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Relationship between Beta Estrogen Receptor Polymorphism and Psychosocial Stress on Menopausal Women

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Abstract

Menopause is a normal stage of development experienced by elderly women. This study aimed to evaluate the association between polymorphisms of ERα & ERβ and the incidence of psychosocial stress on menopausal women. This research was conducted in BLU dr. Wahidin Sudirohusodo and affiliate hospitals of Department of Obstetrics and Gynecology Medical Faculty of Hasanuddin University in Makassar, using cross-sectional design. Obtained sample of 102 subjects who met the inclusion criteria and examined polymorphisms receptors ERβ. Data were statistically analyzed using the chi square test with p value <0.05. Results showed that respondents who experienced psychosocial stress has the most ERβ polymorphism homozygous dominant AA. There is ERβ polymorphism relationship with the occurrence of psychosocial stress in menopausal women.

Keywords: Polymorphism of estrogen receptor α Pvull; α Xball; β; psychosocial stress; menopause.

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1. Introduction

An increasing concern on the treatment and improvement of health services focused on prevention, also increase the overall survival rate for patient. Thus, most of women will spend a third of their lives in the post-menopausal period. Specifically, on US census in 2010, there are nearly 42 million women aged 55 years old and older. The menopausal transition and the life spent in postmenopausal period will bring many quality of life issues also prevention and management of disease in this periods [1].

Life expectancy in indonesia, also has increased until reaching the age of 70 years starting from the year 2000 until now. This resulted in an increase in the number of postmenopausal women in Indonesia. Age of women enter menopause period ranging between 45-55 years. Thus if menopausal period occured at around the age of 50 years, it is predicted that Indonesian women will experience menopause around 20 years of her life, which is almost a third of her life [2].

The term menopause refers to a point that is one year after the cessation of menstruation. Postmenopause is the period after that point. The average age of women who had their last menstrual period was 51.5 years, but the cessation of menstruation due to ovarian disorders can occur at any age [2].

Menopause comes from the Latin word "*mensis*", which means the moon and from the Greek word "*pausis*", which means stop. Menopause is a phenomenon in women's lives characterized by the cessation of the menstrual cycle due to a decrease in ovarian function that resulted in decreased production of the estrogen. Menopause is the most significant physiological changes in middle aged women that is around 40-65 years [3].

Eventually over time, menopausal women may experience isolation (loneliness). Decrease of the individual ability of hearing, seeing or any other activity makes women felt loss of attention and support from her social environment [4]. Depression is a continuation of the stress that is not resolved. Problems that can cause stress include the aspects of physiology, psychology, social and sexual aspects, but it is different for every woman so that the stress level is also diverse [5]. Currently the direct relationship between psychological symptoms and hormonal changes such as decreasing of estrogen level is not well esthablished. Stress, education level, ethnicity, socio-economic factors affecting the prevalence of partner status and the onset of menopausal symptoms and depression [6] .

Logically, individuals who have problems with mood can have such a mechanism. In one same study conducted in US with 34 subjects of perimenopausal women with symptoms of major and minor depression were treated with 50 mg estradiol transdermal show changes that independently in vasomotor symptoms. This experiment shows the use of estrogen beneficial for the treatment of women who experience clinical depression. This conclusion is supported by the success of treatment in depression for postpartum women using estradiol as the treatment [7].

Many factors can affect levels of estrogen in a woman. The activity of estrogen in the brain are mediated through activation of intracellular, transmembrane and membrane receptors of estrogen that binds to its receptor through non-genomic mechanisms. Estrogen receptor (ER) has two subtypes, namely α subtype and β subtype (ERβ).

Both receptors are located in brain areas and associated with cognitive and emotional function. Estrogen, as a steroid hormone, acts by increasing gene expression in the cell nucleus. There are two important estrogen receptors. The alpha-receptor (ERα) is responsible for estrogen effects on cognitive functions, whereas the beta-receptor (ERβ) is responsible for the serotonergic system and emotional processes. Mood, cognition and neuronal health are associated with the effect of estrogen on the central nervous system. Estrogen increases serotonin levels by reducing monoamine oxidase, which catabolizes serotonin, by separating tryptophan bound to albumin essential for serotonin synthesis, and by increasing serotonin transport.

Menopausal women often experience physical and emotional problems which occurs due to declining of estrogen levels that can cause psychosocial stress. Psychosocial stress which can not be resolved will cause anxiety and even can cause symptoms of depression. However, this situation does not occur in all menopausal women.

Research on the relationship of psychosocial stress with estrogen receptor polymorphisms is still lacking, so that researchers interested in conducting research aimed to evaluate the association between polymorphisms ERβ on the incidence of psychosocial stress menopausal women.

2. Material and Method

2.1 Place & Duration of study

This study was conducted in a teaching hospital in Makassar which is BLU Dr. Wahidin Sudirohusodo Hospital as wel as others public and private hospital that are included in the teaching hospital networks. The study was conducted from April 2015 until the number of samples are met.

