg/dl)	98.85±61,4	90.6±57.5

Based on statisticaly result table 2 shows on breast cancer women in the aged < 50 years that estradiol concentration increased difference in the aged > 50 years and on the same condition followed with the decreased cholesterol , Trygliserida and HDL- cholesterol to be low too in the age < 50 years difference in the age > 50 years.

Chi-square examination between for two group results with $p < 0.04$ ($p < 0.05$).

Table 3: The Difference of estradiol levels and HDL-cholesterol levels between of Subjects with breast cancer

Variables	Age Group	
	< 50 yr	> 50 yr
Estradiol (pg /dl)	106.9±95.8	25.6±34.8
HDL cholesterol(mg/dl)	30.4±15.1	32.1±18.7

Estradiol concentration increased in the aged group 40-49 years and decreased in the age group 50-59 yr. HDL- cholesterol in the aged group 40-49 years and 50-59 years to be decreased. In the aged < 50 years estradiol concentration to be decreased and in the aged > 50 years estradiol to be low and HDL – cholesterol increased in the aged > 50 years.

Table 4: Estradiol dan Lipid Profile between normal and breast cancer women

	Group I		Group II		Group III		Group IV	
	Normal	BC	Normal	BC	Normal	BC	Normal	BC
Estradiol(pg/dl)	47.9±20	98.3±67,48	48.8±21	114.2±107.2	8.6±4.2	33.4±43.98	3.2±1.5	18.9±9
Total cholesterol (mg/dl)	201±38	139,2±74	208±40	151.0±91.7	234±63	120±97.7	240±42	171.6±68.9
HDL chol l(mg/dl)	56±5	32.2±15.8	52±14	28.6±15.3	47±10	25.9±21.6	39±15	38.3±15.7
LDL chol (mg/dl)	129±38	86.6±53.6	131±55	101.2±66	156±60	78.2±63.7	160±36	111±44.6

Group I, 30 – 39 years Group II, 40 – 49 years Group III, 50 – 59 years Group IV,> 60 years

BC, breast cancer

It was found that in normal patients the estradiol content which decreased significantly between the age group of 40-49 years with the content of 48.8 ± 21 ng/ml and the age group of 50-59 years with the content of 8.6 ± 4.2 pg/m [6,7]. Table 4 demonstrate the differences in the age groups II and III, in which the was the decrease in estradiol content from 40-49 years (114 ± 107.2) to 50-59 years (22.4 ± 43.98), the differences was significant between pre-menopause and menopause. Syukur and his colleagues found the difference of HDL levels among normal patients. There was significant decrease in HDL –cholesterol levels in the age group of 30-39 years, 40-49 years, and 50-59 years with the content value of 56 ± 5 mg/dl, 52 ± 14 mg/dl, and 39 ± 15 mg/dl respectively. While in breast cancer patients this condition was also found in the age group of 30-39 years (32.2 ± 15.8), 40-

49 years (28.6 ± 15.3), and 50-59 years (25.9 ± 21.6) which decreased so that there was significant difference between pre-menopause and menopause. Therefore, it could be concluded that there was the correlation of Group II with Group III toward the incidence of breast cancer which was associated with the increase in ages with estradiol content and HDL-cholesterol content. Current research the correlation of body weight with breast cancer was not obvious or in negative stage, the low level of HDL-cholesterol had been associated with the risk for breast cancer. The 27-hydroxycholesterol (27HC) could function as estrogens and could increase the proliferation of breast cancer cells in ER (+). High cholesterol, and LDL became obesity co-morbidity which was probably the independent risk factor for breast cancer, and the mechanism of pathology in which it was based had made it relatively new to be defined [7,8,9]. The incidence of breast cancer rarely occurs in a person who is below 30 years old in accordance with multistep cancer progression model of Knudson [10,11]. The decrease in HDL-cholesterol in the age group of > 50 years was related to the period of menopause and to the post-menopause. The older a person is, the more he has the risk for being affected by cancer. Breast cancer usually occurs in women who are over 50 years old and who have undergone menopause. About 80% of the cases in breast cancer occur in women who are over 50 years old. In the post-menopause women, the risk factor for obesity can increase the incidence of breast cancer with positive Estrogen Receptor (ER) of > 50% where the obesity population in the United States is > 40% and it increases rapidly in women over 60 years old. Supplementary independent risk factor for breast cancer occurs in women in the post-menopause in which morbidity with obesity exists; it can also cause damage/change in cholesterol metabolism [12,13]. In the pre-menopause women, about two-thirds of women who suffer from breast carcinoma are < 50 years old who have positive ER expression.

