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HOW WERE STRESS FAMILY AND INSR (INSULIN RECEPTOR) EXPRESSION IN POLYCYSTIC OVARY SYNDROME (PCOS) INSULIN RESISTANT IN MADURESE TRIBE?: INDONESIA

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ABSTRACT

Introduce: Polycystic ovary syndrome (PCOS) is characterized by menstrual disorders (amenorrhea/oligomenorrhea), hirsutism, the appearance of acne, alopecia, and biochemical examination results showed an increase in androgen (testosterone). This study aimed to analyze family stress and expression of the gene INSR in insulin-resistant polycystic ovary syndrome (PCOS) in Madura.
Methods: This is an analytic observational study with a study design case-control, the sample of 50 patients (PCOS cases) and 50 healthy women (Non-PCOS) in Madura Tribe, the research instrument used Kempe Family Stress Inventory (KFSI) and using in-depth interviews, followed by screening for SGOT, SGPT, BUN, Creatinin and GDA, PCR and RFLP on the INSR gene and continued sequencing on 10 samples.
Result: The results of family stress reaction family with isolation, low self-esteem or depression average score was 56.4 (SD=4.23). The gene INSR had TT genotype 16.67%, TC 63.33%, and CC 20.00%, the control group had TT genotype 38.24%, TC 44.12%, and CC 17.65%, while in the case group T allele 48.33%, C allele 51.67% and T allele control group 56.67% and C allele 43.33%, based on statistical test chi-square in case and control groups, both genotype and allele had no significant difference (P-value> 0.05).
Conclusion: The results showed family stress reaction with isolation, low self-esteem or depression. The expression result of INSR gene yielded was no difference between PCOS and healthy women. It needs to be continued because of racial differences between ethnic groups, especially in Indonesia.

Keywords: Stress Family, Polycystic ovary syndrome, Genes, INSR, Insulin Resistance

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INTRODUCTION

Polycystic ovary syndrome (PCOS) is associated with increased psychological distress in clinical populations. We aimed to assess depression, anxiety, and perceived stress in women with and without PCOS in a large community-based sample and investigate the role of stress in contributing to and mediating the relationship between PCOS, depression, and anxiety. Polycystic ovary syndrome (PCOS) is one of the most common endocrine disorders in women of reproductive age, which is characterized by menstrual disorders (amenorrhea/oligomenorrhea), hirsutism, acne, alopecia, and biochemical examination results showed an increase in androgen (testosterone)1,2

The etiology of this observed increased prevalence of depression and anxiety in PCOS is still unclear3. Possible explanatory factors that have been investigated as a source of distress include visible features such as excess weight, clinical hyperandrogenism (hirsutism, acne, or androgenic alopecia) medical consequences such as infertility, concerns relating to diagnosis, and fear regarding long-term health complications4. Based on the diagnostic criteria National Institutes of Health 1990, the prevalence of PCOS is 6% to 10% of reproductive age women, but prevalence with Rotterdam criteria is twice as high5. Based on research conducted in Sampang and Bangkalan districts, around 4.7-5.2% of reproductive age women, and 60% of infertility cases are due to PCOS6.

The Iranian Turkish woman revealed that no link between the gene INSR and PCOS6 was confirmed by studies that the gene INSR was associated with PCOS7. This insulin resistance affects hypothalamic-pituitary pathway so that it stimulates adrenal cortisol and affects ovaries, androgens, and cortisol increases inhibiting aromatization that affects FSH and immature follicles, decreased estrogen, increased LH, and decreased SHBG(Sex hormone-binding globulin) so that ovulation does not occur and oligomenorrhea and amenorrhea appear in PCOS women8

The role of genetic factors in PCOS is very supportive, genes involved in the etiology of syndrome have not been fully investigated until now9. Genetic risk factors can detect

PCOS early, thus avoiding other risk factors that can aggravate PCOS. In this study, the gene was INSR carried out in PCOS patients of Madurese tribe. In Madurese tribe, it has potential for PCOS because they have a poor lifestyle, consuming high carbohydrates, and marriage tends not to be mixed so that bias in research can be controlled. In Asia and other countries, the results are still conflicting so that further research is needed, for the results of hormone levels are still conflicting and it has not been found with certainty what markers indicate PCOS. The purpose of this study was to analyze family stress and gene expression INSR in insulin-resistant polycystic ovary syndrome (PCOS) in Madurese tribe.

