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The Role of CYP17 Gene Expression Towards Imbalance of Estrogen and Progesterone Hormones at Luteal Phase in Marriageable Girls

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ABSTRACT

Background: Menstruation process occurs on marriageable girls as an indication that ovary has been in function. One of the menstruation phases is known as luteal phase. At this phase, a kind of problems sometimes happens, like imbalance of estrogen and progesterone hormones. Both are steroid hormones. CYP17 is a crucial enzyme for biosynthesis of steroid.

Research purpose: to identify the roles of CYP17 gene expression towards imbalance of estrogen and progesterone hormones at luteal phase in marriageable girls.

Research method: adopting non-experimental design using *cross sectional* approach to 40 samples.

Conclusions: there is role between CYP17 gene expression and estrogen hormone levels. There is role between CYP17 gene expression and progesterone hormone levels. There is role between CYP17 gene expression and imbalance of estrogen and progesterone hormone.

Keywords: *CYP17, estrogen, progesterone, marriageable girls.*

INTRODUCTION

Adolescence is an age of change or transition from childhood into adulthood[1]. On marriageable girls, menstruation occurs as an indication that ovary has been in function. It happens due to the unfertilized ovum by the sperms so that it disintegrates endometrium cells in womb. Menstruation cycle averagely is in 28 days. One of the phases is known as luteal phase[2].

During luteal phase, granulose cells in corpus luteum evolve smooth endoplasmic reticulum of the vast intracellular producing estrogen and progesterone cells[3]. Frank in Rimma stated that high level of estrogen and disturbance of estrogen and progesterone imbalance becomes the cause of problems on luteal phase[4]. A research showed that 23% to 31% of reproductive-aged women got problem at luteal phase and it affects their daily lives. It causes mood nuisance and complication[5]. Approximately 13% to 19% of reproductive-aged women got nuisance at luteal phase[6].

A research published in 2011, the *International Journal of Biological and Medical Research*, indicated that of 300 students, 67% of them got problem at luteal phase. It was caused by estrogen hormone, at reproductive age started from puberty years, is secreted 20 times or more affected by gonadotropin hypophyse hormone[3]. Some in small quantities are from acetyl coenzyme A, a multiple molecule which is able to combine to shape appropriate steroid nucleus.

P450 17alpha-hydroxylase/17,20-lyase (CYP17) is a microsomal enzyme catalyzing two different activities: 17alpha-hydroxylase and 17,20-lyase, which is essential for adrenal and gonad steroid biosynthesis[7]. Cytochrome

P-450c17α mediates between activity of steroid 17α–hydroxylase converting pregnenolone becomes dehydroepiandrosterone and activity of 17,20-lyase producing androstenedione from progesterone[8].

Researches related to CYP17 have been conducted, such as Jahromi in Teheran, Iran who showed that excessive androgen in mouse at prenatal period caused increase on LH hormone level, prime regulator for steroidogenesis in granulose and theca cell. In this cell, LH is bound to LH receptor and activate cAMP (*cyclic adenosin monophosphate*)[9]

To learn about the further role of Cyp17 gene expression and estrogen and progesterone hormone imbalance, a study about CYP17 gene expression, estradiol level of serum, and progesterone was then conducted. This study identify the roles of CYP17 gene expression towards imbalance of estrogen and progesterone hormones at luteal phase on marriageable girls.

RESEARCH METHOD

This research applied non-experimental design using *cross sectional* approach, which is a monitoring executed once as the time decided by researcher considering whether or not relations between dependent and independent variables. (Sugiyono, 2009).

This research was conducted at STIK GIA Makassar and Molecular Biology and Immunology Lab, Medical Faculty, Hasanuddin University.

Population of this research is marriageable girls with luteal phase (students of STIK GIA Makassar)

Sampling applied the nonprobability one using purposive sampling technique, which is a technique based on specific considerations.

