INAJOG

Research Article

The Impact of Educational Intervention of High-Risk Pregnancy and High-Risk Childbirth on Knowledge, Attitude, and Behavior in Recognizing Danger Signs in Pregnancy: A Single Blind Clinical Trial

Dampak Edukasi Mengenai Kehamilan Risiko Tinggi dan Persalinan Risiko Tinggi terhadap Pengetahuan, Sikap, dan Perilaku dalam Mengenali Tanda Bahaya Kehamilan: Sebuah Uji Klinis Tersamar Tunggal

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Abstract

Objective: To assess the impact of additional educational interventions given to pregnant women in identifying high-risk pregnancy and high-risk childbirth by measuring changes in knowledge, attitudes and behaviors in the pregnancy and after childbirth.

Methods: This study was a single blind clinical trial conducted at obstetrics out-patient clinic of Dr. Cipto Mangunkusumo Hospital, Jakarta. Total sample were 52 responders, for the 26 responders to the control group and 26 responders to the intervention group. The research instrument was a questionnaire form, which include knowledge, attitude and behaviour of a number of 48 questions. Analysis was performed using SPSS 20 with bivariate analysis.

Results: We did not found significant differences in a range of age (30.65 ± 29.38 with 1.20 ± 0.75), education (both groups showed a high level of education) and employment for both groups. We found significant differences on knowledge, behaviour (p = 0.001 and = 0.042, respectively) on the first antenatal care compared with after childbirth.

Conclusion: The educational intervention gives significant impact in attitude and behaviour.

[Indones J Obstet Gynecol 2017; 5-2: 69-72]

Keywords: attitude, behaviour, educational intervention, knowledge

Abstrak

***Tujuan**: Untuk menilai dampak intervensi edukasi tambahan yang diberikan pada ibu hamil dalam mengenal kehamilan risiko tinggi dan tanda bahaya persalinan dengan menilai adanya perubahan pada pengetahuan, sikap dan perilaku pada awal kedatangan dan sesudah persalinan.*

***Metode**: Penelitian ini merupakan penelitian uji intervensi tersamar tunggal yang berlokasi di poli kebidanan Rumah Sakit Dr. Cipto Mangunkusumo, Jakarta. Total sampel sebanyak 52 responder, masing-masing 26 responder untuk kelompok kontrol dan 26 responder untuk kelompok intervensi. Instrumen penelitian berbentuk kuisioner yang meliputi pengetahuan, sikap dan perilaku sejumlah 48 pertanyaan. Media edukasi tambahan yang diberikan pada kelompok intervensi menggunakan media lembar balik yang dikeluarkan oleh HOGSI dan USAID.*

***Hasil**: Penelitian didapatkan untuk karakteristik dari dua kelompok tidak didapatkan perbedaan bermakna dilihat dari rentang usia (30,65 ± 1,20 dengan 29,38 ± 0,75), pendidikan (kedua kelompok menunjukkan tingkat pendidikan tinggi) dan pekerjaan. Pada kedua kelompok didapatkan adanya perbedaan pengetahuan, sikap dan pengetahuan (nilai p <0,001). Perbandingan antara kedua kelompok kontrol dan intervensi sesudah persalinan memberikan hasil yang secara statistik berbeda bermakna pada sikap dan perilaku (p = 0,001 dan p=0,042).*

***Kesimpulan**: Asuhan antenatal dengan intervensi edukasi menggunakan alat bantu dapat meningkatkan sikap dan perilaku pada ibu hamil.*

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***Kata kunci**: intervensi edukasi, pengetahuan, perilaku, sikap*

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INTRODUCTION

According to data acquired by the Indonesian Demographic and Health Survey (IDHS) on 2007 and Riskesdason 2010, Maternal Mortality Rate (MMR) in Indonesia was 228 per 100,000 live births.[1-3] Compared with MMR years before, in 1991 as many as 390 per 100,000 live births and in 2002-2003 was 253 deaths per 100,000 births, the number had dropped, however the maternal

mortality rate in Indonesia was still relatively high. Indonesia's MMR is still quite far from the target of the fifth MDG (Millennium Development Goals) which in 2015 is expected only 102 per 100,000 live births.[2,4,5]

One of the interventions supposed to decrease the MMR are appropriate antenatal care services, delivery assistance by health provider, adequate care for high-risk pregnancies, family planning, reducing the rate of unsafe abortion and post abortion care, and programs for behavior change (raise awareness) among reproductive aged women.[3,5-8]

Antenatal care given by health care was expected to give result in a healthy pregnancy and delivery and decreased neonatal morbidity.[7,8-12] This study is aimed to look at the impact of additional educational intervention on high-risk pregnancy and childbirth danger signs.[13] We expect that the impact of giving the intervention will give changes in knowledge, attitude and behaviour in recognizing danger signs of labor and high-risk pregnancies.

METHODS

This study was a single blind randomized clinical trial with control group involving pregnant women who underwent a routine ANC at obstetrics clinic, Dr. Cipto Mangunkusumo Hospital during the period of August 2015 to August 2016. The responders were all pregnant women who underwent antenatal care at Obstetrics Clinic, Dr. Cipto Mangunkusumo Hospital.

Samples were divided into two groups; control group and intervention group. Sample allocation was performed by using block randomization. Both groups would receive antenatal care from the obstetrics clinic. The intervention group would be given additional education by using sheet issued by HOGSI and USAID, while the control group was not given additional education. After we obtained patients' consent, we would obtain the initial data derived from the interview. Patients would be followed during pregnancy and also participated in labor. After delivery, the patients would be interviewed again with a questionnaire to assess knowledge, attitude and behavior.

Data Analysis

Analysis the statistical differences between two groups would be analyzed using paired T-test/ Wilcoxon. The statistical differences in the control group before and after the intervention would also be analyzed using the same hypothesis tests. Data are presented in the mean, SD, mean difference, CI from the difference between the mean and the p-value.

RESULT

We obtained a total of 52 patients to be responders in this study. The responders met our inclusion criteria and were willing to follow the course of the study. Responders were divided into two groups randomly selected using excel windows, into a control group and the intervention group. Each of the group was consisted of 26 responders. The control group was provided with education in accordance with the provision of education in obstetric clinic while the intervention group was provided with additional education. The responders were guided to fill out a questionnaire consisted of 48 questions at the beginning of the ANC and after the childbirth.

The results of our study showed that the mean age of the control group was 29.38 ± 0.75 years and the treatment group was 30.65 ± 1.20. Education revealed high-level education in both groups (57.6% of control group and the treatment group 50%). In the control group 38.5% of responders are primigravid and 23.1% of responders in the treatment group was primigravida.

Having obtained two sets of questionnaires from each responder, we continued the process of coding and data processing using Microsoft Excel and for statistical calculations performed with the SPSS program. We made the classification level of knowledge, attitudes and behaviour of responders into categories of good, average and bad based on a subjective judgment.

We performed data analysis of the knowledge, attitudes and behaviour of both groups, to find out any significant difference between each groups. There were significant differences in knowledge, attitude, and behaviour of the control group compared on the first visit and after childbirth ($p<0.001$). We also obtained a significant difference result in knowledge, attitude and behavior among intervention groups ($p<0.001$).

We analyzed between two groups on the 1st time visit and after childbirth. We found significant difference in attitude and behavior.

Table 1. Knowledge and Behavior Comparison of both Groups

Parameter	Mean (SD)	Deviation (SD)	CI 95%	p
Knowledge of control groups	14.42 (2.74)	4.73 (0.87)	2.97 - 6.48	0.336
Knowledge of intervention group	19.15 (3.5)			
Behaviour of control groups	19.34 (3.12)	2.57 (3.12)	0.27 - 4.87	0.042
Behaviour of intervention group	21.92 (4.93)			

Table 2. Attitude Comparison of both Groups

Parameter	Median (min-max)	p
Attitude of control groups	18 (11 - 26)	0.001
Attitude of intervention group	22 (15 - 34)	

DISCUSSION

Knowledge is defined as the result of knowing that occurs after a person perform the sensing process of an object, which will underlie her perform a specific action. The higher the education level, the greater ability to receive information.[14] Prior knowledge in both groups was poor, amounted to 92.3% and 100% for the intervension group and control, respectively. Our result was aligned with Athanaseet al in Tanzania[15], that the majority of pregnant women who did not receive antenatal care did not know the risk and complication possibly occur due to hypertension in pregnancy. Our research revealed that the knowledge of the responders was in the middle and high level, thus making the responders able to receive counseling and education given to antenatal care quickly.

Knowledge between the two groups after intervention were collected after the mother gave birth showed that antenatal care performed by using the print media can help improve the mother's knowledge. In each group there is an increased level of knowledge before and after the antenatal care, but there is no significant increase between the two.

The use of the print media tools antenatal care can help health care providers, yet so far there has been no study comparing the group given the tools and the control group who underwent antenatal care without tools.[16] In our study, there was an improvement of knowledge in each group, where we found intervention group had higher knowledge than the control group (9.96 vs. 4:57). We found significant differences between the two groups with $p = 0.001$ for the attitude and $p = 0.042$ for behavior. In literature searches, we could not find any research that follow - include changes in attitudes and behaviour at antenatal care using media tools.

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Research Article

Iron Deficiency Anemia, Cystitis, and Bacterial Vaginosis Increase the Risk for Developing Preterm Labor

Anemia Defisiensi Besi, Sistitis dan Bakterial Vaginosis Meningkatkan Risiko Terjadinya Kelahiran Prematur

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Abstract

Objective: To prove the association of iron deficiency anemia, cystitis, and bacterial vaginosis as risk factors for preterm labor.

Methods: This study was an observational case-control study conducted at the Polyclinic and IRD of Obstetrics and Gynecology Sanglah Denpasar General Hospital Center between September and December 2015. The sample selection was done by consecutive sampling with total sample as many as 35 case samples (women with preterm pregnancy) and 35 control samples (women with aterm pregnancy).

Results: Pregnant mothers with iron deficiency anemia had 4 fold increased risk of developing preterm labor (OR = 4.04, 95% CI = 1.15 to 14.16, p = 0.023) compared to those without iron deficiency anemia. Pregnant mothers with cystitis had 4 fold increased risk of preterm labor (OR = 4.00, 95% CI = 1.25 to 12.80, p = 0.016) compared to those without cystitis. Pregnant mothers with bacterial vaginosis had 3 fold increased risk of preterm frequency (OR = 3.24, 95% CI = 1.22 to 8.63, p = 0.017) compared to those without bacterial vaginosis.

Conclusion: Iron deficiency anemia, cystitis, and bacterial vaginosis increase the risk for developing preterm labor.

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Keywords: bacterial vaginosis, cystitis, iron deficiency anemia, preterm labor

Abstrak

***Tujuan**: Untuk membuktikan hubungan anemia defisiensi besi, sistitis, dan bakterial vaginosis sebagai faktor risiko kelahiran prematur.*

***Metode**: Penelitian ini bersifat observasional dengan studi case-control yang dilakukan di Poliklinik dan IRD Kebidanan dan Penyakit Kandungan RSUP Sanglah Denpasar, yang dilakukan mulai bulan September 2015 hingga Desember 2015. Pemilihan sampel dilakukan dengan cara berurutan sampling dengan sampel penelitian sebanyak 35 sampel kasus (ibu hamil preterm) dan 35 sampel kontrol (ibu hamil aterm).*

***Hasil**: Ibu hamil dengan anemia defisiensi besi mempunyai peningkatan risiko 4 kali terjadinya kelahiran prematur (OR = 4,04, IK 95% = 1,15-14,16, p = 0,023) dibandingkan dengan ibu hamil tanpa anemia defisiensi besi. Ibu hamil dengan sistitis mempunyai risiko 4 kali lebih terjadinya kelahiran prematur (OR = 4,00, IK 95% = 1,25-12,80, p = 0,016) dibandingkan dengan ibu hamil tanpa sistitis. Ibu dengan vaginosis bakterial memiliki peningkatan risiko 3 kali terjadinya kelahiran prematur (OR = 3,24, IK 95% = 1,22-8,63, p = 0,017) dibandingkan dengan ibu hamil tanpa vaginosis bakterial.*

***Kesimpulan**: Anemia defisiensi besi, sistitis, dan vaginosis bakterial meningkatkan risiko terjadinya kelahiran prematur.*

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***Kata kunci**: anemia defisiensi besi, kelahiran prematur, sisititis, vaginosis bakterial*

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INTRODUCTION

The Millennium Development Goals targeted to reduce two-thirds mortality rate of infants and toddlers than 1990's frequency which is 20 per 1,000 live births until 2015. Currently, the infant mortality rate is still high, which is amounted to 67 per 1,000 live births. The major cause of high infant mortality rate is preterm labor. Premature babies have 35 times higher risk of death compared to aterm babies.[1]

According to WHO, the worldwide prevalence of anemia in pregnancy is 55% and will likely increase linearly in accordance to increasing gestational age. In Indonesia, the prevalence of anemia among pregnant women is 63%[2], whereas, 46.2% of pregnant women in Bali suffer from anemia.[3]

Other than iron deficiency anemia, other factors that may affect premature labor include urinary tract infection (cystitis) and bacterial vaginosis. UTI is associated with adverse pregnancy, including

preterm labor, fetal growth retardation, and stillbirth.[4]

Bacterial vaginosis is a gram-positive streptococcus bacteria, also known as Streptococcus agalactiae. Approximately 40-70% of pregnant women with bacterial vaginosis will likely spread the pathogenic bacteria to their babies vertically ie during vaginal labor. This occurs due to retrograde bacterial infection from the vagina into the uterus which later penetrate into the placental membrane, thus may lead to premature labor, stillbirth, and miscarriage.[5]

METHODS

This research was an observational case-control study conducted at the Polyclinic and IRD of Obstetrics and Gynecology Sanglah Denpasar General Hospital Center during the period from September 2015 to December 2015. The sample selection was done by consecutive sampling with total sample as many as 35 case samples (women with preterm pregnancy) and 35 control samples (women with aterm pregnancy). Mean values were compared between groups using independent T test, $p < 0.05$ was considered statistically significant.

RESULT

Demographic characteristics of the subjects are presented in Table 1. No significant differences were observed.

Association between Iron Deficiency Anemia and Preterm Labor

Chi-Square test was used to determine the association between iron deficiency anemia and preterm labor (Table 2).

Iron deficiency anemia was significantly associated with preterm labor ($p = 0.023$) OR = 0.023). In addition, subjects with iron deficiency anemia had 4 fold increased risk of developing preterm labor (OR = 4.04, 95% CI = 1.15 to 14.16, $p = 0.023$) compared to those without iron deficiency anemia.

Association between Cystitis and Preterm Labor

Chi-Square test was used to determine the association between cystitis and preterm labor (Table 3).

Table 1. Demographic Characteristics of the Subjects

Variable	Preterm Pregnancy (N=35)		Aterm Pregnancy (N=35)		p
	Mean	SD	Mean	SD	
Age (Years)	25.37	5.53	26.89	6.33	0.290
Parity	0.74	1.07	1.03	1.04	0.261
Gestational Age	31.74	3.09	38.63	1.42	0.001

Table 2. Association between Iron Deficiency Anemia and Preterm Labor

		Group		OR	CI 95%	p
		Case	Control			
IDA	Positive	12	4	4.04	1.15-14.16	0.023
	Negative	23	31			

Table 3. The Association between Cystitis and Premature Labor

		Group		OR	CI 95%	p
		Case	Control			
Cystitis	Positive	14	5	4.00	1.25-12.80	0.016
	Negative	21	30			

Cystitis was significantly associated with preterm labor. Subjects with cystitis had 4 fold increased risk of preterm labor (OR = 4.00, 95% CI = 1.25 to 12.80, p = 0.016) compared to those without cystitis.

Association between Bacterial Vaginosis and Preterm Labor

Chi-Square test was used to determine the association between bacterial vaginosis and preterm labor (Table 4).

Bacterial vaginosis was significantly associated with preterm labor (p = 0.017). In addition, subjects with bacterial vaginosis had 3 fold increasing risk of preterm frequency (OR = 3.24, 95% CI = 1.22 to 8.63, p = 0.017) compared to those without bacterial vaginosis.

DISCUSSION

We found that subjects with iron deficiency anemia had 4 fold increased risk of developing preterm pregnancy compared to those without iron deficiency anemia. Anemia may lead to complications in preterm neonates, including low Apgar score and fetal distress. Anemia in the second trimester and third trimester may can cause premature parturition, antepartum haemorrhage, intrauterine growth restrisction, intrapartum asphyxia, gestosis, and cardiac decompensation.[6] This research was supported by the results of Karasahin et al. research, (2006) which suggested that pregnant women with anemia had four times higher risk of developing premature labor and 1.9 times higher risk of having low birth weight (LBW) baby compared to nonanemic pregnant women.[7]

Chi-Square test results demonstrated that pregnant women with cystitis had 4 fold increased risk of preterm frequency compared to those without cystitis. Cystitis is an infection involving the kidney, ureter, bladder, or urethra, while urinary tract infection (UTI) is a general term that indicates the presence of microorganisms (MO) in the urine.[8] In a study involving 25.746 pregnant women with UTI disorders, it is reported that the majority pregnant women developed complications such as premature birth, gestational hypertension, anemia, and amnionitis.[9] Similar to our study, Dimetry et al found that the risk of developing preterm labor were higher in people mothers who had histories of UTI during pregnancy.[10] The study also revealed pregnant mothers suffering from UTI during had 2.2 times higher risk of developing premature labor compared to those without history of (RR = 2.2; 95% CI = 1.35 to 3.58). [10]

Chi-Square test result showed that bacterial vaginosis had 3 fold increasing risk of preterm frequency compared to those without bacterial vaginosis. Bacterial vaginosis was associated with increased risk of developing preterm labor (OR 1.5; 95% confidence interval, 1.2 to 1.9).[11] The prevalence of bacterial vaginosis was 16%, and the rate of preterm birth was 5.2%. Bacterial vaginosis was significantly associated with low birth weight (OR 1.95, 95% CI 1.3 to 2.9), premature birth of low birth weight babies (OR 2.5, 95% CI 1.6 to 3.9), and preterm labor (OR 2.4, 95% CI 1.4 to 4.1).[12]

CONCLUSIONS AND RECOMMENDATIONS

Iron deficiency anemia, cystitis, and bacterial vaginosis increase the risk for developing preterm labor. To prevent premature labor, it is recommended that mothers should undergo complete blood count, urinalysis, and vaginal swab during antenatal care visit. Thus, iron deficiency anemia, cystitis, and bacterial vaginosis, could be detected earlier, and appropriate therapy may be given.

Table 4. Association between Bacterial Vaginosis and Preterm Labor

		Group		OR	CI 95%	p
		Case	Control			
Bacterial Vaginosis	Positive	23	13	3.24	1.22-8.63	0.017
	Negative	12	22			

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Research Article

Endothelin-1 Levels in Pregnant Women with Severe Preeclampsia and Normal Pregnant Women

Kadar Endothelin-1 di Ibu Hamil dengan Preeklamsia Berat dan Ibu Hamil Normal

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Abstract

Objective: To determine the ratio of serum endothelin-1 levels between severe preeclampsia and normotensive pregnancy.

Methods: Observational analytic study using cross-sectional. Sixteen woman with normal pregnancy and sixteen others with severe preeclampsia who met the inclusion and exclusion criteria, were tested for ET-1. The serum was analyzed at Prodia Laboratory, Manado. The ET-1 level was examined using ELISA (R&D Systems, Inc., Minneapolis, MN 55413, USA). The data obtained was analyzed using SPSS software version 20.0.and discussions were held using the existing literature theory.

Results: The mean and median levels of endothelin-1 plasma in patients with severe preeclampsia is 2:46 ± 1:44 pg/ml, 1:09 ± 0:26 pg/ml, whereas in normotensive pregnancy is 1:03 ± 0:26 pg/ml, 1.95 ± 1:44 pg/ml with p < 0:05 (0000).

Conclusion: There was a significant difference between endothelin-1 level in severe preeclampsia and normotensive pregnancies.

