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## Maternal Age, Abortion and Aneuploidy

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Epidemiologic studies demonstrated that fertility begins to decline while women reached age 25. This is due to a decline in oocyte quality such as aneuploidy rather than merely decreasing oocyte number. The decline in oocyte quality becomes clinically relevant for women from their mid-30s. The prevalence of infertility increases from 1% at age 25 to 55% at age 45. While 75% of women attempting conception at age 30 conceive within 12 months, by age 40 this has declined to 44%. Further, 20% of women who conceive at age 35 will have a spontaneous abortion. The monthly fecundity resulting in live-birth rate among women aged 30, 35 and 40 year is 17%, 12% and 5% respectively.<sup>1</sup>

The age-associated decline in female fecundity and increased risk of spontaneous abortion are largely attributable to abnormalities in the oocyte. The meiotic spindle in the oocyte of older women frequently exhibits abnormalities in chromosome alignment and micro tubular matrix composition. Higher rates of single chromatid abnormalities in oocytes, as well as aneuploidy in pre-implantation embryos and ongoing pregnancies, are observed in older women. The higher rate of aneuploidy is a major cause of increased spontaneous abortion and decreased live birth rates in women of advanced reproductive age.<sup>2</sup>

Currently efforts to screen the risk of chromosomal or genetic abnormalities are through pre-implantation genetics screening (PGS) in IVF program.

New technologies for genetics and chromosomal abnormalities screening are based in personal genomics: comparative genomic hybridization (CGH), microarray-based CGH, multiplex real-time polymerase chain reactions (PCR), digital PCR, real time PCR, single nucleotide polymorphism (SNP) and next generation sequencing (NGS). One of today's challenges is to perform multiple analyses on each embryo, not only for multiple mutations, but also for a combination of diagnosis and aneuploidy screening, perhaps thereby creating a broader testing platform that can be used in all patients.<sup>2,3</sup>

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**Research Article** 

## Prevalence of Postpartum Anxiety and Depression after Intrapartum Oxytocin

## Kejadian Ansietas dan Depresi Postpartum pada Pemberian Oksitosin Intrapartum

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#### Abstract

**Objective**: To know the prevalence of postpartum anxiety and depression in patients who received intrapartum oxytocin.

**Method**: Across-sectional observational study was conducted in Dr. Cipto Mangunkusumo hospital. Observation used Edinburgh Postnatal Depression scale and Beck Anxiety Inventory before delivery day, on the first and fourteenth day of postpartum.

**Result**: Of 112 patients, we found the prevalence of mild and moderate anxiety were 94.6% and 5.4%. There were no anxiety women before delivery and on the first day of postpartum. The prevalence of mild and moderate anxiety on the fourteenth day of postpartum were 83% and 16.9%. There was no severe anxiety found at those time. On the other hand, the prevalence of postpartum depression on the first and fourteenth day of postpartum were 31.3% and 32.1%. There was no association between exogenous oxytocin and postpartum anxiety also depression. In multivariate analysis, we found that women with low self image were more prone to postpartum depression was associated significantly to self image (OR 0.17, 95% CI 0.07-0.83), low income (OR 10.35, 95% CI 1.72-62.45) and pregnancy plan (OR 0.17, 95% CI 0.06-0.53)

**Conclusion**: The patients who received intrapartum oxytocin are more prevalent to have mild anxiety. The prevalence of depression before delivery day, on the first and fourteenth day of postpartum are similar. In statistic, there is no relationship between intrapartum oxytocin administration and postpartum anxiety or depression.

[Indones J Obstet Gynecol 2016; 4-2: 59-63]

Keywords: anxiety, depression, oxytocin intrapartum, postpartum

#### Abstrak

*Tujuan*: Mengetahui prevalensi kejadian kecemasan dan depresi pascamelahirkan pada pasien yang mendapat oksitosin intrapartum.

**Metode**: Studi observasi dengan desain potong lintang dilakukan di RSUPN Dr. Cipto Mangunkusumo. Observasi dengan menggunakan Edinburgh Postnatal Depression scale dan Beck Anxiety Inventory sebelum melahirkan, hari ke-1 dan 14 pascamelahirkan.

Hasil: Dari 112 pasien, prevalensi kecemasan ringan dan sedang sebesar 94,6% dan 5,4%. Tidak ada yang mengalami kecemasan sebelum melahirkan dan satu hari pascamelahirkan. Prevalensi kecemasan ringan dan sedang pada hari 14 pascamelahirkan sebesar 3% dan 16,9% dan tidak ada yang menjadi kecemasan berat. Sementara itu, prevalensi depresi pada hari ke-1 dan 14 pascamelahirkan sebesar 31,3% dan 32,1%. Tidak ada hubungan yang bermakna antara cara pemberian, dosis kumulatif dan lama pemberian oksitosin dengan kecemasan dan depresi pascamelahirkan pada hari ke-1 dan 14. Dari hasil analisis multivariat, hanya citra diri saja yang berpengaruh terhadap kejadian kecemasan pascamelahirkan (OR 0,16; IK 95% 0,06-0,46). Sementara itu, depresi pascamelahirkan berhubungan dengan citra diri (OR 0,17; IK 95% 0,01-0,83), penghasilan rendah (OR 10,35; IK 95% 1,72- 62,45) dan keinginan hamil (OR 0,17; IK 95% 0,06-0,53).

Kesimpulan: Pasien yang mendapat oksitosin intrapartum lebih sering mengalami kecemasan ringan. Kejadian depresi sebelum melahirkan, hari ke-1 maupun 14 pascamelahirkan memiliki prevalensi yang serupa. Secara statistik, tidak ada hubungan yang bermakna antara pemberian oksitosin intrapartum dengan kecemasan atau depresi pascamelahirkan.

[Maj Obstet Ginekol Indones 2016; 4-2: 59-63]

Kata kunci: depresi, intrapartum, kecemasan, oksitosin, pascamelahirkan

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## INTRODUCTION

Oxytocin is a neuropeptide known as the "maternal hormone" due to its activation during pregnancy and lactation. The role of oxytocin during pregnancy and lactation is to improve the function of the reproductive and maternal behavior.<sup>1</sup> Some studies found that oxytocin receptors were distributed in various areas in the brain<sup>2</sup> and associated with sexual behavior, maternal and social attitude. They could also response to the stressor.<sup>3</sup> Oxytocin has anxiolytic effect and may delay the hypothalamus-pituitary-axis responsiveness to adrenal (HPA). The effect is very dependent on the gender, species, duration of therapy, and the brain areas.

During prenatal and postpartum, women have more risk for anxiety and depression. The prevalence of anxiety on the postpartum period is increased compared to women in the general population. Postpartum anxiety and depression impact to maternal and child development.<sup>4</sup>

#### 60 Valentine and Kayika

Various experimental studies about the role of oxytocin to anxiety and depression have been carried out on animals; however, there are only few studies conducted in human.<sup>5,6</sup> One study showed that women getting exogenous oxytocin intravenously and intramuscularly at birth had the decreased of anxiety and increased of social function in the observation on the second day, second month, and sixth month postpartum.7 Unfortunately, there was still few studies finding the central effect of exogenous oxytocin, especially on the anxiolytic and depression effects. Therefore, this study was carried out to know the prevalence of postpartum anxiety and depression in patients who received intrapartum oxytocin and the relationship between exogenous oxytocin to postpartum anxiety and depression.

### **METHODS**

This observational study was a cross-sectional design on the aterm pregnant patients planned to vaginal birth and received oxytocin during labor in Tangerang and Fatmawati General Hospital from December 2013 to May 2014. The subjects who fulfilled the inclusion criteria and agreed to participate in the study were asked to fill out questionnaires before and after delivery (on the first and fourteenth day of postpartum). The questionnaire was consisted of Beck Anxiety Inventory and the Edinburgh Postnatal Depression Scale (EPDS), which had been validated and proven their reliabi-lity in assessing anxiety and depression. The ethical committee from Faculty of Medicine University of Indonesia had approved the study protocol. We conducted a descriptive categorical data analysis to look at the prevalence of postpartum anxiety and depression in both study groups at three times measurement. Statistical analysis was performed with Mann-Whitney test to see the relationship between oxytocin and postpartum anxiety also depression. Multivariate analysis with stepwise logistic regression analysis method was done to control the confounding variables of age, parity, unplanned or unwanted pregnancy, socioeconomic status, selfimage, problems in the household, as well as the emotional and social support from her husband and family.

### RESULTS

There were 112 subjects in this study. The age of them was ranged from 16 to 44 years old with a

median value of 29.5 years old. The majority of subjects (80.4%) had secondary level, 52.7% was multipara and 78.6% had moderate self-image and didn't plan for this pregnancy. The delivery process accompanied by her husband was 73 subjects (65.2%) (Table 1).

### Table 1. Characteristics of Subjects

Characteristics	Total n (%)
Age, Median (years old)	29.5 (16-44)
Age group, n (%)	
<20 years old	5 (4.5)
20-30 years old	56 (50)
31-40 years old	46 (41.1)
>40 years old	5 (4.5)
Education status, n (%)	
Low	19 (17)
Moderate	90 (80.4)
High	3 (2.7)
Economic status, n (%)	
Low	10 (9.1)
Moderate	94 (84.6)
High	8 (7.2)
Total number of pregnancies, n (%)	
Primipara	40 (35.7)
Multipara	59 (52.7)
Grande multipara	13 (11.6)
Pregnancy plan	
Yes	87 (77.7)
No	25 (22.3)
Self-image	
Low	24 (21.4)
Moderate	88 (78.6)
Any conflict in family	
Yes	8 (7.1)
No	104 (92.9)
Wanted pregnancy	
Yes	101 (90.2)
No	11 (9.8)
Any husband companion during labor	
Yes	73 (65.2)
No	39 (34.8)
Oxytocin	
IV-IM	81 (72.3)
IM	31 (27.7)

## Cumulative dose of oxytocin

Duration of administration, median (range) (hours)	6.5 (1-27)
>20 U	65 (58)
20 U	1 (0.9)
15 U	16 (14.)
10 U	30 (26.8)

In this study, the prevalence of mild and moderate anxiety in patients who received intrapartum oxytocin were 94.6% and 5.4%. None experienced severe anxiety prenatal and on the first day of postpartum. After 14 days of postpartum, patients having moderate anxiety increased to 16.3% and no subjects suffered from severe anxiety. In subjects receiving oxytocin intravenously and intramuscularly, the prevalence of mild and moderate anxiety at admission were 93.8% and 6.2%. Meanwhile, the prevalence on the fourteenth day of postpartum were 85.1% and 14.8%. In subjects getting oxytocin intramuscularly only, the prevalence of mild and moderate anxiety at admission were 96.7% and 0.9%; and after 14 days of postpartum, the prevalence became 77.4% and 22.7%.

In this study, we found that the prevalence of depression at admission, 1 day and 14 days after delivery in patients receiving intrapartum oxytocin were 29.5%, 31.3% and 32.1% respectively. In subjects who received oxytocin intravenously and intramuscularly, the prevalence of postpartum depression at admission, 1 day and 14 days after delivery were 27.1%, 33% and 34.5%, respectively. In subjects getting oxytocin intramuscularly, the prevalence of postpartum deprevalence of postpartum depression before delivery day, on the first and fourteenth day of postpartum were 35.4%, 25.8% and 25.8%, respectively.

We found no relationship between the mode of administration (IV and IM or IM only), cumulative dose and length of administration and postpartum anxiety on the first and fourteenth day of postpartum (Table 2 and 3).

Table 2.	Relationship between Mode of Administration, Cumulative Dose, and Duration of Administration of
	Intrapartum Oxytocin on Postpartum Anxiety.

	Da	Day-1 postpartum		Day	y-14 postpartum	
	Mild anxiety	Moderate anxiety	р	Mild anxiety	Moderate anxiety	р
Mode of administration						
Intravenous and intra- muscular oxytocin (%)	96.3	3.7	0.21*	85.2	14.8	0.33**
Intramuscular oxytocin only (%)	90.3	9.6		77.4	22.6	
Oxytocin cumulative dose (IU)	30 (10-50)	20 (10-35)	0.46**	30 (10-45)	15 (10-50)	0.32**
Duration of administration (hours)	6.5 (1-27)	12 (6-18)	0.26**	6.5 (1-18)	7.25 (6-27)	0.51**

\*Fisher; \*\*Chi-square

**Table 3.** Relationship between Mode of Administration, Cumulative Dose, and Duration of Administration of Intrapartum Oxytocin on Postpartum Depression.

	Day-1 postpartum		Day-14	Day-14 postpartum		
	No depression	Depression	р	No depression	Depression	р
Mode of administration						
Intravenous and intra- muscular oxytocin (%)	54	27	0.44*	53	28	0.37*
Intramuscular oxytocin only (%)	23	8		23	8	
Oxytocin cumulative dose (IU)	30 (10-35)	30 (10-50)	0.15**	30 (10-35)	30 (10-50)	0.19**
Duration of administration (hours)	6.5 (1-18)	12 (2-27)	0.38**	6.5 (1-18)	10 (2-27)	0.49**

\*Chi-square; \*\*Mann-Whitney

## DISCUSSION

In this study, we found that the prevalence of depression at admission, 1 day and 14 days after delivery in patients who received intrapartum oxytocin were 29.5%, 31.3% and 32.1%; respectively. The prevalence of postpartum depression in the general population in other countries was varied from 10 to 34%. The prevalence of postpartum depression in Asia was between 11% and 60.8%.<sup>9</sup> Alfiben et al. found that postpartum depression prevalence in Dr. Cipto Mangunkusumo hospital was 33%.<sup>10</sup>

In this study, the assessment was carried out on the first and fourteenth day of postpartum. In a previous study conducted by Alfiben et al. in RSCM, they found that the EPDS evaluation at 48 hours postpartum and 2 weeks postpartum were not statistically significantly, so they suggested to perform only one assessment only.<sup>9</sup> The aim of assessment using EPDS at two weeks postpartumin in this study was to exclude the postpartum blues which usually happened and lasted until the two weeks after delivery.

