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CORRELATION OF mRNA ALPHA RECEPTOR ESTROGEN (ERα) AND BETA RECEPTOR ESTROGEN (ERβ) EXPRESSION IN ADENOMYOSIS

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ABSTRACT--- *Adenomyosis is a condition in which endometrial tissue in the myometrium is hypertrophy and reactive. Adenomyosis is one of the gynecological problem that have an impact on a woman's social life because very painful symptoms during menstruation and infertility. The exact pathogenesis of adenomyosis is still unclear. The estrogen is thought to be envolved in adenomyosis. Estrogen works on two estrogen receptors, ERα and ERβ. Estrogen* ***receptors*** *thought to be an important step in the occurrence of adenomyosis. The exact mechanism regarding the role of ERα and Erβ is still being investigated. Research that analyzes the comparison of ERα and Erβ mRNA expression on the incidence of adenomyosis has never been done in Indonesia. This study aims to analyse the* ***c****orrelation of mRNA ERα and ERβ Expression in Adenomyosis. Research was done at educational hospital in Makassar from January to August 2019. an observational study with cross sectional study approach, a sample of 15 people diagnosed with Adenomyosis and 15 people diagnosed not Adenomyosis. Tissue were examine in the Pathology Anatomy Laboratory and Erα/Erβ mRNA expressions in Molecular Biology and Immunology Laboratory using Real Time PCR method. The Result of this study is ERα and ERβ mRNA expression increased in the adenomyosis group compared to the non-adenomyosis group. Pearson correlation showing strong positive correlation between ERα mRNA and ERβ mRNA expression with p-value of <0.0001 and correlation coefficient (r) of 0.692. In Adenomyosis, the expression of ERβ is higher than Erα, with an average Erβ mRNA expression value of 9.85 ±1.44, and mRNA Erα expression of 8.09 ±1.21.* ***so*** *there is a significant relationship between mRNA ERα and Erβ expression on the incidence of adenomyosis, especially more on Erβ*

Keyword -- *Adenomyosis, ERα mRNA expression, Erβ mRNA expression*

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I. INTRODUCTION

The mechanism underlying the occurrence of adenomyosis is still unclear (Garcia and Isaacson, 2011). Adenomyosis was first discovered by Bird et al in 1972, commonly found in multiparas aged 40-50 years characterized by clinical symptoms such as pelvic pain and abnormal uterine bleeding which certainly greatly affects the quality of life of patients (Sebastiano Campo, 2012, leyendecker et al., 2009, Qin et al., 2012

The incidence of adenomyosis varies according to the way the examination and case findings. Clinically found 10-20%, while based on histopathology found 20-40%. More than 80% of patients with adenomyosis have an abnormal uterus, 50% in conjunction with uterine myoma, 11% with endometriosis, and 7% with endometrial polyps (Rajuddin and Jacoeb, 2008).

The causes of adenomyosis are due to global disorders of the endometrium involving the extracellular matrix (ECM), proliferation, apoptosis, and steroid hormone signals (C. N. Herndon et al., 2016). Steroid hormones are involved in the pathogenesis of adenomyosis (Benagiano et al., 2012). Increased levels of local estrogen produced by adenomyosis cells may be responsible for hypertrophy in the myometrium and hyperplasia in the endometrium. Furthermore, various studies have shown that the use of high-dose progesterone can temporarily induce regression of adenomyosis (Senturk and Imamoglu 2015; Benagiano et al., 2012). In recent years, tissue injury and repair mechanisms have been considered as major events in the development of adenomyosis where these events involve the production of local estrogen in an estrogen-sensitive environment (Leyendecker et al., 2009; Leyendecker and Wildt, 2011).

Tissue-specific reactions to the estrogen hormone are caused by the presence of intracellular protein receptors. Different forms of network will provide different responses. These receptors mainly work in the process of DNA transcription through various mechanisms. Estrogen receptors consist of Alpha Estrogen Receptors (ER-α) and Beta Estrogen Receptors (ER-β). Estrogen acts on these two estrogen receptors. These estrogen receptors are present in the ovarian surface epithelium and are responsive to estrogen. ERβ is a predominant estrogen receptor in normal ovaries.