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Keywords: endothelin-1, normotensive, preeclampsia

Abstrak

***Tujuan**: Mengetahui perbandingan kadar endothelin-1 antara kehamilan preeklamsia berat dan kehamilan normotensi.*

***Metode**: Penelitian kuantitatif observasional analitik menggunakan pendekatan potong lintang. Dilakukan pemeriksaan kadar endothelin-1 pada 32 sampel ibu hamil yang memenuhi kriteria inklusi dan eksklusi, terdiri atas 16 kehamilan preeklamsia berat dan 16 kehamilan normotensi. Analisis sampel dilakukan di Laboratorium Prodia Manado. Pemeriksaan kadar endothelin-1 menggunakan metode ELISA. Data yang diperoleh diolah dengan menggunakan perangkat lunak SPSS versi 20.0 dan dilakukan pembahasan menggunakan teori kepustakaan yang ada.*

***Hasil**: Rerata dan median kadar endothelin-1 plasma pada penderita preeklamsia berat yaitu 2,46 ± 1,44 pg/ml, 1,09 ± 0,6 pg/ml, sedangkan pada kehamilan normotensi yaitu 1,03 ± 0,26 pg/ml, 1,95 ± 1,44 pg/ml dengan nilai p < 0,05 (0,000).*

***Kesimpulan**: Terdapat perbedaan signifikan kadar endothelin-1 antara kehamilan preeklamsia berat dengan kehamilan normotensi.*

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***Kata kunci**: endothelin-1, normotensif, preeklamsia*

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INTRODUCTION

Two of the maternity care quality indicators are maternal and perinatal mortality. Approximately 600,000 women die from pregnancy-related disorders annually, and 98% of the deaths occurs in the developing countries. The Maternal Mortality Rate (MMR) in Indonesia is still far from the Millennium Development Goals (MDGs) target. The 2015 MMR target was 102 per 100,000 live births, while the infant mortality rate (IMR) target was 23 per 1.000 live births.[1-4]

Preeclampsia is a pregnancy specific condition characterized by placental and maternal response dysfunction to systemic inflammation, accompanied with endothelial activation and coagulation. Preeclampsia is a major cause of morbidity and mortality of pregnant women and newborns, occurred in approximately 2-8% of all pregnancies in the world. The majority of preeclampsia onset is late (> 34 weeks), and 10% is early (< 34 weeks).[5-7]

Several risk factors for preeclampsia include primigravida, hyperplacentosis, aged < 20 years or > 35 years, previous history of preeclampsia/ eclampsia, obesity, multiple pregnancies, medical abnormal history due to endothelial dysfunction such as chronic hypertension, and pregestational diabetes mellitus.[5,7,8]

Preeclampsia is usually started by a decrease in utero-placental perfusion due to a cytotrophoblast abnormal invasion in uterine spiral arteries. A placental hypoxia will cause a decrease in placental perfusion. In such circumstances, an imbalance between pro-angiogenic and antiangiogenic factors occur. There are two antiangiogenic proteins produced excessively in preeclamptic patients, which include soluble Fms-like tyrosine kinase-1 (sFlt-1) and soluble endoglin where ET-1 as a mediator of hypertension. Due to increased free radical and cytokine, decreased proangiogenic factors (VEGF and PIGF), and uncontrolled increase in lipid peroxide, vascular endothelial dysfunction occurs.[9,10]

ET-1 is a potent vasoconstrictor produced by endothelial cells, macrophages, fibroblasts, and cardiac myocytes. It is a family of peptide composed of 21 amino acids with two intramolecular disulfide bonds. Increased ET-1 level plays an important role in the development of hypertension in pregnancy, which may later progress to preeclampsia.[6,11,12] Several studies suggested that preeclamptic subjects with higher ET-1 levels had poorer prognosis. We aimed to compare ET-1 serum levels between severe preeclampsia and normotensive pregnancy.

METHODS

We conducted a cross-sectional study to compare the ET-1 serum in severe preeclampsia and normotensive pregnancies. This study was conducted at the Obstetrics and Gynecology Department of the Prof. dr. R. D. Kandou Hospital, Manado, and the sister teaching hospitals in Manado, during the period of October 2015 to November 2015.

The study population were all pregnancy outpatients with severe preeclampsia and those with normal blood pressure at the hospitals. The inclusion criteria were pregnant women with gestational age above 20 weeks, diagnosed with severe preeclampsia and were willing to participate in research. The exclusion criteria were pregnant women with chronic illnesses: diabetes mellitus, chronic hypertension, renal disease, thyroid disease, and patients who had received the cholesterol medication, twin pregnancies, and those who refused to participate in the study. The control group was the normotensive pregnant women with the same gestational age. We collected the sample by consecutive sampling, where every patient who met the study criteria would be included in the study until the minimum number of samples met. Statistical analyses were conducted using SPSS. T test was used for numerical variables.

RESULTS

The total number of subjects was 32, consisted of 16 normotensive pregnant women and 16 severe preeclamptic women. Demographic characteristics of the subjects are presented in Table 1.

Tabel 1. Demographic Characteristics of the Subjects

Characteristics	Normal pregnancy		Severe preeclampsia	
	n	%	n	%
Mother age				
< 35 years old	14	88	8	50
≥ 35 years old	2	12	8	50
Parity				
Primigravida	8	50	6	38
Multigravida	8	50	10	62
Gestational age				
Preterm	5	31	6	38
Aterm	11	69	10	62
Education				
Primary school	0	0	1	7
Junior high	3	19	3	19

Characteristics	Normal pregnancy		Severe preeclampsia	
	n	%	n	%
High school	13	81	10	62
Diploma	0	0	0	0
Graduate	0	0	2	12
Occupation				
Public servant	1	6	2	13
Private employee	0	0	0	0
Student	2	13	1	6
Housewife	13	81	13	81
History of Preeclampsia				
Negative	16	100	13	81
Positive	0	0	3	19
Body Mass Index				
≤ 25	12	75	10	62
> 25	4	25	6	38
Infant Birth weight				
≤ 2500	3	19	6	38
> 2500	13	81	10	62

Table 2. Comparison of the Characteristics between Subjects with Preeclampsia and Normal Pregnancy

Variables	Normal pregnancy			Severe preeclampsia			p
	Mean	Median	SD	Mean	Median	SD	
Age	26.19	27.5	7.26	30.88	34.5	6.95	0.51
Parity	1.75	1.5	1.54	3	2.5	2.03	0.16
Systole	112.5	110	7.75	168.13	160	21.67	0
Diastole	73.13	70	4.79	113.13	110	15.37	0
AST	21	19	6.55	36.44	28.5	31.46	0.138
ALT	12	11.5	3.5	24.81	19.5	21.69	0.043
Hemoglobin	11.41	11.1	1.47	10.66	11.65	3.95	0.669
Platelet	284125	266000	60202	294688	294500	78408	0.672
BMI	22.94	21.5	5.29	24.25	23.5	4.09	0.196
Birth weight	2806.25	3050	831.44	2653.13	2750	642.77	0.16

We found no significant difference between the preeclamptic population and the normotensive group ($p > 0.05$) for the following variables: maternal age, parity, AST, the number of hemoglobin and platelets, BMI and birth weight. There were significant differences between the preeclamptic and normotensive groups for these variables: systolic, diastolic and the levels of ALT ($p < 0.05$).

From Graph 1, the obtained results mean and median distribution of ET-1 appears to be higher severe preeclampsia (2.46 ± 1.44 pg/ml, 1.95 ± 1.44 pg/ml) compared to normotensive pregnancy (1.03 ± 0.26 pg/ml, 1.09 ± 0.26 pg/ml). This result demonstrate increased levels of ET-1 in severe preeclampsia compared to normotensive pregnancy.

The Mann-Whitney statistical test showed that there were significant differences between the levels of endothelin-1 between the severe preeclamptic and the normotensive groups (p = 0.000). This means that the concentration of endothelin-1 affects the occurence of severe preeclampsia.

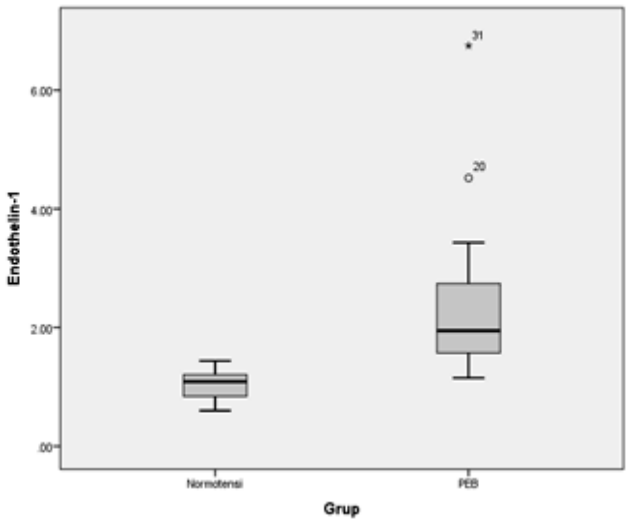

Figure 1. The ET-1 Correlation between Severe Preeclampsia and Normotensive Pregnancy

DISCUSSION

Approximately 5-10% of pregnant women experienced a syndrome known as preeclampsia. In women with normotensive pregnancies, a thorough physiological alterations and body system adaptation occur. In cardiovascular system, ET-1 receptor blockage maintains the vascular tone. Pregnant women with risk factors, such as maternal disease and oxidative stress, the angiogenic factor were dysfunctional.[14]

Multiple evidence suggested that ET-1 was one of the preeclampsia pathophysiological factors. When the preeclampsia and the normotensive groups were compared, an elevated ET-1 in preeclampsia group was found. It indicates that the concentration of ET-1 affects the occurrence of severe preeclampsia.[11]

Early incident in preeclampsia occurs due to decreased utero-placental perfusion, which happens because of remodelling failure of the spiral arteries and endothelial dysfunction. Placental hypoxia will lead to decreased placental perfusion. In such circumstane, an imbalance factors between proangiogenic and antiangiogenic occurs. In preeclampsia, there are two antiangiogenic proteins produced excessively in the maternal circulation: soluble Fms-like tyrosine kinase-1 (sFlt-1) and soluble endoglin where ET-1 as a mediator of hypertension. Due to increase in free radical and cytokine causing oxidative stress and decrease in proangiogenic factors VEGF, PIGF and an uncontrolled increase in lipid peroxide. This causes vascular endothelial dysfunction that is disintegration of endothelial structure and function.[9,10]

Increase in endothelin-1 in the circulation of pregnant women have an important role in the occurrence of hypertension in pregnancy and may develop into preeclampsia. In several different studies, preeclamptic patients with higher ET-1 level in the maternal circulation had poorer prognosis.[11,15]

The obtained results mean and median distribution of ET-1 appears to be higher in severe preeclampsia compared with normotensive pregnancies. The interpretation result indicates that the presence of increased levels of ET-1 in severe preeclampsia compare with normotensive pregnancies. This means that the concentration of endothelin-1 affects the occurence of severe preeclampsia.

Croom et al conducted are search on the serum level of ET-1 in normal pregnancy and preeclampsia. This study demonstrated increased levels of ET-1 in preeclampsia compared to normal pregnancy. In normal pregnancy, the level of ET-1 0.5 pg/ml (1st trimester), 1.1 pg/ml (3rd trimester), and 1.7 pg/ml (at birth). Whereas in patients with preeclampsia, level of ET-1 1.9 pg/ml (before delivery) and 3.5 pg/ml (at birth). Slowinski et al found a very strong relationship between the level of ET-1 in patients and preeclampsia at 24 weeks and 36 weeks of pregnancy compared to normotensive pregnancy. The level of ET-1 was higher in preeclamptic patients at both gestational ages (1.07 ± 2.00 vs 0.54 ± 0.56 pg/ml, p=0.045 at 24 weeks and 0.75 ± 1.20 vs 0.44 ± 0.45 pg/ml, p=0.023 at 36 weeks).[16,17]

Several studies have shown that ET-1 is associated with the development of preeclampsia. The levels of ET-1 in preeclamptic patients increased significantly compared to normal pregnancy. ET-1

is a potent vasoconstrictor that can be used as predictors of hypertension and preeclampsia. Increase in ET-1 indicated vasospasm progressivity accompanied with decreased renal plasma flow and utero-placental blood flow. Finally, elevated ET-1 level is responsible formitogenic activity reported on preeclampsia.[6,13]

Aggarwal et al found that ET-1 level in preeclampsia was higher compared to normotensive pregnancy (1.52 ± 0.55 vs 0.88 ± 0.35 pg/ml, p<0.001). Nova et al found that ET-1 was highest in patients with HELLP syndrome than without HELLP syndrome and the lowest in normotensive group.[13,18]

Kamoi et al suggested that ET-1 level in normotensive pregnant women was lower than non pregnant women (0.6 ± 0.1 vs 1.5 ± 0.3pg/ml) and higher in women with pregnancy-induced hypertension (1.9 ± 0.3 pg/ml). After delivery, increased levels of ET-1 would go back to its normal level in line with decreased blood pressure in all patients. Meanwhile, in pregnant women with chronic hypertension, the ET-1 levels were slightly higher compared to normotensive pregnant women (0.9 ± 0.3 pg/ml, p<0.01). In a study of 6 patients with pregnancy induced hypertension (PIH), 2 patients with pregnancy induced proteinuria (PIP) without hypertension and 7 normotensive pregnant women which was conducted by Ohya et al, subjects with PIP had the highest levels of ET-1, while the normotensive pregnant population had the lowest levels (4.4 ± 0.5 vs 4.5 ± 0.6 vs 1.5 ± 0.2 pg/ml). One week after delivery, ET-1 level in PIH and PIP were still high. After one month, the level of ET-1 in PIH population began to decline, whereas the PIP population still had high ET-1 levels.[19,20]

In the severe preeclamptic group, four subjects had complications, in which two subjects developed suffered from eclampsia, one subject had eclampsia and one subject suffered from eclampsia with HELLP syndrome. Of these subjects, four them had their ET-1 levels increased significantly, which may indicate further endothelial damage in preeclampsia. These findings are consistent with a study conducted by Nova et al, which found that ET-1 level were significantly elevated in patients with HELLP syndrome compared without HELLP syndrome. However, this needs to be done with greater samples to assess the association between complications of preeclampsia with high levels of ET-1.[13]

This study has some limitations that may affect the results. Counfounding factors including level of urea, creatinine and other biochemical mediators as predispositions of preeclampsia, were not controlled. In addition, we did not examine other biochemical factors that were expected to influence ET-1 levels to our subjects. Family physicians and maternity care provider should educate patient and families to do antenatal care regularly to detect high risk pregnancies, including the early signs of preeclampsia, therefore prevention and treatment can be done as early as possible and is expected to reduce maternal and perinatal morbidity and mortality. Further studies with larger sample size are required to obtain the causal association between ET-1 and the complications of preeclampsia.

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Research Article

Anti Müllerian Hormone as a Predictor of Metabolic Syndrome in Polycystic Ovary Syndrome

Hormon Anti Mülleri sebagai Prediktor Sindrom Ovarium Polikistik Metabolik

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Abstract

Objective: To evaluate whether Anti Müllerian Hormone (AMH) can be used as a predictor of metabolic syndrome in Polycystic Ovarian Syndrome (PCOS).

Methods: This cross-sectional study was conducted in Yasmin Clinic, Dr. Cipto Mangunkusumo General Hospital Jakarta between June and December 2012. Forty-one patients diagnosed with PCOS based on Rotterdam Criteria were enrolled. Secondary were was taken from medical record..

Results: A total of 22 subjects were involved in this study. Mean AMH level in the metabolic syndrome group is compared to the non-metabolic syndrome group (10.72±6.23 ng/ml vs 7.97±4.50 ng/ml, p=0.12). AMH was strongly associated with HDL, triglyceride and insulin resistance (r-value of -0.29, 0.23, and 0.21 respectively, p< 0.05).

Conclusion: AMH can be used as a predictor of metabolic syndrome in PCOS.

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Keywords: anti müllerian hormone, metabolic syndrome, polycystic ovarian syndrome

Abstrak

***Tujuan**: Untuk mengevaluasi apakah Anti Müllerian Hormone (AMH) dapat dijadikan prediktor untuk sindroma metabolik pada pasien Sindroma Ovarium Polikistik (SOPK).*

***Metode**: Studi ini merupakan studi potong lintang yang dilakukan di Klinik Yasmin Rumah Sakit Dr. Cipto Mangunkusumo Jakarta pada bulan Juni hingga Desember 2012. Empat puluh satu persen pasien didiagnosis SOPK berdasarkan kriteria Rotterdam. Data sekunder didapatkan dari rekam medis.*

***Hasil**: Pada studi ini terdapat 22 pasien yang mengalami sindroma metabolik. Nilai rerata kadar AMH pada pasien SOPK dengan sindroma metabolik ditemukan lebih tinggi dibandingkan dengan pasien tanpa sindroma metabolik (10,72±6,23 ng/ml vs 7,97±4,50 ng/ml, p=0,12). Hasil penelitian ini juga menunjukkan bahwa AMH berhubungan dengan HDL, trigliserida, dan resistensi insulin dengan nilai r -0,29, 0,23, dan 0,21 (p < 0,05).*

***Kesimpulan**: AMH dapat menjadi salah satu parameter untuk memprediksi sindrom metabolik pada pasien SOPK.*

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***Kata kunci**: anti müllerian hormone, sindroma metabolik, sindroma ovarium polikistik*

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INTRODUCTION

Anti Müllerian Hormone (AMH), also known as Mullerian inhibiting substance, is a member of the transforming growth factor β family of growth and differentiation factors. AMH is known to have an essential role in folliculogenesis. AMH inhibits the growth of primordial follicles, and inhibits the sensitivity of antral follicles to follicle-stimulating hormone (FSH) during cyclical recruitment. Thus, AMH is believed to play an important role in the regulation of the ovarian follicular growth. In addition, AMH is widely used as an ovarian reserve marker.[1,2] To date, the raise of AMH level found in polycystic ovarian syndrome (PCOS) is believed due to increased expression of the promoter gene. In addition, its level increases up to two to three times compared to normal women worldwide.[3,4] In addition, the more severe PCOS phenotype one person has, the higher level of plasma AMH is detected.[4,5]

PCOS is one of the diseases frequently found in reproductive aged women. It affects both endocrine and metabolic system. In fact, 46% women with PCOS has shown association in the development of metabolic syndrome in later life ($p<0.0001$).[6] A study in Korea surprisingly demonstrated that young women with PCOS (mean age of 26.5 years old) had a three-fold

increased risk of developing metabolic syndrome compared to normal population.[7] However, due to many panels of examinations should performed to diagnose metabolic syndrome and not all patients present with the same characteristics, sometimes the diagnosis of metabolic syndrome is disregarded.

Currently, the relationship between level of AMH with the risk of cardiovascular event, particularly metabolic syndrome, remains unknown. Several studies suggested that the disturbance in lipid profile in patient with PCOS was not associated with body mass index.[8] However, Skalba et al found that AMH was significantly associated with total cholesterol, LDL and HDL.[9] Legro et al found that numerous PCOS patients with metabolic syndrome were under diagnosed, which may lead to inadequate therapy.[10] Therefore, a panel of examination that can predict the risk of cardiovascular event in PCOS is needed to be carried out, therefore clinicians will be able give adequate treatment to patients with PCOS.

METHOD

A cross sectional study design was used. The inclusion criteria were women of reproductive age (15-45 years) who met the diagnostic criteria for PCOS based on Rotterdam 2003 in Yasmin Clinic, Dr. Cipto Mangunkusumo General Hospital during the period of June to December 2012 and agreed to participating in the research. Patient who consumed hormonal therapy or uses insulin-sensitizing agents within three months before, and had done ovarian drilling was being excluded. The patient would be diagnosed as having PCOS according to the Rotterdam consensus,[11] which were the finding of 2 out of the 3 following criterias; oligo-and/or an ovulation, hyper androgenism, defined as hirsutism (Ferriman-Gallwey score > 8), or minor signs including acne, seboborrhea, and/or testosterone > 3 nmol/l and/or androstenedione > 12 nmol/l, and criteria for polycystic ovary by ultrasound examination (minimum of 12 follicles with 2-9 mm diameter in each ovary, and/or increasing ovarian volume with a minimum size 10 mm^3).