MATERIAL AND METHODS

The first step conducted on this research was explorative with a questionnaire and the second step with the approach of case-control. The research instrument used was Kempe Family Stress Inventory with the sensitivity of KFSI was calculated at 80%. The specificity was 89.4%, positive predictive value was 52.5%. The negative predictive value was 96.8%. The sample size was calculated using power analysis. The sample size is 1) the power of statistical test (1-β). 2) Level of significance (α), and the population effect size (γ)10. Therefore, the sample size in this study has estimated the significance at 0.05 a power of 0.80 and small effect size of 0.25 requiring a sample size of 100 families for the study. The questionnaire of this study was developed by the researcher based on literature review of Kempe Family Stress Inventory. The instruments included 10 items: 1) Family was beaten or deprived, 2) Family has criminal or mental illness record or substance abuse history, 3) Family suspected of abuse in the past, 4) Family with isolation, low self-esteem, or depression, 5) Multiple stresses or crises, 6) Violent temper outbursts, 7) Rigid, unrealistic expectation, 8) Harsh punishment, 9) Child is being felt difficult to perceived by family. 10) Unwanted or at risk for poor bonding. The Kempe Family Stress Inventory consisted of 10 items with third degrees were normal. Mild (rate as 5 if one or more applies). Severe (rate as 10 if one or more applies). The sensitivity of KFSI was calculated at 80%. Specificity was 89.4%. The positive predictive value was 52.5%. The negative predictive value was 96.8%. The second step with the approach case-control, independent variable INSR gene, and SHBG hormone levels and dependent variable insulin resistance Polycystic ovary syndrome (PCOS) in Madurese tribe, INSR gene is a single base change that occurs in the population> 1%, in strand DNA INSR was in exon 17, the product was PCR a 317 bp fragment which appeared as band with UV irradiation on ELP examination, followed by RFLP and analysis of the results RFLP in the form of homozygous, heterozygous and continued with sequencing. The number of respondents was 100, in the case group as many as 50 and in the control group at about 50 according to inclusion and exclusion criteria, in this study, selected cases were new insulin-resistant Polycystic ovary syndrome (PCOS) patients, native from Madura, aged 20-40 years, have no kidney and liver physiological disease, have 2 clinical symptoms of PCOS and have been diagnosed with PCOS from ultrasound results by obstetricians and controls are healthy women who already have one child without pregnancy program and are not insulin resistant, aged 20-40 years and native from Madura, are not used hormonal contraception in last 3 months, did not have blood sugar disease, kidney, and liver physiological disorders, regular menstruation with an interval of 21-35 days. This research is ethical and accepted by the ethics of the STIKes Ngudia Husada Madura study with No.715 / KEPK-NHM / EC / II / 2020.

A case group is a group of new patients diagnosed with PCOS from the hospital in Madura, then it divided into case and control groups. Interviewed to determine the original three generations of Madurese using Pidegree, blood samples were taken from SGOT, SGPT, BUN, creatinine, and blood sugar screening. Blood draw 3-5 days of menstruation in the morning and fasting. If the sample falls into inclusion criteria, then the examination is continued with Peripheral blood mononuclear cells (PBMC) from separated blood, then PBMC DNA is extracted and amplified in the INSR gene area using restriction enzyme PmlI with PCR technique. PCR polymorphism of the gene INSR 35 cycles, denaturation of 930C around 45 seconds, annealing 560C around 30 seconds, and extension 720C around 45 seconds. PCR gene INSR primary forward 5'-CCA AGG ATG CTG TGT AGA TAA G-3 'and reverse 5'-CCA ACA GAG GAC TCT TGG TCT-3'. If PCR, RFLP products, and sequencing of 10 samples of each gene were found, statistical tests were used Chi-square, Mann Withney to determine the difference between two groups of cases and controls.

RESULTS

3.1. First Step, explorative with a questionnaire

First Step, explorative data as demographic data. Family stress and polycystic ovary syndrome (PCOS) insulin resistance patients in Madurese tribe used was Kempe family stress inventory. Demographic data can be seen as below:

Table 1 Demographic data of the family and PCOS Patient. Family: Age 69 years (n = 69%). The mean of career 28.20 years, living in one house 22.40 years. Mostly employed (n = 67%). The majority education was Primary School (n = 57%). Majority Residences were Rural (n = 78%). Most Relationships were Couples (n = 67%). Demographic data by Mean ± SD. Age PCOS (26.50 ± 6.03); Age Non-PCOS (31.25 ± 6.56). The Weight of PCOS (64.72 ± 14.45); Weight Non-PCOS (55.05 ± 6.13), The Height PCOS (155.7 ± 6.47); Height Non-PCOS (154.5 ± 4.81), Body Mass Index (BMI) PCOS (27.06 ± 4.63); IMT Non-PCOS (24.68 ± 2.26). Duration of illness PCOS (Mean = 3.4 years). P-value <0.05 is Age, Weight, Body Mass Index (BMI) showing significant difference and only Height there is no difference between PCOS and Non-PCOS.