There were no association among the mode of oxytocin administration, cumulative dose and length of administration with postpartum anxiety. These findings differred from the results by Jonas, et al. which showed that women who got exogenous oxytocin during labor either IM or IV had the lower risk to be anxiety and aggression; as well as, they stated that exogenous oxytocin was related to the increased socialization, including maternal behavior.<sup>7</sup> Apart from that, there were two studies finding a negative correlation between plasma levels of oxytocin and anxiolytic effects; meanwhile it described a positive relationship between plasma oxytocin level and positive feelings in non-pregnant women.<sup>11,12</sup> Other studies also found a negative correlation between the incidence of postpartum depression and oxytocin. Women with low level of oxytocin level during pregnancy had increased risk of postpartum depression. Increased incidence of depression at 2 weeks after delivery occurred in individuals with lower plasma oxytocin concentration.<sup>13</sup>

Whether plasma oxytocin level can be used as a prediction for central oxytocin level, activity level in the brain, and its relationship with the social and emotional behavior is still unclear. One study mentioned that plasma oxytocin level did not reflect the availability of oxytocin in the central. Central oxytocin release was not necessarily related to the release of oxytocin into the peripheral; therefore, the relationship among psychological variables should be interpreted cautiously.<sup>3,14</sup>

Neurohypophysis is a major contributor to the plasma oxytocin level, especially during childbirth and breastfeeding. This release is not affected by the blood-brain barrier; this hormone is released directly from the circuit magnoseluler into the capillary. Either endogenous or exogenous oxytocin has a poor penetration to the blood-brain barrier. In physiological condition, there are differences in plasma oxytocin concentration and cerebrospinal fluid. Level of oxytocin shows circadian cycle in the cerebrospinal fluid, but not in the other way around. The difference in oxytocin concentration or release pattern in body fluid, including cerebrospinal fluid versus plasma, is influenced by various peptides. In addition, oxytocin also has a short halflife in the blood, between 3 and 9 minutes.<sup>14</sup>

Study about antidepressants or anxiolytic effect of exogenous oxytocin is still limited. The recent study showed that there was a negative effect from exogenous oxytocin on depression postpartum.<sup>15,16</sup> However, the role of exogenous oxytocin on maternal behavior is quite clear, although recent evidence states that the effect may still be confounded by endogenous oxytocin level produced during the mother-baby bonding time. In addition, individual factors, such as variation of the oxytocin receptor function, production of endogenous oxytocin, bonding between mother and baby should be considered. These factors affect individual responsiveness to exogenous oxytocin. Gonadal hormone, especially estrogen also affects the regulation of endogenous and exogenous oxytocin.<sup>17</sup> It is still unknown whether the administration of exogenous oxytocin can interact with the level of endogenous hormone oxytocin or other endogen hormone.<sup>18</sup>

## CONCLUSION

The patients who received intrapartum oxytocin are more prevalent to have mild anxiety. The prevalence of depression before delivery day, on the first and fourteenth day of postpartum are similar. In statistic, there is no relationship between intrapartum oxytocin administration and postpartum anxiety or depression.

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**Research Article** 

## **Profile of Maternal Referral Cases**

Profil Rujukan Kasus Ibu

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### Abstract

#### Abstrak

**Objective**: To explore the demography of maternal referral cases in Dr. Cipto Mangunkusumo Hospital (RSCM) along with the accuracy of referral. We also aim to evaluate the types of referral, origin of referral, referring healthcare facility and quality of referring healthcare facility.

**Method**: The design of this study was a cross sectional design which described the accuracy of obstetrics referred cases in Emergency Unit Dr. Cipto Mangunkusumo Hospital from 2013 to 2014.

**Result**: The total referred obstetric cases in 2013 was 1,645 patients. It was consisted of 1,307 appropriate (79.5%) and 338 inappropriate (20.5%) referred cases. Primary healthcare and general hospital were the most often referring cases to RSCM during two consecutive years. The top three cases referred to RSCM in both 2013 and 2014 were preterm premature rupture of membrane (PPROM), continued by severe preeclampsia and preterm labor.

**Conclusion**: The number of referral cases in Indonesia is considered high, particularly in RSCM as the tertiary healthcare facility. There are still a high number of inappropriate referrals originating from primary healthcare facilities, pointing to the fact that the referral system is not running according to design or plan. To improve the quality of referral system, proper monitoring and evaluation of referral should be performed by local health department.

[Indones J Obstet Gynecol 2016; 4-2: 64-66]

Keywords: maternal case, referral system

**Tujuan**: Mengetahui gambaran rujukan kasus ibu yang terjadi di RSCM beserta ketepatan pelaksanaan rujukan. Selain itu dapat ditemukan sebaran jenis dan daerah asal rujukan kasus, fasilitas pelayanan kesehatan perujuk, serta kualitas fasilitas pelayanan kesehatan dalam merujuk kasus.

*Metode:* Studi kuantitatif desain potong lintang menggambarkan ketepatan kasus rujukan ibu ke IGD RSCM pada tahun 2013-2014.

**Hasil**: Rujukan kasus Ibu 2013 sebanyak 1.645 pasien, 20,5% tidak tepat rujuk. Asal fasilitas pelayanan kesehatan perujuk terbanyak adalah Pusat Kesehatan Masyarakat (Puskesmas) dan RS Umum Daerah (RSUD). Jenis kasus yang dirujuk terbanyak adalah ketuban pecah dini, preeklamsia berat dan persalinan preterm.

Kesimpulan: Jumlah kasus rujukan di Indonesia masih tinggi, khususnya di RSCM sebagai fasilitas kesehatan tersier. Masih banyak rujukan yang tidak tepat berasal dari pelayanan kesehatan primer. Hal ini menandakan bahwa rencana sistem rujukan yang belum tepat. Untuk meningkatkan kualitas sistem rujukan, perlu dilakukan monitoring dan evaluasi oleh dinas kesehatan setempat.

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Kata kunci: kasus maternal, sistem rujukan

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## INTRODUCTION

Hospital is an institution that provides curative and also preventive health services. A hospital must be prepared for the necessary infrastructure covering all requirements of medical personnel and adequate funding to meet the public needs.<sup>1,2</sup>

The prevailing referral system in Indonesia is based on health ministry policy in 1978. Unfortunately, the management system has not been appropriately applied in this situation such as the mess of referral system. It is due to the failure of policy maker to set the referral system and guideline. Thus, we need a new innovation to solve this problem.<sup>3</sup> The referral system will be effectively worked through the close relationship between health care services and the society so that people can reach the health services easily. It will create the cost effectiveness in approaching the primary and secondary health services.<sup>4,5</sup> A good and effective referral system assures the achievement of optimum performance.<sup>6</sup> In fact, there are a lot of complaints such as inappropriately and always refer also no return referral to primary health care. These cause the overload of patients in referral hospital so that the services become ineffective and inefficient.<sup>7,8</sup>

The recording, reporting and giving feedback system are not well coordinated in a hospital, even

in Dr. Cipto Mangunkusumo hospital (RSCM) as the center of referral. A study conducted in RSCM about the emergency referral system revealed that there were improper referral steps showed by the high rate of referral coming from primary health care (38%).<sup>9</sup> Several problems included the incomplete data of the referral notes and no early assessment of the cases.<sup>10,11</sup>

In the era of universal health coverage, it is mandatory to carry out appraisals of the referral program based on a quantitative approach. This aims to determine the events occurred in health facilities at the lower level and the condition of patients referred. This approach can also give the suggestion to determine the health policy in multilevel referral system for the tertiary hospital. The system can reduce the number and kind of referral cases in RSCM as tertiary hospital.<sup>12</sup>

### METHODS

This descriptive quantitative study used the crosssectional design. The study was conducted toward maternal referral cases patients who met the inclusion and exclusion criteria in Dr. Cipto Mangunkusumo Hospital from 2013 to 2014.

Data collection was carried out between January and July 2015 using total sampling method so that all eligible samples were included in the data analysis. The variables consisted of the accuracy of referral, type of referral, origin of referral and the referring healthcare facility. Determination of referral accuracy was based on type of referral case, level of referral, regionalization of referral and competence of referring healthcare facility. We inputted all data to be analyzed in Microsoft Excel. We analyzed the descriptive data in categorically and the result was presented in frequency and percentage on either table or graphic.

### RESULTS

The study showed that there were an increase number of improper referrals as many as 289 cases in 2014. The percentage rose from 20.5% in 2013 to 27.0% in 2014. The top three cases referred to RSCM in both 2013 and 2014 were preterm premature rupture of membrane (PPROM), continued by severe preeclampsia and preterm labor. Apart from that, the number of cases was also significantly increased from 632 cases in 2013 to 1,119 cases in 2014 (Table 1). Table 2 compared the ap-

propriate and inappropriate of maternal referral cases in RSCM during 2013-2014.

Table 1. The Maternal Referral Cases in 2013 - 2014

Most frequent Cases (n(%))			
Tear	PPROM	Severe Preeclampsia	Preterm
2013	240 (14.6)	227 (13.8)	165 (10.1)
2014	479 (20.3)	393 (16.7)	247 (10.5)

**Table 2.** Comparison of Appropriate and InappropriateMaternal Referral Cases in RSCM in 2013-2014.

Year	Total Materna	l Referral Cases	Total	
(Total)	Appropriate	Inappropriate	iotai	
2013 (1645)	1307 (79.5%)	338 (20.5%)	1645	
2014 (2357)	1730 (73%)	627 (27%)	2537	

Some health care facilities referred the maternal cases to RSCM including: general hospital, primary health care, clinic, midwives and also private hospital. Primary health care and general hospital were the most often referring cases to RSCM during two consecutive years. The data were similar in each region. In 2013, the regions which referred the most number of cases were as follows: East Jakarta (500 cases), Central Jakarta (336 cases) and unnamed regions (332 cases). Meanwhile, outside Java only ever referred for 1 case in 2013. In 2014, Central Jakarta (742 cases) became the most often referring cases, followed by East Jakarta (573 cases) and Bogor Tangerang Bekasi (258 cases). Also, the least frequently referring cases in 2014 came from outside Java only for 16 cases.

**Table 3.**Healthcare Facilities Presenting most ReferralCases in 2013-2014

Year	<b>Referring Healthcare Facility</b>	Total (n(%))
0040	Primary healthcare	736 (44.7)
2013	General hospital	383 (23.3)
0044	Primary healthcare	684 (29.0)
2014	General hospital	399 (16.9)

## DISCUSSION

In 2014, maternity cases referred to RSCM increased to 712 cases (43.0%) compared with 2013.

The increase of it was influenced by the legalization of uni-versal health coverage in Indonesia which managed by Badan Pengelola Jaminan Sosial (BPJS) from January 1<sup>st</sup> 2014. Besides, it was caused by the inappropriate national referral system so that there was an accumulation of maternal cases in RSCM. According to Murray, the proper pattern of referral had to follow the pyramidal format of health services, starting from the lowest to the highest level.

A referral case is correct if it meets the criteria of case type, stage of case, regionalization of case and the competence level of healthcare facility. Unless it meets the criteria mentioned, the referral should be deemed inappropriately. In 2014, the number of inappropriate cases rose from 20.5% to 27.0%. The particular problem was probably caused by the fact that the bypassed referral of the secondary healthcare facility directs to RSCM as a result of geographical consideration. Apart from that, the fact that the secondary healthcare facility was not being manned by the required specialist doctor on site and also inadequate facilities that could handle the emergency situation.

## CONCLUSION

The number of referral cases in Indonesia are considered high, particularly in RSCM as the tertiary healthcare facility. There are still a high number of inappropriate referrals originating from primary healthcare facilities, pointing to the fact that the referral system is not running according to design or plan. The low quality of referral is influenced by several factors such as the absence of referral standard operating procedure which complies with the national referral guidance in 2012, the absence of referral proper recording, limited cooperation among several referral stages, inadequate referral transportation means, the absence of specialist doctors on site and unavailable proper emergency operation theatre. Based on interviewing with patients, they expressed disappointment with the quality of service in the referring healthcare facilities because of time consuming process, unavailable transportation, the absence of specialist doctors on site and other unclear reasons of referrals.

## RECOMMENDATION

To improve the quality of referrals, each primary health care must have a clear and detailed referral standard operating procedure on the management of referral and return referral. Proper monitoring and evaluation of referral should be performed by local health department.

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**Research Article** 

## A1298C Polymorphism of Fetal Methylenetetrahydrofolate Reductase (MTHFR) Gene as a Risk Factor for Spontaneous Abortion

Polimorfisme A1298C Gen Metilentetrahidrofolat Reduktase (MTHFR) Fetus sebagai Faktor Risiko Aborsi Spontan

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#### Abstract

**Objective**: To investigate the role of A1298C polymorphism of fetal methylenetetrahydrofolate reductase (MTHFR) gene in spontaneous abortion.

**Method**: The case control study design recruited 96 subjects in Siti Fatimah and Pertiwi mother and child hospital, Dr. Wahidin Sudirohusodo, Pelamonia, Bhayangkara, Syekh Yusuf, Haji and Labuang Baji hospital from March to September 2014. All subjects fulfilling the inclusion criteria were taken tissue samples from mothers experiencing spontaneous abortion and blood samples from normally born baby. The data were analyzed using Pearson chi-square with significant rate of 5% (p<0.05).

**Result**: There were 49 samples as case group consisting of 36 samples (62.1%) with mutant genotype of MTHFR gene (1298AC + 1298CC) paired with 22 samples (37.9%) for the control group and also 13 samples (34.2%) with normal genotype gene (1298AA) from case group paired with 25 samples (65.8%) from control group.

**Conclusion**: A1298C polymorphism of fetal MTHFR gene has correlation to the rate of spontaneous fetal abortion.

[Indones J Obstet Gynecol 2016; 4-2: 67-69]

Keywords: A1298C polymorphism, spontaneous fetal abortion

#### Abstrak

**Tujuan**: Untuk mengetahui peran kejadian polimorfisme A1298C dari gen metilentetrahidrofolat reduktase (MTHFR) fetus pada abortus spontan.

**Metode**: Penelitian dengan desain kasus kontrol dilakukan dengan mengambil 96 subjek di RSIA Siti Fatimah dan Pertiwi, RS Dr. Wahidin Sudirohusodo, RS Pelamonia, RS Bhayangkara, RSUD Syekh Yusuf, RSUD Haji, RSU Labuang Baji selama periode Maret hingga September 2014. Seluruh subjek yang memenuhi kriteria inklusi diambil sampel jaringan dari hasil aborsi spontan dan sampel darah dari bayi yang lahir normal. Analisis data menggunakan tes Pearson chi-square dengan angka kemaknaan 5% (p<0,05).

Hasil: Dari hasil penelitian didapatkan 49 sampel untuk kelompok kasus dan 47 sampel untuk kelompok kontrol. Sebanyak 36 sampel (62,1%) dengan mutasi genotip gen MTHFR (1298AC+1298CC) dari kelompok kasus dan 22 sampel (37,9%) sebagai kelompok kontrol. Selain itu, 13 sampel (34,2%) dengan gen genotip normal (1298AA) dari kelompok kasus dan 25 sampel (65,8%) dari kelompok kontrol.

Kesimpulan: Polimorfisme A1298C dari gen MTHFR memiliki hubungan dan berpotensi untuk terjadinya abortus spontan.

[Maj Obstet Ginekol Indones 2016; 4-2: 67-69] **Kata kunci**: abortus spontan, polimorfisme A1298C

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## INTRODUCTION

Spontaneous abortion (miscarriage) is used to explain the spontaneous end of pregnancy during the first 20 weeks and considered as a common failure of pregnancy. Some studies stated that 10-25% of all pregnancies would be ended as spontaneous abortion, whereas 50-75% of those cases were caused by chromosomal abnormality at the level of embryo or fetus.<sup>1-3</sup>

There are several predisposing factors of abortion, such as parity and maternal age. As a risk factor, the rate of abortion is increased after 40 years old compared to below 30 years old. Another factor which also contributing is the heterogeneity between maternal and paternal chromosome. Genetical code analysis in spontaneous abortion cases showed a role of certain enzymes in metabolic crisis pathway, including methylenetetrahydrofolate reductase (MTHFR) fetal gene.<sup>4,5</sup>

Recent studies in literature showed an involvement of gene mutation in MTHFR C677T and A1298C as a risk factor of fetal death in recurrent spontaneous abortion, particulary in MTHFR homozygote related to fetal viability. Moreover, Moeljono in his thesis stated that gene mutation of A1298C was a risk factor towards spontaneous abortion cases, and its impact as a risk factor had been proven to be unrelated with parental genetic, as opposite within C677T gene mutation where spontaneous mutation occured along with hereditary pattern of the parents.<sup>6,7</sup>

Therefore, this study is aims to analyze the role of A1298C polymorfism of fetal MTHFR gene in spontaneous abortion.