According to the American Heart Association,[12] metabolic syndrome is diagnosed when someone fulfill three among five diagnose criteria which are blood pressure ≥ 130/85 mmHg, triglyceride ≥ 150 mg/dl, fasting blood glucose ≥ 100 mg/dl, HDL-C < 50 mg/dl, and waist circumference ≥ 80 cm (Asian women).

Secondary data derived medical records were used to obtain the data of the subjects including AMH levels and all panels to diagnose metabolic syndrome: blood pressure, triglyceride, HDL-C, fasting blood glucose, and waist circumference.

Data were processed using Windows SPSS version 11.0. Results were presented as mean ± SD. Relationship between each variable to AMH level was analyzed with independent t-test, p-value of < 0.05 would be considered statistically significant.

RESULT

Using the Rotterdam criteria, 41 subjects were diagnosed with PCOS. Among these patients, twenty-two subjects were confirmed to have metabolic syndrome while nineteen had normal metabolic profile. Characteristics of the subjects in the study are presented in Table 1.

Table 1. Baseline Characteristics of the Subjects

	Metabolic syndrome (n=22)	Non metabolic syndrome (n=19)	p
Age (years)	30.14 ± 2.88	31.11 ± 3.43	0.33
BMI (kg/m^2)	29.62 ± 4.62	26.89 ± 4.11	0.05
Waist circumference (cm)	91.80 ± 9.72	87.00 ± 7.19	0.08
Fasting blood Glucose (mg/dl)	102.45 ± 29.45	89.16 ± 6.64	0.06
HDL (mg/dl)	42.36 ± 6.99	48.68 ± 11.93	0.04
Triglyceride (mg/dl)	195.27 ± 10.41	101.26 ± 4.69	0.001
AMH (ng/ml)	10.72 ± 6.23	7.97 ± 4.50	0.12

Mean age between patients who had metabolic syndrome was younger than non metabolic syndrome (30.14 ± 2.88 vs 31.11 ± 3.43). Significant results in the metabolic syndrome group were found in HDL level (42.36 ± 6.99 vs 48.68 ± 11.93, p = 0.04) and triglyceride (195.27 ± 10.41 vs 101.26 ± 4.69, p < 0.001). Despite no significant association was found, the level of AMH was higher in the metabolic syndrome group compared to the non - metabolic syndrome group (10.72 ± 6.23 vs 7.97 ± 4.50, p = 0.12).

Independent t-test was performed to determine the association of AMH between each of variable of metabolic syndrome. Variables such as BMI, waist circumference and fasting blood glucose were not significantly associated with AMH, whereas both HDL and triglyceride were significantly associated with AMH (p < 0.05 [Table 2]).

Table 2. p-value of AMH as Predictor of each Variable of Metabolic Syndrome

	AMH p-value
BMI	0.05
Waist circumference	0.08
Fasting blood glucose	0.06
HDL	0.04
Triglyceride	0.01

DISCUSSION

We found no significant difference between metabolic syndrome and non metabolic syndrome in PCOS. Moreover, metabolic syndrome can be seen as early as 30 years old. This finding is consistent a previous study conducted Park which demonstrated that young PCOS Korean women with mean age of 26.5 years old were three times at risk in developing metabolic syndrome in later life.[7]

In this study, AMH was higher in the PCOS group. This is consistent with previous studies.[3,4] In addition, the metabolic syndrome population had higher AMH levels compared to the non-metabolic syndrome group. (10.72 ± 6.23 vs 7.97 ± 4.50, respectively). Despite the non-sigfniciant difference (p=0.12), 25% difference in clinical view might be considered as a significant finding.

Numerous studies found that increased plasma AMH level was significantly correlated with body weight.[13,14] However, we found no significant association between BMI and AMH level (p=0.05), which is consistent with a previous study conducted by Skalba et al.[13]

To date, there is a new finding that AMH can replaced one of the criteria diagnosis of Rotterdam, such as polycystic ovarian morphology. Wiweko et al found that AMH was proven to be significant predictor of PCOS (cutoff value = 4.19ng/ml).[4] More over, AMH can be used of predictor of therapeutic response in PCOS.[15] AMH was found to have significant association with fasting insulin, glucose, in women with or without PCOS. However, this study did not demonstrate significant association between fasting blood glucose with level of AMH (p = 0.06).

It is well known that metabolic syndrome is related to the development of cardiovascular risk in later life. PCOS is not only disease of reproductive system but also a disease of metabolic. 46% women with PCOS suffer from metabolic syndrome.[6,8] In addition, PCOS was associated with the development of dyslipidemia in obese women due to the increment of androgen level which may lead to atherosclerosis.[10,16] We found that AMH was significantly associated with HDL and triglyceride, which is in line with a previous study conducted by Skalba et al. Based on these findings, we suggest that AMH can be used as potential predictor of metabolic syndrome in PCOS. Our limitations include limited sample size. Further studies are required to make the result more useful, to treat PCOS more holistically.

CONCLUSIONS

AMH level is associated with two markers of metabolic syndrome, triglyceride and HDL. This study demonstrates that AMH might be used as a potential predictor of metabolic syndrome in PCOS. Thus clinician can be aware and treat it as early as it can to prevent women with PCOS from developing cardiovascular event.

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Research Article

Post-laparoscopic GnRH-agonist Therapy does not Improve Spontaneous Conception Rates of Women with Endometriosis

Terapi GnRH-agonis Post-laparoskopi tidak Meningkatkan Angka Kehamilan Spontan pada Perempuan dengan Endometriosis

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Abstract

Objective: To investigate the reproductive benefits of combining laparoscopic surgery with GnRH-agonist hormonal therapy in treating women with endometriosis.

Methods: This is a non-randomized prospective analytic study of patients with endometriosis conducted in a private hospital in Bandung during the period of January 2014 to December 2015, whom were later followed up after 12-24 months for assessment of post-surgical reproductive performance. A total of 121 patients were enrolled, 60 of which received post-surgical GnRH-agonist hormonal therapy.

Results: We discovered that 56 women (46.3%) eventually achieved spontaneous conception after surgery but upon further analysis, it was discovered that GnRH-agonist played no significantly meaningful role in improving the spontaneous pregnancy rates of these patients (OR 1.539; 95% CI 0.750-3.159; p-value 0.239). Furthermore, even though there was a wide range as to when they achieved conception, those untreated with hormonal therapy tended to conceive far more quickly than those who were (5.91 ± 6.28; 8.56 ± 4.24; p-value: 0.011).

Conclusion: Post-laparoscopic GnRH-agonist administration to women with endometriosis does not significantly improve their chances of spontaneous conception. In fact, such administration seems to delay it.

[Indones J Obstet Gynecol 2017; 5-2: 87-93]

Keywords: endometriosis, GnRH-agonist, laparoscopy

Abstrak

***Tujuan**: Untuk menyelidiki efek reproduktif dari terapi kombinasi operasi laparoskopi dan terapi hormonal GnRh-agonis dalam manajemen perempuan dengan endometriosis.*

***Metode**: Penelitian ini adalah studi analitik prospektif tidak teracak pada pasien-pasien dari sebuah rumah sakit swasta di Bandung dari tahun 2014-2015 yang menderita endometriosis dan kemudian diikuti hingga 12-24 bulan untuk mengevaluasi performa reproduktif post-operatif mereka. Terdapat 121 pasien pada penelitian ini, 60 di antaranya mendapatkan terapi hormonal GnRH-agonis post-operatif.*

***Hasil**: Penelitian ini menemukan bahwa 56 pasien (46,3%) berhasil hamil setelah dilakukan laparoskopi tetapi disimpulkan bahwa GnRh-agonis tidak memainkan peranan signifikan dalam meningkatkan angka kehamilan dari pasien-pasien ini (OR 1,539; 95% CI 0,750-3,159; p 0,239). Selain itu, meskipun terdapat variasi cukup luas terkait kapan pasien-pasien ini berhasil hamil, mereka yang tidak mendapatkan GnRH-agonis cenderung berhasil hamil lebih cepat dibandingkan mereka yang dapat (5,91 ± 6,28; 8,56 ± 4,24; p 0,011).*

***Kesimpulan**: Pemberian GnRH-agonis post-laparoskopi pada pasien-pasien dengan endometriosis tidak meningkatkan kemungkinan mereka hamil dan sebaliknya, cenderung menunda kehamilan.*

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***Kata kunci**: endometriosis, GnRH-agonis, laparoskopi*

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INTRODUCTION

Endometriosis is a gynecologic disease that stems from the presence of uterine endometrial tissue (both glands and stroma) outside the uterine cavity.[1] These ectopic tissues are often planted on the pelvic viscera, e.g. ovaries, and the peritoneum. It is a disease of primarily women of reproductive age. While studies vary on its prevalence in the general population, it is estimated that endometriosis adversely affects 1 in every 10 women.[2] Endometriosis acquaints itself with numerous risk factors, e.g. infertility, early age at menarche, shorter menstrual cycle length, history of endometriosis in 1st degree relative and diet high in fat and alcohol.[1] Nevertheless, there are numerous protective factors against endometriosis, e.g. use of oral contraceptive drugs, increased BMI, exercise and diet high in vegetables and fruits.[1,2]

Despite being a common gynecologic disease, the exact cause of endometriosis remains elusive and the available theories seem to offer only part of the explanation. The most widely supported theory is the transplantation theory proposed by Sampson which assumed that retrograde menstruation into the peritoneal cavity allowed the implantation of sloughed endometrial tissue on the pelvic viscera and/or peritoneum.[1,2] This, together with heightened inflammatory state, reduced apoptosis and evasion from NK cells predation, have allowed the endometrial tissue to not just implant but also persist and flourish in their foreign territories.[2,3] There are other theories, including the coelomic metaplasia and the induction theories, which attempt to explain the histogenesis of endometriosis and it seems more likely that an eclectic mix of the various theories available today underlies the true pathomechanism of endometriosis.[1]

To date, endometriosis has many classification systems, but the original one, proposed for the ASRM remains the benchmark today. This classification divides endometriosis into 4 groups, ranging from stage I (minimal endometriosis) to stage IV (severe endometriosis). There are other systems, such as the Endometriosis Fertility Index (EFI) primarily used to predict pregnancy rates of patients based on their endometriosis severity, and the Enzian classification for classifying deep infiltrative endometriosis.[4]

Endometriosis adversely affects the quality of life of many females as it primarily produces pain. Various types of pain have been reported, including dysmenorrhea, dyspareunia, dyschesia and non-menstrual pelvic pain.[5] Even more unfortunate has been the revelation that the painful symptoms are only part of the misfortune befalling these patients as endometriosis is also linked to infertility.[6,7]

There are several causes of infertility, conveniently divided into 3 groups: ovulatory dysfunction (20-40%), tubal and peritoneal pathology (30-40%) and male factors (30-40%).[8] Endometriosis causes ovulatory dysfunction, induces a heightened inflammatory state in the peritoneum and fertilization-hampering changes in peritoneal fluid while also disrupting the hormonal interaction in the uterus.[7]

Fortunately, the ESHRE has published a guideline for the management of endometriosis, in the hope of providing relief and also restoring the reproductive function of the patients.[9,10] The ESHRE recommends laparoscopic surgery as a means to remove the endometriosis lesions and also reduce endometriosis-associated pain.[9] Another justification for surgery comes from the observation that endometriosis appears to progress in 30-60% of patients within a year of diagnosis and it is not possible to predict which patients' endometriosis will worsen. Fortunately, surgery is deemed to live up to its hype as spontaneous conception is to be expected within the 1st year post-surgery.[11]

There is also a widespread practice today of adding adjuvant medical therapy in the form of GnRH-agonist injections after laparoscopic surgery for these patients. This hormonal therapy should reduce and/or postpone endometriosis recurrence by inactivating, but not eliminating, the remaining microscopic endometriosis lesions.[12,13] Current evidence states that this regimen is more effective than surgery alone in reducing the symptoms and recurrence of endometriosis but its effects on increasing pregnancy rate are still up to debate.[14,15]

Therefore, this study aims to evaluate the reproductive performance of patients with endometriosis who underwent operative laparoscopy 12-24 months post-surgery and also the association, if any, between post-surgical GnRH agonist administration and pregnancy rates. To our knowledge, this is the first study in Indonesia that attempts this approach on endometriosis patients.

METHOD

This is a non-randomized prospective analytic study involving a cohort of patients in a private hospital in Bandung who was operated by a single physician between January 2014 and December 2015 for various complaints, e.g. dysmenorrhea and inability to conceive and subsequently discovered to harbor endometriosis as one of the post-surgical diagnoses. Surgical treatment was performed in a standardized manner following hospital protocols. Laparoscopy was performed under general anesthesia using a 4-port approach. When an ovarian endometrioma(s) was discovered, ovarian cystectomy began with adhesiolysis. Once the ovary was mobilized, the ovarian cortex was grasped with forceps and incision of the cortex was made. If the cyst was opened and

there was a spillage, peritoneal irrigation as performed. Upon cyst decompression, its wall was exposed, inspected and finally stripped from the normal ovarian tissue. Should there be any bleeding from the cortex after stripping, such bleeding zones were coagulated using the bipolar forces. The endometrioma was then removed through a 10-mm trocar. When a peritoneal endometriosis was encountered, endometriosis removal was done through ablation. Adhesiolysis was first performed to obtain proper visualization of the lesions. The lesions were then destroyed using either bipolar coagulation or laser vaporization. Vaporization was continued until no pigment remained to be seen.[16] Documentation of endometriosis location and rASRM stage was undertaken. All the surgeries were undertaken by a single physician (T.D) to minimize operator bias. Routine follow-up consisted of an obligatory post-operative visit at 1-2 weeks where a physical examination was done and patients were questioned regarding their bowel and bladder functions. Planning on whether to treat the patient with an adjuvant hormonal therapy (i.e., GnRH-agonist injections) was made during this consultation and should a patient be recommended to undergo such therapy, a repeat consultation was arranged at 2-4 weeks during which she would receive her first GnRH-agonist injection. Patients would normally receive either 3 or 4 monthly injections, and the decision to assign which regimen to which patients was subject to the physician's (T.D) clinical assessment.

Women identified as having endometriosis of any rASRM stage were then contacted by telephone from November to December 2016 (on at least 2 separate days for those who didn't respond to our first call) and were requested, by phone, for their consent to participate in the study and divulge their post-surgical reproductive performance. The following items were collected during the interviews: any pregnancy and/or abortion post-surgery; any additional GnRH-agonist injection(s) post-surgery for those whose records were incomplete and/or who had to receive their monthly injections elsewhere due to various reasons; and details of the pregnancy and its outcome for those who did conceive which included the last menstrual date, mechanism of pregnancy (spontaneous conception, intrauterine insemination (IUI), IVF/ICSI)), gender of the child, weight and length of the child at delivery, date of delivery, means of delivery and site of delivery.

Initially, 166 eligible patients were identified from our 2014-2015 databases to participate in this study. However, 45 patients had to be opted out due to one or more of the following reasons: the patient did not respond to telephone calls on 2 separate days; the patient's contact numbers were inactive, out of reach or incorrect; the patient already had children prior to surgery and did not desire to conceive again; the patient was already > 42 years old at the time of surgery; the patient was widowed prior to surgery and did not remarry; the patient was unmarried until the time of follow-up and the patient's successful conception was by assisted reproduction technology (ART). In the end, 121 patients were included in the final cohort for analysis.

Data were then recorded in a purpose-built database on Microsoft Excel with subsequent statistical analysis undertaken with Statistical Package for Social Sciences (SPSS), version 21. An 80% power was assigned at the beginning of the study, which determined the sample size for each group. Shapiro-Wilk's test was then used to determine the data's normality. Chi-squared and Mann-Whitney U tests were later used to analyze the data. Finally, probability values of less than 5% were considered statistically significant.

RESULTS

Table 1. Demographics of Study Population

Variables	GnRH-agonist (+)	GnRH-agonist (-)	p-value (Sig.)
Age during surgery (mean SD)	29.88 ± 3.87	31.62 ± 3.93	0.014
Pre-surgical reproductive history			
Parturition			
0	52	56	
≥ 1	8	5	

Variables	GnRH-agonist (+)	GnRH-agonist (-)	p-value (Sig.)
Abortion			
0	52	56	
≥ 1	8	5	
GnRH-ag inj.			
≤ 3	27	N/A	
≥ 4	34	N/A	
Post-surgical reproductive history			
ongoing pregnancy	12	5	
parturition	15	16	
abortion	4	4	
Timing of pregnancy post-surgery* (mean ± SD)	8.56 ± 4.24	5.91 ± 6.28	0.011
range of timing	(3-21)	(0-26)	
Pregnancy outcome (parturition)	15	16	
Gender			
Male	7	13	
Female	8	3	
Average weight (g)	3008.67 ± 326.49	2980 ± 397.06	0.770
Average length (cm)	49.13 ± 1.25	48.38 ± 1.79	0.232
Status at delivery			
preterm	4	5	
term	9	10	
postterm	2	1	
Weight status at delivery			
SGA	1	2	
AGA	14	14	
LGA	0	0	

**some patients' details who had abortion were missing, so calculation of the average timing could not include all 56 patients*

Table 2. Spontaneous Conception Rates among Study Population

	Pregnant (+)	Pregnant (-)	Total
GnRH-agonist (+)	31	29	60
GnRH-agonist (-)	25	36	61
Total	56	65	121

Odd-ratio: 1.539 (95% CI 0.750 - 3.159), p-value: 0.239

From January 2014 to December 2015, 166 patients underwent fertility-preserving operative laparoscopy by a single surgeon for varying states of endometriosis (stages I-IV) at a private hospital in Bandung, Indonesia. Follow-up was performed over the course of 2 months, from November to December 2016. Data were retrieved from 121/166 patients (72.9%), whom were then divided into two groups: those who received GnRH-agonist injections after surgery and those who didn't. On average, those who received GnRH-agonist injections were younger at the time of

surgery than those who didn't (p-value 0.014). Then, for both groups, most women had never conceived prior to surgery (108/121 patients, 89.3%). Among those who received GnRH-agonist injections, the number of women who received 3 monthly injections or less (27 patients) was slightly lower than those who received 4 monthly injections or more.

After surgery, the reproductive performances of the two groups were compared. Firstly, of those whom are currently pregnant, two-thirds come from those who received GnRH-agonist injections post-surgery (12 to 5, respectively). Secondly, about the same number of patients from both groups have delivered a child after undergoing laparoscopic surgery (15 and 16, respectively). Finally, the same number of patients from both groups have experienced an abortion after the surgery (4 and 4, respectively).

Thus, in total 56 patients managed to spontaneously conceive after surgery. However, when the association between the administration of GnRH-agonist (regardless of the number of injections received by the patients) and spontaneous pregnancy rates was investigated, the Chi-squared test revealed no significant role to be played by GnRH-agonist injections in increasing spontaneous pregnancy rate (OR 1.539; 95% CI 0.750-3.159; p-value 0.239).

Of those who did conceive though, there was a wide range as to when they managed to conceive. Some conceived immediately after surgery (a patient managed to conceive within a single month after surgery) while others had to wait significantly longer to achieve pregnancy (the longest was 26 months). On average, though, those who did not receive GnRH-agonist injections tended to conceive significantly more quickly than those who did (5.91 ± 6.28; 8.56 ± 4.24; p-value: 0.011).