Table.2 Stress reaction is shown in Table 2

Table 2 showed that study findings revealed that overall family stress reaction with Isolation, Low Self-esteem, or Depression average score was 56.4 (SD = 4.23) and for the lowest stress reaction that Unwanted or at-risk poor Bonding average score was 3.4 (SD = 1.5). The second highest stress reaction that was Child is being felt difficult to perceived by family average score was 46.7 (SD = 5.2) and Rigid Unrealistic Expectation 44.1 (SD = 3.3). Consequently, average Mild stress score was 24.43 (SD = 4.7). The severe average score was 3.85 (SD = 2.45). Family has a Criminal or Mental Illness Record or Substance Abuse History average score was 12.24 (SD = 3.7), Family Suspected of Abuse in the

Past average score was 7.7 (SD = 1.7), Multiple Stresses or Crises average score was 8.4 (SD = 2.4). Violent Temper Outburst's average score was 5.2 (SD = 3.5). Harsh Punishment average score was 5.3 (SD = 1.4).

Based on the results of research on INSR gene expression using PCR-RFLP

Table 1. Distribution of Demographic data

Family Characteristics N (100)	N =%
Age (Years)	
12-25	69 (69)

26-45			31 (31)
Carer			28.20 years
Living in one house			22.40 years
Occupation			
Full time/part time			67 (67)
Unemployed/retired			33 (33)
Education			
Primary School			57 (57)
Junior high schools			17 (17)
Senior high schools			10 (10)
University			16 (16)
Residences			
Urban			78 (78)
Rural			22 (22)
Relationships: families are patients			
Couples			67 (67)
Parents			12 (12)
Brothers/sisters			21 (21)
Patient Characteristics PCOS N (100)	PCOS (Mean ± SD)	Non-PCOS (Mean ± SD)	P-value
Age (Years)	26.50 ± 6.03	31.25 ± 6.56	0.000
Weight (Kg)	64.72 ± 14.45	55.05 ± 6.13	0.001
Height (Cm)	155.7 ± 6.47	154.5 ± 4.81	0.547
Body Mass Index (BMI)	27.06 ± 4.63	24.68 ± 2.26	0.001
Duration of illness (PCOS)	3.4 years		

Source: Premier

Table 2. Stress reaction

Stress Reaction	Mean ± SD
Normal	3.28 ±5.6
Mild	24.43 ±4.7
Severe	3.85 ± 2.45
Parent was Beaten or Deprived	3.5 ± 2.12
The family has a Criminal or Mental Illness Record. or Substance Abuse History	12.24 ± 3.7
Family Suspected of Abuse in the Past	7.7 ± 1.7
Family with Isolation. Low Self-esteem. or Depression	56.4 ± 4.23
Multiple Stresses or Crises	8.4 ± 2.4
Violent Temper Outbursts	5.2 ± 3.5
Rigid Unrealistic Expectation	44.1 ± 3.3
Harsh Punishment	5.3 ± 1.4
Being felt difficult to perceived by family	46.7 ± 5.2
Unwanted or at Risk for Poor Bonding	3.4 ± 1.5

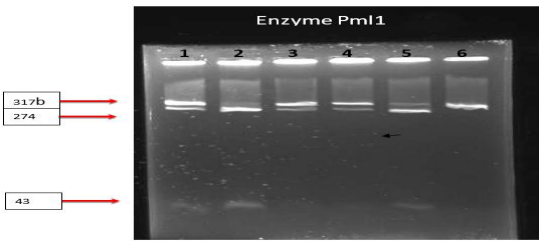


Figure 1 Case Group (PCOS)

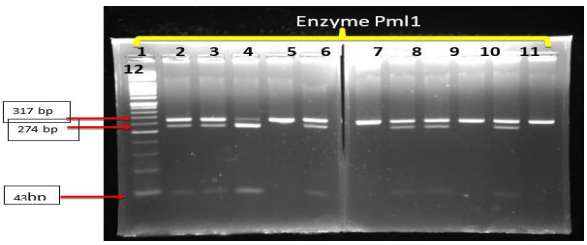


Figure 2 Results PCR control group (non-PCOS)