Total of sample was acquired from the large formula sample for correlative analytical research, it is:

$$n = \left\{\frac{(Z\alpha+Z\beta)}{[(1+r)/(1-r)]}\right\}^2 + 3$$

$$= \left\{\frac{(1,28+1,28)}{[(1+0,4)/(1-0,4)]}\right\}^2 + 3$$

$$= \left\{\frac{2,56}{[0,5\ln[2,33]}\right\}^2 + 3$$

$$= 36,6+3$$

$$= 39,6$$

$$= 40$$

According to calculation above so the large of sample taken was 40 which is proper to inclusive criteria. (Dahlan, 2010)

Inclusive and exclusive criteria

Its inclusive criteria are:

1. Childbearing-aged women with luteal phase
2. Menstruation cycle is in 28-35 days

Its exclusive criteria are:

1. Applying hormonal therapy
2. Alcoholic history
3. Reproductive system disorders history
4. Not signing informed consent
5. Not cooperative

Research tools and Materials

1. Form of Food Frequency Questionnaire
2. Stationery
3. RT-PCR
4. Spoit 10 cc
5. Blood samples
6. KAPA SYBR "Fast one-step qRT-PCR kit
7. Commercial kit: DIAsource E2-EASIA
8. Primer gen CYP17, PCR System 7300 (Applied Biosystems, USA) with SYBR Green

RESEARCH RESULTS

Table 1. Distribution of marriageable girl respondents' characteristics

Characteristics	Frequency	%
Age		
18-21	26	65
22-24	14	35
Menstruation Cycle		
28-30 days	21	52.5
31-35 days	19	47.5
Menstruation Duration		
3-5 days	24	60
6-7 days	16	40
Estrogen Level		
Low	10	25
High	30	75
Progesterone Level		
Low	10	25
High	30	75

***Cont...* Table 1. Distribution of marriageable girl respondents' characteristics**

Estrogen & Progesterone Combination		
High Estrogen, High progesterone	20	50
High Estrogen, low progesterone	10	25
Low Estrogen, high progesterone	10	25
Low Estrogen, Low progesterone	0	0
CYP17 gene expression High Low	 30 10	 75 25
Total	40	100.0

Based on the characteristics of estrogen level, majority of them (30 respondents) were categorized in high level (75%), and 10 respondents (25%) were low. According to the progesterone level, 30 respondents (75%) were in high category, while 10 of them (25%) were in low category. Meanwhile according to combination of estrogen and progesterone, they mostly (20 respondents) had high level of both estrogen and progesterone (50%), 10 respondents (25%) had high estrogen but low progesterone category, contrary 10 respondents (25%) had low estrogen but high progesterone category, and there was no respondent with low category on both estrogen and progesterone.

Table 2. Role of CYP17 gene expression towards estrogen level in marriageable girls

	Estrogen level				**n**	**%**	***p***	**r**
	High	**%**	**Low**	**%**				
CYP17 gene expression High Low	 27 3	 90 30	 3 7	 10 70	 30 10	 100.0 100.0	0.000	0.600

Based on Table 2 about high CYP17 gene expression (total 30 respondents), 27 respondents (90%) had high estrogen level, while 3 of them (10%) had low estrogen. Meanwhile low CYP14 gene expression (total 10 respondents), 7 respondents (70%) had low estrogen level, while 3 of them (30%) had high estrogen. From the tables, the data showed that there is role of CYP17 gene expression towards estrogen levels. The trend is positive and strong.

Table 3. Role of CYP17 gene expression towards progesterone level in marriageable girls

	Progesterone Level				**N**	**%**	***p(r)***
	High	**%**	**Low**	**%**			
CYP17 gene expression High Low	 20 10	 66.7 100	 10 0	 33.3 0	 30 10	 100 100	 0.018 (-0.333)

Based on table 3: high CYP17 gene expression (total 30 respondents), majority (20 respondents) had high progesterone level (66,7%) while 10 of them (33,3%) had low progesterone. Meanwhile from 10 respondents with low CYP17 gene expression, all of them (100%) had high level.