Of those who delivered, both groups yielded notable results. Firstly, those who received GnRH-agonist injections had more female children while those who did not, delivered predominantly male children. Secondly, there were no significant differences on the average weights and lengths of the children at delivery across the two groups (p-value 0.770 and 0.2332, respectively). Finally, most of the deliveries were at term (19/31; 61.3%) and had normal weights at delivery (28/31; 90.3%)

DISCUSSION

Endometriosis is a benign gynecologic disease defined by the ectopic presence of endometrial tissue outside the uterus.[8] It affects the pelvic viscera and/or peritoneum and characteristically, it is often present in the most declivitous parts of the pelvis, such as the Douglas pouch. Its characteristic symptom is pain, manifesting as dysmenorrhea, dyspareunia, dyschesia and non-menstrual pelvic pain.[5] The symptoms may be so severe that it degrades the patient's quality of life (QoL) as well as debilitating the patient from performing her daily activities. It is this painful characteristic of endometriosis that has prompted ESHRE to recommend laparoscopic surgery as a means to both diagnose and treat endometriosis.[9] ESHRE recommends operative laparoscopy to both relieve the patient of endometriosis-associated pain as well as preserve the patient's fertility. An Australian study by Abbott et al lent support to this ESHRE recommendation as patients who underwent surgery for endometriosis reported significant improvement for the above-mentioned 4 pain symptoms, the patient's QoL and sexual pleasure. Relieved of endometriosis, the patients later should try to conceive naturally for at least 12 months prior to resorting to assisted reproductive technology (ART). This is a recommendation of at least 2 studies from France and Australia.[5,11]

In the present study, 56 out of 121 patients (46.3%) managed to conceive naturally. This is a comparable rate of conception when compared to a study in Australia in which they analyzed the spontaneous conception rate among nurses who underwent laparoscopic surgery for endometriosis and got a spontaneous conception rate of 46.5% (66/142 patients).[10] In addition, our results are slightly more superior to another similar study in Korea, in which they analyzed the natural conception rate among women who had their endometriosis surgically removed.[17] The Korean study exhibited a 41.4% conception rate but a major difference was their follow-up period, which was limited to only 12 months post-laparoscopy. Had they extended the follow-up period to match ours (12-24 months post-laparoscopy), it would be realistic to assume their natural conception rate would have been higher.

In our present study, our patients were divided into two equally sized groups: those who received

GnRh-agonist monthly injections post-surgery and those who didn't. The GnRH-agonist used in this study was leuprolide, falling under two brand names: Tapros and Divalin . Of those who received the injections, slightly more patients (34 patients) received at least 4 injections than those who received only 3 injections or less (27 patients). When this exposure was accounted for and compared towards the natural conception rates of each group's members, it was revealed that the administration of a GnRH-agonist did not significantly improve natural conception rates. This is in agreement with many other studies that have also been conducted throughout the years.[14,18-21] A study in Germany by Alkatout et al even went a step further, by analyzing conception rates from women with endometriosis treated with hormonal therapy alone, surgical therapy alone, and combined surgical-hormonal therapy. The results they had led to the same conclusion, that there were not any significant differences in the natural conception rates across the 3 different groups.[14] In fact, this result is to be expected, as the primary rationale to prescribe GnRH-agonist injections after laparoscopic surgery is to prevent endometriosis recurrence by inactivating any remaining microscopic endometriosis lesions and not to directly improve fertility.[22,23] The mechanism by which GnRh-agonist administration prevent sendometriosisre currence is by abolishing the pulsatile release of follicle-stimulating hormone (FSH) and luteinizing hormone (LH), thereby inactivating the ectopic endometrial lesions which would otherwise grow along the pulsatile FSH and LH secretion.[24] However, it is important to stress that such hormonal therapy only function to inactivate and not eliminate the ectopic endometrial lesions. GnRH-agonist and all forms of hormonal therapy place the ectopic endometrial tissue in a quiescent state and as long as the drug is administered, the patient may expect to remain free of endometriosis symptoms.[13]

It is interesting to note that upon further analysis, there is a significant difference in the average waiting time to pregnancy after surgery between those who were treated with GnRH-agonist and those who were not. In our results, those who did not receive GnRH-agonist injections had to wait significantly less to conceive (5.91 ± 6.28) than those who did (8.56 ± 4.24), p-value: 0.011. This result does support the views expressed in other studies, which recommended patients to attempt natural conception for at least 12 months post-surgery before requesting the help of ART.[10,11]

However, as with all other scientific studies, our study is still replete with limitations from various aspects. The first is selection bias, introduced by the non-randomized nature of this study and also the specific population from which the study participants were recruited, as they all came from a single private hospital. The second limitation was the relatively small cohort of patients this study could work with (only from 2014-2015) and this small pool was further hampered by non-responders, which would have introduced non response bias. Another limitation was with the presence of possible confounders, e.g. male factor infertility and the presence of other gynecologic comorbidities. Finally, missing details from the medical records also disallowed us from stratifying the endometriosis diagnosis into the rASRM classification.

CONCLUSION

To conclude, post-laparoscopic administration of GnRH-agonist to women with endometriosis does not significantly improve their spontaneous conception rates. In fact, those who had post-laparoscopic GnRH-agonist injections tended to wait longer before eventually conceiving.

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Research Article

The Efficacy of Monoplant® and Indoplant® as Contraceptive Methods: A Comparative Study

Efektivitas Susuk Monoplant® dan Indoplant® sebagai Kontrasepsi: Sebuah Studi Komparative

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Abstract

Objective: To determine the effectiveness, safety, and time of insertion between Monoplant® with Indoplant® to prevent pregnancy.

Methods: Data were collected from November 2015 until May 2016 in Raden Saleh Clinic. A total of 153 patients met the inclusion and exclusion criteria for the study and were divided into 77 patients who received Monoplant® and 76 patients received Indoplant®. The study period was 6 months.

Results: The data obtained showed no significant difference in the effectiveness of both contraceptive methods. In addition, side effects such as menstrual disorders and weight gain did not differ significantly in those study groups. However, the time of insertion between Monoplant® and Indoplant® was siginificantly different (162.91 + 197.04 + 49.81 seconds versus 44.96 seconds, p<0.001). For complications such as skin irritation, inflammation, there are no differences between Monoplant® (0.0%) and Indoplant® users (0.0%).

Conclusion: There are no significant differences in efficacy and side effects using Monoplant® and Indoplant® during the 6-month follow-up. However, the insertion time of Monoplant® is shorter compared to Indoplant®'s. Monoplant® can be considered for use as contraception with the effectiveness and side effects are almost the same, but with shorter time of insertion compared to Indoplant®.

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Keywords: contraception, implant, indoplant®, monoplant®

Abstrak

***Tujuan**: Untuk mengetahui efektivitas, keamanan penggunaan, dan waktu penyisipan antara Monoplant® dibandingkan dengan Indoplant® untuk mencegah kehamilan.*

***Metode**: Pengambilan data dilakukan sejak November 2015 hingga Mei 2016 di Klinik Raden Saleh. Sebanyak 153 pasien memenuhi kriteria inklusi dan eksklusi penelitian dan dibagi menjadi 77 pasien yang menerima susuk Monoplant® dan 76 pasien menerima susuk Indoplant®. Penelitian dilakukan secara kohort prospektif hingga observasi selama 6 bulan.*

***Hasil**: Data yang didapat menunjukkan tidak terdapat perbedaan bermakna pada efektivitas yaitu kejadian hamil pada penggunaan Monoplant® dan Indoplant®. Selain itu, efek samping seperti gangguan haid dan kenaikan berat badan tidak berbeda bermakna pada kedua kelompok penelitian. Namun, waktu penyisipan antara Monoplant® dan penyisipan Indoplant® (162,91 + 49,81 detik vs 197,04 + 44,96 detik, p<0,001) berbeda secara berkmakna. Untuk komplikasi sepert iritasi kulit, peradangan, tidak terdapat perbedaan komplikasi pada saat penyisipan Monoplant® (0,0%), dan Indoplant® (0,0%).*

***Kesimpulan**: Tidak terdapat perbedaan bermakna pada efektivitas serta efek samping pada penggunaan Monoplant® dan Indoplant® selama 6 bulan follow-up. Namun, waktu penyisipan lebih singkat untuk penggunaan Monoplant® dibandingkan Indoplant®. Dapat dipertimbangkan untuk menggunakan Monoplant® sebagai implan untuk kontrasepsi dengan efektifitas dan efek samping yang hampir sama, namun waktu penyisipan yang lebih singkat dibanding Indoplant®.*

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***Kata kunci**: indoplant®, kontrasepi, monoplant®, susuk*

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INTRODUCTION

Every contraceptive method has advantages and disadvantages. The advantages of implant are high effectiveness, long-term usage, convenient usage, minimal maintenance, quickly returned after releasing the implant, minimal metabolic effects, and relatively low cost. Limitations of the implant are menstrual disorders, requiring surgical procedures for insertion and removal, and no providing of protection against sexually transmitted infections. The side effects are the main reason that women stop using implants. The side effects include irregular or prolonged bleeding, headaches, mood swings, weight gain, depression or anxiety, acne, abdominal discomfort, and pain in the area of insertion.[1-8]

Indoplant® has already been marketed in Indonesia since 2005 and gets good response among family planning users in Indonesia. Indoplant®, consistsed of two rods, is reported to have an effective and safe contraceptive based on research.[9] Monoplant® is a contraceptive implant containing the same hormonal content as Norplant® but have different packaging because it consists only one rod. Monoplant® is expected to be one of the more desirable contraceptive method because the packaging is simpler which is expected easier insertion and removal. Therefore, it is needed to study further about comparison of the effectiveness and safety of new contraceptive implant that consists of one rod, which is Monoplant®, with Indoplant®.

From the background, it can be formulated whether there are differences in effectiveness, safety, and the time of insertion between Monoplant® compared with Indoplant®. This study aims to determine the effectiveness, safety, and time of insertion between Monoplant® with Indoplant® to prevent pregnancy.

METHODS

A double-blind randomized clinical trial was used. The affordable population were implant acceptors in Family Planning Clinic, RSUPN Dr. Cipto Mangunkusumo from August 1st, 2015 until August 31st, 2018. Inclusion criteria of this study were reproductive age women between 20 and 35 years, healthy, not in a pregnant, had active sexual intercourse, not used hormonal contraception within 6 months, explained and signed informed consent, were willing to do repetitive visits, and were only using implant as a contraceptive during the three years period of study. Exclusion criteria were having a family history of any type of cancer, abnormal uterine bleeding, cardiovascular problem, mental disorders, tuberculosis, frequent headaches, history of liver disease or active liver disease, using drugs that induce liver enzymes, having high blood pressure, and severe hirsutism.

Data were collected using consecutive sampling. If the corresponding patients were available, they would be recruited as a research subject. Processing and data analysis were performed using the software "SPSS for Windows" version 20.0.

RESULTS

After the data were collected prospectively in Obstetrics and Gynecology clinic, RSCM, we obtained 153 subjects with 77 subjects of Monoplant® and 76 subjects of Indoplant®. At 1-month follow-up, there were three subjects that is loss-to-follow-up and 16 subjects had not yet reached the period of 1 month during the study period. At 3-month follow-up, there were two subjects who dropped out of the study because they developed intolerable side effects including drastic weight gain and continuous spotting. At 6-month follow-up, there were four subjects that can be assessed when writing this report. Characteristics of the subjects are presented in Table 1.

Table 1. Characteristics of the Subjects

Characteristics		**Monoplant® (n=77)**	**Indoplant® (n=76)**
Age	18-19 years old	0 (0.0)	1 (1.3)
	20-24 years old	15 (19.5)	15 (19.7)
	25-29 years old	22 (28.6)	26 (34.2)
	30-34 years old	35 (45.5)	28 (36.8)
	35-40 years old	5 (6.5)	6 (7.9)
Weight (kg)		58.78 ± 11.59	56.99 ± 12.52
Height (cm)		155.31 ± 6.42	154.93 ± 5.25
BMI (kg/m^2)		24.37 ± 4.57	23.70 ± 4.74

Characteristics		Monoplant® (n=77)	Indoplant® (n=76)
Blood pressure (mmHg)	Systolic	115.06 ± 12.51	116.47 ± 14.45
	Diastolic	75.90 ± 9.43	75.64 ± 10.71
Parity	P0	2 (2.6)	0 (0.0)
	P1	21 (27.3)	25 (32.9)
	P2	34 (44.2)	30 (39.5)
	P3	15 (19.5)	20 (26.3)
	P4	3 (3.9)	0 (0.0)
	> P4	2 (2.6)	1 (1.3)
Length of menstruation	< 3 days	0 (0.7)	1 (1.3)
	3-7 days	73 (94.8)	72 (94.7)
	> 7 days	4 (5.2)	3 (3.9)
Lactation	No	40 (51.9)	36 (47.4)
	Yes	37 (48.1)	40 (52.9)
Last contraceptive method	Pill	14 (18.2)	13 (17.1)
	Implant	6 (7.8)	12 (15.8)
	Injection	34 (44.2)	30 (39.5)
	IUD	3 (3.9)	2 (2.6)
	Condom/diaphragma	0 (0.0)	2 (2.6)
	Withdrawal	0 (0.0)	0 (0.0)
	Lactational amenorrhea method (LAM)	3 (3.9)	2 (2.6)
	Never	17 (22.1)	15 (19.7)

Normal distribution of data presented in mean ± standard deviations; abnormal distribution data presented in median (minimum - maximum); categorical data are presented in the amount (percentage).

From these results, the most common of last contraceptive method used by both groups as much as 34 subjects (44.2 %).

The effectiveness of a contraception assessed through the ability to prevent pregnancy. In both groups of the study, the subjects assessed as pregnant or suspected pregnant. The result of the analysis are shown in Table 2.

Table 2. Comparison of Effectiveness between Monoplant® and Indoplant®

Follow-up	Research group	N	Pregnant or suspected pregnant		p value
			Yes	No	
1 month	Monoplant®	69	0 (0.0)	69 (100.0)	-*
	Indoplant®	68	0 (0.0)	68 (100.0)	
3 months	Monoplant®	32	0 (0.0)	32 (100.0)	-*
	Indoplant®	30	0 (0.0)	30 (100.0)	
6 months	Monoplant®	1	0 (0.0)	1 (100.0)	-*
	Indoplant®	3	0 (0.0)	3 (100.0)	

** Chi-Square test can not be conducted because the result of variable dependent is only 1 category*

Weight of the subjects were assessed at the beginning of the implant insertion, 1 month, 3 months, and 6 months follow-up. The number of subjects who reached 1-month follow-up was 35 subjects for Monoplant® and 37 subjects for Indoplant®. Both group had increments in body weight, ie 0.85 kg for Monoplant® and 0.5 kg to Indoplant®. For the 3-month follow-up, insertion of 1 and 3 months of Monoplant®, there were increments of 0.27 kg and 0.57 kg from initial body weight. After the insertion of 1 month and 3 months of Indoplant®, weight tended to be stable. In four subjects who had reached the 6-month follow-up, one Monoplant® subject gained 0.5 kg since the use of the implant and stable 3 months onward. For three Indoplant® subjects, two of which gained 2 kg and 1 subjects gained 1 kg. It should be noted that there was one Monoplant® subject who dropped our study because she gained 11 kg in one month. The average weight gained in 3000 acceptors in China that used Norplant® was 2.5 kg.[11] Within three years of usage of LNG implant, the mean weight changed as much as 0.6 to 0.8 kg, of which 49.6 and 52.2% women in the study had gain 1 kg or more, while 29.5 and 29.7% decreased body of 1 kg or more.[12]

At the time of follow-up, menstrual disorders of both groups of the study were assessed. The result of the analysis is shown in Table 3.

Insertion time between Monoplant® and Indoplant® were assessed. The insertion time for Monoplant® and Indoplant® is 162.91 ± 49.81 seconds and 197.04 ± 44.96 seconds consecutively. This difference is significant based on statistical test (p value <0.001). At the time of insertion, complications were also assessed. The results showed no complications such as skin irritation, infection, inflammation, or anaphylactic reaction in Monoplant® (0.0%) and Indoplant® (0.0%).

DISCUSSION

Table 2 suggested that there was no contraceptive failure at Monoplant® group (0.0%) and Indoplant® (0.0%). Thus, it can be concluded that there is no difference in effectiveness between Indoplant® and Monoplant®. Another studies showed that there were two Indoplant® subjects (0.7%) who experienced pregnancy during 36 months[9] and no pregnancy found in 30 Monoplant® subjects at 6 months follow-up.[10]

Table 3. Comparison of Menstrual Disorders in Monoplant® and Indoplant®

Follow-up	Characteristics		Monoplant®	Indoplant®	p value
1 month	Dismenorrhea	Yes	6 (8.7)	24 (2.9)	0.274
		No	63 (91.3)	30 (97.1)	
	Menstruation	Amenorrhea	26 (37.7)	27 (39.7)	0.931
		Shorter than usual	9 (13.0)	8 (11.8)	
		Normal/usual	21 (30.4)	18 (26.5)	
		Longer than usual	13 (18.8)	15 (22.1)	
3 months	Dismenorrhea	Yes	1 (3.1)	0 (0.0)	1.000[a]
		No	31 (96.9)	30 (100.0)	
	Menstruation	Amenorrhea	9 (28.1)	10 (33.3)	0.937[b]
		Shorter than usual	3 (9.4)	3 (10.0)	
		Normal/usual	8 (25.0)	8 (26.7)	
		Longer than usual	12 (37.5)	9 (30.0)	
6 months	Dismenorrhea	Yes	0 (0.0)	0 (0.0)	-*
		No	1 (100.0)	3 (100.0)	
	Menstruation	Amenorrhea	1 (100.0)	1 (33.3)	1.000
		Shorter than usual	0 (0.0)	0 (0.0)	
		Normal/usual	0 (0.0)	2 (66.6)	
		Longer than usual	0 (0.0)	0 (0.0)	

[a] T-dependent test, [b] Fisher test, [*] Chi-Square test can not be conducted because the result of variable dependent is only 1 category

There were no differences in menstrual disorders between Monoplant® and Indoplant® at 1, 3, and 6 months follow-up. In addition, there was one subject using Monoplant® who dropped out due to exessive spotting. In both groups, there were increased percentage of subjects with longer menstrual duration. This is contrast with a study conducted by Affandi B[9] in Indonesia which compared Norplant® and Indoplant®. It is reported that were an increment in the percentage of subjects with shorter periods than usual from the beginning, 12, 24, and 36 months follow-up. This may occur due to differences in measurement time in our study, where the follow-up were conducted at 1, 3, and 6 months, while Affandi B[9] are at 12, 24, and 36 months.

CONCLUSION

Both implant have the same effectiveness, safety, no differences in weight gain and menstrual disorders during 1, 3, and 6 months follow-up. However, the insertion of Monoplant® are faster than Indoplant®. Monoplant® can be used as a contraceptive method with the same effectiveness and safety as Indoplant®, yet with shorter insertion time.

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Research Article

The Impacts of Pelvic Floor Dysfunction Counseling to Knowledge Level and Attitude of Pregnant Women with Gestational Age above Thirty Six Weeks in the Selection of Delivery Method

Dampak Penyuluhan Disfungsi Dasar Panggul terhadap Pengetahuan dan Sikap Ibu Hamil lebih dari Tiga Puluh Enam Minggu dalam Pemilihan Metode Persalinan

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Abstract

Objective: To determine the level of knowledge about pelvic floor dysfunction before and after counseling in term pregnant women and knowing whether a difference a change of attitude in the selection method of delivery before and after counseling.

Methods: This study design using pre - post test. At the beginning of our study provide some sort of written test to determine the initial knowledge of participants prior to the extension and the selection of the desired method of delivery. Having obtained the results of the test, followed by education about pelvic floor dysfunction. Then do the post-test to determine the level of knowledge of the subject and mode of delivery that would be pursued. The study took place between February and May 2016 in 5 Public Health Center (PHC) in Jakarta that PHC Warakas (North Jakarta), PHC Tanah Abang (Central Jakarta), PHC Cengkareng (West Jakarta), PHC Jatinegara (East Jakarta) and PHC Jagakarsa (South Jakarta).

Results: A total of 102 study subjects who began the study gave the results of the pretest mean 71 ± 10.49 (p<0.0001) and post test results of 80.725 ± 7.7 (p<0.0001). Of the 102 subjects who began the study, there were two people who had previously chose method of delivery by caesarean section turned into vaginal.

Conclusion: There is a change scores better in knowledge about pelvic floor dysfunction after counseling. There was no significant difference between selecting the desired method of delivery before the after counseling.