### **METHODS**

The analytical study through case control approach analyzed the role of A1289C polymorphism of fetal MTHFR gene in spontaneous abortion. The samples were taken from several hospitals, such as Dr. Wahidin Sudirohusodo, Pelamonia, Labuang Baji, Bhayangkara, Haji and Syekh Yusuf hospital, St. Fatimah and Pertiwi mother and child hospital. The samples were collected to be further measured using PCR in NECHRI laboratory.

All spontaneous abortion were taken as the case and term babies as the control. The sample were conception tissues as the result of curretage procedure from women with spontaneous abortion and umbilical cord blood from normally born baby. Data analysis used SPSS program through Pearson chi-square test and odds ratio to determine the correlation between two characteristics of nominal and ordinal data. The significant rate was 5% (p<0.05).

## RESULTS

In case group, 14 of 49 samples of women were more than 30 years old; meanwhile in control group, 14 of 33 samples were more than 30 years old. About 25 samples in case group and 29 samples in control group were multigravida. In Shapiro Wilk analysis, the data between two groups were normally distributed (p>0.05).

The distribution of natural homozygote genotype of 1298AA (couple allele A) among case group was 13 samples (26.5%) compared to 25 samples (53.2%) in control group; meanwhile homozygote genotype of mutant 1298CC (couple allele C) among case group was 12 samples (44.9%) and 4 samples (8.5%) in control group. The rest was heterozygote genotype of 1298 AC.

Table 2.	Correlation between A1298C Polymorphism of
Fetal MTH	IFR Gene Towards Spontaneous Abortion

Variable	OR (Exp B)	95% CI	p value
Parity			
Multigravida	1.6	0.2-2.4	0.47
Primigravida	1		
Gestational age			
$\leq$ 12 weeks	2.1	1.1-4.1	0.02
$\geq$ 12 weeks	1		
Maternal age			
< 30 years	1.2	0.2-3.9	0.87
$\geq$ 30 years	1		

## DISCUSSION

In this study, there was not association between case and control group according to maternal age and parity. Theoretically, the increasing of women age is in accordance with the risk of genetical abnormality. The increasing of maternal age in 3<sup>rd</sup> or 4<sup>th</sup> decade goes along with oocyte aging. As a risk factor, the condition of primordial follicle in reserve oocyte is stated to be able to reproduce some egg cells with lower quality of follicles.

As opposite, the highest number of spontaneous abortion cases in this study was found in less than 30-years-old women both in case and control group, although the maternal age had not related to the rate of spontaneous abortion. Some studies showed a risk enhancement equal to maternal parity in spontaneous abortion. This phenomenon was caused by reproductive compensation (the failure of pregnancy could be related to some attempts of repetitive conception in multigravida) and short interval between each maternal pregnancy. Along with this study, there was no significant correlation found between maternal parity and spontaneous abortion.

 Table 1.
 Comparison between Polymorphism Genotype of Mutant Fetal MTHFR Gene A1298C (1298AC + 1298CC) and Normal (1298AA) in Case and Control Group

Feotus MTHFR genotype 1298	Case Group (n=49) %	Control Group (n=47) %	p value*	OR (95% CI)
AC + CC	36 (62.1)	22 (37.9)	0.008	3.2 (1.3-7.4)
AA	13 (34.2)	25 (65.8)		

\*Pearson Chi Square

The frequency of genotype A1298C polymorphism of fetal MTHFR gene was showed by couple alleles of A, C, and AC. The highest frequency of genotype among case group was in couple allele C. sequantially followed by mutant heterozygote and normal or couple allele A. According to statistical analysis, if genotype 1298AC and 1298CC were united as one and compared to genotype 1298AA, the result of this would be statistically significant with p value around 0.008 and OR 3.2. This basically showed that a role of A1298C polymorphism of fetal MTHFR gene was related to the rate of spontaneous abortion. Study by Kim Y revealed that A1298C polymorphism of fetal MTHFR gene was related to the risk enhancement of gene chromosomal abnormality in spontaneous abortion. According to some studies in MTHFR, the two homozygote mutations both MTHFR 1298CC and 677TT stated to be related to the reducing of DMA metilation level which could lead to hypometilation in DNA.

Theoretically, domain 1298 of MTHFR gene works in genetical regulation centre. Chronologically, there are two mechanisms for the influence from enzyme mutation of MTHFR gene. The first mechanism occurs through MTHFR enzyme as a catalyst of homocystein to metionin (an important amino acid for body), where the defect of MTHFR gene can lead to hyperhomocysteinemi. The second mechanism happens in genetical regulation, namely abnormal MTHFR gene. The failure of DNA metilation can lead to further genetical change and bring more tendency to worsen the current condition and even end up as fetal death.

The role of A1298C polymorphism of fetal MTHFR gene in spontaneous abortion can be correlated with some theories. Generally, abortion is started by fetal death which can be caused by the abnormality of fetus, embryo, and zygote growth. Chromosomal abnormality, particularly trisomal abnormality becomes the cause of 50-75% from spontaneous abortion. Moreover, the death of conception which occured in the first trimester of pregnancy happens to be greatly influenced by fetus genome as the main determinant.

Other factors influencing the risk of spontaneous abortion are folic metabolism, vein thrombosis, and hyperhomocysteinemi. Folic metabolism and vein thrombosis impact to process of embryo genesis. The abnormality of enzyme activity acts to control this folic metabolism; therefore it can lead to the abnormality or even the death of fetus. The recent study done in Indonesia by Moeljono ER stated that A1298C polymorphism of fetal MTHFR gene had an important role in the rate of spontaneous abortion, yet there was a little difference in the type of mutant genotype which considered to have more impact. Therefore, further study should be conducted to know this issue particularly in Indonesia.

## CONCLUSION

A1298C polymorphism of fetal MTHFR gene has a correlation with the rate of spontaneous abortion. Further studies to assess the A1298C polymorphism of fetal MTHFR gene in both parents and fetus should be conducted to enrich the information regarding this correlation.

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**Research Article** 

## **Transforming Growth Factor** β1 **and Tropoelastin Expression in Uterine Prolapse**

*Ekspresi Transforming Growth Factor*  $\beta$ *1 dan Tropoelastin pada Prolaps Uteri* 

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#### Abstract

**Objective**: To know the correlation of the expression of transforming growth factor beta (TGF- $\beta$ 1) and tropoelastin in uterine prolapse.

**Method**: A cross-sectional study of 30 subjects suffered from uterine prolapse in the Department of Obstetrics and Gynecology Dr. Mohammad Hoesin hospital Palembang. The study was conducted since December 1<sup>st</sup>, 2014 until July 31<sup>st</sup>, 2015. The sample was from the sacrouterine ligament and immunohistochemical examination was conducted to see the expression of TGF- $\beta$ 1 and tropoelastin.

**Result**: Of the 30 subjects obtained, the expression of TGF- $\beta$ 1 was on 30 subjects consisting of 18 (60%) for weak expression and 12 (40%) for strong expression. Meanwhile, the strong tropoelastin expression was on 18 subjects (60%) and weak tropoelastin expression on 12 subjects (40%). There was a positive correlation between TGF- $\beta$ 1 and tropoelastin expression with moderate correlation (p=0.014; r=0.44).

**Conclusion**: There is a positive correlation between the TGF- $\beta 1$  and tropoelastin expression of sacrouterine ligament in uterine prolapse with moderate correlation.

[Indones J Obstet Gynecol 2016; 4-2: 70-74]

**Keywords**: transforming Growth Factor Beta 1, tropoelastin, uterine prolapse

#### Abstrak

**Tujuan**: Untuk mengetahui korelasi ekspresi transforming growth factor beta 1 (TGF-\31) dengan ekspresi tropoelastin pada prolaps uteri di Rumah Sakit Dr. Mohammad Hoesin Palembang.

**Metode**: Sebuah penelitian potong lintang dari 30 subjek penderita prolaps uteri yang dilakukan di Departemen Obstetri dan Ginekologi Rumah Sakit Dr. Mohammad Hoesin Palembang, Penelitian dilakukan sejak 1 Desember 2014 sampai dengan 31 Juli 2015. Sampel berasal dari ligamentum sakrouterina dan dilakukan pemeriksaan imunohistokimia untuk melihat ekspresi TGF-\B1 dan tropoelastin.

**Hasil**: Dari 30 subjek didapatkan ekspresi TGF $\beta$ 1 kuat pada 12 (40%) subjek, ekspresi TGF $\beta$ 1 lemah pada 18 (60%) subjek, ekspresi tropoelastin kuat pada 18 (60%) subjek dan ekpresi tropoelastin lemah pada 12 (40%) subjek. Dilakukan uji korelasi non parametrik dan didapatkan korelasi positif dengan nilai p=0,014 dan r=0,444. Derajat korelasi sedang.

Kesimpulan: Terdapat korelasi positif antara ekspresi TGF-\B1 dengan ekspresi tropoelastin pada ligamentum sakrouterina pasien dengan prolaps uteri dengan derajat korelasi sedang.

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Kata kunci: prolapsus uteri, Transforming Growth Factor Beta 1, tropoelastin

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### INTRODUCTION

Uterine prolapse is the descent of the uterus from its normal position in the pelvic cavity into the vaginal canal or even out of the vagina. It is caused by the weakness of the muscles, fascia, and supporting ligaments.<sup>1</sup>

According to *Badan Pusat Statistik* (BPS), the life expectancy of Indonesian women will increase from 66.8 years in 2010-2015 to 72.2 years in 2030-2035. The longer life expectancy is, the more effort to improve the quality of life. Therefore, it is necessary to change the passive treatment paradigm of the pelvic organ prolapse (POP) to active preventive treatment.<sup>1-3</sup>

The prevalence of uterine prolapse was varied. In 2002, the Women's Health Initiative (WHI) reported the prevalence of uterine prolapse was 14% in women 50-79 years old; meanwhile, study in Dr. Hasan Sadikin hospital in 2006 found there were 30/1,455 cases (2.1%) of uterine prolapse and 13 of them was treated by vaginal hysterectomy. Fauzi A and Anhar K reported 43 cases of uterine prolapse in Dr. Mohammad Hoesin hospital Palembang during 1999-2003.<sup>3-6</sup>

Several risk factors of uterine prolapse are age, occupation, weight, parity, type of delivery, vaginal delivery using a vacuum or forceps, birth weight, surgical history, history of medical illness and menopausal status. Actually, all of the risk factors may cause damage to the basic pelvic connective tissue, especially the cardinal and sacrouterine ligament.<sup>6,7</sup>

Sacrouterine ligament supporting and maintaining the uterus makes it in the appropriate position of the pelvic. Fixation function of the uterus is essential to prevent the further POP. Sacrouterine ligament is the first level in the pelvic support system according to Delancey. Sacrouterine ligament is composed of cells, extra cellular matrix consisting of fibers (collagen, elastin and reticulin), proteoglycans and glycoproteins. Abnormalities of the connective tissue of the pelvic floor and vaginal seem to have an important role in the pathophysiology of basic pelvic disorders.<sup>7-11</sup>

Transforming Growth Factor Beta 1 (TGF- $\beta$ 1) has a key role in the regulation of the extra cellular matrix and enzymes component. This TGF- $\beta$ 1 increases elastin matrix regeneration in vascular smooth muscle cells and a layer of skin fibroblasts.<sup>1</sup> Elastin is a protein in the extracellular matrix that affects the tension and elasticity of the tissue. Elastin plays an important role in maintaining the pelvic organs in order to be in the normal position. Women with genetic metabolic disorders, such as cutis Laxa, showed an increased risk for the occurrence of POP.<sup>11-16</sup>

Takacs P in 2011 reported a positive correlation between the expression of TGF- $\beta$ 1 and mRNA expression of elastin in the tissues of the vagina without pelvic floor disorders. In vitro, TGF- $\beta$ 1 is a potential regulator of the elastin production from the extra cellular matrix not only in pathological states, but also in the normal condition.<sup>14</sup> Previous studies revealed that the TGF- $\beta$ 1 increased the production of elastin in fibroblasts and vascular smooth muscle cells via mRNA elastin stabilization.<sup>17-19</sup> In addition, a decrease of the expression of elastin found in fibroblasts was derived from cardinal ligaments in POP women.<sup>1</sup>

Based on background above, this study aims to determine the correlation of TGF- $\beta$ 1 expression and tropoelastin expression of the sacrouterine ligament in women with uterine prolapse.

## METHODS

The cross sectional study was carried out at the Department of Obstetrics and Gynecology Dr. Mohammad Hoesin hospital Palembang. The study was performed from December 1<sup>st</sup>, 2014 to July 31<sup>st</sup>, 2015 whereas only 30 subjects met the inclusion criteria.

The inclusion criteria were uterine prolapse women who were willing to sign an informed consent; while, the exclusion criteria were patients with connective tissue disorders or were undergoing the estrogen replacement therapy. They were doing the gynecological examination to determine the stage of uterine prolapse based on Pelvic Organ Prolapse Questionnaire (POP-Q) system.

We took the sacrouterine ligament in distal portion along 1 cm through total vaginal hysterectomy. We fixed the ligament into 10% buffered formalin, did the paraffin blocks manufacture, hematoxylin and eosin staining and also immunohistochemical examination to assess the expression of TGF-B1 and tropoelastin. Examination of TGF-B1 expression used monoclonal antibodies ab66403 and optimization of 1:75 was performed as the control. For the expression of tropoelastin, we used the monoclonal antibodies ab21598 with optimization of 1: 100. Mixture was read by a light microscope (Olympus BX 51 brands) with 400 times magnification and wide field of view 0.65 mm. The expression of TGF-B1 and tropoelastin were assessed by using a score based on the proportion of smeared cells and the intensity of light brown staining to dark brown. The interpretation was positive 1 (weak) if the smeared brown/vaguely into the cytoplasm extra cellular matrix  $\leq 10\%$  and positive 2 (strong) if the smeared dark brown/ darkness of the cytoplasm and the extra cellular matrix of >10%.