Table 7: The Difference in the aged group and reseptor ER/PR Receptor in the breast cancer women

	Ages	ER+/PR+	ER+/PR-	ER-/PR+	ER-/PR-
I	30-39 years	3	0	2	1
II	40-49 years	6	0	4	6
III	50-59 years	2	0	2	3
IV	>60 years	2	3	1	5
	Total	13	3	9	15
	Persentase	32.5%	7.5%	22.5%	37.5%

Multiple positive tumors (55% to 65% of breast cancer) have better prognosis and response to hormonal therapy. This group is also associated with older age, lower level, smaller size tumor, and lower mortality.

Multiple negative tumors which are the second biggest group (18-25%), 85% of them are level 3 tumor; it is related to high recurrence level, low resistance, and unresponsive to hormonal therapy. Mean while, for single positive group, the consequences of ER+/PR- (12-17 %) and ER-/PR+ (1-2%) are not understood well. This

group can be related to high level of histopathology, bad prognosis, and big size tumor [13].

Table 8: The Difference between Age Group and HER-2/neu Receptors in each Age Group in the Age of Breast Cancer Cases

	Umur	HER-2/neu(+1)	HER-2/neu (+2)	HER-2/neu(+3)
I	30 – 39 yr	1	1	5
II	40 – 49 yr	1	0	8
III	50 – 59 yr	0	0	4
IV	>60 yr	0	2	3
	Persentase	5,5%	8,3%	55,5%

Human epidermal growth factor receptor-2 onkogene ERBB2 (which is commonly called as HER-2) encodes epidermal growth factor receptor (EGFR) family from tyrosine kinase and is located in chromosome 17q21. The gene is very important for differentiation, adhesion, and motility cells. HER-2 is positive in about 18-20% of breast cancer. Positive HER-2 is commonly associated with bad differentiation, metastasis to lymph nodes, recurrences, and high mortality rate so that it has bad prognosis.

Other researchers point out that high HER-2/neu expression is associated with high level of histopathology, decreasing resistance, decreasing response to methotrexate and hormonal receptor modulators, and increasing response to dexorubicin. It is also associated with bigger size tumor, metastasis to lymph nodes, and worse resistance.

The risk for being affected by breast cancer a woman who is 50 years old and who will have the risk for being affected by breast cancer in all her life is 11%, while a woman who is 70 years old and who will have the risk for being affected by breast cancer is only 7% [13.14]. In the following research on Polish female population with breast and ovarian cancers with the history of family, it was found that there were three BRCA1 genes which were mutated; some of the important ones had been detected, including 538insG, 185delAG, and C61G in exons 20.2 and 5 [14].

5. Conclusion

From the explanation of the Tables above, it could be concluded that

1. Age groups II-III (40-60 years old) were the period of breast cancer development which was associated with the drastic decrease in estradiol hormone and in HDL-cholesterol;
2. This condition was correlated with the age group of pre-menopause, menopause, and post-menopause;
3. There was no SNP polymorphism, due to the differences in location, race, lifestyle, and environment.

The next stage was the examination of PCR DNA in cancer tissues/blood and sequencing. Based on the analysis

on the result of sequencing, it was found that there was no mutation or polymorphism in the SNP. Of the 35 samples which had been analyzed, all of them had normal alleles (wild type).

References

[1] Azhar,M,Natalia, D.,Syukur,. S, Vovien, Jamsari, (2015) Gene Fragments that encodes inulin hydrolysis enzyme from genomic Bacillus licheniformis:Isolation by PCR technique using new primers International, Journal of Biological Chemistry 9 (2) ,pp.59,

[2] Abbas AK, Lichtman AH, Pillai S. 2007. Immunity tu Tumors, in Cellular and Molecular Immunology, 6th Ed. Philadphia: WB Saunders, pp. 397-418.