In other studies it was found that increased levels of ERβ or decreased levels of Erα. Increased expression of ERβ is an important step in the occurrence of adenomyosis, although the exact mechanism of the role of ER α and ERβ is still being investigated.

Based on the findings of the ERα gene expression, many researchers conducted a gene expression study on the incidence of epithelial ovarian cancer and found a hypothesis that ERα gene expression affects the occurrence of epithelial ovarian cancer. This expression can affect the ovarian epithelium through regulation of cell proliferation and apoptosis, hence cell growth this ovarian epithelium will change and endometriosis occurs. This is the basis for this research.

Several studies on the expression of these two genes have also been carried out several times, ERα or ERβ alone or both. As Indrasari has conducted research on the expression of Estrogen receptor beta rs 1271572 in epithelial ovarian carcinoma, Wang et al conducted an ERα expression study in breast carcinoma and Hayashi et al conducted a study by comparing ERα and ERβ expression in breast carcinoma. But until now no one has done research on expression either ERα, ERβ or comparing both of them in endometriosis in Indonesia. (Indrasari, 2012. Hayashi, 2003. Wang, 2014.)

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Research that looks for an association between the expression of estrogen receptor Alpha and Beta mRNA genes in the incidence of adenomyosis has never done in Indonesia. This research generally aims to find out the expression of mRNA ERα and ERβ on adenomyosis and non adenomyosis patients and to find out the correlation between mRNA ERα and ERβ expression in Adenomyosis. This research is intended to see whether there are similarities in the expression patterns of population genes in Indonesia, especially in Makassar, with previous studies. Understanding the molecular mechanism of estrogen in the occurrence of endometriosis and the use of biomarkers for predictors of the tendency to experience adenomyosis is very necessary in understanding the pathogenesis of this disease.

II. METHODOLOGY

This research was conducted at Hasanuddin University hospital and Wahidin Sudirohusoso Hosital RSWS from 1 November 2018 – 31 August 2019. The sampling technique used was the purposive sampling method consisting of 30 samples of patients with two groups: 15 samples with adenomyosis and 15 samples with non adenomyosis. The population of this research is all patient women with adenomyosis and non adenomyosis through histopathological examination. The sample in this study were women with adenomyosis and women with no adenomyosis who have performed surgeries that meet the inclusion criteria and have signed the consent letter. who are categorized into two criteria as follows: 1) Age (20-35 years old, and >35 years old), 2) Parity (1-3 children, >3 children).

In this study interviews were conducted to obtain information about the characteristic and general circumstance of the subjects such as name, age, number of children, medical history, and previous operation history. Furthermore, tissue endometrial sampling of respondents took from operation for mRNA gene estrogene receptor expression. then taken the endometrial tissue subsequently carried out an examination of immunology and molecular biology laboratory Faculty of Medicine, Hasanuddin University, Makassar, Indonesia, using the Molecular technique that is Real Time Polymerase Chain Reaction (RT-PCR). Primers for real-time PCR (5'-GAGTGGGTCTGTGACGAGGAT-3' and 5'-GGTTGTATAGGATGCGAGGCT3') and b-actin (5'-G ATCATTGCTCCTCCTGAGC-3' and 5'-ACTCCTGCTTGCTGATCCAC-3') were designed using software PRIMER3 and purchased from Shanghai Sangon Biological Engineering Technology & Services CO., Ltd Data analysis with Independent T-Test were tested SPSS version 20 statistic program.

In the last activity, this study deal to conduct a comparative analytic research to find out the comparison of estrogen receptor alpha and beta expression level in women with adenomyosis and without adenomyosis. To do this, this study used a nested case control research design.