2.2 Study Design

The method used for this study is analytic survey with cross-sectional study

2.3 Population and Sample

The study population were all women between 40 – 60 years old who came to the teaching hospital and its networks in Makassar. Study samples are all of the study population who met the inclusion criteria.

2.4 Data Collection Method

Study sample who signed the agreements of participation in the study will recorded into patient data, and filled out a questionnaire that has been set. Peripheral blood was collected from each patient and control in an EDTA-containing tube. Genomic DNA was extracted from peripheral blood lymphocytes using an Illustra blood genomicPrep Mini Spin Kit (GE Healthcare Life Sciences, USA), according to the manufacturer's instructions. Molecular analysis of the *ER*b gene (MIM 601663/ Genbank ID 2100) +1730 G/A polymorphism (rs4986938) was performed according to the protocol of Lee and cols.. (2007) (12), with modifications. The primers used

were: 5´-TTTTTGTCCCCATAGTAACA- 3´ (forward) and 5´-AATGAGGGACCACAGCA-3´ (reverse). PCR reaction was carried out in a final

volume of 25 μL, containing 1X buffer, 2.5 mM of MgCl2, 0.1 mM of each dNTP, 50 nM of each primer, 1U Taq Polymerase (Invitrogen), and 200 ng of DNA. Amplification was performed with an initial denaturation step at 95oC for 7 minutes, followed by 35 cycles of: denaturation at 95oC for 45 seconds, annealing at 53oC for 1 minute, extension at 72oC for 1 minute, and a final extension step at 72oC for 7 minutes. PCR products were analyzed for restriction fragment length polymorphism (RFLP) by using 5U of AluI restriction enzyme at 37°C overnight and visualized in 2% agarose gel stained with ethidium bromide under UV light. A G/A exchange at nucleotide 1730 in exon 8 introduces a recognition site for AluI. Digestion by AluI produces one band of 307 bp in the normal ERb sequence (GG); three separate bands of 307, 240, and 67 bp, respectively, in the heterozygous polymorphism (GA); and two separate bands of 240 and 67 bp, respectively in the homozygous polymorphism (AA). A random subset (~20% of samples) was repeated by qPCR to verify the results. Detection of the *ER*b +1730 G/A polymorphism (rs4986938) was made by TaqMan real-time PCR, using the Rotor-Gene Q 6 plex Platform (QIAGEN, Valencia, CA, USA). Commercially available Taqman primers and probes for *ER*β +1730 G/A polymorphism were used (C__11462726_10, Applied BiosystemsR, Foster City, CA, EUA). Assays were performed with Taqman Universal Master Mix (Applied Biosystems R, Foster City, CA, EUA), with 50 ng of DNA per reaction. PCR conditions were as recommended by the manufacturer: initial denaturation at 95°C (15 min), followed by 40 denaturation cycles at 95°C (15 sec), and a final annealing/extension cycle at 60°C (1 min). The chi-square test was used to compare allele and genotype frequencies between groups. Statistical tests of significance and $\chi 2$ analysis were carried out using SPSS for Windows 20.0 (SPSS, Inc., Chicago, IL). All

p-values were two-tailed and 95% confidence intervals (CIs) were calculated. A p-value < 0.05 was considered statistically significant.

2.5 Data Analysis Technique

The sample data obtained are recorded in the research form, and then analyzed using chi–square test, then the results are presented in tables and narrative accompanied by an explanation.

3. Results

The analytic survey study has been conducted using cross sectional study to evaluate the relationship between polymorphisms ERβ on the incidence of psychosocial stress in menopausal women. This research was conducted in a teaching hospital in Makassar which is Dr. Wahidin Sudirohusodo Hospital and its network hospitals. The study was conducted from April 2015 until the number of samples met.

The results showed that most respondents aged are over 50 years (69.6%), with educational level over 9 years (66.6%), unemployment (78,4%), and obesity (39,2%) (Table 1). Distribution of study variables showed that many distribution of research variables do not experience psychosocial stress (64.7%).

Table 1: Respondents Characteristics

NO	Characteristics	Number (n)	
		n = 102	%
	Age (year)		
1	≤ 50	31	30,4
	> 50	71	69,6
2	**Education**		
	≤ 9 year	34	33,3
	> 9 year	68	66,6
3	**Occupation**		
	Not work (IRT)	80	78,4
	Work	22	21,6
4	**Stress**		
	No	66	64,7
	Yes	36	35,3
5	**Bone density**		
	Normal	34	33,3
	Osteopenia	68	66,7
6	**IMT (Kg/m^2)**		
	< 18,5	7	6,9
	18,5 – 22,9	27	26,5
	22,9 – 24,9	28	27,5
	≥ 25	40	39,2
7	**Beta Receptor**		
	GG	70	68,6
	GA	18	17,6
	AA	14	13,7

