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FOLLICLE STIMULATING HORMONE RECEPTOR Ser680Asn POLYMORPHISM IN WOMEN WITH POLYCYSTIC OVARY

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Abstract

Polymorphisms at codon 680 (Ser680Asn) of FSH receptor gene affected the basal FSH levels and sensitivity of FSH receptor to FSH exogenous in women undergoing assisted reproduction program in some ethnics in the world, while it could not proved in others. This study aims to determine the association of polymorphisms Ser680Asn in patients with polycystic ovary (PCO) in Indonesian population. The study involved 30 PCO patients and 30 normal women. Polymorphisms analysis was performed by PCR - RFLP. ELISA was used to detect FSH level. Statistical analysis was done by chi - square and t tests. This study shows the homozygote variant Ser/Ser is significantly different in distribution between patients and normal women where it is more frequent in PCO women. In normal women, the three allelic variant distributed normally. There is no significant different in FSH level in three genotipe between patients and normal women. Conclution: There is a significant different in genotype distribution of FSH receptor gene between PCO patients and normal women for polymorphism at codon 680 but it was not associated with FSH level.

Keyword: polymorphism, FSH receptor gene, exon 10

INTRODUCTION

Follicle stimulating hormone (FSH) plays an important role during the folliculogenesis. FSH plays a role in the stimulation of granulosa cells to produce estrogen via induction of aromatase activity (Fauser et al., 2005). FSH activity is mediated by the FSH receptor. FSH receptor is a member G-protein receptor. These receptor cluster is characterized by the transmembrane domain consisting of seven α-helix membrane connected to the three extracellular and three intracellular loops (Simoni *et al*., 2002 Themmen and Huhtaniemi, 2000). FSH receptor gene is located on chromosome 2p21-16 (Simoni *et al*., 2002).

It has been known, there are several mutations in the FSH receptor gene. The mutations were detected in the promoter region (Simoni *et al*., 2002; Wunsch *et al*., 2005) and the structural gene region (Simoni *et al*., 1999). In exon 10 of the structural gene, it was found two point mutations at position 919 and 2039 (codons 307 and 680) (Simoni *et al*., 1999). Polymorphisms in exon 10 resulted in two major alleles with similar frequency in the population of Caucasia, namely Thr307 - Asn680 and Ala307 - Ser680 (Simoni *et al*., 1999; Simoni *et al*., 2002). However, the same studies conducted by some researchers in the world gave the discrepancies results. The frequency of polymorphisms depends on ethnic. Liu *et al*. (1998); Conway *et al*. (1999); Simoni *et al*. (1999) found no different distribution of both variants between normal and infertile men and women. However, other studies found significant differences in the distribution of allelic variants between patients and controls (Sudo *et al*., 2002 Laven *et al*., 2003; Dolphin *et al*., 2011).

FSH receptor gene polymorphism at position 680 in exon 10 affecting the serum levels of

FSH and the sensitivity of FSH receptor to FSH stimulation in Caucasia women undergoing assisted reproduction program. In women with normal ovarian undergoing assisted reproduction program, it was found that the basal levels of FSH and FSH ampuls needed for ovarian stimulation depends on the FSH receptor genotype. Women with genotype Ser/Ser have higher levels of FSH and needed more FSH ampuls than women with genotype Asn/Asn (Perez *et al*., 2000, de Castro *et al*., 2005; Gerasimova *et al*., 2010). Further study on Italian women with polycystic ovary also showed the higher responsiveness of Ala307Thr women than normoovulatory women (Dolfin *et al*., 2011). The aim of this study is to assess the prevalence of the three allelic variants (Ser680Ser, Ser680Asn, Asn680Asn) in women with polycystic ovary and normal women and to clarify if the allelic variant could influence the level of FSH serum.

RESEARCH METHOD

Screening women with polycystic ovary

Screening is done by the doctor hospital to see the development of follicles using ultrasonography (USG). The patients included in this study when there were not the normal of follicular development detected or there was not ovulation.

The control samples

The control samples were women with normal menstruation and have children.