III. RESULT

Table 1 shows that the age of respondents in the control grup were from ,<20 years and >35 years of age as many as 12 respondents (80%), while the age of 20-35 years were 3 respondents (20%). While the age of respondents in the adenomyosis group was <20 year and >35 years old as 8 respondents (53%), while the age 20-35 years old as 7 respondents (47%). While based on parity shows that the highest percentage are respondents with 1-3 persons in the adenomyosis group at 12 respondents (80%) while respondents with parity greater than 3

people in the adenomyosis group at 3 resepondents (20%). While in the group of control, parity was 1-3 people are 5 respondents (33%) and parity >3 are 10 respondents (67%) .

Table 2 shows that the average expression of mRNA gen ERα on the uterine tissues of the adenomyosis was 9.14 Ct with a standard deviation of 0.65 Ct, whereas in control group was 7.05 Ct with a standart deviation of 0.53 Ct. Based on Statistical analysis results showed LL= 1.651 And UL= 2.533 With a value of p=0.0001 means that the average expression of mRNA gen ERα in the uterine tissues is expressed higher in the adenomyosis group and lower in control group.

Table 3 shows that the average expression of mRNA gen ERβ on the uterine tissues of the adenomyosis was 11.057 Ct with a standard deviation of 0.604 Ct, whereas in control group was 8.641 ct with a standart deviation of 0.893 Ct. Based on Statistical analysis results showed 95% Cl with LL= 1.846 And UL= 2.986 With a value of p= <0.0001 means there is a difference in the average of mRNA gen ERβ expression in the adenomyosis group compared to the control group so that it can be concluded that there is a relationship between mRNA gen ERβ expression with adenomyosis. This is clearly seen in the graph which shows that the average expression of mRNA gen ERβ in the uterine tissues is expressed higher in the adenomyosis group and lower in control group.

Table 4 shows that the average expression of mRNA gen ERβ on the uterine tissues of the adenomyosis is expressed higher than the average expression of mRNA gen ERα where the value of ERβ expression was 9.85 ± 1.44 while the value of ERα expression 8.09 ± 1.21.

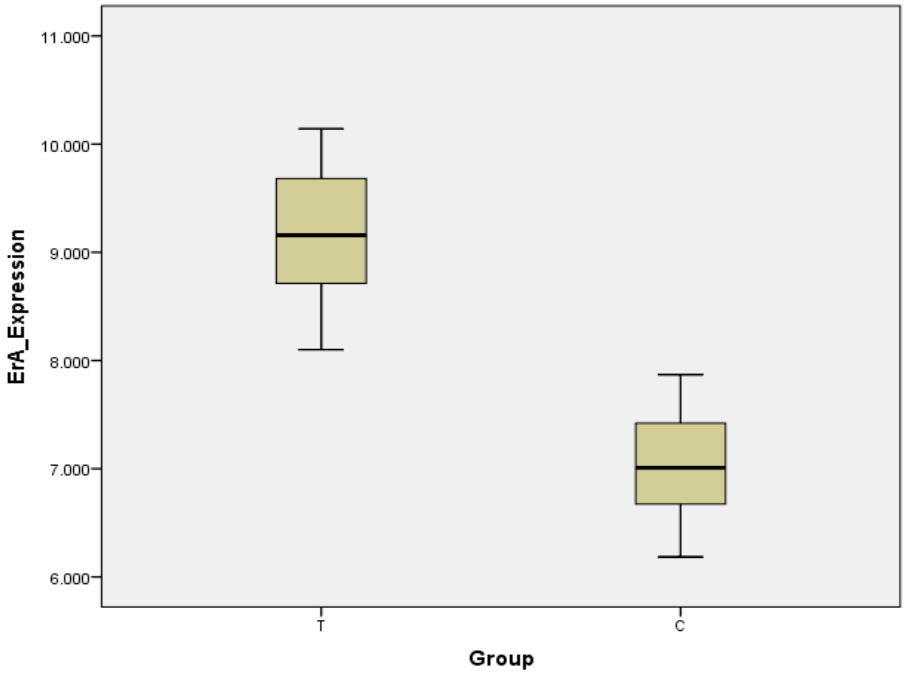
Tabel 5 From the result of the correlation analysis pearson correlate between mRNA gen ERα and ERβ expresson is 0.692. This shows how strong connections between mRNA ERα and mRNA ERβ. While the connection direction is R positive, meaning the higher the ERα expression, the higher ERβ to the adenomyosis patient. Because the significance score (0.0001<0.05), we could get the connection as significant between the mRNA ERα and mRNA ERβ.