[3] Bolton Jl and Thatcher GRJ. 2008. Potential Mechanisms of Estrogen Quinone Carcinogenesis, Chem Res Toxicol, pp. 21 (1):93-101

[4] Baudi F, Quaresima B, Grandinetti C, Cuda G, Faniello C, Tassone P, Barbieri V Bisegna R, Ricevuto E, Conforti S, Viel A, Marchetti P, Ficorella C, Radice P, Costanzo F,Venuta S.2001. Evidence of a Founder Mutation of BRCA1 in a Highly Homogeneous Population from Southern Italy with Breast/Ovarian Cancer. Hum Mutan; 18(2), pp. 163.

[5] Donal P. Mc Downell, Sungkee Park, Matthew T. Goulet, Jeff Jasfer, Suzane E, Wardell, Ching-Yi Chong, Jhon D. Haris, Jhon D Guyton, and Erik R. Nelson. 2014. Obesity, Cholesterol Metabolism and Breast Cancer Pathogenesis Cancer Res, pp. 4976-4982

[6] Ellis IO, Schinitt SJ, Sastre GX, et al. 2003. Invasive Breast Carcinoma in World Health Organization Classification of Tumors Pathology & Genetics Tumors of the Breast & Female Genital Organs. IARC, pp. 13-59

[7] Ginting, S,F, Syukur S, Ibrahim, S, Tjong, D, H, and Fachrial E, 2015, Lipid profile and estradiol analysis between pre-menopose and post-menopause woman in Medan areas of North Sumatra, Indonesia, journal of Chemical and Pharmaceutical Research,7(10), 505-508

[8] Ibrahim AM. Ali RM. Ali MM, Azary SS, Hassan AK. 2011. Hormonal Changes in Egyptian Patients Suffering from Prostate Cancer and Benign Prostate Hyperplasia. NY Scj; pp. 4(3):18-26

[9] Knudson AG, 2001. Two Genetic Hits (more or less) to Cancer. Nat Rev Cancer 1, pp. 157-162

[10] Lacey J, Kreimer A , Buys S, Marcus P, Chang S , Leitzmann M Hoover R Prorok P, Berg C., Hartge P, Prostate, Lung, Colorectal and Ovarian (PLCO) Cancer Screening Trial Project Team. Breast cancer epidemiology according to recognized breast cancer risk factors in the Prostate, Lung, Colorectal and Ovarian (PLCO) Cancer Screening Trial Cohort.BMC Cancer 2009, pp. 9:84-92

[11] Lee A, Park WC, Yim HW, Lee MA, Park G, Lee KY. 2007 Expression of c-erbB2, cyclin D1, and

Estrogen Receptor and their Clinical Implication in the Invasive Ducal Carcinoma of the Breast. Japan Journal of Clinical Oncology 37 (9), pp708-714 Meads,C.Ahmed,I, Riley,R.D.2012. A Systematic Review of Breast Cancer Incidence Risk Prediction Models with Meta Analysis of their Performance. Breast Cancer ResTreat; 132(2), pp. 365-377

[12] Syukur S, Syafrizayanti, Zulaiha S, (2017). Virgin Coconut 0il Increase HDL, lower triglyceride and fatty acids profile in blood Serum of mus musculus, Research journal of Chemical and Pharmaceutical Research 8(2) pp 1077.

[13] Sihombing, B., Jamaludin, Cong, D,H, Ibrahim, S., Syukur, S., (2015) Immunohistohemical detection of p53 protein as a prognostic indicator in prostate carcinoma, Journal of Chemical and Pharmaceutical Research 7(9)

[14] Grzybowska E, Zientek H, Jasinska A. High Frequency of Recurrent Mutations in BRCA1 and BRCA2 Genes in Polish Families with Breast and Ovarian Cancers. Human Mutan 2000, pp. 16: 482–90.