## RESULTS

Table 1.	Subject Characteristics
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Number	Percentage
0	0
10	33.3
15	50.0
4	13.3
1	3.3
0	0
10	33.3
20	66.7
	Number           0           10           15           4           1           0           10           20

72 Alvilusia	et	al
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Menopausal status					
Premenopause	1	3.3			
Menopause	29	96.7			
Type of delivery					
Never	0	0			
Spontaneous	30	100.0			
vacuum/Forceps	0	0			
Sectio caesarean	0	0			
History of giant baby					
Not known	11	36.7			
< 3500 g	7	23.3			
>3500 g	12	40			
Body Mass Index					
Normal	16	53.3			
Over weight	14	46.7			
Degree of uterine prolapse					
II	6	20.0			
III	15	50.0			
IV	9	30.0			

**Table 2.** Description of TGF- $\beta$ 1 and Tropoelastin Expression on Sacrouterina Ligament

No.	Expre of TG	Expression of TGF-β1		ssion oelastin
Subject <sup>–</sup>	Strong	Weak	Strong	Weak
1.		+1		+1
2.		+1		+1
3.		+1		+1
4.		+1		+1
5.		+1		+1
6.	+2		+2	
7.		+1		+1
8.	+2		+2	
9.		+1	+2	
10.	+2			+1
11.		+1	+2	
12.		+1	+2	
13.		+1		+1
14.		+1		+1
15.		+1		+1
16.	+2		+2	
17.		+1		+1
18.	+2		+2	
19.	+2			+1
20.	+2			+1
21.		+1		+1

Indones J
Obstet Gvnecol

22.		+1		+1
23.		+1	+2	
24.		+1		+1
25.	+2			+1
26.	+2		+2	
27.		+1		+1
28.	+2		+2	
29.	+2		+2	
30.	+2		+2	

**Table 3.** Correlation of TGF- $\beta$ 1 and Tropoelastin Expression on Sacrouterine Ligament.

		Tropoelastin expression		
		Strong (%)	Weak (%)	
TGF-β1	Strong	8 (66.7)	4 (33.3)	
expression	Weak	4 (22.2)	14 (77.8)	
Total		12 (40.0)	18 (60.0)	

The subject characteristics, description of TGF- $\beta$ 1 and tropoelastin expression on sacrouterine ligament, and the correlation between both of them were shown in Table 1, 2 and 3; respectively. The statistical test result showed there was a relation-ship between the expression of TGF- $\beta$ 1 and tropoelastin (p=0.024). Non-parametric test performed and found that there was a positive correlation between the expression of TGF- $\beta$ 1 and tropoelastin in sacrouterine ligament with moderate correlation (p=0.014; r=0.44).

### DISCUSSION

The imbalance between the synthesis and degradation of matrix extracellular components can cause POP.<sup>20</sup> In our study, we found there were 6 samples obtained the different expression between TGF- $\beta$ 1 and tropoelastin; it indicated the existence of another cytokine that induced the expression of tropoelastin.<sup>20</sup>

Cytokines which can regulate the components of the extracellular matrix are TGF- $\beta$ 1, thrombospondin I (TSP-I), matrix metalloprotease (MMPs), insulin growth factor (IGF-I), basic fibroblast growth factor (bFGF), Heparin binding Epidermal Growth Factor (Hb-EGF), Epidermal Growth Factor (EGF), Transforming Growth Factor Alpha (TGF- $\alpha$ ), Tu-



mor Necrosis Factor Alpha (TNF- $\alpha$ ). All of these cytokines may be involved in the regulation of elastin.<sup>20-22</sup> Further studies should be conducted to find the role of cytokines in the regulation of extra cellular matrix, especially on the tropoelastin components.

The correlation between the expression of TGF- $\beta$ 1 and tropoelastin did not explain the causal relationship of the two components. The weakness of our study was that we did not do the comparative assay of the TGF- $\beta$ 1 and tropoelastin expression in sacrouterine ligament from patients without uterine prolapse.

## CONCLUSION

There is a positive correlation between the TGF- $\beta$ 1 and tropoelastin expression of sacrouterine ligament in uterine prolapse with moderate correlation.

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**Research Article** 

## Factors Related to the Number of Antral Follicles on In-Vitro Fertilization (IVF)

Faktor yang Terkait dengan Jumlah Folikel Antral pada Fertilisasi in Vitro

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#### Abstract

**Objective**: To determine factors which are related to the number of antral follicles on infertile patients.

**Method**: This cross sectional study was conducted in In-Vitro Fertilization (IVF) clinic of Graha Tunjung, Sanglah hospital, Bali. All fertile patients following the IVF program were calculated the number of antral follicles in both ovarian using transgene USG. This sample was recruited by random sampling from April 1<sup>st</sup>, 2001 to April 30<sup>th</sup>, 2011. We analyzed the data using Chi square test through SPSS for Windows 17.0 version.

**Result**: Of 102 samples, the mean of patients' age was 32.9% (SD 4.6) years old. From 72 patients (70.6%) experienced above 3 years of infertile period, the primary infertile was on 69 patients (67.7%). There was a relationship between patients' age and the number of antral follicles significantly (prevalence ratio (PR) 1.41; 95% CI 1.11-1.79). Meanwhile, the number of antral follicles and type of infertile (PR 1.02; 95% CI 0.76-1.37) also infertile period (PR 0.95; 95% CI 0.72-1.27) were not associated significantly.

**Conclusion**: Patients' age has an association with the number of antral follicles on IVF.

[Indones J Obstet Gynecol 2016; 4-2: 75-77]

**Keywords**: age, infertile, infertile period, number of antral follicles and type of infertile

#### Abstrak

**Tujuan**: Mengetahui faktor yang berhubungan dengan jumlah folikel antral pada pasien infertil.

**Metode**: Studi potong lintang ini dilaksanakan di Klinik Bayi Tabung Graha Tunjung, RSUP Sanglah, Bali. Sampel penelitian ini adalah semua pasien infertil yang mengikuti program fertilisasi in vitro dengan menghitung jumlah folikel antral pada kedua ovariumnya menggunakan USG transvaginal. Pengambilan sampel dilakukan dengan cara random sampling mulai dari 1 April 2001 hingga 30 April 2011. Analisis data memakai uji Chi Square dengan bantuan SPSS untuk windows versi 17.0.

Hasil: Dari 102 sampel penelitian rerata usia pasien adalah 32,9% (SD 4,6) tahun. Pasien yang mengalami infertil lebih dari 3 tahun adalah 72 (70,6%) orang dengan infertil primer sebanyak 69 (67,7%) orang. Terdapat hubungan antara usia ibu dengan jumlah folikel antral secara bermakna (PR 1,41; IK 95% 1,11-1,79). Tidak terdapat hubungan antara jenis infertil dengan jumlah folikel antral (PR 1,02; IK 95% 0,76-1,37). Tidak terdapat hubungan antara lama infertil dengan jumlah folikel antral (PR 0,95; IK 95% 0,72-1,27).

Kesimpulan: Usia pasien berhubungan dengan jumlah folikel antral pada fertilisasi in vitro.

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Kata kunci: infertile, jumlah folikel antral, lama infertile, tipe infertile dan usia

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## INTRODUCTION

Infertility is defined as not getting pregnant (conceive) after one year of unprotected intercourse. Nowadays, it becomes the concern of many married couple. A survey conducted by Statistics Centre Agency in 1995, the infertility rate in Indonesia was 12%.

Assisted Reproductive Technology (ART), especially in-vitro fertilization (IVF) or well-known as "test tube baby" is one way to solve the infertility in advanced. Unfortunately, there are several factors influencing the success of this IVF such as age, infertility period, and type of infertility.<sup>1,2</sup> The advanced technology in IVF has enabled us to assess how much the possibility of success gained on every patients and it can determine the proper time to follow the program. It is very important to perform a diagnostic examination based on evidence based to escalate the chance to get pregnant. The easy and important examination to predict the success of stimulating ovarian is the number of antral follicles. Meta-analysis study reported that the number of antral follicles was an excellent predictor in ovarian stimulation. The increase of age was associated with the number of antral follicles; however, study carried out in San Francisco rejected this statement.<sup>1,3,4</sup>

Indones J Obstet Gynecol

According to the previous results above, this study aims to determine factors contributing to the number of antral follicles to increase the success rate of IVF.

## METHOD

This cross sectional study was conducted in IVF clinic of Graha Tunjung, Sanglah Hospital, Bali. All fertile patients following the IVF program were calculated the number of antral follicles in both ovarian using transgene USG. This sample was recruited by random sampling from April 1<sup>st</sup>, 2001 to April 30<sup>th</sup>, 2011. We analyzed the data using Chi square test through SPSS for Windows 17.0 version.

## RESULTS

Of 102 infertile patients joining to the IVF program in IVF Graha Tunjung Sanglah Hospital Bali, the mean of age was 32.9 (SD 4.6) years old and the infertile period was 6.1 (SD 4.0) years.

Table 1 explained the relationship of age, type of infertile, and infertile period with the number of antral follicles on IVF. From Chi-square test, only age had association with the number of antral follicles with prevalence ratio (PR) 1.41 (95% CI 1.11-1.79; p=0.016).

## DISCUSSION

The result showed that 45 patients (44.1%) younger than 36 years old had less than 10 antral follicles on IVF and only 29 patients (28.4%) had more than 10 follicles. Other study pointed out that the number of antral follicles on young female was 3-11 per ovarian. Antral follicles calculated for superovulation process on both ovarian were the primordial follicles which would be developed to be-

come a mature follicle. The ideal number of antral follicles on both ovaries after IVF was 11-30 follicles; less than 5 follicles signed poor response and more than 30 follicles indicated excellent response.

The older the patients are, the less possibility to get conceive. A study revealed that before 24 years old, the fertility rate of women reached 100% and it decreased gradually as the increase of age and finally, after 50 years old, the fertility rate became 0%. Another study showed that women would experience the decrease of fertility at 37.5 years old, whereas the pregnancy rate over 1 year under 31year-old women was 74% and they fell into 54% above 35-year old women.<sup>1,3-7</sup> This fact is due to the lack number of primordial follicles. The acceleration loss of follicle occurred at 37 years old and raised after 10 to 12 years of menopause. The number of antral follicles sized  $\geq 2 \text{ mm}$  which was assessed by transvaginal USG decreased 60% between 22 and 42 years old. In accordance with the number of antral follicles, the ovum quantity and quality was worsened started from 30 years old and much more after 40 years old.

The impaired of fecundity increased from 2% at the age of 15-19 years old to 28% at the age of more than 35 years old.<sup>6</sup> The decrease number of antral follicles was in parallel to the decrease in the quality of the oocyte. Similar study had been conducted showing that there was a relationship between age and the number of antral follicles, which there was a decrease in the number of antral follicles from 0.35 to 0.95 per year. According to a study in India, it had been found that the difference in the number of antral follicles were associated with age probably because there were differences in race and geography of the region. There was a significant decrease in fertility in line with the increase of age. Around 11% women did not get

Table 1.	Relationship of Age,	Type of Infertile,	and Infertile I	Period with the	Number of Antra	al Follicles on IVF.
		- )				

	Number of Follicles					
Characteristics		≤ <b>10</b>	>10	PK	95% CI	р
Age	>36 years old	24	4	1.41	1 1 1 1 70	0.016
	≤36 years old	45	29		1.11-1.79	0.016
	Primary	47	22	1.02		0.004
Type of Infertile	Secondary	22	11		0.76-1.37	0.884
Infertile period	>3 years	48	24	0.95		0 = 40
	≤3 years	21	9		0.72-1.27	0.743

Factor related to the number of antral follicles 77

pregnant after 40 years old and 87% was infertile at 45 years old.<sup>8-11</sup>

The result from this study revealed that the type of infertility did not affect the number of antral follicles as either primary or secondary infertility in married couples. It was clearly showed that the number of antral follicles did not have an association with the type of infertility because one year did not describe how long the couple was married and tried to get pregnant. It could happen at 25vear-old women who would not like to have a child after one year of marriage although they had good number of antral follicles. Apart from that, other same age women who suffered from diseases, for example Polycystic Ovarian Syndrome (PCOS), had many number of antral follicles, but it looked the small size. The duration of infertility did not provide information on whether the problem either in male or only female, even both of them. There might be heavy biological problem if the duration was longer than 3 years.<sup>12-18</sup>

## CONCLUSION

Patients' age has an association with the number of antral follicles on IVF.

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**Research Article** 

## Postplacental IUD Insertion Using Ring Forceps versus Push and Push Technique

Insersi IUD Pascaplasenta Persalinan Pervaginam Menggunakan Teknik Ring Forceps dan Teknik Push and Push

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#### Abstract

**Objective**: To compare IUD-endometrium (ED) distance and the incident of malposition postplacental CuT-380A IUD insertion in vaginal delivery between ring forceps technique and push and push technique.

**Method**: This study was a double-blind randomized control trial, performed in September 2014 until March 2015 at Dr. Kariadi Hospital. Ring forceps and push and push insertion technique groups consisted of 25 subjects in each group. Follow-up was performed at 1-2 weeks, 6-8 weeks and >12 weeks after insertion.

**Result**: The mean of IUD-ED distance in push and push group was shorter (but not statistically significant) than ring forceps group. The IUD-ED distance was at 1-2-week follow-up 4.1 (2.2) vs. 4.9 (3.4) mm; p=0.208, at 6-8-week follow-up: 2.6 (1.8) vs. 3.2 (3.7) mm; p=0.452, and at > 12-week follow-up: 0.9 (0.8) vs. 1.0 (0.9) mm; p=0.427, respectively. Malposition was found in 1-2-week follow-up, but the IUD was changed to the normal position (sagital position in uterine fundus) at 6-8-week and >12-week follow-up. Up to 3 months of follow-up, there was no occurrence of perforation, expulsion or pregnancy in both groups. Most of subjects (56% in the ring forceps, 68% in push and push groups) did not feel painful during IUD insertion.

**Conclusion**: Push and push insertion technique clinically tends to produce IUD-ED distance shorter than ring forceps technique. Both techniques are comfortable, safe and effective.

[Indones J Obstet Gynecol 2016; 4-2: 78-87]

**Keywords**: immediate postplacental IUD insertion technique, IUDendometrium distance, IUD malposition, push and push technique, ring forceps technique

#### Abstrak

**Tujuan**: Membandingkan jarak IUD-endometrium (ED) dan kejadian malposisi pada insersi IUD CuT-380A pascaplasenta pada persalinan pervaginam antara teknik 'ring forceps' dan teknik 'push and push'.

**Metode**: Penelitian ini merupakan uji klinis tersamar ganda, dilakukan pada bulan September 2014 hingga Maret 2015 di RSUP Dr. Kariadi. Kelompok 'ring forceps' dan kelompok 'push and push' masing-masing terdiri dari 25 subjek. Pemantauan dilakukan pada 1-2 minggu, 6-8 minggu dan >12 minggu pascainsersi.

Hasil: Rerata jarak IUD-endometrium kelompok 'push and push' lebih pendek daripada kelompok 'ring forceps', tetapi tidak bermakna secara statistik Masing-masing pada 1-2 minggu pascainsersi 4,1 (2,2) vs 4,9 (3,4) mm; p=0,208, 6-8 minggu pemantauan: 2,6 (1,8) vs 3,1 (3,7) mm; p=0,452 dan pada >12 minggu: 0,9 (0,8) vs 1,0 (0,9) mm; p=0,427. Kejadian malposisi ditemukan dalam 1-2 minggu pemantauan (satu subjek dalam setiap kelompok), tetapi pada pemantauan 6-8 minggu dan >12 minggu telah berubah menjadi posisi normal (posisi IUD sagital pada fundus uteri). Sampai dengan 3 bulan pemantauan tidak didapatkan kejadian perforasi, ekspulsi maupun kehamilan pada kedua kelompok. Sebagian besar subjek (56% pada kelompok 'ring forceps' dan 68% pada kelompok 'push and push') setelah insersi dilakukan menyatakan bahwa prosedur insersi terasa tapi tidak nyeri.