Genomic DNA isolation

Genomic DNA isolated from 1 mL of blood of patients and normal people using the Wizard DNA purification kit (Promega) following standard procedures. The genomic DNA obtained were stored at 4°C until subsequent analysis.

Polymerase chain reaction-Restriction fragment length polymorphism (PCR- RFLP)

PCR- RFLP asay was done to determine the FSH receptor genotype. Primer used to amplify FSH receptors gene were: F: 5'- CTGTGTTCACTTTGGACATGTTG - 3' and R: 5'- GACGTTGGAAGATACCAGGTTG - 3 ' (Perez *et al*., 2000). PCR performed in a PCR machine type Whatman Biometra T Personal (Germany) with PCR conditions refer to Perez *et al*. (2000). PCR products was digested using BsrI restriction enzymes and electrophorated in 2 % agarose gel. Gel stained with ethidium bromide (10 μg/ml) and visualized under ultraviolet light. Wild-type allele (Asn) is single band, the length of a fragment is 288 bp and allele of variant (Ser) is double band, the length of fragment are 178 bp and 90 bp.

Measurement of blood FSH levels by ELISA Method.

Measurement of FSH level of patients and normal women performed in the Biomedical laboratory, Faculty of Medicine, Andalas University, Padang.

Statistical Analysis

Analysis of the genotype distribution of the patients and normal women performed with Chi - Square test. Analysis of differences in FSH levels between genotypes performed with one way anova.

RESULT AND DISCUSSION

Amplification of FSH Receptor Gene Exons 10

Amplification is restricted to nucleotide 1624 till 2143 of exon 10 of FSH receptor gene using genomic DNA as a template and a pair of primers. Forward primer consisted of 5'- CTGTGT-TCACTTTGGACATGTTG-3 ' and reverse primer 5'- GACGTTGGAAGATACCAGGTTG- 3 '. In this study we got PCR product 288 pb in length as shown in Figure 1. The Figure 1 shows that the amplification was done successfully on samples of patients and control. This is evidenced by the presence of one band in each well and the thickness of the band is good. Amplification of the FSH receptor gene exon 10 was performed on 30 samples of polycystic ovary and 30 normal women.

Genotype distribution at position 680 of FSH Receptor Gene Exon 10

The results of the analysis of genotypes at position 680 in the patient samples showed that the variant Asn/Asn was 17 % (5 people). Variant Asn/Ser was 43 % (13 people) and the variant Ser/Ser was 40 % (12 people). From this data it appears that the frequency of genotype Ser/Ser is

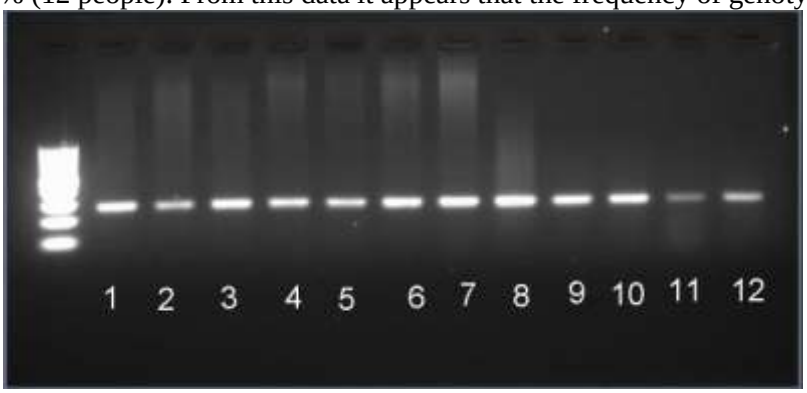

Figure 1. PCR products of the FSH receptor gene (288 bp) in 2 % agarose gel. M = 100 bp DNA ladder, numbers 1-12 are FSH receptor genes of polycystic ovary woman.

much higher than the frequency of genotype Asn/Asn. In the control women, in contrast, the distribution of the three genotypes followed the Hardy - Weinberg law. Of the 30 control samples analyzed, 23 % (7) had a variant Asn/Asn, 60 % (18 people) had a variant Asn/Ser, and 17 % (5 people) have a variant Ser/Ser. The distribution of the three genotypes in patients and controls can be seen in Figure 2.