Table 1. Characteristic of adenomyosis patients and controls

Variables	Control		Adenomyosis	
	N	(%)	N	(%)
Age				
<20 year and >35 year	12	80	8	53
20-35 year	3	20	7	47
Parity				
1-3	5	33	12	80
>3	10	67	3	20

Table 2.Comparison of mRNA ERα expression of endometrial tissue in the adenomyosis group and control

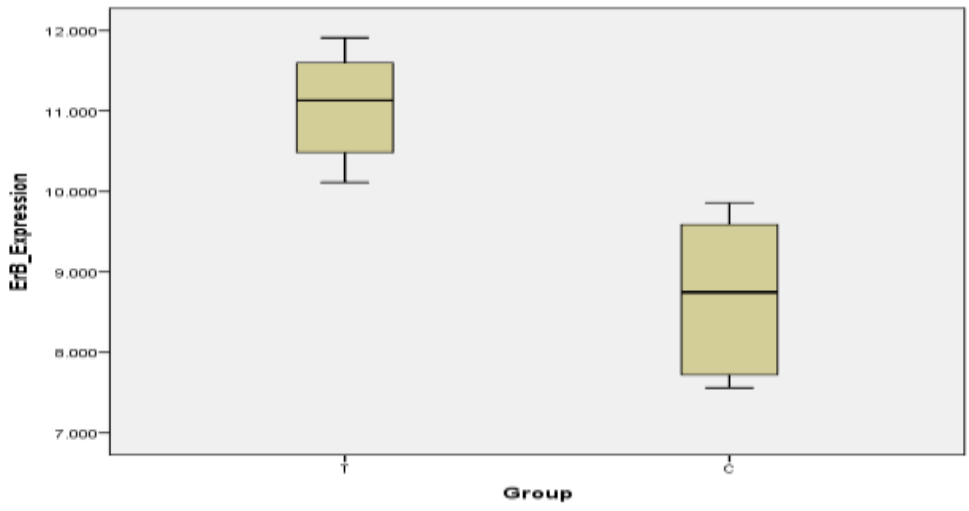
mRNA ERα expression	n	Mean	SD	95% CI (LL-UL)	P value
Adenomyosis	15	9.14	0.65	(1.651-2.533)	<0.0001
Control (non Adenomyosis)	15	7.05	0.53	(1.651-2.534)	



Box Plot Diagram of comparison of mRNA ERα expression of endometrial tissue in the adenomyosis group and control

Table 3: Comparison of mRNA ERβ expression of endometrial tissue in the adenomyosis group and control

mRNA ERβ expression	n	Mean	SD	95% CI (LL-UL)	P value
Adenomyosis	15	11.057	0.604	(1.846-2.986)	<0.0001
Control (Non Adenomyosis)	15	8.641	0.893	(1.842-2.991)	