Kesimpulan: Teknik insersi push and push cenderung menghasilkan jarak IUD-endometrium yang lebih pendek daripada teknik ring forceps. Kedua teknik tersebut merupakan prosedur yang nyaman, aman dan efektif.

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Kata kunci: IUD pascaplasenta, jarak IUD-endometrium, malposisi IUD, teknik insersi 'ring forceps', teknik 'push and push'

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## INTRODUCTION

The high rate of growth population (1.49%) and maternal mortality rate (MMR) (359/100,000) in Indonesia increase the awareness of family planning program. Long acting reversible contraceptives (LARC) device is one of choices for the family planning program.<sup>1-3</sup> High unmet need, short birth spacing and high discontinuitation rates are asso-

ciated with the increased risk of maternal and perinatal morbidity and mortality.<sup>4</sup> Intrauterine device (IUD) as a long acting reversible contraceptive device is the best choice for postpartum women.<sup>5</sup> This device has some advantages including not interfering with lactation, performing as soon after placental delivery, protecting against unwanted pregnancy, maintaining birth spacing, not disrupting to mothers activity while taking care of their babies.<sup>6,7</sup> Additionally, IUD also has high effectivitity, safety, and excellent reversibility.<sup>8</sup>

According to study by *Badan Koordinasi Keluarga Berencana Nasional* (BKKBN) about postpartum and post-miscarriage contraceptive service at 22 hospitals in 14 provinces from 2008 to 2009, the rate of using contraception was only 5-10%. It proved that the women lacked of concern to this service.<sup>9</sup>

The side effects and complications of IUD are not protecting from sexual transmitted diseases, more prone to malposition and perforation. The most frequent cause of IUD failure is an expulsion. Factors that affect the occurrence of expulsion is the clinical skills competency of operator and timing of insertion.<sup>10,11</sup> The insertion of IUD can be performed immediately after placental delivery/ immediate post placental insertion (IPPI), 48 hours postpartum/immediate postpartum (IPP), 4 to 8 weeks postpartum/late postpartum insertion and interval insertion.<sup>8,12,13</sup> Most of women still prefers to interval insertion, due to low expulsion rate (3-13%) compared with postpartum insertion, IPPI (9.5 to 12.5%), IPP (25-37%). Meanwhile, late postpartum insertion is not recommended because of high rates of expulsion and perforation.<sup>14</sup> However, postpartum insertion, especially IPPI, has some advantages compared with interval insertion, namely increasing the participation rate because it is inserted directly postpartum, minimalizing the painful sensation and certainly, patients feel safety.

An observational cohort study in Dr. Cipto Mangunkusumo General Hospital in 1994, the doctors inserted the MLCU-250 IUD using fore and middle fingers to the uterine cavity as soon as possible after placental delivery. After three-month follow-up, the expulsion rate was 7.1% of the 75 subjects; however, the loss to follow-up patients reached 40%.15 Xu in 1996 compared between postplacental CuT-380A IUD insertion using finger and ring forceps; the result showed there were no significant differences in the numbers of IUD expulsion (13.3% for finger and 12.7% for ring forceps).<sup>16</sup> Since 2009, in Dr. Kariadi Hospital Semarang, Hary Tjahjanto introduced two techniques of postplacental CuT-380A IUD insertion by blinding method. In the beginning, insertion technique that had been used on postplacental service was using ring forceps (10 inches or 25.5 cm length) and a

new modification insertion technique was applied using combination of ring forceps and standard IUD inserter (standard inserter tube and plunger rod). It is known as push and push technique. During insertion procedure, insertion consists of three steps to puss the ring forceps and standard inserter into uterine cavity to reach the center of uterine fundus. Automatically, the IUD horizontal arm enters the narrow gap between the anterior and posterior wall of uterine fundus; finally, it can attach to the endometrium of the uterine fundus. The prospective cohort study including 108 subjects with the length of follow-up  $\geq 12$  months, showed the satisficating results (no occurrence of pregnancy, continuation rate reached 94.1%, and expulsion rate was low (2.86%). There was no perforation reported and only 5.6% patients lost to follow-up.<sup>17</sup> The aim of this study is to compare IUD to endometrium distance and the incident of malposition postplacental CuT-380A IUD between ring forceps and push and push technique.

## **METHODS**

This double blind randomized controlled trial study was performed in Obstetrics and Gynecology Department Dr. Kariadi Hospital Semarang from September 2014 to March 2015. There were 50 women consisting of 25 women for each group (ring forceps and push and push technique). The inclusion criteria were women delivering vaginally, approving to be the participants and willing to undergo the procedure until 3 months of follow-up for the IUD insertion. Apart from that, we included all women with gestational age  $\geq$ 37 weeks, Hb  $\geq$ 8 g%, body mass index <40 kg/m<sup>2</sup>, birth weight <4,000 g, the residence of the Semarang to ease the follow-up. The exclusion criteria were premature rupture of membranes  $\geq 18$  hours, body temperature  $\geq$ 38°C, purulent vaginal discharge, tumors or genital tract malignancy, postpartum hemorrhage, total perineal rupture, history of diabetes mellitus, blood clotting disorders, and failure of using IUD previously.

## **Insertion Procedures**

## Push and push insertion technique

**Preparation:** Cutting the IUD strings about 6 cm from the end of vertical stem or in the middle of a long string. The string and vertical stem is inserted

into the tube IUD inserter, the horizontal arm of the IUD remains outside of the tube inserter yet. Entering the plunger rod into the inserter tube, clamped inserter tube within a position tip of ring forceps in line with the horizontal arm or slightly lower than the outer edge of ring forceps tip (Figure 1). Cleaning with the antiseptic solution for the pubic area, labia, perineum, vaginal wall and cervix.

**Procedures:** Firstly, exploring the uterine cavity for the rest of amnion membrane and residual blood clot by using the fore and middle fingers of the right hand (or dominant hand). In supine position, inserting the two fingers into the vagina up to the fingertips touching the edge of the muscle wall of the uterine corpus (fibromusculair junction/FMJ). Fore and middle fingers widen opening the FMJ circle, push down the palms (keep in supination position) to open the vagina. By using the no-touch technique, the left hand hold the ring forceps, bring the ring forceps to insert the tip of the forceps ring along the base of the fore and middle fingers between the fingers until it reaches the circular opening of FMJ. After the end of ring forceps moves toward the uterine cavity, using the fingers of the right hand (first, fourth, and fifth fingers) to hold the ring forceps to maintain the position of it (Figure 2). Then, using the left hand to push the ring forceps to move more getting into uterine cavity, while the fingers of the right hand direct and maintain the position of the ring forceps. After that, the left hand presses the fundus and using the right hand to push the ring forceps to move more getting into uterine cavity. Performing repeatedly until the end of the ring forceps reaches the fundus and we can feel the pressure on palpation of the fundus using the left hand. The next step is holding the inserter by the left hand, opening the ring forceps using the right hand (opening width 1-2 cm), and pushing the inserter tube to the uterine fundus wall. After that, holding and maintaining inserter position by using left hand, removing ring forceps, and pushing the inserter tube using right hand so that the tube inserter tip moves into the narrow gap between the anterior and posterior uterine fundus wall in conjunction with fundus control using the left hand. Holding the plunger rod using the right hand, followed by pulling inserter tube so that the proximal end of the tube touches ring the plunger rod. Finally, pulling out the plunger rod out of inserter tube and the inserter tube from the uterine cavity.

Besides, we enter the ring forceps slowly into the uterine cavity and push the ring forceps and stardard inserter after the insertion of the IUD in the center of fundus. The ring forceps and the inserter are inserted into the uterine cavity to reach the fundus. Then, when the ring forceps is opened, the inserter is encouraged to move in the gap of fundus wall and after the ring forceps is removed from the uterine cavity, the inserter is driven again to make sure the attachment on the uterine fundus wall. Pushing the ring forceps or inserter tube must be accompanied by fundus palpation on the abdominal wall with left hand. It aims to ensure the position of ring forceps tip on the fundus and prevent perforation.

We have to make sure that the uterine cavity has been confirmed cleaned from blood clots and rest of the amnion to prevent the expulsion during puerperium period. Therefore, this insertion is not limited to the first 10 minutes after delivery of the placenta.



Figure 1. How to put the IUD in the inserter tube.



Figure 2. Fingers position of the right handfor holding the ring forceps.

## **Ring Forceps Insertion Technique**

Insertion technique using ring forceps is performed in the same way with the push and push technique. The difference is the instrument used only ring forceps without the standard inserter. The insertion of IUD is performed by residents who are considered competence to perform the postplacental CuT-380A IUD insertion.

## **Data Analysis**

We did the computerized randomization for the fifty sealed envelopes containing the CuT-380A distribution from Indonesia National Population and Family Planning Boards (BKKBN), sheets of informed consent, control cards, sheets of patient follow-up and insertion techniques. If there were subjects fulfilling the criteria, the operators took the envelope and performed the insertion technique in accordance with techniques which have been listed in the envelope and the operators filled out the study data sheet. In addition to requiring state of clean uterine cavity, we prescribed the uterine tonic contraction intramuscular injection of oxytocin after delivering the baby, metilergometrin maleate intavaginally, and also intramuscular or intravenous injection of oxytocin during or after delivering the placenta. It was to reduce the risk of expulsion of IUD. Furthermore, to ensure that uterine involution was maintained, we administered the metilergometrin maleate tablets (1/2-1 tablet three times a day for 1-2 weeks). After insertion, subjects were interviewed about their experience of pain during insertion. The operator recorded the insertion interval and birth outcomes in patient follow-up sheet. The subjects of the study were given a control card to be taken on the next follow-up period.

Patients went for control for the first follow-up (FU1) at  $1^{st}-2^{nd}$  weeks, second follow-up (FU2) at  $6^{th}-8^{th}$  weeks and third follow-up (FU3) at  $\ge 12^{th}$ 

weeks (3 months) after insertion. In each followup, a physical examination and an ultrasound were undergone to determine the IUD position and the distance of the IUD-ED, and also the adverse events related to the insertion technique. This study used intention to treat analysis. Follow-up following postpartum IUD insertion could be done clinically by ultrasonography (USG). Some studies stated that the ultrasound was better in follow-up the position of IUD than clinical examination. Evaluation of the IUD position was measured by assessing IUD-endometrium (IUD-ED) distance as gold standard.<sup>18-21</sup> Therefore, we analyzed the postpartum IUD insertion techniques with push and push and ring forceps technique.

The IUD-ED distance was conducted using abdominal ultrasonography. The malposition of IUD was when the IUD was not located in the middle of the uterine cavity (located in the lower segment of the uterus, cervix, rotated, the influx of part of the body or arms IUD into the myometrium) by ultrasonography. Insertion interval was the time between delivery of the placenta with the completion of IUD insertion in minutes. We did the bivariate analysis to determine the difference between the IUD-ED distance among both groups using Independent t-test and Mann-Whitney test. The significance was determined based on the value of p <0.05. Malposition event and other side effects were reported. Overall data were analyzed using SPSS.



Figure 3. Systematic Overview of Uterine Sagital Sonography with intrauterine IUD.<sup>22</sup>

Show some distance proportions: 1: IUD-fundus, 2: Myometrium thickness, 3: Endometrium thickness, 4: IUD-endometrium, 5: IUD-myometrium. (Taken from: Faundes D et al. No Relationship Between the IUD Position Evaluated by Ultrasound and Complaints of Bleeding and Pain. Contraception. 1997; 56: 43-7)

## RESULTS

## **Characteristics of the Subjects**

Based on the clinical characteristics of the subjects (Table 1), there were no statistically significant differences in the characteristics of age, BMI, occupation, education, parity and gestational age between the two insertion techniques. The 20% subjects preferred to use postplacental IUD contraception

during antenatal visit, while the rest chose to use in the hospital (at admission or during labor). The clinical characteristic variables showed significant differences between two insertion groups (p<0.05). The characteristic of clinical features and outcomes of labor were not statistically significant differences between the two insertion groups in terms of premature rupture of the membrane (PROM) incidence, vaginal delivery types and birth weight.

Charactoristics	Ring forceps group		Push and push group		
character istics	Mean (SD); median (min-max)	n (%)	Mean (SD); median (min-max)	n (%)	- P
Age (years old)	27.0 (6.3); 27.0 (16.0-36.0)		27.7 (5.9); 28.0 (15.0-39.0)		0.613ª
BMI (kg/m <sup>2</sup> )	26.7 (4.3); 25.7 (22,0-38,3)		25.0 (3.1); 24.6 (19.2-35.1)		0.107 <sup>a</sup>
Occupation					
Housewife		19 (76.0)		13 (52.0)	
Labor		3 (12.0)		1 (4.0)	0.140 <sup>b</sup>
Private employee		3 (12.0)		7 (28.0)	
Governm. employee		0 (0.0)		4 (16.0)	
Education					
Elementary		2 (80)		1 (4.0)	
Junior high school		5 (20.0)		4 (16.0)	0.742 <sup>b</sup>
Senior high school		15 (60.0)		14 (56.0)	
University		3 (12.0)		6 (24.0)	
Parity					<b>0.833</b> ª
0		13 (52.0)		11 (44.0)	
1		7 (28.0)		9 (36.0)	
> 1		5 (20.0)		5 (20.0)	
Gestational age	38.6 (1.5); 38.0 (3743.0)		39.0 (1.1); 39.0 (37.0-41.0)		0.193ª
PROM					0.196 <sup>b</sup>
No		16 (64.0)		21 (84.0)	
Yes:	7.5 (3.2); 8.0 (3.0-12.0)	9 (36.0)	5.5 (1.7); 5.0 (4.0-8.0)	4 (16.0)	0.260 <sup>c</sup>
< 6 hours		2 (8.0)		3 (12.0)	
≥6 hours		7 (28.0)		1 (4.0)	
Choose IUD					
ANC		0 (0.0)		10 (40.0)	
Inpatient		9 (36.0)		2 (8.0)	0.002 <sup>b</sup>
During labor		7 (28.0)		7 (28.0)	
Delivery		9 (36.0)		6 (24.0)	
Type of delivery					
Spontaneous		19 (76.0)		20 (80.0)	
Vaccum extraction		5 (20.0)		5 (20.0)	$1.000^{b}$
Breech		1 (4.0)		0 (0,0)	
Birthweight (gr)	2,916.0 (409.7); 3,000.0		3,000.6 (363.5); 3,000.0		0.415 <sup>a</sup>
	(2,100.0-3,600.0)		(2,300-3,600)		

Table 1. Clinical Characteristics of the Subjects.

a. Mann-Whitney test, b. Pearson chi-square test, c. Independent t-test

## **Insertion Procedure**

In Table 2, we obtained an average insertion interval between ring forceps group (4.8 minutes) and push and push group (6.6 minutes). Insertion interval on all subjects in both study groups was within a maximum of 10 minutes after placental delivery. The ease of insertion mean score in ring forceps group was 7.5; while in push and push group was 6.7. The ease of insertion in both groups had a statistically significant difference (p<0.05) whereas the ring forceps technique was easier than push and push technique.