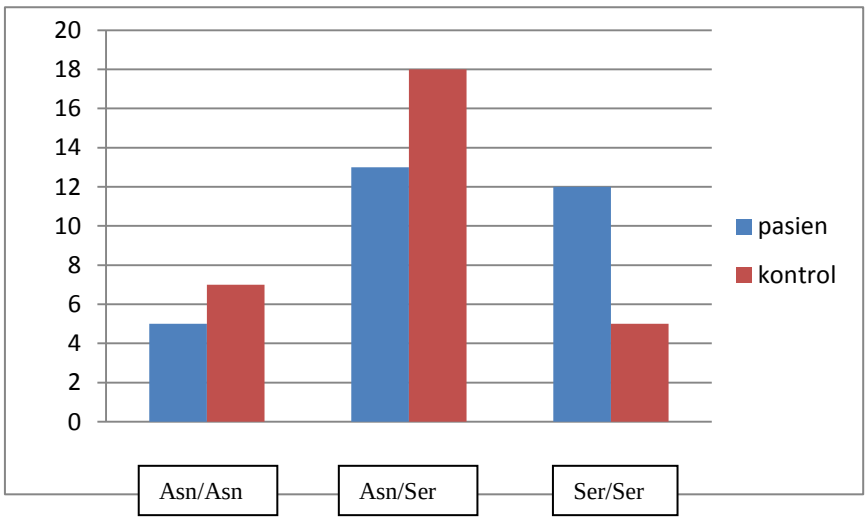

Figure 2. Distribution of FSH receptor genotype at position 680 of FSH receptor gene exon 10 in women polycystic ovary syndrome and normal women

Figure 2 shows that the frequency of Ser/Ser in polycystic ovary women is much higher than normal women (controls). The chi-square analysis showed a significant difference in genotype

frequency of Ser/Ser between polycystic ovary women and normal women. In addition to genotyping analysis, in this study we also analyzed the prevalence of polycystic ovary alleles in patients and normal women (Tabel 1).

Tabel 1. Distribution of allele variants of the FSH receptor in polycystic ovary and normal women.

Group	**Alel (%)**	
	Asn	**Ser**
Normal women	53	47
Polycystic ovary women	39	61

Table 1 shows that the percentage of alleles in polycystic ovary women is higher than normal women. The results of the chi square test showed a significant difference between these two variants.

Clinical characteristics of the patients and normal women

In this study we also measured the clinical characteristic of patients that consist of age, body weigth, and FSH levels. Data is shown in Table 2.

Table 2. Clinical characteristics of patients and normal women based on genotype in position 680 of the FSH receptor gene

Characteristic	Sampel	Asn/Asn	Asn/Ser	Ser/Ser
Age (year)	Pasien	32,5 ± 2,5	33,4 ± 3,6	32,1 ± 1,7
	Normal	33,4 ± 3,1	33,5 ± 2,6	32,7 ± 2,7
Body weight (kg)	Pasien	52 ± 2,5	54 ± 2,1	53 ± 2,4
	Normal	54 ± 2,2	53 ± 2,9	55 ± 2,3
FSH level (IU/L)	Pasien	5,9 ± 1,2	5,7 ± 2,4	6,4 ± 1,9
	Normal	8,2 ± 3,9	6,7 ± 1,7	7,4 ± 1,5

In all three clinical characteristic observed (Table 2), we did not find the significant different of values between patient and normal women. However, FSH level of patients with Ser/Ser tend to higher than other two genotypes.