Table 2: Receptor distribution of beta estrogen to the sample Characteristics

	Receptor Estrogen Beta			
	GG (70)	GA (18)	AA (14)	P
Age (year)	56.3 ± 10.82	56.1 ± 11.71	59.9 ± 11.63	0.53
Education (year)	11.4 ± 3.01	11.4 ± 2.30	9.8 ± 3.62	0.17
Blood sugar (mg/dL)	106.3 ± 29.29	137.9 ±118.02	120.9 ± 35.45	0.10
Body weight (kg)	155.8 ± 6.02	154.9 ± 4.56	153.4 ± 3.15	0.53
Body Height (cm)	57.6 ± 11.38	59.8 ± 8.44	55.6 ± 8.67	0.30
IMT (kg/m^2)	23.6 ± 3.85	24.9 ± 3.31	23.5 ± 3.01	0.40

Table 3: Relationships of Polimorfisme Receptor Estrogen Beta with the Stress Psychosocial on Menopause women

Psycho social Stress	Receptor Beta								*p*
	GG		GA		AA		Total		0,035
	n	%	n	%	n	%	n	%	
Yes	23	32,9	4	22,2	9	64,3	36	35,3	
No	47	67,1	14	77,8	5	35,7	66	64,7	
Total	70	100,0	18	100,0	14	100,0	102	100,0	

The relationship of Beta receptor polymorphism with psychosocial stress in menopausal women showed that respondents who experienced psychosocial stress mostly have the polymorphism of ERβ AA dominant homozygous (64.3%). While respondents were not experiencing psychosocial stress mostly have a dominant homozygous polymorphism ERβ GA (77.8%). Results obtained from chi square test are $p = 0.035$ ($p < 0.05$), which showed association between ERβ polymorphism and psychosocial stress in menopausal women (Appendix, Table 3).

4. Discussion

This study indicates that respondents who experienced psychosocial stress mostly have ERβ dominant homozygous polymorphisms AA. There is a relationship between ERβ polymorphism with the occurrence of psychosocial stress in menopausal women [8]. Psychological and cognitive symptoms may occur during the menopause transition include depression, mood swings, poor concentration and memory disorders. Although a lot of women assume that the change was associated with age or due to worsening of premenstrual syndrome (PMS), these symptoms can actually occur due to changes in reproductive hormones [2, 9, 10]. Related to neuroendocrine response, ERα and ERβ work oppositely on different neuronal populations within or near the PVN. There is some evidence support that the regulation of the neuropeptides promoter directly controlled by ERβ, such as CRH. For the behavioral response, ERβ appears to work through brain circuits involving the amygdala. However, further research is still needed to ensure the cellular and molecular mechanisms used by ERα and β to regulate the stress response [11].

There is a lot of evidence suggesting ERβ mediate estradiols anxiolytic function in the central nervous system. In ovariectomized female rats, that had been given specific agonists of pharmacologically ERβ, diarylpropionitrile (DPN), instead of agonists of ER-α specific propyl-pyrazole-triol (PPT) could reduces behavioral anxiety as measured on an open area, *elevated plus maze (EPM),* and the light-dark box. In addition to reducing anxiety, DPN not only increases anxiety behavior in mice that had been ovariectomized, but also led to a decrease in the reactivity of the HPA axis due to stress. Decrease stress reactivity is indicated by a decrease in plasma CORT

and ACTH levels and activation of c-fos gene early in the ventricular nucleus (PVN) of the hypothalamus, a region in the brain that has the highest expression of ERβ. Adminisitered non-selective ER antagonist, tamoxifen, block the action of inhibitory DPN, confirming the role of ERβ in modulating stress reactivity [11 - 14]. This study showed that respondents who experienced psychosocial stress mostly have the beta estrogen receptor polymorphism AA dominant homozygous (64.3%), while respondents who did not experience psychosocial stress mostly has the estrogen receptor beta polymorphisms GA dominant homozygous (77.8%), and with the results obtained from chi square test were p = 0.035 (p <0.05) indicating there is relationship between beta estrogen receptor polymorphism with the occurrence of psychosocial stress in menopausal women.

5. Conclusion and Recommendation

Author concluded that there is no relationship between ERα Pvull, ERα Xbal polymorphism with the occurrence of psychosocial stress on women menopasuse. There is a correlation between Erβ polymorphism with the occurrence of psychosocial stress in menopausal women. There is no interaction between the Erα and Erβ polymorphism with the occurrence of psychosocial stress in menopausal women. Author suggests further studies looking at the relationship of estrogen receptor polymorphism with other psychosocial variables. The need for the design of another study in analyzing the interaction of estrogen receptor polymorphism with incidence of psychosocial stress in order to obtain a more accurate insight of the mental status in menopausal women on psychiatric interventions in the future.

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