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Keywords: fecal incontinence, pelvic floor dysfunction, sexual dysfunction, stress urinary incontinence, uterine prolapse

Abstrak

***Tujuan**: Untuk mengetahui tingkat pengetahuan seorang ibu hamil > 36 minggu yang datang ke puskesmas di wilayah DKI Jakarta tentang disfungsi dasar panggul dan apakah edukasi yang diberikan akan mempengaruhi pemilihan metode persalinan yang akan dipilihnya.*

***Metode**: Penelitian ini menggunakan desain pre - post tes. Pada awal penelitian kita memberikan semacam tes tertulis untuk mengetahui pengetahuan awal peserta sebelum dilakukan penyuluhan dan pemilihan metode persalinan yang diinginkan. Setelah didapatkan hasil tes, dilanjutkan dengan pemberian edukasi tentang disfungsi dasar panggul. Kemudian dilakukan post tes untuk mengetahui tingkat pengetahuan subjek penelitian dan cara persalinan yang akan ditempuh. Penelitian ini berlangsung pada bulan Februari hingga Mei 2016 di 5 Puskesmas Wilayah DKI Jakarta.*

***Hasil**: Sebanyak 102 subjek penelitian yang mengikuti penelitian ini memberikan hasil mean pre-tes 71± 10,49 (p<0,0001) dan hasil post-tes 80,725± 7,7 (p<0,0001). Dari 102 subjek yang mengikuti penelitian ini, setelah mereka mendapat penyuluhan dan pengetahuan yang memilih metode persalinan secara pervaginam sebanyak 97 subjek (95,1%) dan memilih metode persalinan secara seksio sesarea sebanyak 5 orang (4,9%).*

***Kesimpulan**: Bahwa terdapat perbedaan skor pengetahuan setelah dilakukan penyuluhan dan terdapat perubahan pemilihan metode persalinan antara sebelum dan sesudah penyuluhan.*

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***Kata kunci**: disfungsi dasar panggul, disfungsi seksual, inkontinensia fekal, prolaps uteri, stres inkontinensia urin*

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INTRODUCTION

A woman has 11% lifetime risk for developing pelvic floor dysfunction. Sometimes, when pelvic floor dysfunction is severe enough, surgery is indicated. More than 300.000 surgeries had been perfomed to treat the condition. Pelvic floor dysfunctions may lead to urinary inconticence, fecal incontinence, obstruction in miction or defecation, pain in perineal area, and bulging in vaginal area (specific symptoms clinically related with pelvic organ prolapse) and sexual dysfunction.[1]

As the incidence of pelvic floor dysfunction increases, the demand of cesaeran section in Nigeria also increases. There are several reasons, including labor pain (68.9%), afraid of problems that may occur during labor (60.1%), afraid of fecal incontinence incidence (20.2%) and afraid of urinary incontince incidence (16.8%).[2]

Pregnancy and labor are the major risk factors in the development of pelvic floor dysfunction. Never the less, women with risk factors have greater chance to develop pelvic floor dysfunction. Budi I Santoso concluded that risk factors could be determined from birth weight of the baby, history of episiotomy, second stage of labor duration, and history of perineal laceration.[3]

Inaccurate knowledge and understandings will make the wrong choice of delivery mode. Researchers believe that with good, correct, and comprehensive counseling, a woman will be able to choose mode of delivery more rationally and not based on the fear of pelvic floor dysfunction. Discussion about pelvic floor dysfunction needs early recognition of pelvic floor dysfunction risk factors.[4]

Uterovaginal prolapse can be equated to a herniation, which is the protruding of uterus into, or through the vagina caused by weakening of pelvic floor muscles and ligaments and fascia supporting the uterus. Many factors affecting strength and integrity of pelvic floor fascia will affect pelvic floor function. These factors can be congenital anomaly (such as hyperelasticity of collagen components of fascia) or environmental factors (such as distention or rupture of fascia during labor or weight bearing). Muscles in pelvic floor will experience degradation and denervation along with time. This denervation process is the cause of weakening of pelvic floor muscle. Usually, if a women experienced prolonged second stage of labor, cesarean section can protect from this injury. There is still a debate regarding cause from labor, some believe the rise from the stretch of distal pudendal nerve up to alcock canal in spina schiadica or from crushing injury on neuromuscular junction.

Urinary incontinence is a condition that can disturb all aspects in quality of life of a woman. There is an assumption that loss of bladder control due to labor and ageing is a normal thing. Generally definition and majority of urinary incontinence are when pressure of bladder exceeds pressure in urethra due to weakening urethra sphincter mechanism (urodynamic urinary incontinence) or due to high detrusor pressure (detrusor overactivity, neurogenic detrusor overactivity).

Consensus regarding classifications for sexual dysfunction in women which are sexual desire disorder where there is hypoactive sexual desire disorder, the diminished or lack of sexual fantasy and/or desire or urge to do sexual activity that persists or recurs, which cause personal distress; and there is sexual aversion disorder: rejection or avoidance of sexual contacts with partner that persists or recurrents, which caused personal distress.

There are two components which have major role in the incidence of fecal incontinence, which are anal sphincter (internal and external) and puborectalis muscle. Contraction from smooth muscle of internal anal sphincter can lasts long, in charge of closing anal canal up to 85%, and work 24 hours non stop, including when sleeping. If there is a damage in internal anal sphincter, then it is enough to cause fecal incontinence. External anal sphincter will help internal sphincter during certain sudden times; which is when there is elevation of intra abdominal pressure such as when coughing, sneezing, and more.

METHODS

This study used cross sectional as a study design to measure the change in knowldege score and method of delivery mode choice. In the beginning of the study, we gave a written test to know the initial knowledge of subjects before counseling was performed and choice of mode of delivery wanted. After we got the tests result, it was continued with counseling about pelvic floor dysfunction. Then post tests was done to determine level of knowledge of subjects and delivery mode they would undergo.

This study was conducted in public health centers (PHC) in DKI Jakarta, which include Warakas PHC in North Jakarta, Jatinegara PHC in East Jakarta, Tanah Abang PHC and Maternity Hospital, Cengkareng PHC in West Jakarta, and Jagakarsa PHC in South Jakarta. These public health centers were chosen for having maternity hospital facility and having the most antenatal care visitors

comparing with PHC in same area in DKI Jakarta. This study was conducted for 4 months, starting from February 2016 to May 2016.

The inclusion criteria were all pregnant mother with gestational age > 36 weeks that had never attend any class or counseling about pelvic floor dysfunction, had not known about method of delivery that would be planned, agree to participate in the study by signing informed consent, and had educational level from junior high school and above. The exclusion criteria were are all pregnant women with gestational age > 36 weeks with disorders that blocked birth canal such as tumors, huge hemorrhoid that may delay labor, patients with preeclampsia and eclampsia, patients that had known method of delivery planned was per abdominam labor, patients who were unwilling to be interviewed, and patients who couldn't communicate properly. All other clinical condition would be considered by main researcher of this study.

Plan of data analysis of this study was used to know the level of knowledge before and after education and selection of method of delivery that would be chosen by subjects before and after counseling. For the first data analysis, the score was obtained from subjects from test results before and after counseling. Analysis used for these data based on paired numeric comparative analysis was paired t-test if data distribution was normal. If the data distribution was not normal, we used Wilcoxon test. In this study, the expected results were mean of each group, mean difference between group, and confidence interval from the mean difference. For the analysis of selection of method of delivery chosen by subjects where subjects choice categorized into two categories, so for this data the test that will be used is McNemar test. In this study result expected were proportion in each group, comparison of proportion between group, confidence interval and p value of the comparison.

Table 1. Demographic Characteristics of the Subjects

Variable	n (%)	Variable	Mean ± SD
Education		Age	29.59 ± 6.2
SMP (junior high)	40 (39.2)		
SMU (senior high)	50 (49.0)		
D3	4 (3.9)		
S1	8 (7.8)		
Employment		Body weight (kg)	64.26 ± 1.07
Employed	30 (29.4)		
Unemployed	72 (70.6)		
Gravida		Body height (meter)	1.54 ± 6.05
Primigravida	38 (37.3)		
Multigravida	64 (62.7)		
History of delivery			
Yes	60 (58.8)		
No	42 (41.2)		
History of complication			
Yes	4 (3.9)		
No	98 (96.1)		
Family income (rupiah)			
< 1 million	7 (6.8)		
1 million - 2.5 million	65 (63.7)		
2.5 million - 5 million	27 (26.4)		
> 5 million	3 (2.9)		

RESULTS

As many 102 respondents fulfilled inclusion criteria. All respondents performed pretest, continued with attending counseling, and ended with perfoming posttest. Demographic characteristic of subjects are presented in Table 1.

There was significant difference between respondent's knowledge about pelvic floor dysfunction before and after counseling. In this study, we achieved that pre-test score with mean 72 and post-test score mean was 80. There was 9 subjects with lower level score of knowledge, 84 subjects with higher level score of knowledge, and 9 subjects with same results.

There was significant difference of subject's delivery methods between before and after counseling. From 102 subjects, there was 9 subjects choosing abdominal delivery methods before counseling but after the counseling, 2 subjects changed their option to choose vaginal delivery method.

DISCUSSION

A total of 102 subjects were included in this study. Most of the subjects was high-school education (49%), not working (70.6%), multigravida (62.7%), history of delivery (58.8%), absence of complications history (96.1%), and family income was about Rp 1.000.000 - 2.500.000 (63.72%).

Onkokwo NS, et al, reported that 39.6% pregnant woman from 843 subjects choosing cesarean section as a delivery method though there was no medical indication or comorbid disease that was indicated for a cesarean section. This is associated with health care facility type and educational status of subjects. In this study, most of the pregnant woman was high-school graduate (49%) and unemployed (70.6%). This research was done in primary health care facility which with the recent applied reference system, the researcher was easier to get the subjects who met the inclusion criteria than in secondary or tertiary health care facilities. In this research, compared to the previous study, the researcher not only use the physician's information, but also use the education material standardized by IUGA, where we aim to reduce mistakes in giving the information.

In this study, we would like to know whether there is improved knowledge of subjects. In this study, there is improvement in post-test score compared to pre-test score, which there was improvement in mean of score about 9.725 ± 2.788 and p 0.0001. This means that most of the subjects had understand the counseling which was given about the pelvic floor dysfunction, so the subjects can acquire the knowledge and consideration about her delivery method. There was 9 subjects who has the lower post-test score compared than the pre-test score which is after the counseling. The researcher analyzed that education failure according to Clinical Training Skills for Reproductive Health Professionals that the success key of effective clinical education determined by: usage of modelling behavior, competency-based training, and human counseling technique.[5]

Table 2. Pre-test and Post-test Score Comparison

Knowledge Level	Median (min, max)	Mean Difference	p
Before counseling	72.0 (36, 96)	9.725 ± 2.788	0.0001
After counseling	80.0 (64.100)		

Table 3. Selection of Delivery Method before and after Counseling

		selection of delivery method after counseling		*Total*	p
selection of delivery method before counseling		**Vaginal delivery**	**Abdominal delivery**		
	Vaginal delivery	93	0	93 (91.17%)	0.063
	Abdominal delivery	2	7	9 (8.83%)	
	Total	95 (93.1%)	7 (6.9%)	102 (100%)	

After the counseling, we examined that pregnant woman choosing cesarean section procedure was 7 from 102 subjects (6.9%). If we correlate with the education factor and health care type affected this study, the result will contradict with the previous study. Most of the pregnant woman has high-school graduate (49.0%), and only a few (7.8%) which were a college graduate. So, from the education history prospective, we can conclude that woman with educational stage as high as high school have an enough consideration to choose their best delivery method.

From seven pregnant women that choose a c-section delivery method, 42.86% are afraid of getting uterine prolapse, 42.86% are afraid of the pain during labor, and the rest are afraid of getting weak pelvic floor muscles and having straining problems. Moreover, two women who had chosen to do a c-section delivery method before have changed their mind and chose pervaginam delivery instead. That's because after getting counseled and knowing the risks of getting pelvic floor dysfunction, the precautions like Kegel exercises, reduce body weight, and change of lifestyle by reduce heavy lifting, respondents switch their delivery method from c-section to vaginal delivery.

Other than that, Indonesia also have a payment system (health insurance) called BPJS (Badan Penyelenggara Jaminan Sosial) that will make sure all the medical treatments are according to indications. If someone choose a c-section delivery method but she doesn't meet the medical indications, then the cost of actions will not be insured by the government, so that the patient must pay the bill herself. The costs of c-section method in Indonesia are relatively more expensive than the normal pervaginam method. Where in our research, there are 7 subjects (6.8%) whose salaries are below one million rupiah, 65 subjects (63.7%) whose salaries are between 1 - 2.5 million rupiah, 27 subjects (26.4%) whose salaries are between 2.5 - 5 million rupiah, and 3 subjects (2.9%) whose salaries are more than 5 million rupiah.

In addition to the causes that are explained before, the other causes of the lack in awareness and rights of choosing a c-section delivery method in Indonesia are the influence from their husband and family in making a decision. The family and the husband are often make decisions that will not endanger the mom and the baby and also relatively cheaper in costs and insured. While in Indonesia, women are more likely to obey and follow what the husband says. This was also discussed by Onkokwo NS, et. al. in his research that discovered the influences from the husband and family are pretty much big, 33.3% from the husband, 17% from the parents, and 11% from the family members.

This study has the advantage that carried out in primary care facilities in each region of the municipality of Jakarta that the sample obtained represents the Primary Health Centre in DKI Jakarta and the study subjects got the knowledge about early detection of signs and symptoms of pelvic floor dysfunction, prevention and treatment of pelvic floor dysfunction. However, there is disadvantage of this study, such as: no standarized education materials so the education materials that we use based on education guidelines from International Urogynecology Association (IUGA) so the reseachers aware that not all the subjects understand well with given content of education materials.

Because the education done in indoor hall and in big groups that each group consist of 10 - 15 people. This study was only done in Primary Health Centre where the subjects mostly use BPJS Kesehatan (Badan Penyelenggara Jaminan Sosial) insurance so the selection method of delivery was still associated with financial problems. It would require further research using more samples and conducted also in secondary and tertiary health centers where it can get more research subjects who use another financing beside BPJS so the selection method of delivery does not depend on financial terms.[6]

CONCLUSION AND SUGGESTIONS

From this study, it can be concluded that there is a difference in knowledge score about pelvic floor dysfunction after counseling, where the score is better. There are 2 out of 102 subjects (1.9%) that change their choice of method of delivery from before to after counseling.

Based on these findings, further study with better method of research and larger population which covered more research location other than primary healthcare facilities need to be done so more comprehensive research data with more variative population can be acquired. Furthermore,

standardized education/counseling guidelines about pelvic floor dysfunction need to be written to determine knowledge in larger population.

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Research Article

Vascular Endothelial Growth Factor-C Serum and Endostatin Serum as Predictors of Lympho Vascular Invasion in Early Stage Cervical Cancer

Hubungan Kadar Serum Vaskular Endothelial Growth Factor-C (Serum VEGF-C) dan Serum Endostatin dengan Invasi Limfo-Vaskuler pada Pasien Kanker Serviks Uteri Stadium Awal

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Abstract

Objective: To determine the relationship of Vascular Endothelial Growth Factors-C (VEGF-C), endostatin and the ratio of VEGF-C/ endostatin with limfo vascular invasion in patient with early stage cervical cancer.

Methods: This study used a cross sectional method. Samples were all patients with early stage cervical cancer who came to the several teaching hospitals of Obstetrics and Gynecology Department Universitas Hasanuddin Medical Faculty that meet the criteria, then we measured the levels of VEGF-C and endostatin.

Results: We get 30 women with cervical carcinoma. The results showed that the serum levels of VEGF-C was higher in limfo vascular invasion positive (p = 0.017); the ratio of VEGF-C/ endostatin higher in limfo vascular invasion positive (p = 0.004); whereas serum levels of endostatin did not differ significantly in limfo vascular invasion positive or negative (p = 0.522).

Conclusion: The level of VEGF-C and VEGF-C/ endostatin ratio was higher in patients with early stage cervical cancer with positive LVSI than negative LVSI.

[Indones J Obstet Gynecol 2017; 5-2: 105-109]

Keywords: cervix uteri cancer, endostatin, limfo vascular invasion, VEGF-C

Abstrak

***Tujuan**: Untuk mengetahui hubungan Vaskular Endothelial Growth Factors-C (VEGF-C) dan Endostatin serta rasio VEGF-C/ endostatin dengan invasi limfo vaskuler pada pasien kanker serviks uteri stadium awal.*

***Metode**: Penelitian ini menggunakan metode cross sectional. Sampel penelitian adalah semua penderita kanker serviks uteri stadium awal yang datang ke Bagian Obstetrik dan Ginekologi Rumah Sakit Umum Pendidikan Universitas Hasanuddin dan jejaringnya yang memenuhi kriteria inklus yang kemudian dilakukan pengukuran kadar VEGF-C dan Endostatin.*

***Hasil**: Kami mendapatkan 30 perempuan penderita karsinoma serviks. Hasil penelitian menunjukkan bahwa kadar serum VEGF-C lebih tinggi pada invasi limfo-vaskuler positif (p = 0,017); rasio VEGF-C/ Endostatin lebih tinggi pada invasi limfo-vaskuler positif (p = 0,004); sedangkan kadar serum endostatin tidak berbeda secara bermakna pada invasi limfo-vaskuler positif ataupun negatif (p = 0,522).*

***Kesimpulan**: Kadar VEGF-C dan rasio VEGF-C/Endostatin lebih tinggi pada pasien kanker serviks uteri stadium awal dengan LVSI positif dibandingkan dengan LVSI negatif.*

[Maj Obstet Ginekol Indones 2017; 5-2: 105-109]

***Kata kunci**: endostatin, invasi limfo-vaskuler, kanker serviks uteri, VEGF-C*

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INTRODUCTION

Cervical cancer is the second type of cancer in women in the world and most cancers occur in women in developing countries. Lymph node metastasis in pelvic, size of tumor, depth invasion of the stroma, the invasion of parametrial and emboli limfo vascular will affect the recurrence and distant metastasis as well as a prognostic factor in early stage cervical cancer.[1,2]

Radical hysterectomy with pelvic lymphadenectomy was performed for cervical cancer stage IB and IIA. 5-years cure rate in patients without lymph node metastasis is 70-90% and is decreased to 40-60% in patients with lymph node metastasis. There was a reported in early-stage cervical cancer patients who undergo radical histerectomy and bilateral pelvic lymphadenectomy is detected as many as 83% of the total invasion limfo vascular, 77% are stage IB and from that stage, there are

21% got lymph node metastasis. Women with positive pelvic lymph nodes have recurrence risk and should be treated aggressively.[1,3]

Tumor growth, neovascularization and metastasis depend on the ability of cancer cells to invade the tissue that consist extracellular matrix degradation and basal membrane structure. Tumor invasion and metastasis are also crucial step in determining the aggressiveness of the cancer and the cause of death due to cancer. Molecular understanding that leads to metastasis as well as the complex interaction between the host cell metastasis, important in deciding more effective cancer therapy. Family Vascular endothelial growth factor (VEGF) VEGF-A, B, C, D, E is particularly important because it causes angiogenesis and limfangiogenesis which will further lead to tumor growth and metastasis of cancer.[4-6]

Several studies have been carried out by comparing the VEGF with endostatin in several human cancers, Shaarawy et al (2001) conducted a study of postmenopausal women: 72 endometrial cancers, 27 endometrial hyperplasia and 30 healthy female controls and compare their levels of VEGF and endostatin at each in each case. It was found that the serum levels of VEGF in endometrial hyperplasia (142 ± 18 ng/ml) and endometrial cancer stage I (291 ± 22 ng/ml), II (623 ± 68 ng/ml) and stage III- IV (1527 ± 119 ng/ml) was significantly higher than the average of the controls (12 ± 1.6 ng/ml). Serum levels of endostatin on endometrial hyperplasia (149 ± 19 ng/ml), endometrial cancer stage I (320 ± 41 ng/ml), II (644 ± 86 ng/ml) and stage III-IV (1253 ± 114 ng/ml) also significantly higher than the average of the controls (13 ± 2.4 ng/ml). Elevated VEGF values above the normal levels achieved in nonmalignant state by 7% (stage I), 37% (stage II) and 100% (stage III-IV) of endometrial cancer. While endostatin was respectively 37%, 59% and 100%.[7-9]

These results suggested that both of the biomarker levels in the circulation were associated with staging of the tumor. Serum levels of VEGF and endostatin were significantly decreased after treatment, but will rise again if there is a relapse. The ratio of VEGF - endostatin was higher in advanced stage compared with early stage endometrial cancer. This shows that the balance between angiogenic stimulators with angiogenic inhibitor can affect metastasis and tumor progression.[7,10]

The objective of this study is to determine the relationship of VEGF-C, endostatin, ratio VEGF- C/ endostatin with limfo vascular invasion in patients with early-stage cervical cancer.