In this study, we analyzed polymorphism at position 680 of FSH receptor gene in polycystic ovary women compared to normal women. It is shown that the frequency of genotype Ser/Ser higher in women with polycystic ovary compared to normal women. This is consistent with the study of Perez *et al*. (2000) and Sudo *et al*. (2002). However, in this study we did not found the significant difference in blood FSH levels among three genotypes, both in patient and normal women. This is not in accordance with the study of Perez *et al*. (2000) who found that basal FSH level of patients with genotype Ser/Ser higher than basal FSH level of patients with genotype Asn/Asn. Different results was also indicated by Dolfin *et al*. (2011) who analyzed the FSH receptor gene polymorphism at codon 307 (Ala307Thr). They indicated that the frequency of polymorphism Ala/Thr higher in patients compared to normal women. Furthermore, they showed that patiens with genotype Ala/Thr are more sensitive to exogenous FSH than normal women. We suggest that incompatibility of FSH level results of this study compared to previous studies is likely due to the time difference in the measurement of FSH hormone. In the study of Perez *et al*. (2000)

and Dolfin *et al*. (2011) measurements were done to find the patient's basal FSH levels. Basal FSH levels were measured on days 1-3 of ovarian cycle, whereas in this study the measurements of FSH level performed at an unspecified time. It is necessary to do further study to determine whether there are significant different in the receptor sensitivity among three genotypes of patients of polycystic ovary. For this purpose it is needed to measure basal FSH levels (days 1-3 ovarian cycle).

FSH is essential for normal function of the reproductive system. Caused an important function in follicle growth and steroidogenesis in women and spermatogenesis in men, FSH receptor mutations in genes can affect reproductive capacity, especially in women (Conway, 2000; Gromoll *et al*., 1999). Aittomaki *et al*. have reported six Finnish families that have two or more women with gonadal dysgenesis and primary amenorrhoea, and suggests that there has been a mutation in the FSH receptor Ala189Val which is the cause of the incident of gonadal dysgenesis and the primary amenorrhoea (Aittomaki *et al*., 1995). In contrast, an activation mutation in the FSH receptor has been reported in a hypovisectomyzed man who maintained his normal testes volume and fertility although it is not producing gonadotropin (Gromoll *et al*., 1996). The heterozygous Ala567Gly amino acids causes the activity of mutant receptor becomes ligand independent.

Polymorphism at codon 680 was first confirmed when Aittomaki et al. identify mutations that cause loss of FSH receptor function in patients with ovarian dysgenesis due to mutations Ala189Val (Aittomaki et al., 1995). Although the base change is referred to as polymorphism, however, it was not clear whether its isoformnya affect FSH receptor function and may further impact on growth and steroidogenesis in ovarian follicles. In addition, it is also not known whether these polymorphisms are associated with gynecological diseases. Because polymorphism occurs at the end of the C domain of the receptor, which plays an important role in the process of coupling with G proteins and intracellular phosphorylation followed by desensitization, in this study we look at the frequency of polymorphisms in polycystic ovary women and compared to the normal women. To determine whether the FSH receptor isoforms have different sensitivity to the hormone FSH, the analysis is done by grouping data based on genotypes. The results of this study have shown that there are significant differences in genotype frequencies in patients with polycystic ovary. This raises the suspicion that there are differences in the level of FSH receptor responsiveness to FSH and subsequent impact on the development of follicles in the ovaries. However, from the results of measurements of FSH hormone levels in each genotype patient samples found no significant differences among the three genotypes, although there is a tendency FSH hormone levels are higher in patients with genotype Ser/Ser. This is likely due to the incorrect time of the FSH hormone level measurements. However, once again we have the data indicate a trend to higher levels of FSH hormone in patients with genotype Ser/Ser genotype compared to other two genotypes. This indicates that polymorphisms in the FSH receptor influence the sensitivity of receptor to the FSH.

CONCLUSION AND SUGGESTION

Conclution

There are differences in genotype Asn680Ser frequencies between polycystic ovary women and normal women. In patients with polycystic ovary, serine allele found in the highest number and significantly different with asparagines allele. In normal women, the distribution of three genotypes followed Hardy - Wainberg law. There were no significant differences in blood FSH levels in all three genotypes in both normal and polycystic ovary women. However, it was found a tendency that blood FSH levels of patients with Ser/Ser is higher than other two genotypes.

Suggestion

This research should be continued to determine the association of FSH receptor gene polymorphisms with basal FSH levels in patients with ovarian dysgenesis, anovulatory ovaries, and others.

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