In the ring forceps insertion procedure technique, after touching the tip of fundus wall, we released the IUD; meanwhile, in the push and push insertion technique, the forceps was opened after touching uterine fundus, then the inserter tube and plunger rods together could still be pushed in again closer to the fundus. The mean length distance the entry of inserter in final push was 1.9 cm. The majority of subjects in both groups stated that they did not feel painful during the insertion procedure. Insertion pain in both groups was different, but it was not statistically significant (p>0.05).

# Postplacental IUD insertion 83

## **Follow-up**

## Loss to follow-up

Of 25 subjects in the ring forceps group, at the first follow-up 23 (92.0%) subjects attended, at  $2^{nd}$  follow-up 22 (88.0%) subjects attended, and 21 (84.0%) subjects attended at  $3^{rd}$  follow-up. Meanwhile, in the push and push group, the subjects came to the first, second, and third follow-up were 24 (94.0%), 22 (88.0) and 20 (80%); respectively. Therefore, the overall percentages of loss to follow-up on were 6.0%, 12.0% and 18.0%; respectively for the first, second and third follow-up.

## **Expulsion and perforation**

We did not find the expulsion and perforation incident.

## **IUD-Endometrium distance**

The distance difference was not statistically significant (p > 0.05).

### **Table 2.** Insertion Process Characteristics.

Insertion techniques **Ring forceps group** Push and push group р Mean (SD); median (min-max) Mean (SD); median (min-max) n (%) n (%) Insertion interval (minutes) 4.8 (1.5); 5.0 (3.0-10.0) 6.6 (1.7); 7.0 (3.0-10.0) <0.001<sup>a</sup> 7.5 (0.7); 8.0 (6.0-9.0) 6.7 (0.7); 7.0 (5.0-8.0) Easiness (1-10) < 0.001ª Length distance the entry of 1.9 (0.6); 2.0 (1.0-3.0) inserter in final push (cm) Insertion pain 5 (20.0) Not feel 4 (16.0) Feel but not pain 14 (56.0) 17 (68.0) 0.670<sup>b</sup> Uncomfortable 5 (20.0) 3 (12.0) Pain 1 (4.0) 1 (4.0)

a. Mann-Whitney test, b. Pearson chi-square test

#### **Table 3.** IUD-Endometrium (IUD-ED) Distance.

	Insertion		
IUD-ED (mm)	Ring forceps	Push and push	р
	Mean (SD); median (min-max)	Mean (SD); median (min-max)	
1st Follow-up (FU1)	4.9 (3.4); 5.8 (0.0-13.0)	4.1 (2.2); 4.2 (0.0-8.1)	0.208ª
2 <sup>nd</sup> Follow-up (FU2)	3.2 (2.3); 3.7 (0.0-7.0)	2.6 (1.8); 2.5 (0.0-6.6)	0.452 <sup>b</sup>
3rd Follow-up (FU3)	1.0 (0.9); 1.2 (0.0-2.7)	0.9 (0.8); 1.1 (0.0-2.7)	0.427 <sup>a</sup>

a. Mann-Whitney test, b. Independet t-test

### 84 Tjahjanto and Rizal

Indones J Obstet Gynecol



**Figure 4.** Abdominal USG Appearance in a Patient of Push and Push Technique Group at the First Follow-up in Rotation, However, at the Second and Third Follow-up, the IUD Position was Back to Normal.



**Figure 5.** Abdominal USG Appearance in a Patient of Ring Forcepss Group at the First Follow-up, IUD was Located Close to the Opening of Internal Ostium of Uterine Cervix; However, at the Second and Third Follow-up, The IUD Position was Back to Normal.

Malposition occurrence happened in each insertion groups on the first follow-up. In the group of push and push technique, malposition occurred in a rotation of intrauterine IUD position, so that it was not placed in midsagital of the uterus (Figure 4); while in the ring forceps insertion group, malposition occured at the position of the IUD in the lower uterine segment approach internal ostium of uterine cervix (Figure 5). At the second and third follow-up, we did not find the malposition.

At three months follow-up in both insertion groups technique, we did not get the incidence of expulsion, perforation or pregnancy. There were two subjects in the ring forceps insertion group and one in the push and push insertion group who asked to remove the IUD at the third follow-up. There-fore, the continuation rate for three months of follow-up in the ring forceps insertion group was 90.4% and 95% for the push and push insertion group.

### DISCUSSION

Global reference manual in Postpartum Intrauterine Contraceptive Device (PPIUD) stated that IUD insertion required three instruments consisting of a vagina speculum, a ring forceps and a long placental forceps (Kelly placenta forceps, 12 inches in length). Vaginal speculum was for visualizing the cervix by depressing the posterior wall of the vagina, ring forceps was for grasping the anterior lips of the cervix and placental forceps was to grasp IUD and for the IUD insertion to the uterine cavity.<sup>23</sup>

Two techniques in this study had implemented a new inovation of IUD insertion through only one instrument (ring forceps). The vaginal speculum and ring forceps function was replaced by the middle and fore fingers in a supine position. The placenta forceps for clamping and inserting the IUD was replaced by ring forceps. The benefit of the tube inserter and plunger rod on push and push technique was that the IUD could be placed as close as possible to the endometrium of uterine fundus. Therefore, this application could be performed for postpartum contraceptive services which did not require the placenta forceps and gynecology bed.

Two retrospective cohort studies about postplacental IUD using in Dr. Kariadi Hospital in 2013 showed that the typical use rate was 0.2%, the continuity rate was 92.3%, the expulsion rate was 1.4% and no perforation reported. Accordance with the study, in 2014, the typical use, continuity, expulsion rate were 0%, 98.1% and 0.8%, respectively. They did not find the perforation occurrence.<sup>24,25</sup> Our study was a double-blinded randomized controlled trial where the patient and examiner of ultrasound did not know the IUD insertion procedure that had been used thereby reducing the bias after procedure due to restriction of activity in both groups. Selection bias could be anticipated through random allocation using randomization techniques using computers. Bias that might arise in the variable characteristics of the study subjects such as age, BMI, parity, gestational age at birth, early rupture of amniotic membrane, occupation and education, and the type of labor. All of the bias could be excluded by looking to the statistic where there were no differences in both treatment groups.

Counseling for using contraception postpartum must be integrated during antenatal visits. In our study, the majority of subjects received the counseling while in the hospital, but the continuity rate was still high. Study by Xu et al about 3 months follow-up postplacental IUD insertion, the continuation rate was 87.7%.<sup>16,26,27</sup>

Our study revealed that push and push insertion technique required a longer time than the ring forceps technique. This was because the technique of push and push needed preparatory stages starting from entering IUD string, vertical stem and the plunger rod into the inserter tube, also using ring forceps to grasp the inserter tube. However, push and push technique had advantages in terms of more able to put the IUD as close as possible to the endometrial uterine fundus. The distance of IUD-ED at 6 weeks of follow-up was 10 mm. A distance of more than 10 mm could be at risk of spontaneous expulsion, but it was easier to be lifted.<sup>28,29</sup> Other studies mentioned that the IUD-ED distance of 7 mm was the maximum distance which was safe in relation to the incidence of side effects of pain and bleeding. Our analysis obtained the differences of IUD-ED distance in both treatment groups; however it was not statistically significant. Our study found the average distance of the IUD-ED on the ring forceps and push and push group in three periods of follow-up was less than 7 mm, which meant that both techniques had low risk of side effects of pain, bleeding and spontaneous expulsion.<sup>22</sup> The IUD-ED distance in both insertion technique would be reduced as the involution of the uterus without the occurrence of spontaneous expulsion at 12-week follow-up. The mean distance of IUD-ED in both insertion techniques was not significant difference, but the maximum IUD-ED distance at 1-2-week and 6-8-week post insertion was shorter in push and push insertion technique group. These results proved that the technique of push and push by using a combination ring forceps and standard inserter, the IUD could be pushed closer to the fundus after the ring forceps being removed.17

Retrospective cohort study in our hospital in 2013, IUD-ED distance in each follow-up period among 1,555 women at < 6 weeks was 6.2 mm, among 1,209 women at 6 wks-<3 months was 5.8 mm and among 928 women at 3-<6 months was 5.6 mm.<sup>25</sup> In this study, we did not find the expulsion incident (Table 4).

Multicenter comparative trial study in China comparing hand insertion (470 subjects) with ring forceps insertion (440 subjects) of CuT-380A IUD found the six-month expulsion rates per 100 women were 13.3 for hand insertion and 12.7 for instrument insertion.<sup>16</sup> Cohort study by Eroglu, et al in 2006, among 82 women who had obtained IPPI, the rate of expulsion at 8-week post insertion was 22 events (25.8%) consisting of 13 (15.8%) of partial expulsion and 9 (10.0%) of complete expulsion. At 6-month post insertion from 61 women, they obtained 4 (6.5%) expulsion, 1 (1.6%) partial expulsion and 3 (4.9%) complete expulsion.<sup>30</sup> A prospective randomized control trial by El Betalgy, et al, compared the early (within 48 h) insertion in normally delivered women between the CuT-380 IUD (150 subjects) and Multiload 375 IUD (375 subjects) using Kelly's forceps. The expulsion rates were relatively high for both IUD; 15.0% in CuT-380 compared to 14.9% in Multiload 375 insertion. In CuT 380 IUD group, at 6-week follow-up, there was 5/143 (3.4%) IUD expulsion and at 6 months, there was 8/125 (6.0%) expulsion.<sup>29</sup> Meanwhile, a cohort study in Dr. Kariadi Hospital using insertion

technique of push and push, there was no expulsion at 1-2 week post insertion. The incidence of expulsion were 2 (1.9%) and 2 (1.9%) at 3- and 6-month follow-up, respectively. At 12-month follow-up they found 3 of 102 subjects (2.9%) experiencing expulsion.<sup>17</sup> In 2013 from the same hospital which covered 609 subjects, the expulsion rate was 1.4%. In 2014 with the number of subjects 305, the expulsion rate of was 0.8%. Dr. Kariadi Hospital is a referral and teaching hospital so that the postpartum family planning services will be undergone by the residents who are learning to gain the competence in IUD insertion post placental.<sup>24,25</sup>

Malposition in postplacental insertion occured because the large capacity of the uterus and cervix opening width at the time of insertion and the residual the blood clot at the early puerperium.<sup>19</sup> Malposition was not associated with postpartum insertion.<sup>31,32</sup> In our study, malposition happened in one subject of each treatment group in 1-2 week follow-up, but in 6-8-week and >3-month followup, the IUD already changed to the normal position due to the involution of the uterus and miometrium contraction.<sup>33,34</sup>

The weakness of this study was the ultrasound follow-up of each subject was performed by a single examiner only. Apart from that, the followup of the subjects should be conducted by interclass correlation method. However, the subjectivity of the follow-up results of ultrasound in measuring IUD-ED distance could be reduced because the ultrasound examiner did not know the type of IUD insertion technique used. The loss to follow-up at the first, second, and third follow-up were 6.0%, 12.0% and 18.0%. We had anticipated this events by adding 20% of the minimum number of samples.

### CONCLUSION

There are differences in the IUD-ED distance between the CuT-380A IUD insertion postplacental vaginal delivery using the technique of push and push and ring forceps technique; however it is not statistically significant. Clinically, in push and push insertion group, IUD-ED distance tend to be shorter. Push and push IUD insertion technique can continue its use for producing IUD-ED distance which is likely to be closer to the endometrium uterine fundus.

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Follow-up period	< 6 weeks	6 wks - < 3 mo.	3 mo < 6 mo.	6 mo < 9 mo.	9 mo < 12 mo.	12 mo < 24 mo.
Number of IUD user	2,231	2,066	1,713	1,397	1,188	745
Expulsion event	6	27	23	5	3	4
Expulsion rate	0.3%	1.2%	1.3%	0.4%	0.3%	0.8%

**Table 4.** Expulsion Rate of Postplacental IUD User in Each Follow-up Period.

Source: Research paper in 24th Annual Scientific Meeting Indonesian Society of Obstetrics and Gynecology 2014<sup>24</sup>

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**Research Article** 

## Ovarian Tissue Vitrification as a Method for Ovarian Preservation in Women with Cancer: an Analysis of Granulose Cell Apoptosis

## Simpan Beku Korteks Ovarium sebagai Pilihan dalam Upaya Mempertahankan Fungsi Reproduksi Perempuan Penderita Kanker: Sebuah Kajian Apoptosis Sel Granulosa

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### Abstract

**Objective**: To obtain the effective method of ovarian function preservation with granulose cell apoptosis assessment. Ovarian tissue vitrification became a method for ovarian function preservation in women with cancer. This technique can be done anytime without delay on cancer therapy both in prepubertal and unmarried patient. It can also store many primordial follicles. Ovarian tissue vitrification study is still limited to animal test and there are no data about apoptosis assessment after ovarian vitrification in human ovary.

**Method**: This quasi experimental study was held in Department of Obstetrics and Gynecology Faculty of Medicine University of Indonesia - Dr. Cipto Mangunkusumo General Hospital and Fatmawati Hospital Jakarta from March 2012 to May 2015. Ovaries from thirteen women between 31 and 37 years old who underwent oophorectomy with gynecological indication were examined.

**Result**: There was no morphological difference between follicles from fresh and warmed-vitrified ovaries. The mean protein Bax expression on the fresh ovaries assessed in the form of H-score was 1.66 (SD 0.14) compared with 1.68 (SD 0.13) on the warmed-vitrified group (p=0.165). The mean protein Bcl-2 expression on the fresh ovaries examined in the form of H-score was 1.73 (SD 0.10) compared with 1.71 (SD 0.10) on the warmed-vitrified group (p=0.068).

**Conclusion**: Ovarian tissue vitrification does not affect the Bax and Bcl-2 expression on human ovary.

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Keywords: apoptosis, bax, Bcl-2, ovarian tissue vitrification

### Abstrak

**Tujuan**: Untuk memperoleh upaya mempertahankan fungsi ovarium yang efektif dengan penilaian apoptosis sel granulosa. Simpan beku korteks ovarium menjadi pilihan dalam upaya mempertahankan fungsi reproduksi perempuan penderita kanker karena dengan teknik ini dapat disimpan banyak folikel primordial. Penyimpanan ini dapat dilakukan kapan saja saat siklus haid tanpa penundaan terapi kanker serta pada pasien pra-pubertas dan belum menikah. Penelitian simpan beku korteks ovarium masih terbatas pada hewan coba serta belum terdapat data yang menilai kejadian apoptosis sel granulosa pascasimpan beku korteks ovarium manusia yang dilihat dari ekspresi gen terkait apoptosis.