METHODS

The study method was cross sectional. This study was conducted in several teaching hospitals of Obstetrics and Gynecology department Universitas Hasanuddin Faculty of Medicine in Makassar from February 2014 until February 2015. The population were women with early stage of cervical uteri cancer who examined in several teaching hospitals of Obstetrics and Gynecology department Universitas Hasanuddin Medical Faculty in Makassar. Samples who met the criteria and approved the informed consent. Data processing with SPSS. Data analysis use T independent test and Mann Whitney test. Hypothesis testing determined significant if $\alpha<0.05$.

RESULTS

There were 30 samples who met inclusion criteria. In this study, the results of distribution characteristics shown in Table 1.

Table 1. Characteristics Comparison Distribution of the Study Sample

Characteristic	Sample (n=30)	Percentage (%=100)
Age		
< 45 years old	13	43.33
45-55 years old	14	46.67
> 55 years old	3	10.00
Education		
Elementary school	7	23.33
Middle school	11	36.67
High school	10	33.33
University	2	6.67
Occupation		
Housewife	28	93.33
Enterpreneur	2	6.67
Age when first married		
≥ 16 years old	27	90
< 16 years old	3	10

Characteristic	Sample (n=30)	Percentage (%=100)
Parity		
Primipara	2	6.67
Multipara	28	93.33
Frequence of married		
Once	28	93.33
Twice	2	6.67
Contraception use		
DMPA	7	23.33
IUD	1	3.33
Pill	2	6.67
Pill & DMPA	3	10.00
None	17	56.67

Table 2 shows the characteristics of biological distribution and we obtained sample highest LVSI positives and negatives in stage IB 2 each 6 cases (40%), from that sample the most histopathologic type are invasive squamous cell carcinoma 12 cases (80%) and 7 cases (46.67%), respectively. Middle differentiation was the most in LVSI positive or negative.

Table 3 demonstrates the comparison level of VEGF-C, endostatin and ratio VEGF-C/ endostatin. VEGF-C Serum higher in LVSI positive with mean 12720.40 ± 2593.13 that LVSI negative. Serum levels of endostatin higher in LVSI positive with mean value 178.20 ± 33.99, and ratio VEGF-C/ endostatin higher in LVSI positive that LVSI negative with a mean value 71.39 ± 6.16 and P = 0.04.

Table 2. Characteristics of Biological Distribution Study Sample

Biological Characteristic	Sample (n=30)		Persentage	
	LVSI negative (n=15)	LVSI negative (n=15)	LVSI positive (%=100)	LVSI positive (%=100)
Clinical stage				
IB 1	4	6	26.67	40
IB 2	6	6	40	40
II A	5	3	33.33	40
Histopathological diagnosis				
Servical adenocarcinoma	3	6	20	40
Squamous cell carcinoma				
Large cell non keratinizing type	12	7	80	46.67
Large cell keratinizing type	0	2	0	13.33
Differentiation degree				
Well defined	1	3	6.67	20
Moderate	9	9	60	60
Poor defined	5	3	33.33	20

Table 3. Comparison Level of VEGF-C, Endostatin, and Ratio VEGF-C/ Endostatin

Variable	LVSI		p value
	Positive (mean ± SD)	Negative (mean ± SD)	
Level of VEGF- C	12720.40 ± 2593.13	10704.46 ± 16909.86	0.17
Level of endostatin	178.20 ± 33.99	170.46 ± 31.24	0.552
Ratio VEGF- C/ endostatin	71.39 ± 6.16	63.58 ± 7.50	0.04

DISCUSSION

In some other studies showed similar results that cervical cancer frequently found at the age of 45-55 years old, studies by Turah, Hasan and Perfitri. In the United States, the mean age about 52.2 years. Based on study by Aziz MF with gynecologic cancer data in Indonesia on 2009, the peak age of cervical cancer incidence is found in the age range 45-54 years old.

The results showed the sample is usually first married at age ≥ 16 years old. One risk factor for cervical carcinoma is sexual intercourse at an early age. The result similar with study by MF Aziz that suggested that the first sexual intercourse at age < 20 years old had higher risk of developing cervical cancer than doing first sexual intercourse at the age of > 20 years old.

Samples were generally educated middle and high school as well as the daily activities as housewife. While the sample who graduated from college were only two people. This result is accordance with a study conducted by MF Aziz that showed that were less educated had higher risk those who were well educated and those who compared to did not work have higher risk of developing cervical cancer compared to those who work.

In terms of parity, frequency of marriage and contraception usage showed that almost the entire sample were multiparas and frequency of marriage was once and samples generally did not use contraception. This is according to study by Aziz MF which showed that multiparous women with the number of children ≥ 6 have higher risk than primiparous. In addition, it also concluded that who were not taking birth control pills have higher risk than those taking birth control pills.

In this study, the most histopathological type samples were squamous cell carcinoma, large cell non keratinize type. Based on study conducted by AP Vizcaino, around two thirds of cervical cancer types were squamous cell carcinoma and approximately 15% were adenocarcinoma.

The study showed that the level of VEGF-C serum was higher in the LVSI positive (12720.40 ± 2593.13) than LVSI negative (10704.46 ± 1609.86) with p = 0.17. VEGF-C works well on blood vessels and lymphatic vessels and play an important role in the process of angiogenesis, and metastasis limfangiogenesis. Study conducted by Tjandra on 47 patients with early stage cervical cancer found that stage results, the size of lesions > 40 mm, degree of differentiation, limits of the vaginal incision, limfo vascular invasion, levels of VEGF-C > 10066.90 were lymph node metastasis risk factor and they could be used as predictors. Lesions size of > 40 mm, differentiation, parametrial invasion, levels of VEGF-C > 10066.90 were limfovascular invasion risk factor. VEGF-C levels > 10066.90 is likely have 80 times risk get lymphnode metastasis compared to VEGF-C ≤ 10066.90 (p < 0.001) and 12.5 times get limfo vascular invasion (p = 0.022).

Mitsuhashi A in his study concluded that the levels of VEGF-C serum were signs of biomolecular potential for cervical cancer squamous cell type. Yu H did study in 2007-2009 in Qi Lu Hospital, Shandong University on 97 patients with cervical cancer stage Ia-IIa, which 30 of them had positive lymphnode metastasis with the result of VEGF-C, VEGF-D, FLT-4 relating to limfangiogenesis. Similarly, in study by Wang et al on 89 patients (22 cervicitis chronic, 24 CIN, 43 squamous cell carcinomas) with a result higher expression of VEGF-C in tissue, the higher levels in serum (p = 0.024), VEGF-C in serum and tissues associated with clinical stage, tumor size, lymphnode metastasis and not related to the degree of differentiation.

We could see that the levels of endostatin on the LVSI positive (178.20 ± 33.99) was higher than LVSI negative (170.46 ± 31.24), but this result was not significant because the p-value was 0.552. Tjandra found that endostatin levels ≥ 184.5 ng/ml was a protection factor of the invasion limfo vascular but in statistics are also not significant (p = 0.562). Endostatin is an angiogenesis inhibitor. It is produced by our body as a reaction of the malignant tumor through the degradation of the basement membrane. There were elevated levels of endostatin in circulation (with over gene expression in endothelial) less than 2 times can suppress tumor growth by 2- 3 fold. It would require further research on the relationship endostatin with limfo vascular invasion with a larger number of samples.

CONCLUSIONS AND SUGGESTIONS

Levels of VEGF-C and ratio VEGF-C/ endostatin are higher in patients with early-stage cervical cancer

with LVSI positive than negative. Further research on angiogenesis inhibitor and endostatin in cervical cancer with a larger number of samples is required.

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Research Article

Evaluation of the Accuracy of Human Kallikrein-6, Cancer Antigen-125, and Human Epididymis - 4 in Predicting Ovarian Cancer

Evaluasi Akurasi Human Kallikrein-6, Cancer Antigen-125, dan Human Epididymis-4 dalam Memprediksi Kanker Ovarium

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Abstract

Objective: To evaluate the accuracy of hK6, HE4, and CA125 in predicting the malignancy of ovarian mass.

Methods: The design of this study was cross-sectional. This study was conducted in the Obstetrics and Gynecology Clinic, Sanglah Hospital, Denpasar, between the period of September 2014 and August 2016. Samples were all patients with ovarian tumors who underwent surgery at Sanglah Hospital, Denpasar. Data analysis was performed using McNemar and chi square test in SPSS for windows version 17.0.

Results: 22 samples were obtained. P > 0.05 value of age and parity variables indicated no differences between the two groups. There is no accuracy differences (sensitivity, specificity, positive predictive value, negative predictive value) of hK6 compared to histopathology examination in diagnosing ovarian cancer (p = 1). There is no accuracy differences (sensitivity, specificity, positive predictive value, negative predictive value) of HE4 compared to histopathology examination in diagnosing ovarian cancer (p = 1). There is no accuracy difference (sensitivity, specificity, positive predictive value, negative predictive value) of CA125 compared to histopathology examination in diagnosing ovarian cancer (p = 0.687).

Conclusion: There was no accuracy differences (sensitivity, specificity, positive predictive value, negative predictive value) found between hK6, CA125, HE4 compared to histopathology examination in predicting ovarian cancer.

[Indones J Obstet Gynecol 2017; 5-2: 110-113]

Keywords: cancer antigen 125, human epididymis-4, human kallikrein 6, ovarian cancer

Abstrak

***Tujuan**: Untuk mengetahui akurasi hK6, CA125 dan HE4 dalam memprediksi keganasan ovarium pada massa ovarium.*

***Metode**: Rancangan penelitian ini adalah uji diagnostik (cross sectional) yang dilaksanakan di Poliklinik Kebidanan dan Kandungan RSUP Sanglah, Denpasar. Sampel penelitian ini adalah semua penderita dengan tumor ovarium yang datang ke Poliklinik Kebidanan dan Kandungan RSUP Sanglah dan menjalani operasi di RSUP Sanglah, Denpasar. Pengambilan sampel dilakukan dengan cara consecutive sampling mulai September 2014 sampai Agustus 2016. Analisis data memakai uji Chi Square dan McNemar dengan bantuan SPSS for windows 17.0 version.*

***Hasil**: Didapatkan sebanyak 22 sampel penelitian variabel usia dan paritas didapatkan nilai p > 0,05, yang menyatakan bahwa tidak adanya perbedaan antara kedua kelompok. Tidak ada perbedaan kurasi hK6 (sensitivitas, spesivisitas, nilai prediksi positif, nilai prediksi negatif) dibandingkan dengan hasil pemeriksaan histopatologi dalam mendiagnosis kanker ovarium (p=1). Tidak ada perbedaan kurasi HE4 (sensitivitas, spesivisitas, nilai prediksi positif, nilai prediksi negatif) dibandingkan dengan hasil pemeriksaan histopatologi dalam mendiagnosis kanker ovarium (p=1). Tidak ada perbedaan kurasi CA125 (sensitivitas, spesifisitas, nilai prediksi positif, nilai prediksi negatif) dibandingkan dengan hasil pemeriksaan histopatologi dalam mendiagnosis kanker ovarium (p=0,687).*

***Kesimpulan**: Tidak ada perbedaan kurasi antara hK6, CA125, HE4 (sensitivitas, spesifisitas, nilai prediksi positif, nilai prediksi negatif) dibandingkan dengan hasil pemeriksaan histopatologi dalam memprediksi kanker ovarium.*

[Maj Obstet Ginekol Indones 2017; 5-2: 110-113]

***Kata kunci**: cancer antigen 125, human epididymis-4, human kallikrein 6, kanker ovarium*

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INTRODUCTION

Ovarian cancer is a major burden in the field of gynecology oncology, due to high rate of mortality resulted from this cancer.[1] Increasing ratio of morbidity and mortality in ovarian cancer patients is due to progression of disease that shows no symptoms found until metastasis. 70% of women with ovarian cancer are diagnosed at advanced stage. The five year survival rate of ovarian cancer is 85% when diagnosed at early stage (stage I and II), but may decrease to less than 20% if diagnosed at advanced stage (stage III or IV).[2]

Serum of CA125 tumor marker to predict the presence of malignancy in patients with ovarian mass has lower sensitivity and specificity in pre and postmenopausal women.[3] Several studies conducted to diagnose ovarian cancer in patients with ovarian mass using tumor marker HE4 and combination of HE4 and CA125 have shown that HE4 has higher sensitivity and specificity compared to CA125.[4]

Kallikrein 6 gene is a trypsin-like serine protease of human gene, family kallikrein that has great potential to be developed as a tool for early detection for ovarian cancer and various preliminary research have been conducted to support towards it and result of the research can be used as rationale that hK6 can be used as a medium or tool for early detection of ovarian cancer.[5]

Based on explanation elaborated above, assessment of the correlation or relationship between hK6 with ovarian cancer will be performed. This study is expected to be a reference or additional consideration to support usage hK6 as the early detection of ovarian cancer diagnostic.

METHOD

We used cross-sectional study design. This study was conducted at the Obstetrics and Gynecology Clinic, Sanglah Hospital, Denpasar, during the period between September 2014 and August 2016. The subjects were all patients with ovarian tumors who came to Obstetrics Clinic of Sanglah Hospital and underwent surgery in Sanglah Hospital, Denpasar. Data analysis was performed using SPSS for Windows version 17.0.

RESULT

In this study, T-independent test was conducted toward age and parity variable between the two groups. As seen in Table 1, p value> 0.05 of age and parity was obtained, indicated no differences between both groups.

To determine diagnostic test of hK6 toward histopathology in the diagnosis of ovarian cancer, it was analyzed using Chi-Square test. The results of the analysis are presented in the following table.

Table above with 2x2 cross table, showed 80.0% sensitivity, 75.0% specificity, 72.7% positive predictive value, 81.8% negative predictive value, 27.3% false positive, 18.2% false negative values, and 77.3% accuracy. McNemar test showed no accuracy differences of hK6 (sensitivity, specificity, positive predictive value, negative predictive value) compared to histopathology examination in diagnosing ovarian cancer (p = 1.00)

To determine diagnostic test of HE4 compared to histopathology in diagnosis of ovarian cancer, Chi-Square test analysis was conducted. Results are presented in Table 3.

Table 1. General and Parity Characteristics Comparison between both Groups

Risk factor	Malignancy group (n=10)		Benign tumor group (n=12)		p
	Mean	DS	Mean	DS	
Age (year)	52.80	16.72	50.50	14.94	0.737
Parity	2.10	1.10	2.08	1.88	0.981

Table 2. Diagnostic Test of hK6 toward Histopathology in Diagnosing Ovarian Cancer

		Ovarian cancer		Total
		Malignant	Benign	
hK6	High	8	3	11
	Low	2	9	11
Total		10	12	22

Table 3. Diagnostic Test of HE4 toward Histopathology in Diagnosing Ovarian Cancer

		Ovarian cancer		Total
		Malignant	Benign	
HE4	High	7	2	9
	Low	3	10	13
Total		10	12	22

Table 4. Diagnostic Test of CA125 toward Histopathology in Diagnosing Ovarian Cancer

		Ovarian cancer		Total
		Malignant	Benign	
CA125	High	6	2	8
	Low	4	10	14
Total		10	12	22

Table above with a 2x2 cross table showed 70.0% sensitivity, 83.3% specificity, 77.8% positive predictive value, 76.9% negative predictive value, 22.2% false positive, 23.1% false negative values, and 77.3% accuracy. McNemar test showed no differences of accuracy (sensitivity, specificity, positive predictive value, negative predictive value) HE4 compared to histopathology examination in diagnosing ovarian cancer (p = 1.00).

To determine diagnostic test of CA125 compared to histopathology in diagnosis of ovarian cancer, Chi-Square test analysis was conducted. The results of analysis is presented in Table 4.

Table above with a 2x2 cross table showed 60.0% sensitivity, 83.3% specificity, 75.0% positive predictive value, 71.4%, negative predictive value, 25.0%, false positive, 28.6%, false negative values, and 72.7% accuracy. McNemar test showed no accuracy differences (sensitivity, specificity, positive predictive value, negative predictive value) of HE4 compared to histopathology examination in diagnosing ovarian cancer (p = 1.00).

DISCUSSION

Result of the research revealed p value > 0.05 of age and parity variable, suggesting no differences between the two groups.

Diagnostic test of HK6 toward histopathology in diagnosis of ovarian cancer revealed sensitivity, specificity, positive predictive value, negative predictive value, false positive value, negative value, and accuracy of 80%, 75%, 72.7%, 81.8%, 27.3%, 18.2%, and 77.3%, respectively. McNemar test showed p-value of diagnostic tests of hK6 toward histopathology examination in diagnosing ovarian cancer is p = 1.00. It shows that no accuracy differences (sensitivity, specificity, positive predictive value, negative predictive value) of hK6 compared to histopathology examination in diagnosing ovarian cancer. This can be explained that, in ovarian cancer, the increment of hK5, hK6, hK8, hK10, hK11 and hK14 in serum make kallikrein become a potential biomarker. Several studies on the association of hK6 with ovarian cancer showed that among many types of cancer, only in ovarian cancer, hK6 levels in circulation showed remarkable increase.[6]

Diagnostic test of HE4 toward histopathology showed 70.0% sensitivity, 83.3% specificity, 77.8% positive predictive value, 76.9% negative predictive value, 22.2% false positive, 23.1% false negative values, and 77.3% accuracy. McNemar test showed no differences of accuracy (sensitivity, specificity, positive predictive value, negative predictive value) HE4 compared to histopathology examination in diagnosing ovarian cancer (p = 1.00). This result is supported by another research conducted Wang et al which examined HE4 level in the differential diagnosis of pelvic mass in the population of Chinese women. The

study demonstrated that the sensitivity and specificity of HE4 were 86.7% and 98.0%, respectively.[7]

Diagnostic test of CA125 toward histopathology showed 70.0% sensitivity, 83.3% specificity, 77.8% positive predictive value, 76.9% negative predictive value, 22.2% false positive, 23.1% false negative values, and 77.3% accuracy. McNemar test showed no differences of accuracy (sensitivity, specificity, positive predictive value, negative predictive value) CA125 compared to histopathology examination in diagnosing ovarian cancer ($p = 1.00$). In initial report, it is known that level of CA125 increased by about 80% in women with advanced ovarian cancer and only 1-2% in the normal population. While in stage I ovarian cancer, CA125 level increased less than 50%. Specificity of CA125 is also low in differentiating between benign and malignant cases. In a retrospective study of 9233 women, sensitivity 62% of CA125 was obtained.[8]

CONCLUSION

There were no accuracy differences (sensitivity, specificity, positive predictive value, negative predictive value) between hK6, CA125, HE4 compared to histopathology examination in diagnosing ovarian cancer. Each of hK6, CA125 and HE4 value can be used as an ovarian cancer biomarker.

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Research Article

Human Papilloma Virus Self-testing as an Alternative Method for Cervical Cancer Screening

Uji Pemeriksaan Mandiri Human Papilloma Virus sebagai Metode Alternatif untuk Skrining Kanker Serviks

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Abstract

Objective: To determine the level of acceptance of self Human Papilloma Virus (HPV) examination and to compare the level of sensitivity and specificity of self HPV examination with Fluid Based Cytology on precancerous cervical lesion and cervical cancer.