Table 4. Role of CYP17 gene expression towards imbalance of estrogen and progesterone hormone in marriageable girls

	Imbalance of estrogen and progesterone hormone								n	%	*p* r
	High Estrogen, high progesterone	%	High Estrogen, low progesterone	%	Low Estrogen, high progesterone	%	Low Estrogen, Low progesterone	%			
CYP17 gene expression											
High	17	56.7	10	33.3	3	10	0	0	30	100.0	0.004
Low	3	30	0	0	7	70	0	0	10	100.0	0.408

From the table, the data gained that there is a role of CYP17 gene expression towards imbalance of estrogen and progesterone hormone. The trend is positive and weak.

Based on table 5: combination of both high estrogen and progesterone (20 respondents), majority (13 respondent) experienced moderate premenstrual syndrome (PMS) (65%), 6 respondents (30%) experienced mild PMS, and 1 respondent (5%) experienced severe PMS. In combination of high estrogen coupled with low progesterone (10 respondents), majority (7 respondents) experienced moderate PMS (70%), 2 respondents experienced severe PMS (20%), and 1 respondent (10%) experienced mild PMS.

Table 5. Role of imbalance of estrogen and progesterone hormone towards PMS level in marriageable girls

	PMS level						n	%	*p*	r
	Severe PMS	%	Moderate PMS	%	Mild PMS	%				
Imbalance of estrogen and progesterone hormone										
High Estrogen, High progesterone	1	5	13	65	6	30	20	100.0		
High Estrogen, low progesterone	2	20	7	70	1	10	10	100.0	0.010	-0.369
Low Estroge, high progesterone	2	20	8	80	0	0	10	100.0		
Low Estrogen, progesterone	0	0	0	0	0	0	0	0		

Moreover, in combination of low estrogen coupled with high progesterone (10 respondents), majority (8 respondents) experienced moderate PMS, 2 respondents (20%) experienced mild PMS, and no respondent experienced mild PMS. No respondent had both low level of estrogen and progesterone.

DISCUSSION

According to result of the research, the data shows that there was role of CYP17 gene expression and estrogen hormone level. The higher the CYP17 gene expression, the higher the estrogen level. It is relevant with opinion of Onland-Moret et al. In his research, it was found that there was role of CYP17 in steroidogenesis, in which one result of the process is estrogen hormone.

Respondents with both high level of CYP17 gene expression and estrogen are 27 respondents (90%). It is caused by the high food supply of respondent related to consumption of soy cake (9 respondents consumes > 1 time/day). Soy cake is a kind of food containing

isoflavone, a factor II (6,7,4-trihydroxyisoflavone) fermented from soy which is rich of isoflavone called phytoestrogen due to its similarity to estrogen[10]. Structure of isoflavone is almost the same as chemical structure of estrogen[11]. Besides respondents often consume tofu which is a product of soy containing estrogen. This affects the high of estrogen level and CYP17 gene expression.

Increasing estrogen levels during the luteal phase triggers an increase in prostaglandins and causes an inflammatory response, which will cause uterine muscle spasm and systemic complaints such as nausea, vomiting, flatulence and headaches. Strong uterine contractions cause in reduced blood flow to the uterine muscles, resulting in reduced oxygen intake into the tissue that causes ischemia. The state of ischemia will affect the release of oxygen reactive resulting tissue damage and pain[12].

This research is also in line with Weston's opinion that CYP17 is a microsomal enzyme that is important for adrenal and gonadal steroid biosynthesis Activity of 17alpha-hydroxylase produces 17OHPreg and 17OHP and plays a major role in glucocorticoid synthesis, in this case the androgen[13] . Activity 17.20-lyase produces DHEA and Δ4A from 17OHPreg. Zhang, et al. in Kempna, said that stimulation in the cAMP / PKA pathway (protein kinase A) increases phosphorylation CYP17. (Kempná et al., 2015) The activity of each phosphoprotein result comes from a balance of phosphorylation and dephosphorylation regulated by phosphatase and kinase. According to Pandey in Kempna,[13], protein phosphatase 2A (PP2A) dephosphorylates CYP17 thereby reducing lyase and androgen synthesis. This has an impact on the reduction of the estrogen hormone[13]