Table 3. Distribution of genotype and allele frequencies of genes INSR

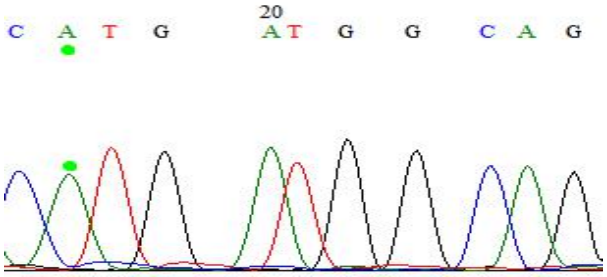
genotype	Case (PCOS)		Non-PCOS		P-value
		N%		N%	
INSR Gene					
TT	8	16:00	12	34.00	0118
TC	30	60.00	28	56.00	
CC	12	24.00	10	20.00	
Allele					
T	48	48.00	57	57.00	0.326
C	52	52.00	43	43.00 the	

Results of frequency distribution of genotypes and alleles in the gene INSR can be seen on statistical analysis of the genotype and allelegene INSR showed no significant difference (P> 0.05) between PCOS group and non-PCOS group.

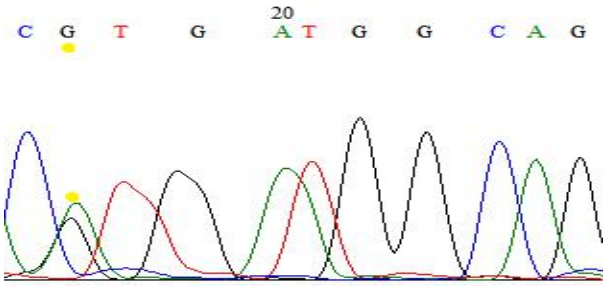
Figure 3. Sequencing results of 10 samples PCR-RFLP results of the INSR gene with Pml1 enzyme in the PCOS case group were obtained. PM 25-30 and in the control group 15-25, represented by TC (heterozygous) band

shown by band 317 bp, 274 bp, 43 bp, TT (Homozygous) shown by band 317 bp, CC (Homozygous) shown by band 274 bp, and 43 bp.

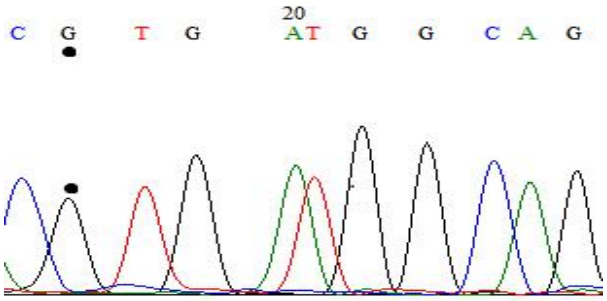
Genotype T



Genotype C / T



Genotype C



DISCUSSION

The present study assessed for the first time depression and anxiety symptoms and perceived stress in adult women with PCOS in a large community-based cohort study. Women reporting PCOS reported an increased prevalence of depression and anxiety symptoms, perceived stress, self-reported medical diagnoses of depression, anxiety, or other major mental illnesses, and treatment for psychological conditions or mental illness. Based on the result families stress reaction with PCOS is felt like a psychological burden revealed that Family with Isolation, Low Self-esteem or Depression. Researcher reported here an increased prevalence of clinical depression of 27.3% and 18.8% and anxiety of 50.0% and 39.2% in women with and without PCOS, respectively, compared with 7.1–8% and 18% in the general population4,11. In agreement with previous research11, women with PCOS had an increased risk of clinically significant depression and anxiety symptoms (1.39- and 1.37-fold respectively). These increased odds were lower than reported in a recent meta-analysis (4.03 and 6.88-fold)12, which might because meta-analysis included clinical-based studies while our study used a community-based sample. Many factors in women may contribute to depression including hormonal and biological (eg infertility, childbirth, and premenstrual syndrome) and psychosocial (eg:stress, socio-economic advantage, and violence) factors. This suggests an independent confounding relationship of stress both with PCOS status and with depression or anxiety and that higher depression and anxiety in PCOS may be related to higher stress levels in PCOS. Mediation analysis gave further clarification as stress-mediated for a large proportion of the relationship between PCOS and both depression and anxiety. This could suggest that stress can have a relevant direct effect on depression and anxiety symptoms in women with PCOS, rather than other factors considered up to now. This is consistent with prior research on the relationship between stress, chronic illness, and psychological morbidity in the general population. This may explain why previous studies have not found any direct causal relationship between PCOS and depression or anxiety13. Researcher considered in this study BMI, infertility, and socio-demographic variables as specific risk factors for depression for the general population. While some of these factors have been previously considered in investigating the relationship between PCOS and psychological variables14, to our knowledge, this is the first community-based study considering all of them together in one analysis. In the present study, association between PCOS, depression, and anxiety was attenuated but maintained on adjustment for BMI, infertility, and socio-demographic variables. This indicates that although the presence of overweight or obesity and infertility may worsen depression and anxiety as reported in general population, PCOS status is likely to have an independent effect on psychological function4.14. This might related to visible features, the frustration of having a chronic condition, or the perceived risk of future health complications15,16