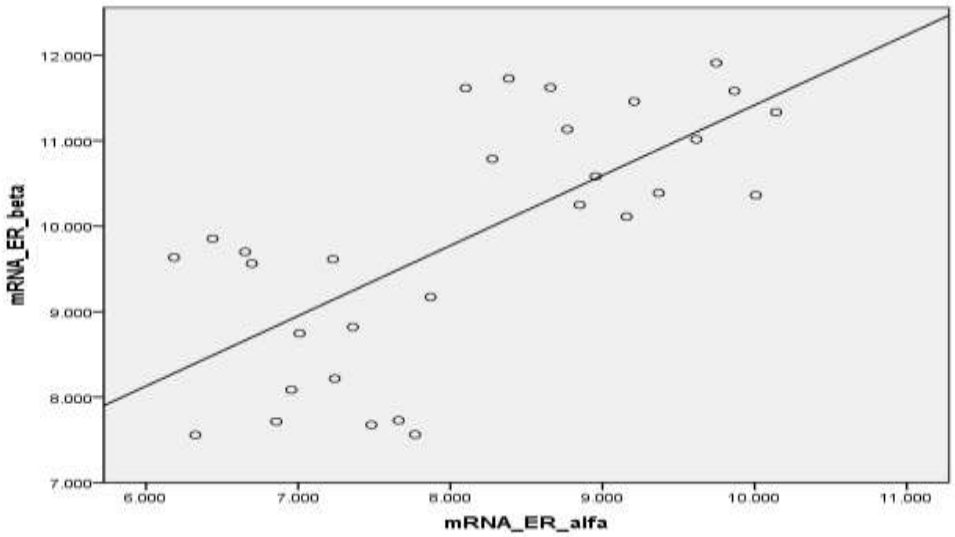
Box Plot Diagram of comparison of mRNA ERβ expression of endometrial tissue in the adenomyosis group and control

Table 4. Characteristic of mRNA ERα expression and mRNA ERβ expression in the adenomyosis group and control

	Mean	Std. Deviation	N
Erα_mRNA	8.09	1.21	30
Erβ_mRNA	9.84	1.44	30

Table 5 : The correlation of mRNA ERα expression and mRNA ERβ expression

	Ekspresi Erβ_mRNA	
	P	R
Ekspresi ErA_Mrna	<0.0001	0.692



IV. DISCUSSION

In this study, Adenomyosis is mostly in the age group of 20-35 years, namely 7 people (47%). This is due to reproductive age between the ages of 20-35 years where active estrogen is produced by the ovary (adenomyosis is one of the diseases that depend on estrogen). This study is different from the study conducted by Meice Fitriana who wrote that the characteristics of many adenomyosis sufferers in reproductive adult age (> 35 years)

Based on parity in this study, in the case of adenomyosis most commonly found in the group of women who have children (parity) between 1-3 people, 12 people (80%) while in the non-adenomyosis group the most found in the group of women who have more than 3 children Other studies have shown differences in women with fibroid adenomyosis and only fibroids. Women with fibroids and adenomyosis experience pelvic pain and dysmenorrhoea, have many children, a history of uterine surgery, compared to women with fibroids alone. adenomyosis is related to the amount of parity. It is estimated that about 80% of adenomyosis is multiparity. Because there are too many deliveries, the mechanism of TIAR (Trauma Injury And Repair) arises so that the boundary between the endometrium and myometrium is weak, which can lead to adenomyosis. (Leyendecker, 2012)

This study generally aims to determine the expression of Alpha and Beta Estrogen Receptors in adenomyosis and non-adenomyosis. Based on the statistical analysis results with Pearson correlation analysis in Table 5, the p value is <0.0001 and the correlation coefficient (r) is 0.692 so it can be concluded that strong correlation between

mRNAα expression and ERβ mRNA. The direction of the positive relationship means that the higher the ERα, the higher the expression of mRNAβ in adenomyosis patients.

This is consistent with an estrogen dependent study which states that estrogen plays an important role in the pathogenesis of adenomyosis (Simmen and Kelley, 2016). Increased expression of estrogen receptor mRNA causes inflammation adenomyosis and angiogenesis. This supports the theory of TIAR (tissue injury and repair).

Where is known The biological effects of estrogen are mediated by estrogen receptors. So the higher the estrogen receptor, the more important it will be in the tissues that are affected by estrogen. When hyperestrogens occur, endometrial tissue hyperplasia and hypertrophy will form.