Methods: The analytical method used in this research was cross sectional with purposive sampling. This study is conducted at Wahidin Sudirohusodo Hospital and its affiliation from October 2014 to May 2015 with 101 subjects. The data measurement used self HPV examination tool and fluid based cytology kit. The data collected through questionnaires before and after the examination. The laboratory examination was carried out using the way of HPV Genotyping primary system (MY09/11) method Wl LTS-06, which is able to detect 35 types of HPV viruses.

Results: A total of 101 subjects were involved in this study. The subjects were divided into two groups: the abnormal and normal group (n - 50/51). The acceptance level of self HPV examination was 62.37% (n = 63/101). Majority of subjects (88.21%) were ready to repeat the same examination in the future. The level of sensitivity and specificity for self HPV were (56% (95%CI (41.25 - 70.01%)) vs 98% (95%CI (89.55 - 99.95%)), p=<0.000001, while the level of sensitivity and specificity for fluid based cytology were (40% (95%CI (26.41 - 54.82%)) vs 98% (95%CI (89.35 - 99.95%)), p=0.000002.

Conclusion: HPV self-testing is very potential to be used as an alternative method for cervical cancer screening.

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Keywords: cervical cancer, cervical cancer screening, human papilloma virus, sensitivity, specificity

Abstrak

***Tujuan**: Mengetahui tingkat penerimaan pemeriksaan HPV Mandiri dan membandingkan tingkat sensitivitas dan spesifisitas pemeriksaan mandiri HPV dengan sitologi berbasis cairan, pada lesi pre kanker derajat tinggi dan kanker serviks.*

***Metode**: Penelitian merupakan analitik cross sectional secara purposive sampling, single center di RS Wahidin Sudirohusodo dan Afiliasinya pada Bulan Oktober 2014-Mei 2015 berjumlah 101 responden. Pengukuran data menggunakan alat pemeriksaan HPV mandiri serta kit sitologi berbasis cairan. Pengumpulan data menggunakan kuesioner sebelum dan setelah melakukan pemeriksaan. Pemeriksaan laboratorium dilakukan dengan menggunakan cara HPV Genotyping sistem primer (MY09/ll) metode Wl LTS-06, mampu mendeteksi 35 tipe virus HPV.*

***Hasil**: Keseluruhan responden berjumlah 101 orang, terbagi menjadi kelompok abnormal/normal (n=50/51). Tingkat penerimaan pemeriksaan HPV mandiri sebesar 62,37% (n=63/101). Sebagian besar responden (88,21%) bersedia mengulangi pemeriksaan serupa di masa mendatang. Tingkat sensitivitas dan spesifisitas yang didapatkan untuk HPV Mandiri (56% (95%CI (41,26 - 70,01%)) vs 98% (95%CI (89,55 - 99,95%)), p=<0,000001, sedangkan tingkat sensitivitas dan spesifisitas untuk sitologi berbasis cairan (40% (95%CI (26,41 - 54,82%)) vs 98% (95%CI (89,35 - 99,95%)), p=0,000002.*

***Kesimpulan**: Pemeriksaan HPV mandiri ini sangat potensial untuk dijadikan metode pemeriksaan alternatif untuk pemeriksaan skrining kanker serviks.*

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***Kata kunci**: human papilloma virus, kanker serviks, sensitivitas, skrining kanker serviks, spesifisitas*

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INTRODUCTION

Cervical cancer is the third most common cancer in the world. It is the only cancer that can be identified early thus making it able to be prevented. Cervical cancer is the fourth cause of death due to cancer in the world. In 2008, it estimated that 530,232 women were diagnosed with cervical cancer worldwide and 275,000 of them died.[1] The majority of cervical cancer (85%) occurred in developing countries, including Indonesia. The prevalence of women with cervical cancer in Indonesia is fairly high; 40-45 new cases were found daily with number of deaths reached 20-25 people while women at risk of cervical cancer was 48 millions.[2] Data from health department demonstrated that regions with the highest

number of cervical and breast cancer were Makassar, district of Gowa, Wajo, Bone, and North Luwu. In 2009, it was found that 97 cervical cancer cases was in hospital and 177 cases was in primary health care.[3]

Human Papilloma Virus (HPV) infection is a significant event for the occurrence of cervical cancer. It is estimated that 50-80% of sexually active women will be infected by HPV in their life and approximately 80% will be infection-free in 2 years and will not cause cancer. Persistent HPV infection is one of the predispositions of dysplasia and cervical cancer. The course of HPV infection developing into cervical cancer may take up to 10-20 years. HPV infection process which later becomes precancerous is mostly asymptomatic.[4-6]

Cytology examination has become cervical cancer screening standard for more than 50 years. This examination evaluates cell morfology abnormalities from cervical epithelial specimen.[7] This examination often results in false negative due to inadequate sample and poor procedure standard. Liquid-based examination may improve this disadvantage. The high false negative result of this examination leads to reevaluation in the interval period of time.[8-10]

HPV DNA examination has the advantage of very high negative predictive value, even towards adenocarcinoma precursor.[11] Human papilloma virus has a high sensitivity in detecting high degree precancer lesion and has high positive predictive value.[12] The effectiveness of this cervical cancer screening programme may be increased by the use of HPV self examination. According to a study in Netherlands, an HPV self examination tool, Evalyn brush, has a sensitivity and specificity of 81.5% and 66.4%, respectively.[13] Efforts on early detection in high risk women are organized into a screening programme or an opportunistic screening. Management and early detection of precancer lesions are 'see and treat' programme and histopathology-based-diagnosis.

A good screening examination should be acurate, highly reproducible, cheap, easy to use, easy to monitor, highly accepted, and safe.[14]

Examination to be used should have been through long period of evaluation and tested in real life. Clinical application of the proposed HPV DNA examination may be as single primary screening instrument or combination with cytology and monitoring women with precancer lesion who has received treatment in order to predict the success of therapy.

High sensitivity means that HPV examination also has high negative predictive value. Negative result of examination may prolong the need to repeat the cervical cancer screening up to 5-8 years.[13] Cytologic examination is often constrained by the lack of infrastructure, particularly in developing countries. Alliance for cervical cancer prevention seeks for alternatives other than cytologic examination, such as acetic acid visual inspection and HPV DNA test. According to a study conducted by Qiao et al, HPV DNA examination and liquid based cytology had higher sensitivities than acetic acid visual inspection.[12]

Self examination of vaginal or cervical specimen sampling has developed in recent years. Self examination derived from vaginal specimen has the inability of the specimen for cytologic examination. However, vaginal specimens are very suitable for HPV examination sample because the result do not significantly differ from those of liquid based cytology or cervical specimen. In self examination, women would collected their own specimen sample using several tools including brush, tampon, and vagina rinse instrument. Some research showed that self vaginal specimen sampling was sufficient for laboratory analysis, both delivered through liquid or dry media.[15]

The ability to detect high risk HPV from self HPV examination or liquid based cytology examination shows no significant differences. This explains that there is no difference in the ability to detect HPV between self examination and examination by paramedics. One advantage of self examination is it does not need paramedics to be performed and ensure privacy. Based on user experiences, this examination is considered easy to use. As cervical cancer screening tool, this tool will improve the outreach of screening on women who has not or rarely checked. Most cervical cancer are found in women who never or rarely check.[16-18] We aimed to know the level of acceptance of HPV self examination and compare the sensitivity and specificity of HPV self examination and liquid based examination on high degree pre cancer lesion and cervical cancer.

METHODS

A cross sectional study design was used. This study was conducted at several teaching hospitals affiliated with the department of Obstetrics and Gynecology, Faculty of Medicine, Hasanuddin University, Makassar from October 2014 until May 2015.

The subjects were women who experienced spontaneous abortion and normal term delivery in several teaching hospitals of Obstetric and Gynecology Department Universitas Hasanuddin Faculty of Medicine in Makassar. Samples were obtained from blood samples of the mothers who met the inclusion criteria.

Method of collecting data

Samples were selected based on an assessment of researchers that met the eligible inclusion criteria with purposive sampling method between 2 group. Data were obtained by self HPV examination tool, liquid based cytology kit, and before and after examination questionnaire. Laboratory test was done by QI LTS-06 method of primary system (MY09/11) HPV Genotyping which was able to detect 35 types of HPV.

Data analysis

Data were analyzed using SPSS. Diagnostic 2x2 table test is used to determine the capability of each tool.

RESULTS

A total of 101 subjects were involved in this study. The subjects were divided into abnormal/normal group (n=50/51). The most age group percentage was the age group of 40-49 year, 40.58% (n=41/101). The respondent was mostly at the education level of high school/on the equal degree, 54.45% (n=50/101). Majority of the subjects were housewives, 73.26% (n=74/101). There were 23 subjects who work as private or civil employees. Most of the subjects was multiparous, 88.12% (n=89/101), did not use contraception, 57.42% (n=27/101). Majority of respondents complained of leucorrhoea, 45.54% (n=46/101), 56.5% was abnormal and 43.5% was normal. Majority of the normal population had no complain (n=24/ 51.47%) before the examination, whereas majority of the abnormal group complained of leucorrhoea (26/50.52%). Majority of the respondents did not know about cervical cancer screening, 79.2% (n=80/101). The major result of Pap smear test of the normal and abnormal group were folicular cervicitis [(n=34/51, 66.67%) and (23/50, 46%), respectively].

Acceptance rate of self HPV examination was 62.37% (n=63/101). As many as 37.62% (n=38/ 101) of the respondent suggested the examination was difficult/could not do the examination by herself because it was difficult to recognize or to insert the instrument (n=27/38, 71.05%). 92 subjects (92/101, 91.01%) did not find difficulties to perform the examination, 54.3% of which was abnormal and 45.7% was normal. However there was 5 subjects who reported pain, 1 subject reported bleeding, 2 subjects failed to used the tool. Of 89 subjects (n = 89/101), 88.12% would repeat HPV testing self-sampling and 12 subjects (n = 12/101) 11.8% would not repeat this test. From those who were willing to repeat the examination in the future because it was easy to use (n = 75/101) 84.23%.

Half of our subjects were highly educated. 64% stated that this tool were ease to use. 84 subjects did not know anything about cancer screening, 66.2% managed to use this tool easily.

The sensitivity and specificity were obtained for HPV Self sample collection test is 56% (95% CI (41.25 to 70.01%)) vs 98% (95% CI (89.55 to 99.95%)), with positive predictive value of 68, 25%, 95% CI (22.75 to 96.43%); 96.73% negative predictive value, 95% CI (20.9 to 99.28%); Accuracy of 0.78, 95% CI (0.68 to 0.85); Compliance Test Kappa 0.543, 95% CI (0.365 to 0.72); p <0.000001.

While the sensitivity and specificity for liquid-based cytology is 40% (95% CI (26.41 to 54.82%)) vs 98% (95% CI (89.35 to 99.95%)); Positive predictive value of 60%, 95% CI (13.54 to 93.35%,; 95.6% negative predictive value, 95% CI (89.32 to 98.74%); Accuracy 0.69, 95% CI (0.59 to 0.78); test the suitability of Kappa = 0.38, 95% CI (0.22 to 0.54); p = 0.000002.

The results of diagnostic test liquid-based cytology (LBC) in cervical cancer and precancerous lesions of the 16 samples were defined as abnormal by 76.19% who tested positive on HPV testing independently (HPV-SSC) and 23.8% expressed negative. While 79 normal results showed that

83.54% had negative on HPV testing independently (HPV-SSC) and 16.45% tested positive. The results of the suitability test results of both tests Kappa value = 0.524 95% CI (0.28 to .768).

All HPV virus detected in the abnormal group were the high-risk HPV types, such as type 16, 18, 31, 33, 45, 51, 53. In the normal group, we obtained one sample who was detected as low risk HPV type.

DISCUSSION

This study evaluated the use of HPV self sample collection test in Makassar as a new diagnostic tool. Our evaluation was done by comparing the histopathology, thus we may determine the ability of each new diagnostic screening tool. The Acceptance rate was fairly, good (62.37%). This finding is consistent with several studies (73%[17]; 87%[18]; 77.1%)[19].

Approximately 91.01% of the subjects did not have difficulties in doing the examination and 88.12% of the subjects were willing to repeat a similar examination in the future. Some parts of the tools fell apart and left in the vagina were the short comings of the tool integrity and expected to have an impact on the design and improvement of educational plan using the tool. Subjects who admitted to have difficulties to perform self-HPV testing said that there was no denial of the usage of these tools which we were considered suitable with our society culture or religion. HPV testing self-denial rate was found relatively high in all age groups (30 to 58.8%), and employment (25 to 41.9%). This result is understandable because HPV testing is a new independent examination. We may increase the acceptance rate by considering on education and make this as a routine examination.

Cytology has become gold standard examination for cervical cancer screening more than last 50 years. This examination evaluates the presence of morphological abnormalities from epithelial cervical specimens. Conventional cytological examination has a sensitivity of 30-87% and specificity of 86-100% for detecting high-grade precancerous lesions. Smear results are often unsatisfactory with high false negative value. Later, liquid-based cytology was developed. Its sensitivity and specificity are 80% and 98%. The number of samples found unsatisfactory on LBC decreased 11.45%.[20] Several studies found the level of sensitivity of liquid-based cytology 13% higher than the conventional Pap smear test. These results suggested that liquid-based cytology had a higher level of sensitivity and specificity levels lower than Pap smear test.[20] On the use of routine screening cytological examination only have a level of sensitivity ranged from 47-62% and a specificity ranged from 60-95%. A meta-analysis of the results revealed similar results to those obtained in this study, the rate reaches 97-100% specificity and sensitivity is only 29-56%.[21] However, these results still show a sensitivity level much lower than the results of research in general. The new technology for cervical cancer (NTCC) and Netherland Thin Prep versus Conventional Cytology (NETHCON) indicates that there is no difference in the detection capability CIN2 / 3 on both methods.[22]

The level of sensitivity of HPV DNA tests for the detection of CIN2 + was better than cytology (94% vs 65%).[21,23] In this study, the sensitivity of self-HPV testing is higher than liquid-based cytology with a difference of 16%, the specificity found similar on both tests. A study in China demonstrated that self-HPV testing had a sensitivity of 86.2% and a specificity of 80.7% while liquid-based cytology had lower sensitivity in detecting CIN2.[24] High risk HPV DNA can be identified 99.7% of cervical cancers and 95% of high-grade precancerous lesions.[25,26] The results of this study showed different results that only high risk HPV types identified (n = 16/32) 50% of cervical cancers and (n = 6/19) 31.57% of high-grade precancerous lesions. All types of HPV detected in the abnormal group were high risk HPV types. HPV DNA PCR method used in this study was GMP09 / 11. The use of PCR method has been shown to have higher sensitivity for detecting high-risk HPV compared to HC2. According to the results of one RCT, amplification method GPM09 / 11 had very low sensitivity level, which accounted for 49%.[26] Therefore, our decision to use similar method could potentially cause low levels of sensitivity.

The usage of certain fixation media and specimen processing of cells contained in the media might influence our results. This might be due to the lack of uniformity filtration process and the possibility of not drawing a specimen of cells in the remaining media.[20] Methods of sample collection for HPV testing can be done through swab, brush, tampon or lavage. The various methods mentioned

showed 78-81% sensitivity rate for the swab or brush, 67-94% for tampons, and <81% for lavage.[27] The collection method used in this study is brush, and it's likely affect the validity of the test. The prevalence of high-risk HPV 62.3% (95% CI: 53.7 to 70.2) was detected in the examination without media fixation and 56.2% (95% CI: 47.6 to 64.4) in the use of media fixation.[28] Sample collection without using fixation was likely to affect the results of our study.

Population and national study which evaluate the level of acceptance, economic impact, the accuracy and precision tools, awareness, adherence screening and suitability level of HPV examination results need to be conducted before using this HPV testing as a screening method in institutions.[27] This study has not been able to ascertain with certainty the validity of the examination, however, the examination is potential to be used as an alternative method of screening.

CONCLUSIONS

Cervical cancer has a long course of the disease, yet it can be prevented. Self-testing of HPV the latest potential modality for cervical cancer screening that corresponds to the cultural, economic, human resources and geographics in Indonesia.

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Research Article

Human Papilloma Virus L1 Gene Methylation as a Potential Biomarker for Precancerous Cervical Lesion: a Preliminary Report

Metilasi Gen L1 Human Papilloma Virus sebagai Biomarker Potensial untuk Lesi Serviks Prekanker: Sebuah Laporan Pendahuluan

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Abstract

Objective: To determine whether HPV L1 gene methylation can be used in triage of precancerous cervical lesions. The main objective is to determine the genotype of HPV in cervical precancerous lesions and to determine the percentage, the sensitivity, specificity, positive predictive value, negative predictive value, and likelihood ratio of DNA HPV L1 methylation in precancerous cervical lesions.

Methods: A number of 57 samples of paraffin blocks (FFPE) from precancerous lesions and cervical cancer biopsies in the Department of Pathology Faculty of Medicine-Cipto Mangunkusumo General Hospital that had been re-evaluated by the pathologist, underwent extraction of HPV DNA. The genotypes of HPV DNA were examined using primers GP5 / 6 and specific HPV 16, HPV 18 and HPV 52 probes and analyzed by real time PCR. Sequencing was performed on samples with unknown HPV DNA type that were detected using the specific probes to determine the type of HPV. Bisulfite conversion procedure was then performed for the samples that met the inclusion criteria.

Results: There were 30 samples (52.6%) with CIN 1, 12 samples (21.1%) CIN 2, 9 samples (15.8%) CIN 3 and 6 samples (10.5%) of cervical cancer. Most of the samples were 36-45 years (35.1%). Of the total 57 samples, 55 samples were successfully extracted and determined the DNA genotyping of HPV (96.5%). HPV 16 infections both in the form of single or multiple was found to be 76.36%. The samples were mostly dominated by co-infection of HPV16 and 18 (49.1%) followed by HPV 16 (24.6%) and HPV 18 (14.0%). Based on the sequencing results there were other types of high risk HPV infection found: HPV 33, HPV 35, HPV 58 and also undeterminate risk HPV 53 and low risk HPV 54. After several procedures of optimization for methylation examination of HPV DNA L1 there was thin band found in electrophoresis procedure in 8 of 42 samples (19%) of HPV 16 after bisulfite conversion but once it was purified there weren't any band found so we can not proceed to the stage sequencing. Until now we are still in the stage of optimizing the methylation procedure.

Conclusion: HPV 16 infection were most commonly found in the form of single or multiple. Co-infection of HPV 16 and 18 were found in the majority of the samples. There were no significant correlation between HPV type and the severity of cervical lesions. Until now, the examination of DNA methylation HPV L1 already obtained eight samples of HPV 16 with a thin band on electrophoresis but the result could not be concluded because it is still in the process of optimization.