Researcher also report here for the first time the confounding and the mediating effect of Polycystic ovary syndrome (PCOS) such as (amenorrhea/oligomenorrhea), hirsutism, the appearance of acne, alopecia and the results of biochemical examinations showed an increase in androgens (testosterone)16. Several factors influence including genetic and environmental factors, environment including obesity, foods with high carbohydrates and inadequate activity which causes weight gain which results in obesity and insulin resistance which affects the occurrence of hyperinsulinemia and hyperandrogenic which results in shrinkage of the egg resulting in PCOS, in several studies that have been carried out with genetic research on different ethnicities such as Chinese, Caucasian but said there was no significant association of FSHR gene (Ala370Thr), INSR gene and LHCGR gene (10–13). This is because PCOS is influenced by several factors of differences in race, ethnicity, genes, obesity which affects polygenic and environment were also a risk factor for PCOS16,17,24,25

Based on this study, it was found that there was no significant difference in INSR gene between PCOS women and healthy women. Research in Madurese Tribe showed that the highest genotype was heterozygous compared to homozygous in INSR gene. Research on INSR gene will get different results if it is carried out in places with different ethnicities and races. Based on research on Turkish women, it was found that INSR gene with enzyme restriction PmlI17, it was found that there was no difference between case and control groups, both genotype and allele18,25. This study is same as our study, it was found that INSR gene had no significant differences in genotype and allele between cases and controls. But seen from the relationship of INSR gene allele with PCOS, it was found that there was a significant relationship with PCOS group. The results of several studies are consistent with this study such as studies on Indian women and Chinese women. Research on Madurese tribe is not in line with research on Iranian women, finding that there is a significant relationship between the INSR gene and PCOS19,20,21

The result of the study in Madurese tribe was in INSR gene also found no significant relationship with PCOS, this is

many factors which affects among them because of racial differences. But although there is no relationship it can be concluded that Madurese women have a risk of PCOS and are also positive for having genes as abroad, and the results of the study also showed that heterozygous genotype is very significant in PCOS women compared to the control group. The INSR study on knockout mice demonstrated extreme insulin resistance. Insulin resistance can affect or stimulate LH secreted from pituitary and production of testosterone from theca cells and activity of cytochrome p450 from granulosa cells thereby interfering with follicular maturation and becoming PCOS. Allele polymorphism INSR is a genetic predisposing factor for PCOS21,24.

In a study conducted in China, there was a significant association of INSR gene with PCOS patients in 677 participants, INSR gene had a risk factor for PCOS, and the association of INSR gene with its clinical examination was also studied, and INSR gene provided the basis for further research as the etiology of PCOS22 , 23,26. The INSR gene has an important role in insulin regulation so that it is considered to be associated with PCOS. In recent years there have been many studies on INSR gene in exon 17 which is in the tyrosine kinase domain. In Chinese population, it was found that there was an increased risk of insulin resistance and associated PCOS in lean patient group based on RFLP analysis26,27,28. Research conducted on Indian women reported that there is an association of INSR gene with the incidence of PCOS29. In obese women, there is insulin resistance resulting in hyperandrogenic that affect follicular development and PCOS, and also convey that pathogenesis of PCOS is different between lean and obese women21,30,31. A study in Korean women reported that there was no significant association between INSR gene and PCOS, the frequency of T allele was higher in PCOS patients than in the normal group. Different studies in Madurese tribe found the C allele to be higher in PCOS patients than in healthy women31,32.

CONCLUSION

The first study, namely Family's stress reaction with PCOS is felt like a psychological burden, showed revealed that Family with Isolation, Low Self-esteem, or Depression carried out in Madurese tribe. In INSR gene and there is no difference in INSR between PCOS and healthy women, it is necessary to carry out further research on genes that are risk factors for PCOS with a larger number of samples and from several ethnic groups in Indonesia.

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