As for some studies conducted on uterine tumors to see the comparison of ERα and ERβ. One of them is the Vallanders study which found higher expression and ERβ levels than ERα, they found ERβ in endothelium-type uterine tumors, mast cells and connective tissue cells while ERα was only found in smooth muscle cells. They also concluded that ERα was not related to the development of uterine tumors. This situation also underlies this study by taking uterine tumors as a comparison, with the results of studies where the expression of Estrogen receptor mRNA is higher in adenomyosis than uterine tumors. This is because adenomyosis occurs in the TIAR mechanism where ERβ is needed to hold in order that the inflammatory reaction that causes tissue damage is not excessive / does not get worse, where adenomyosis occurs in the TIAR process which continues so that the endometrium and myometrium boundaries weaken while in uterine tumors, only the myometrium enlarges. (Gerhard L et al., 2011). So that this is one of the hypotheses evidence that causes why beta receptor adenomyosis is higher expressed. So the ER-β expression may be an important marker of adenomyosis.

The high expression of ERβ mRNA compared to ERα in this study is also related to a study that shows that ER-ekspresi expression is reduced in the endometrial phase of midsecretion in women with adenomyosis, whereas ER-β is increased not only in the endometrium but also in the inner myometrium and the outer myometrium in women with adenomyosis compared with control subjects. (Mehasseb et al., 2011). Although ER-α is accepted as the only type of receptor involved in uterine function, the presence of ER-β in different uterine cells has demonstrated the role of these receptors in their physiology. Although the presence of ER-β has been observed previously in uterine smooth muscle cells, different studies describe ER-β in other cells, such as those in endometrial and stromal glands, as well as in endothelial blood vessels in endometrial vessels. The wide distribution of ER-β may be related to its function in terms of inhibiting ER-α action. (Vallanders et al., 2006) data shows that ER-β is a major estrogen receptor subtype in endometrial endothelial cells and that its expression is regulated by hormones. ER-β is found in all types of endometium cells, including glandular epithelium and stromal cells. ER-β has been reported by several studies as sole ER which is expressed by many specific cells in the endometrium such as endometrial endothelium and uNK cells. (Hapangama et al., 2015)

Higher ER-β expression in the functional gland in the proliferative phase may be related to the incidence of endometrial hyperplasia. However some descriptions indicate that estrogen signaling through ER-β in the functionalist gland may have antiproliferative and antiproapoptotic effects. ER-β expression may be an important marker of adenomyosis. Higher ER-β expression in the adenomyotic uterine myometrium can contribute to the incidence of myometrial hyperplasia and will show clear clinical symptoms according to the degree of Adenomyosis so that high ER-β expression and lack of progesterone receptor expression are related to growth and / or development of adenomyosis. (Mehasseb et al., 2011).

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This is consistent with this study when compared between ER Alpha and ER Beta mRNA expression in the Adenomyosis group.30 respondents from the adenomyosis and non-adenomyosis group obtained higher expression of ER-β in the adenomyosis group than the ER-α expression. With the data obtained the average expression characteristics of mRNA ER-β expression was 9.85 ± 1.44 while mRNA ER-α expression was 8.09 ± 1.21

This situation also underlies this study by taking uterine tumors as a comparison (control group), with the results of studies where the expression of mRNA estrogen receptors is higher in adenomyosis than uterine tumors.

This is because in adenomyosis there is a TIAR mechanism where ERβ is very much needed, where adenomyosis occurs in the TIAR process which continues so that the endometrial and myometrial boundaries are weakened whereas in the uterine tumor, the enlargement is only part of the myometrium. (Gerhard L et al., 2011)

V. CONCLUSION

From the results of this study it can be concluded that there is a significant relationship between ER Alpha mRNA expression with adenomyosis, where the mRNA ER-α expression is higher in the adenomyosis group than the non-adenomyosis (control group). Likewise with mRNA ER-β experiments in the adenomyosis and non-adenomyosis (control) group, the expression of estrogen receptor betha in adenomyosis was higher. However, when we compared to mRNA estrogen receptors Alpha and beta in adenomyosis, the expression of ER-β mRNA is higher than ER-α in adenomyosis.

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