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Keywords: HPV DNA genotype, L1 gene methylation, precancerous cervical lesions

Abstrak

***Tujuan**: Untuk mengetahui apakah metilasi HPV DNA dapat digunakan dalam triage lesi prakanker serviks. Tujuan khususnya adalah untuk mengetahui genotipe HPV pada lesi prakanker serviks dan untuk mengetahui persentase, sensitivitas, spesifisitas, nilai duga positif, nilai duga negatif, dan likelihood ratio metilasi HPV DNA L1 pada lesi prakanker serviks.*

***Metode**: Sejumlah 57 sampel paraffin block (FFPE) hasil biopsi lesi prakanker dan kanker serviks di Departemen Patologi Anatomi FKUI-RSCM dilakukan ekstraksi DNA HPV. Dilanjutkan dengan pemeriksaan genotipe DNA HPV menggunakan primer GP5/6 dan probe khusus HPV 16, HPV 18 dan HPV 52 yang kemudian dianalisis dengan real time PCR. Dilakukan sequencing pada sampel dengan DNA HPV yang tidak dikenal dengan probe spesifik untuk mengetahui tipe HPV. Selanjutnya dilakukan prosedur konversi bisulfit untuk sampel yang memenuhi kriteria inklusi.*

***Hasil**: Terdapat 30 sampel (52,6%) NIS 1, 12 sampel (21,1%) NIS 2, 9 sampel (15,8%) NIS 3 dan 6 sampel (10,5%) kanker serviks. Kelompok usia terbanyak adalah 36-45 tahun (35,1%). Dari total 57 sampel terdapat 55 sampel yang berhasil diekstraksi dan diperiksa genotyping DNA HPV (96,5%). Infeksi HPV 16 baik dalam bentuk tunggal maupun multipel ditemukan sebesar 76,36%. Genotipe HPV yang terbanyak ditemukan adalah ko-infeksi HPV tipe 16 & 18 (49,1%) diikuti dengan tipe 16 (24,6%) dan tipe 18 (14,0%). Berdasarkan hasil sequencing ditemukan infeksi high risk HPV 33, HPV 35, HPV 58, undeterminate risk HPV 53 dan low risk HPV 54 pada masing-masing 1 sampel. Setelah dilakukan beberapa prosedur tahap anoptimasi untuk pemeriksaan metilasi HPV DNA L1, didapatkan band yang tipis pada 8 dari 42 sampel (19%) HPV 16 hasil konversi bisulfit tetapi setelah dipurifikasi masih belum didapatkan adanya band pada proses elektro foresis sehingga belum dapat dilanjutkan ke tahap sequencing. Saat ini masih dilakukan proses optimasi untuk pemeriksaan metilasi HPV DNA L1.*

***Kesimpulan**: Genotipe DNA HPV yang paling banyak ditemukan baik dalam bentuk tunggal maupun multipel adalah HPV 16. Infeksi multipel (ko-infeksi) HPV 16 dan 18 ditemukan pada sebagian besar sampel penelitian. Tidak didapatkan hubungan bermakna antara tipe HPV dan derajat beratnya lesi serviks. Hingga saat ini pada pemeriksaan metilasi HPV DNA L1 sudah didapatkan 8 sampel HPV 16 dengan band yang tipis pada elektro foresis namun belum dapat disimpulkan karena masih dalam proses optimasi.*

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***Kata kunci**: genotipe DNA HPV, lesi prakanker serviks, metilasi gen L1*

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INTRODUCTION

Cervical cancer is still a burden for women around the world. It is the third most common cancer among, with an estimated 528,000 new cases and 35,673 deaths in 2012. Worldwide, cervical cancer mortality rates are substantially lower than the incidence of mortality. The incidence ratio was 50.3%. Most cases are squamous cell carcinoma, followed by adenocarcinoma.[1,2]

In Indonesia, cervical cancer ranks as the second most common female cancer. There are approximately 20,928 new cases of cervical cancer annually in Indonesia.[1,2] Cervical cancer is about 75% of gynecological cancer in Indonesia. It is mostly diagnosed at later stages.[3] Throughout 2005-2010 at Dr. Cipto Mangunkusumo National General Hospital (RSCM), there were 2,298 cervical cancer cases and 66.4% were diagnosed at an advanced stage, generally stadium IIIB.[4] In 2013, according to a report INASGO there were 823 new cases in RSCM.[5]

Human papilloma virus (HPV) which derived from the genus Alphapapillomavirus is the most frequent cause of sexually transmitted infection most frequently worldwide with a broad spectrum of benign and malignant neoplasms.[6] HPV is the cause of 99.7% cervical cancer.[7]

Cervical cancer, precancer lesion and non-neoplastic HPV infection such as *atypical cells of undetermined significance* (ASCUS) and cervical intraepithelial neoplasia (CIN) are diagnosed using cytology examination (Papanicolau test, Pap test), colposcopy and histology examination of tissue biopsy. Methods of screening with cytology (Pap smear) has sensitivity (51-86%) with a high false negative rate (15-45%).[8] Cytological examination can not predict which ones are at risk of becoming malignant, and which are not.

High Risk-HPV DNA detection has become a powerful criteria to improve the sensitivity of screening for cervical cancer. HR-HPV DNA test has a high sensitivity (88-98%) higher than Pap smear (51-86%), yet with lower specificity (83-94%) compared to Pap smear (92-99%).[11] However, since most women who are infected with HPV during their lifetime (> 80%) far exceeded the incidence of cervical cancer (1%) and because the value of HPV DNA testing positive more often indicate a transient infection rather than a development of cervical cancer, the diagnosis of HPV DNA alone is not enough to distinguish benign infections that require intensive treatment. Although more sensitive than cytology, specificity and positive predictive value (PPV) HR-HPV DNA were low compared with cytology. Combined Pap and HPV testing has a sensitivity and specificity values higher with a very high negative predictive value (99-100%) to strengthen the screening of cervical cancer.[8] But the positive predictive value for detection of precancerous lesions is still low and can not differentiate HPV infections will regress with who will experience progression to cancer.

Treatment for abnormal screening tests includes colposcopy vs close-surveillance. However, HPV DNA positive specificity is not sufficient to distinguish precancerous lesions associated with HPV (CIN2 +) and the transients are clinically a benign infection. Colposcopy referral rates were found to be higher after skrining.[9] An unneeded colposcopy examination, can lead to over diagnosis CIN (misclassification) and over treatment. Therefore, a diagnostic test that can differentiate between women who are infected with HR-HPV is transient with risk of progression is necessary.[10-12]

In preventing excessive procedures in patients with abnormal Pap smears who are not at risk for cervical cancer, tests that are sensitive and specific in detecting high-risk patients are required.[6,13-15]

HPV DNA methylation has emerged as a promising biomarker for diagnosis and prognosis of cervical cancer. Some studies show that women with HPV infection, methylation levels increased slowly with increasing duration of persistence of HPV and more increased with the diagnosis of cervical cancer. There is an increasing trend of HPV DNA methylation, especially in L1 and L2 genes in conjunction with the progressivity disease. DNA methylation changes the transcriptional regulation of HPV because of the repression of the expression of cellular genes and viruses may occur with the addition of a methyl group to the residue cytosin. The occurrence of HPV DNA methylation, especially in the L1 and L2 genes will prevent the transcription L1 and L2 with the result of no expression of L1 and L2 which are the components of HPV capsid. The absence of capsid protein L1, as antigen that can be fully accessible during the early stages of lesions intraepithelial squamous lead to circumstances in which the virus can evade the immune system thus the infection will be

retained causing dysplasia which is not recognized by the immune system, then it will proceed further on the transformation malignant.[6,13,14,16-19]

This opens up the possibility to predict which woman has the chance of disease progression into cervical cancer. Thus, HPV DNA methylation can be considered as a potential biomarker in the triage of patients with cervical cancer risk.

This study is conducted to determine whether HPV DNA methylation can be used in triage of cervical precancerous lesions or not. The main objective is to determine the genotype of HPV in cervical precancerous lesions and to determine the percentage, the sensitivity, specificity, positive predictive value, negative predictive value, and likelihood ratio of HPV L1 gene methylation in precancerous cervical lesions. By knowing the correlation of HPV L1 gene methylation in precancerous cervical lesions, it is expected that this examination can be a triage tool to help identify patients with HPV positive who would undergo regression and those at risk of progression into cervical cancer.

METHODS

A cross sectional study design was used to determine the genotype of HPV, assess the sensitivity, specificity, positive predictive value, negative predictive value, and the like hood ratio of HPV DNA L1 methylation in precancerous lesions of the cervix.

The study was conducted at the Division of Gynecologic Oncology, Department of Obstetrics and Gynecology, Faculty of Medicine, Universitas Indonesia - Dr. Cipto Mangunkusumo Hospital, the samples were from the paraffin block bank at the Department of Pathology Anatomy, Faculty of Medicine, Universitas Indonesia - Dr. Cipto Mangunkusumo Hospital and examined in the research and esoteric laboratory Prodia.

The samples were obtained from tha paraffin blocks of tissue biopsies from patients with precancerous lesions and cervical cancer of the Department of Pathology Anatomy. Slides of precancerous lesions and cervical cancer to be sampled underwent re-evaluation by the Pathologist. Samples were taken from formalin fixed paraffin embedded tissue (FFPE) biopsy of cervical precancerous and cervical cancer lesions from Department of Pathology Anatomy. For each sample taken 5 slides of FFPE were obtained. Samples along with the examination request form were then sent to research and esoteric laboratory Prodia. HPV DNA was extracted from formalin-fixed tissue Paraffin Embedded (FFPE) biopsy of the cervix. Genotyping of HPV DNA types (HPV 16, 18 and 52) was performed before proceeding to the examination DNA methylation in genes L1 with Bisulfite sequencing.

RESULTS

The study was conducted at the Division of Gynecology Oncology, Department of Obstetrics and Gynecology, Faculty of Medicine, Universitas Indonesia - RSCM in January-September 2016 and in the Laboratory of Clinical Research & Esoteric Prodia. Samples were taken from the paraffin block biopsies of precancerous lesions and cervical cancer in the Department of Anatomical Pathology, Faculty of Medicine, Universitas Indonesia-RSCM.

Samples were re-evaluated by the Pathologist to ensure the validity of the sample met the criteria of precancerous lesions and cervical cancer. For uniformity of precancerous cervical lesions samples taken were sampled with pure CIN 1, CIN 2, and CIN 3 lesions. Histopathologic type of cervical cancer samples was squamous cell carcinoma of the cervix. There were 57 sample of precancerous lesions and cervical cancer included in this study.

Characteristics of subjects

The mean age of study subjects was 44 years old. There were 30 samples (52.6%) with CIN 1 lesion, 12 samples (21.1%) CIN 2, 9 samples (15.8%) CIN 3, and 6 samples (10.5%) of cervical cancer.

In type 1 CIN lesions, the largest age group as 26-35 years old (10%). In CIN 2, the age group 26-35 years old and 36-45 years old respectively occupies 41.7%. For CIN 3 age group 36-45 years old and 46-55 years old respectively gained 33.3%. In cervical cancer was found most in the age group of 36-45 years old (50%). There was no significant relationship between the age groups with the degree of lesion ($p = 0.525$).

Genotyping HPV

Of the total 57 samples there were 55 samples successfully extracted and detected its HPV DNA

genotype (96.5%). Based on Table 1, most of the samples were co-infected with HPV type 16 and 18 in 28 samples (49.1%) followed by type 16 in 14 samples (24.6%) and type 18 in 8 samples (14.0%).

There were 5 samples that are not detected by the HPV 16, 18 and 52 probe, sequencing was then performed to determine the type of HPV. Based on the results of sequencing there were 1 sample of (1.8%) HPV 33; 1 sample (1.8%) HPV 35; 1 sample (1.8%) HPV 58; 2 samples were not included in the group of high risk HPV: HPV 53 (1.8%) with undeterminate risk and HPV 54 (1.8%) with a low risk potential. HPV type 52 was not found in this study. There are two samples that cannot be amplified in CIN 2 and CIN 3. HPV 16 infections both in the form of single or multiple discovered by 76.36% of the total sample were infected with HPV.

In CIN 1 (56.7%) and in CIN 3 (75%), coinfection of HPV 16 and 18 were mostly found In CIN 2, HPV 16 (54.5%) was the most. In cervical cancer, single HPV infection of 18 and co-infection of HPV 16 and 18 were highest (33.3%).

In CIN 1 there is one sample with HPV 35 infection (3.3%) and one sample of single HPV low risk HPV 54 (3.3%) and 1 sample undeterminate risk HPV 53 (3.3%) infection.

In CIN 3 there were two samples with single infection HPV; HPV 16 (12.5%) and HPV 58 (12.5%). There was no significant relationship between the types of HPV with the degree of lesion ($p = 0.181$).

A total of 55 samples were successfully amplified and detected its HPV typing, but the inclusion criteria for methylation are those with HPV 16, HPV 18 either as a single infection or multiple infection (co-infection of HPV 16 and 18) and HPV 52. There was no HPV 52 found in this study. Therefore, 50 samples were then included for the examination of L1 gene HPV DNA methylation.

HPV Methylation

Bisulfite conversion procedure was performed according to existing protocols followed by methylation PCR procedures to amplify the HPV DNA bisulfite conversion results. The primers used were special primer for HPV16 FP, RP HPV16, HPV18 and HPV18 FP RP, Integrated DNA Technology.[20-22] The next procedure was the detection by gel electrophoresis. After several stages of optimization procedures, there were thin band in 8 of 42 samples (19%) of HPV 16 after bisulfite conversion. Six of them were CIN 1, 1 sample CIN 2 and 1 sample of cervical cancer. However, the samples were polluted with dimers, so the procedure was followed by purification of the samples. After PCR was performed on the purified samples there were no band found on the electrophoresis examination, thus we can not proceed to sequencing. Optimization of HPV 16 and HPV 18 samples are still in progress up currently.

Table 1. HPV Genotyping based on the Histopathology

Genotype	Histopathology				
	CIN 1	**CIN 2**	**CIN 3**	**Cervical Cancer**	**Total**
HPV 16	6 (20%)	6 (54.5%)	1 (12.5%)	1 (16.7%)	14 (25.5%)
HPV 18	4 (13.3%)	2 (18.2%)	0 (0.0%)	2 (33.3%)	8 (14.5%)
HPV 16 & 18	17 (56.7%)	3 (27.3%)	6 (75.0%)	2 (33.3%)	28 (50.9%)
HPV 33	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (16.7%)	1 (1.8%)
HPV 35	1 (3.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (1.8%)
HPV 58	0 (0.0%)	0 (0.0%)	1 (12.5%)	0 (0.0%)	1 (1.8%)
HPV 53	1 (3.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (1.8%)
HPV 54	1 (3.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (1.8%)
Total	30 (100%)	11 (100%)	8 (100%)	6 (100%)	55 (100%)

DISCUSSION

In this study, we obtained 57 samples of paraffin blocks of precancerous lesions and cervical cancer. After extraction of HPV DNA, 55 samples (96.5%) were successfully amplified and performed genotyping. This is a success of its own because the method of detection of HPV in tissue samples is more difficult to do than sitology specimens. This due to formalin fixation which can cause DNA damage, including cross-linking and fragmentasi.[23]

HPV DNA genotyping was done by using primers GP5 + / GP6 + with specific probes HPV 16, HPV 18, and HPV 52. The three HPV typeswere chosen based on the three types of HPV with the highest prevalence in Indonesia on previous studies.[24-27]

In a study involving 11 case control studies from nine countries conducted by Munoz N., et al HPV infection was detected in 90.7% of cases using the primers MY09 / MY011. In this study we used the primers GP5 + / 6 +, and HPV DNA was detected in 96.6% of patients.[28]

In this study, HPV 16 infection was found the most, 76.36% of the total 55 samples, either in the form of single or multiple infections. Most of the samples were infected with multiple HPV infection 16 and 1849.1%, followed by a single infection of HPV 16 (24%) and single infection with HPV 18 (14%).

In a study in Jakarta by de Boer et al, it is reported that HPV 16 (35%) and HPV 18 (28%) were the most common types of cervical cancer.[26] This is some what different from the study in Indonesia conducted Bosch et al in 1995 with the highest prevalence of HPV is HPV 18 (48.9%) in 45 samples followed by HPV 16 as much as 31.9%.[29]

One interesting thing in this study is there was no of HPV 52 on the overall samples examined. This is different to that obtained in previous studies in Indonesia.

The study conducted by the JNI Vet et al in the three regions in Indonesia (Jakarta, Tasikmalaya and Bali) of 2686 samples most were infected with HPV 52 (23.2%), HPV 16 (18%), HPV 18 (16.1%) and HPV 39 (11.8%). Multiple infection was found in 20.7% of samples.[25]

A study by Schellekens et al in Jakarta on 74 cervical cancer specimens obtained three types of HPV mostly infected with HPV 16 (44%), HPV 18 (39%) and HPV 52 (14%). Multiple HPV infections were present in 14.1% of the total HPV positive samples.[24] While the study conducted in Singapore by Sahiratmadja et al in 96 cases of cervical cancer 90% samples were infected with multiple HPV 16 infections both with HPV 18, HPV 45 and HPV 52. Only three samples were infected with single HPV 16.[27]

Most of the samples in this study are co-infected with HPV 16 and 18. This is in line with the cohort by Siddiqa A., et al in Pakistan with samples FFPE tissue biopsies using the primers GP5 + / GP6 +, which are also used in this study, and they found HPV positive in 94, 81% of the samples. Infection with HPV 16 in the form of single or co-infection was found in 64.94% of the samples and HPV 18 was found in 66.23% of the samples. Single infection with HPV 16 was found in 24.68% of the sample and a single infection of HPV in 25.97% of the samples. In this study, 40.26% of samples found positive co-infection with HPV 16 and HPV 18. This is significantly higher than the incidence of co-infection with HPV 16/18 found previously in Pakistan (<2%), in Saudi Arabia (6.7%) and Indonesia (4.1%).[24,30-32]

In this study, we obtained more multiple HPV infection (co-infection with HPV 16 and 18) compared to with single infection. However, one thing to note, in this study we only use three probes specifically for HPV 16, 18 and 52 so co-infection of HPV 16 or 18 other HPV types could possibly present but can not be detected by the probes.

In this study, co-infection of HPV 16 and 17 were mostly found in CIN 1 (56.7%) and CIN 3 (75%). In CIN 2 lesions, HPV 16 (54.5%) was mostly found. In cervical cancer, single HPV 18 infection and co-infection of HPV 16 and 18 were the most found (33.3%). In addition to HPV 16 and 18, in CIN 1 lesion there is one sample with HPV 35 infection (3.3%) and 2 samples were HPV low risk single HPV 53 (3.3%) and HPV 54 (3.3%). Single HPV 58 infection was 12.5% found in CIN 3.

This study was almost in line with the cohort study conducted by Siddiqa A., et al in Pakistan. In the samples of cervical cancer co-infection of HPV 16 and 18 was 34.88%, while a single infection by HPV 16 and HPV 18 were 27.91% and 30.23%, respectively. In high degree lesions (CIN 2 and 3) single infection of HPV 16 and HPV 18 respectively was 18.18%, while the co-infection of HPV 16 and 18 was found 54.54%.[30]

Differences in the prevalence of HPV can be caused by several factors, including the sensitivity of PCR (primary election: MY09 / MY11 versus GP 5 + / 6 +), the quality of the specimen and specimen storage. Thus, the extent of variability that exists is difficult to compare the prevalence of HPV among research.[33]

HPV DNA methylation is a promising biomarker for diagnosis and prognosis of cervical cancer. After HPV DNA genotyping, 50 samples with either single or multiple infection (co-infection) of HPV 16 and 18 were preceded to the examination methylation. Samples with co-infection of HPV 16 and 18 were examined in a both the group of HPV single infection using primers specific for HPV 16 and also with the single HPV 18 group using primers specific for HPV 18.

The bisulfite conversion procedures uses the EZ DNA methylation Kit reagen, Cat No. D5001 Lot.ZRC186674 (Zymo Research Corp., CA, USA). After the bisulfite conversion procedure, PCR procedure was then performed. Primers used for this procedure were HPV16 FP, RP HPV16, HPV18 and HPV18 FP RP, Integrated DNA Technology.[20-22]

After several stages of optimization, we obtained a thin band on 8 samples (19%) HPV 16 but were polluted with dimers. Purification of the samples was then performed. On the electrophoresis reading after the PCR examination of the purified samples there were no band visible. This is likely due to small amount of DNA yield. It can also occur because the samples have been through the stages of bisulfite treatment that alter the components cytosintothiamine, which has a lower binding affinity. HPV detection methods on the tissue samples that have been processed with paraffin are more difficult to examine compared to the citology specimens. This can be caused by formalin fixation can cause DNA damage, including cross-linking and fragmentation.[23]

As in the case of genotyping procedures that needed several stage of optimization, HPV DNA methylation procedures also needs some optimization. We plan to add the volume of templates and make modifications to the protocol procedures as a part of optimization to achieve valid results.

Until now, the optimization process for HPV DNA methylation is still carried out and therefore we can not take final conclusion.

CONCLUSION

HPV 16 infection were most commonly found in the form of single or multiple. Co-infection of HPV 16 and 18 were found in the majority of the samples. There were no significant correlation between HPV type and the severity of cervical lesions. We already obtained eight samples of HPV 16 with a thin band on electrophoresis, however the result could not be concluded because the optimization is still ongoing.

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