**Metode**: Penelitian ini merupakan penelitian kuasi eksperimental yang dilaksanakan di Departemen Obstetri Ginekologi Fakultas Kedokteran Universitas Indonesia - RSUPN Dr. Cipto Mangunkusumo dan RS Fatmawati Jakarta dalam rentang waktu Maret 2012 hingga Mei 2015. Korteks ovarium didapatkan dari tiga belas pasien berusia 31-37 tahun yang menjalani ooforektomi atas indikasi ginekologis.

Hasil: Secara morfologi, tidak terdapat perbedaan folikel dari korteks ovarium segar dengan korteks ovarium pasca vitrifikasi. Rerata ekspresi protein Bax dari korteks ovarium segar yang dinilai dalam bentuk H-score adalah 1,66 (SD 0,14) dibandingkan 1,68 (SD 0,13) pada ovarium pasca vitrifikasi (p = 0,165). Sedangkan, rerata ekspresi protein Bcl-2 dari korteks ovarium segar adalah 1,73 (SD 0,10) dibandingkan 1,71 (SD 0,10) pada ovarium pascavitrifikasi (p=0,068).

Kesimpulan: Simpan beku korteks ovarium terbukti tidak menyebabkan peningkatan ekspresi gen Bax dan Bcl-2.

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Kata kunci: apoptosis, Bax, Bcl-2, simpan beku korteks ovarium

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## INTRODUCTION

Over the past three decades, there has been a remarkable improvement in the survival rates due to the cancer treatment improvement. In United States, more than 650,000 new female cancer cases were estimated to be diagnosed in 2003.<sup>1</sup> Advancing in the diagnosis and treatment of childhood, adolescent and adult cancer have greatly enhanced the life expectancy of premenopausal women with cancer. As a result, there is a growing population of adolescent and adult long-term survivors of childhood cancer.<sup>2</sup> Ovaries, which are endowed with an irreplaceable number of follicles, are extremely sensitive to cytotoxic drugs that induce an irreversible gonadal damage.<sup>1,3</sup>

Ovarian cortex vitrification is one of the promising freezing techniques in order to maintain the reproductive function. Unfortunately, freezing procedure is a process that can cause follicle damage because of the potency to increase the apoptosis process. Assessment of apoptosis by expression of pro and anti-apoptotic proteins can be conducted to determine biomolecular processes occurring intracellular that has been indicated to precede morphological changes. Ovarian cortex vitrification research is still limited in animal experiments and there have been no data to assess the incidence of the granulosa cells apoptosis after human ovarian cortex vitrification.<sup>1,3-5</sup> Therefore, this study aims to obtain the effective method of ovarian function preservation with granulose cell apoptosis assessment.

## METHODS

All experimental procedures were approved by the Ethical Research Committee of the Faculty of Medicine University of Indonesia after obtaining written informed consent. We took the ovaries from six women between 30 and 37 years old who underwent oophorectomy due to cervical or breast cancer. Surgeries were performed in the Acute Tertiary Care Hospital in Jakarta during the period of March 2012 to April 2015.

## **Ovarian Tissue Vitrification**

Ovarian tissue was suspended in 37°C phosphate buffered saline (PBS) and transferred to the laboratory within 15 minutes. A tissue slicer (Square Measure, Kitazato, Shizuoka, Japan) was used to cut the ovarian cortex into pieces measuring 10x10x1 mm.<sup>6,7</sup>

Two chosen section of ovarian cortexes were divided into two groups. The first group was a control group for assessing Bax and Bcl-2 protein expression using immunohistochemical method. The second group was an experimental group frozen by vitrification technique and followed to thawing process, then Bax and Bcl-2 protein expression were assessed with the same immunohistochemical method to be compared with the first group. After being excised, ovarian cortexes were placed in 15 ml of  $\alpha$ -MEM (minimum essential medium). Ovarian cortexes were initially equilibrated in 7.5% ethylene glycol (EG); 7.5% dimethyl sulfoxide (DMSO) for 25 minutes, followed by a se-

cond equilibration in 20% EG, 20% DMSO, 0.5 mol/l sucrose liquid for 15 minutes. Ovarian cortexes were placed in a minimum volume of solution into a thin metal strip and submerged directly into liquid nitrogen.<sup>7</sup>

## Immunohistochemical (IHC) Staining of Bax and BCL-2 Protein in Fresh and Vitrified Human Ovarian Tissue

Bax and Bcl-2 protein staining using IHC were done in fresh and vitrified ovarian cortex from each patient. Immunohistochemical staining was performed using 3 steps polymer detection system of Starr Trek Universal HRP Detection System. The negative control groups were stained using the same method, whereas the first antibody was substituted by PBS. Rabbit anti-human monoclonal Bax and mouse anti-human monoclonal Bcl-2 antibodies were used. All paraffin-embedded samples were deparaffinized and rehydrated. Sections were incubated in 0.5% H2O2 to block endogenous peroxides, and then incubated with blocking background sniper to reduce nonspecific binding. The primary antibody was applied and the sections was kept at room temperature overnight. Universal link (secondary monoclonal antibody from rabbit and mouse) were put on for 15 minutes. After incubating with Trek Avidin-horseradish peroxidase to bind antibody with chromogen, sections were stained with DAB (Diaminobenzidintetraacetic acid) for 5-10 minutes.

The expression of Bax and Bcl-2 proteins was identified as diffuse brown cytoplasmic staining. Ovarian cortex apoptosis was assessed semiquantitatively with Bax and Bcl-2 protein expression in primordial follicle, primary follicle and secondary follicle. The number of granulosa cells stained (Pi) in each follicle were counted and the mean Pi from all follicles examined was calculated. We scored the color intensity as weak, moderate, or strong and the final H-score was calculated with the algorithm: H-score =  $\Sigma$  Pi (i+1).

## **Statistical Analysis**

Statistical analysis was performed using Statistical Program for Social Sciences version 20.0. Saphiro-Wilk test was used to assess the normality distribution. We used paired T-test to compare between H-score.

## RESULTS

We obtained the samples from thirteen patients between 30 and 37 years of age who underwent oophorectomy due to cervical cancer (stage IB) or breast cancer (Table 1). The mean level of antimullerian hormone (AMH) in these patients was 2.9 (SD 1.6) ng/ml.

Table 1. Subject Characteristics

Characteristics	n (%)	Mean(SD)
Age (years old)	-	33 (1.6)
Body mass index (kg/m <sup>2</sup> )	-	23.6 (4.7)
Parity		
Nuliparity	3 (23.1)	-
Primiparity	4 (30.8)	
Multiparity	6 (46.2)	
AMH level (ng/ml)	-	2.9 (1.6)

## Evaluation of Morphology and Apoptosis of Follicles from Fresh and Warmed-Vitrified Ovaries



**Figure 2.** Bax expression on ovarian tissue. A. Bax expression on breast cancer tissue (positive control). B. No visible Bax expression on stromal ovarian tissue (negative control). C. Bax expression on granulosa cells and oocyte with low intensity in fresh ovarian tissue. D. Bax expression on granulosa cells and oocyte with low intensity in warmed-vitrified ovaries. Description: fpr: primordial follicles.



**Figure 1.** Follicles from fresh and warmed-vitrified ovaries. Primordial follicles (A and B), primary follicles (C and D), secondary follicles (D and E). Description: A, C, E: follicles from fresh ovaries. B, D, F: follicles from warmed-vitrified ovaries.



**Figure 3.** Bcl-2 expression on ovarian tissue. A. Bcl-2 expression on breast cancer tissue (positive control). B. No visible Bcl-2 expression on stromal ovarian tissue (negative control). C. Bcl-2 expression on granulosa cells and oocyte with low intensity in fresh ovarian tissue. D. Bcl-2 expression on granulosa cells and oocyte with low intensity in warmed-vitrified ovaries. Description: fpr: primordial follicles, fp: primary follicle.

There were no significant morphological differences between follicles from fresh and warmedvitrified ovaries based on examinatons of basal membrane, granulosa cells and oocyte (Figure 1). This finding also supported by the result of IHC staining of Bax and Bcl-2 protein expression, which also showed no alteration (Figure 2 and 3). The mean H-score for Bax on fresh ovaries was 1.7 (SD 0.1) versus 1.7 (SD 0.1) on warmed-vitrified ovaries (p=0.165). The mean H-score for Bcl-2 on fresh ovaries was 1.7 (SD 0.1) versus 1.7 (SD 0.1) on warmed-vitrified ovaries (p=0.068).

## DISCUSSION

In this study, we performed vitrification of human ovarian tissue according to the methods by Kagawa using DMSO and EG as cryoprotectants.<sup>7</sup> We did not find significantly different in follicles morphology (basal membrane, integrity of follicles and oocyte) between fresh and warmed-vitrified ovarian tissue. Apoptosis could occur through either extrinsic or intrinsic pathways. Bax and Bcl-2 protein were expressed in granulosa cells, oocytes and stromal of both fresh and warmed-vitrified ovaries. However, vitrification did not increase apoptosis via the intrinsic pathway in follicles.

Numerous studies also reported that there were no ultrastructural changes in oocytes, ovarian follicles, and stromal cells in post-vitrification ovarian tissue. Sheiki, et al. found that vitrification was the best method for ovarian tissue cryopreservation. Vitrification liquid used on that experiment was ethylene glycol as a cryoprotectant. There were also no significant differences among oocytes ultrastructure, granulosa cells, and stromal cells from electron microscope observation.<sup>8</sup>

We reported a non-randomized study comparing slow freezing to vitrification on 20 ovarian biopsies. There were no significant differences in the number and morphology of follicles between two groups.<sup>9</sup> Kagawa, Silber and Kuwayama also reported the successful technique using cryotissue method with the results of oocyte viability. They also stated there was no significantly different in morphology of ovarian cortex between fresh ovary and warmed-vitrified ovaries. This method used ethylene glycol, DMSO, and sucrose as cryoprotectants in ovarian cortex vitrification technique.<sup>7</sup>

Assessment of ovarian tissue apoptosis can be performed based on morphology, level of apop-

tosis, and protein expression related to apoptosis in granulosa cells. Excellent assessment makes us understand the biomolecular process that happened intracellular. Protein expression changes precede morphological changes so that not only morphological changes were assessed but it was also impontant to determine the protein expression changes in ovaries after vitrification. The expression of Bax and Bcl-2 were evaluated because vitrification had been reported to initiate the intrinsic apoptosis pathway.<sup>6,10,11</sup>

Cell signaling process both intrinsic and extrinsic pathway regulates the apoptotic process. Extrinsic pathway is initiated by increasing level of death receptor on cell surface.<sup>12,13</sup> Death receptor sends apoptotic signal, such as Fas, tumor necrosis factor receptor (TNFR), interferon (IFN), and TNF related apoptosis-induced ligand (TRAIL).12 Intrinsic pathway is initiated by mitochondria permeability and the release of pro-apoptotic molecule into cytoplasm without initiation from death receptor. Anti-apoptotic protein, such as Bcl-2 and Bcl-x contributes as apoptosis regulator which can be found in mitochondria membrane and cytoplasm. The damage of DNA can cause Bcl-2 and Bcl-x loss from mitochondria membrane and pro-apoptosis protein, such as Bax, also Bax replaces this DNA damage. The decrease of Bcl-2 and Bcl-x protein level are followed by the rise of mitochondria permeability, causing cytochrome C released from mitochondria. Cytochrome c will bind to Apaf-1 protein (apoptotic protease activating factor-1) and caspase-9 and later, this binding will activate caspase cascade and induce cell death.<sup>12,13</sup>

This study showed that vitrification did not affect the morphology of pre-antral follicles. The expression of Bax and Bcl-2 protein on both ovarian cortex was still the same through this vitrication process. Wiweko B also reported that pre-antral follicle vitrification did not increase caspase-3 and FasL expression.<sup>6</sup> There were no significant changes between pro-apoptosis mRNA gen (FasL, Bax, p53, and caspase-3) and anti-apoptosis gen (Bcl-2) in both fresh and warmed-vitrified ovaries. Abdollahi, et al. also reported the similar result.<sup>14</sup>

## CONCLUSION

Ovarian tissue vitrification does not affect the Bax and Bcl-2 expression on human ovary.

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## Impact of Ethinyl Estradiol to Human Telomerase Reverse Transcriptase Activity on Complete Hydatidiform Mole Culture

## Pengaruh Etinil Estradiol terhadap Aktivitas Human Telomerase Reverse Transcriptase (Studi pada Kultur Sel Trofoblas Mola Hidatidosa Komplit)

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### Abstract

**Objective**: To prove the effect of ethinyl estradiol as an activator on human Telomerase Reverse Transcriptase (hTERT).

**Method**: The experimental study was conducted in vitro by using culture of complete hydatidiform mole trophoblast cell. We exposed the culture to ethinyl estradiol in varied doses and measured the concentration of hTERT through RT-PCR quantitative. There were 40 specimens as control group and 20 specimens exposed to ethinyl estradiol in different doses (10, 20, 40 and 80 mcg) as experimental group. The activity of hTERT was measured by RT-PCR and the concentration of it was assessed by ELISA. We analyzed the variables using ANOVA, Turkey post hoc and Pearson correlation test.

**Result**: In control group, the concentration of hTERT was not detected. Meanwhile, the concentration among different doses of ethynil estradiol (10, 20, 40, 80 mcg) was 113,117.5; 114,507.6; 102,193.9; 127.546.1 amoles/ml, respectively. Among experimental group, they were significantly different both using F test (ANOVA) (p=0.001) and Turkey post hoc test (p=0.005). The correlation among group was 0.84 which meant higher level of ethinyl estradiol was correlated with higher activity of hTERT.

**Conclusion**: Ethinyl estradiol impacts to the increase of hTERT activity on complete hydatidiform mole cell culture.

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**Keywords**: complete hydatidiform mole, ethinyl estradiol, human Telomerase Reverse Transcriptase (hTERT)

#### Abstrak

**Tujuan**: Untuk membuktikan pengaruh etinil estradiol sebagai aktivator human Telomerase Reverse Transcriptase (hTERT).

**Metode**: Penelitian eksperimental ini didesain secara in vitro menggunakan kultur sel trofoblas mola hidatidosa komplit. Hasil kultur sel trofoblas mola hidatidosa komplit diberikan etinil estradiol berbagai dosis. Aktivitas hTERT diukur secara kuantitatif dengan RT-PCR. Terdapat 40 spesimen sebagai kelompok kontrol dan 20 spesimen terekspos dengan etinil estradiol berbagai dosis (10, 20, 40, 80 mcg) sebagai kelompok eksperimental. Analisa hasil penelitian menggunakan uji anova, post hoc Turkey dan korelasi Pearson.

**Hasil**: Konsentrasi hTERT pada kelompok kontrol tidak terdeteksi. Konsentrasi hTERT pada pemberian etinil estradiol (10, 20, 40, 80 mcg) ialah 113.117,5; 114.507,6; 102.193,9; 127.546,1 amoles/ml. Uji beda menggunakan uji F (Anova) diperoleh terdapat perbedaan aktivitas hTERT setelah pemberian Ethinyl Estradiol berbagai dosis (p=0,001). Uji komparasi menggunakan post hoc Turkey diperoleh perbedaan bermakna pada berbagai dosis 0, 10, 20, 40, 80 mcg. Pada uji korelasi menggunakan korelasi Pearson diperoleh hasil (p=0,005) dengan koefisien korelasi 0,84 yang membuktikan tingginya kadar etinil estradiol berhubungan dengan tingginya aktivitas hTERT.