Hormonal biosynthesis of adrenal and androgen glucocorticoids is regulated by ACTH which binds to the specific receptor of melanocortin 2 (MC2R) on cell membranes. MC2R is a G protein-coupled receptor (GPCR) that stimulates adenylatecyclase activity and plays a role in the production of cAMPas *a second messenger*. Similarly, sex steroid biosynthesis in gonadal cells is stimulated by FSH and LH binding to specific GPCR in cell membranes that promote cAMP formation. Classically cyclic AMP activates PKA and leads to protein and transcription of the phosphorylation factor. Meanwhile, the cAMP / PKA signaling system plays a role in glucocorticoid synthesis and stress response[13].. High CYP17 gene expression but low estrogen levels can be caused by other factors that play a role in estrogen synthesis, namely the aromatase enzyme (CYP19)[15].

Role between CYP17 gene expression and progesterone hormone levels

According to the results of the study, it was found that there was a role for CYP17 gene expression with progesterone levels. The higher the CYP17 gene expression, the lower the progesterone level. This is in accordance with the opinion of Onland-Moret et al.. In the study, the role of CYP17 in steroidogenesis was found in which one of the results of the steroidogenesis process is the progesterone hormone.

Progesterone is synthesized from pregnenolone, which comes from cholesterol. Cholesterol experienced a double oxidation to produce 22 *R*-hydroxycholesterol and then 20 α, 22 R-dihydroxycholesterol. This vicinal diol is then further oxidized with the loss of the side chain starting at position C 22 to produce pregnenolone. This reaction takes place under the influence of CYP17. The conversion of pregnanolon to progesterone occurs in two steps. First, the 3β-hydroxyl group is oxidized to the keto group. And secondly the double bond is transferred to C4 from C5 via the keto / enoltautomerization reaction. This reaction is catalyzed by 3β-hydroxysteroid dehydrogenase/δ^{5-4}-isomerase.

This study is consistent with Chambo's findings, 2009 that CYP17 polymorphism causes high density in the breast, associated with increasing of biosynthesis of estrogen and progesterone[16]. High CYP17 gene expression but low progesterone levels can be caused by other factors that play a role in the synthesis of progesterone, that is enzyme 3β-hydroxysteroid dehydrogenase (3β-HSD).

Role between CYP17 gene expression and imbalance of estrogen and progesterone hormones

Based on the results of the study, data was obtained that there is a role between CYP17 gene expression and imbalance of estrogen and progesterone hormones. The higher the gene expression, the higher the imbalance between the estrogen and progesterone hormones. The CYP17 gene has two different functions, those are 1 enzyme which only has 17-hydroxylase activity and the other enzyme has 17,20-lyase activity[17]

Taking blood samples to respondents was carried out in the luteal phase, in which at that time LH (Luteinizing Hormone) would cause granulosa cells from the follicles to form the corpus luteum so that it produces large amounts of progesterone and estrogen in small amounts. In women who experience premenstrual syndrome there is an imbalance of the estrogen and progesterone hormones, in which estrogen levels increase and progesterone levels decrease so it affects a decrease in serotonin synthesis which affects changes in mood and behavior[18]. Respondents who have high gene expression and high estrogen progesterone levels may be caused by high activity of the progesterone-forming enzyme namely 3β-HSD.

CONCLUSION

There is a role between CYP17 gene expression and estrogen levels. The high expression of CYP17 gene will cause a lot of estrogen hormone levels to be formed.

There is a role between CYP17 gene expression and progesterone hormone levels. The high expression of the CYP17 gene will cause low levels of the hormone progesterone to be formed.

There is a role between CYP17 gene expression and imbalance of estrogen and progesterone hormones. The high expression of the CYP17 gene will cause a large imbalance of the estrogen and progesterone hormones.

Ethical Clearance- Taken from Medical Faculty committee

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Conflict of Interest – None

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