Kesimpulan: Etinil estradiol dapat meningkatkan aktivitas hTERT pada kultur sel mola hidatidosa komplit.

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Kata kunci: etinil estradiol, human Telomerase Reverse Transcriptase (hTERT), mola hidatidosa komplit

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## INTRODUCTION

Hydatidiform mole as trophoblast disease in pregnancy is a differentiation failure from degeneration of placental hydropic. It is the chorion flakes which are usually seen as bubbles.<sup>1,2</sup> Hydatidiform mole is a part of gestational trophoblastic diseases (GTD). WHO classifies GTD into 6 diseases including gestational trophoblastic diseases (complete and partial hydatidiform mole), invasive mola, choriocarcinoma, the placental site trophoblastic tumor, and an unclassified trophoblastic lesions. Complete hydatidiform (CHM) as a common variant has characteristics as followed widened, edematous, and vesicular lesions of placental chorionic villi, also trophoblastic proliferation in various degrees.<sup>3</sup>

The distribution of GTD is varied worldwide, which higher distribution is found in some parts of Asia, Middle East, and Africa. Study conducted by Harmain in 2010 found the incidence of complete hydatidiform mole in Africa was 11.0 per 1,000 births.<sup>4</sup> Meanwhile, epidemiological study in Ban-

dung showed that the incidence of complete hydatidiform mole was 1:500. Patients with complete hydatidiform mole had 15-20% risk to undergo transformation into malignant gestational trophoblastic disease. Unfortunately, the carcinogenesis process in complete hydatidiform mole is still unknown. Some factors which has been known influencing in the carcinogenesis process are DNA ploidy, expression of phospholipids, oncogenes, tumor suppressor genes, nutrition and hormonal status.<sup>5,6</sup>

Expression from human Telomerase Reverse Transcriptase (hTERT) has an important role in the survival of malignant process. This expression can be detected in complete hydatidiform mole and choriocarcinoma, neither in partial hydatidiform mole nor normal pregnancy. Activation of this enzyme is in conjunction with a malignancy and usually occurs after molar pregnancy.<sup>7,8</sup>

Women with complete hydatidiform mole pregnancy should not get pregnant in a year so that they need contraception methods which can return their fertility as soon as possible after discontinuation of the contraception. The choices are both non-hormonal and hormonal contraception. After complete hydatidiform mole, patients in Indonesia like using condom for their husband as non-hormonal method and pills containing ethinyl estradiol as hormonal method. Previous study showed that the ethinyl estradiol effected to carcinogenesis; however, a meta-analysis study released that in women post mola hydatidiform, the use of ethinyl estradiol on hormonal contraception would cause the recurrence of mola.<sup>9</sup> Meanwhile, Esmaili et al. in 2007 said that ethinyl estradiol had carcinogenesis effect in human.<sup>10</sup>

Tamoxifen triphenylethylene as an anti-estrogen is non steroidal component. Tamoxifen binds to estrogen receptor (ER) in order to minimalize the carcinogenic effect of estrogen. Some studies suggested using anti-estrogen to reduce the occurrence of multiple malignancy. However, the occupation of ER is depended on cellular context and the structure of the ligand. The most important of the biological consequence is whether the activated receptor complex can induce estrogenic or antiestrogenic response. Therefore, we would like to prove the effect of the ethinyl estradiol as an activator on human Telomerase Reverse Transcriptase (hTERT) on complete hydatidiform mole culture.<sup>11</sup>

## METHODS

This was an experimental study conducted between May and July 2012. We obtained the samples from complete hydatidiform mole patients with a bubble of mole. We excluded the patients with negative  $\beta$ -hCG test and failure to grow trophoblast cell after culture.

We took the samples (1 cm<sup>3</sup> CHM tissue) by suction curettage after patients signed the informed consent form. The trophoblast tissue with buble of mole was washed by normal saline (NaCl 0.9%), then put in cord solution (medium for trophoblast tissue sampling), which containing Hank's balance salt solution (HBSS (Sigma H 1641)), gentamycine (Gentamerck), sodium hidrogen bicarbonate (SHB (Sigma), phenol red (Sigma P5530), HEPES Solution (Sigma), and deionized water (WFI Otsuka). We performed the process of culture not more than 12 hours after curettage.

The detailed procedures were firstly; the bubbles of mole were washed by NaCl until free from erythrocytes. After that, we washed twice with Dubbecco's phosphate buffered saline (DPBS) added with a final concentration of 100 U/ml penicillin and 100 mcg/ml streptomycine. After the addition of antibiotics, this solution (DPBS) should be kept at 2-8°C and used within one week. Finally, the bubbles were washed with serum free mellin.

To isolate the cells, the bubbles were incubated in a solution of Collagenase (type 1) 7 mg/10 cc for 2 hours and centrifuged 2,000 rpm for 7 minutes. The supernatant was removed and the pellet was resuspended in serum free myelin. The centrifugation was repeated and the resulting pellet was resuspended in 4 ml culture medium, which containing newborn calf serum (NCS, (Sigma N4637)) and 10% FBS, then incubated in 5% CO<sub>2</sub> incubator, in 37°C. Further, the culture was observed daily under a microscope to detect cell growth. Examination of  $\beta$ -hCG was performed to identify the trophoblast cells.

We divided the CHM trophoblast cultures into 4 experimental group in varying doses of ethinyl estradiol (10, 20, 40, 80 mcg/ml) and 1 control group. The hTERT was measured by QTD rt-PCR (quantitative telomerase device real time PCR (Allied Biotech)) and the concentration of it was measured by ELISA at MIPA laboratory Faculty of Medicine Universitas of Islam Negeri (UIN) Malang. We performed ANOVA test to compare hTERT expression in CHM trophoblast cells and continued by Pearson correlation test to determine the correlation among the groups. Ethical clearance was obtained from Health Research Ethics Committee Saiful Anwar General Hospital no. 132/KILDK-FKUB/05/2012.

### RESULTS

Table 1 showed the hTERT activity in CHM cells in various doses of etinhyl estradiol. The data was in homogeneity and normal distribution (p>0.05). There was a trend of raising concentration of hTERT as the increase dose of ethinyl estradiol compared to the control group in cell cultures exposed to both tamoxifen and without tamoxifen. Interestingly, there was a tendency of decreasing the hTERT concentration in hydatidiform culture without exposure to tamoxifen in the 40 mg of ethinyl estradiol group (Table 1).

For the group of CHM culture which exposed to tamoxifen, hTERT expression did not occur in the group treated with 10 mcg and 20 mcg of ethinyl estradiol. They expressed same as the control group (Figure 1).

We analyzed the hTERT concentration data at various dose groups by using Anova test. The ethinyl estradiol in cell culture without exposure of tamoxifen was significantly different to the level of hTERT concentration among groups (p=0.003). Apart from that, there was significant correlation between the concentration of ethinyl estradiol and hTERT concentration (p=0.005).



**Figure 1.** Amplification Curve of Hydatidiform Mole Culture with Ethynil Estradiol.

Table 1.	hTERT	Activity	in CHN	4 Cells	in V	/arious	Doses	of
Ethinyl Es	tradiol E	Exposure	e					

Estradiol Dose (mcg)	Mean (SD)(amoles/ul)
10 (physiologic)	113117.5 (2.7)
20	114507.6 (4.9)
40	102193.9 (5.5)
80	127546.1 (6.2)
Control (Without EE)	Not detected

## DISCUSSION

Complete hydatidiform mole is characterized by edema of the vesicular placenta chorionic villi without the existence of fetus. The pathophysiology of CHM can derive from eggs which not conceived by haploid sperm or one egg is fertilized by two sperms. Histologically, CHM is seen as trophoblast proliferation with various levels of hyperplasia and dysplasia. Apart from that, the chorionic villi were fulfilled by fluid, which made it swell and there were only a few of blood vessels. Some studies suggested that women with gestational CHM was derived from the paternal karyotype 46XX and a little percentage of triploid or tetraploid maternal. Thus, CHM was occurred not only in pathology, but also in genetic abnormality.

Telomerase (hTERT) is an enzyme associated DNA sequence repetitions (TTAGGG) found in eukaryotic chromosomes. Telomerase prevents the loss of an essential component of DNA, so that every time a doubling of the chromosome causes missing of 100-200 nucleotides to prevent the damage in DNA. Most of the normal human somatic cells telomerase are in inactive state and they have a limited replication capacity. Related to several oncogenes, hTERT activity causes malignancy changes. The role of hTERT in this process is to provide unlimited replication capabilities. Inhibition of hTERT in immortal cells will lead to telomere shortening and apoptotic cell death. This shows that hTERT activity is generally required for making immortal and proliferation of cancer cells.

In the genomic pathway, estrogen binding to its receptor activates the estrogen receptor (ER). This activation happens between estrogen and estrogen response element (ERE). Estrogen response element is an area in the region of the hTERT gene promoter. Association of ER with ERE will cause the hTERT gene expression. In this study, the administration of various doses of ethinyl estradiol was performed. In the control group, we could not detect the hTERT activity. This proved that there was no hTERT activity in trophoblast cell culture. Trophoblast cells which not having hTERT activity was identic to spontaneous regression of mole cell. Giving a dose of 10 mcg, 20 mcg, 40 mcg and 80 mcg EE in trophoblast cell culture resulted the increasing activity of hTERT, whereas the highest activity occurred on 40 and 80 mcg of ethinyl estradiol group.

In the oral contraceptive pills, ethinyl estradiol level is ranged from 20 mcg to 50 mcg, whereas this dose may increase the activity of hTERT. This impacts to a state of post-curettage complete hydatidiform mole, where women should not to become pregnant until one year after the procedure. They are asked to choose hormonal oral contraceptive pill; yet, the pills increase the risk to develop malignant trophoblastic disease (MTD). This event does not happen for all patients taking hormonal oral contraceptive pills because the occurrence of malignancy is also determined by the balance among tumor suppressor gene, oncogene and DNA repair gene.

At the dose of 40 and 80 mcg, ethinyl estradiol increased dramatically the hTERT activity. It could cause the developing of malignant trophoblastic disease complete hydatidiform mole. Therefore, high level of ethinyl estradiol in CHM tissue would increase the activity of hTERT which raised the potency of MTD.

### CONCLUSION

Ethinyl estradiol impacts to the increase of hTERT activity on complete hydatidiform mole cell culture.

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**Research Article** 

## **Bladder Function after Hysterectomy**

Fungsi Berkemih setelah Histerektomi

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#### Abstract

**Objective:** To determine the effect of hysterectomy on bladder function pre- and post-radical hysterectomy in early stage of cervical cancer.

**Method:** This study was a pre-post intervention study. Data were collected through questionnaires from women who had radical hysterectomy and total hysterectomy in Prof. Dr. R. D. Kandou Manado general hospital and other networking hospitals since January 1<sup>st</sup>, 2014 to November 31<sup>st</sup>, 2014. We analyzed the data using Wilcoxon and Mann-Whitney statistical test.

**Result**: There were each 18 respondents for the radical and total hysterectomy group in Prof. Dr. R. D. Kandou general hospital and networking hospitals. The age distribution of radical hysterectomy was 41-45 years old for 44.4%. The parity was dominated by second parity for 38.8%. In pre- and post-surgery, there were significant differences for urinary incontinence disorder (p=0.003), emptying disorder (p=0.008), urinary pain (p=0.034), and total urinary disorder (p=0.001). While, between radical and total hysterectomy, there was no significant difference in bladder function (p>0.05).

**Conclusion:** There is an association before and after surgery to urinary function. However, there is no association between the radical and total hysterectomy group.

[Indones J Obstet Gynecol 2016; 4-2: 97-100]

Keywords: bladder function, cervical cancer, radical hysterectomy

#### Abstrak

**Tujuan:** Untuk mengetahui pengaruh histerektomi terhadap fungsi berkemih pada pasien kanker serviks stadium awal.

Metode: Penelitian ini merupakan studi pre-pascaintervensi. Data dikumpulkan melalui pengisian kuesioner oleh wanita yang telah dilakukan histerektomi radikal maupun total di RSU Prof. Dr. R. D. Kandou Manado dan rumah sakit jejaring lainnya sejak tanggal 1 Januari 2014 hingga 31 November 2014. Kami menganalisis data menggunakan tes statistik Wilcoxon dan Mann-Whitney.

**Hasil:** Terdapat 18 sampel yang telah dilakukan histerektomi radikal dan total di RSU Prof. Dr. R. D. Kandou dan rumah sakit jejaring. Kebanyakan dari yang dilakukan histerektomi radikal berusia 41-45 tahun (44,4%). Kebanyakan sampel pernah melahirkan 2 kali (38,8%). Hasil uji statistik menunjukkan terdapat perbedaan bermakna pre dan pascahisterektomi pada gangguan inkontinensia urin (p=0,003), gangguan pengosongan urine (p=0,008), nyeri berkemih (p=0,034), dan total gangguan berkemih (p=0,001). Pada histerektomi total dan radikal, tidak terdapat perbedaan bermakna pada fungsi berkemih (p>0,05).

**Kesimpulan:** Terdapat hubungan sebelum dan setelah pembedahan pada fungsi berkemih. Tidak ada hubungan diantara kelompok histerektomi radikal dan total.

[Maj Obstet Ginekol Indones 2016; 4-2: 97-100]

Kata kunci: fungsi berkemih, histerektomi radikal, kanker serviks

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## INTRODUCTION

Cancer is one of the most frightening condition in the human life. This condition can impact to both physical and psychological aspects. Although advanced technology in medicine can longer the survival rate of cancer patients, we should pay attention to the quality of life.<sup>1,2</sup>

Cervical cancer as a part of gynecological cancer has a great influence on women's life. The early stage of cervical cancer treatment consists of radical hysterectomy with pelvic lymphadenectomy with or without adjuvant radiation.<sup>1,2</sup> Radical hysterectomy has good survival rate; however, it contributes to several consequences such as infertility, urinary disorders, bowel movement disorders, lymphedema and also sexual disorder.<sup>2-4</sup> Autonomic nerve damage is the consequence of this surgery.<sup>3</sup>

Autonomic nerves in the pelvis can be divided into four components: hypogastric nerves, pelvic splanchnic nerves, inferior hypogastric plexus and branching of three neural structures.<sup>5</sup> Therefore, this study aims to determine the effect of radical hysterectomy on bladder function in pre- and postsurgery condition.

### METHODS

This pre-post interventional study was conducted in Prof. Dr. R. D. Kandou Manado Hospital and other networking hospitals, Manado from January 1<sup>st</sup>, 2014 to November 31<sup>st</sup>, 2014. We included all women who underwent radical and total hysterectomy by assessing bladder function scores before and three months after surgery. This study was carried out in respondents' house by filling the questionnaire. The variables consisted of independent variable (radical and total hysterectomy) and dependent variable (bladder function results).

Data were collected by using a questionnaire translated from Urogenital Distress Inventory (UDI-6 Short Form) questionnaire. It was contained of some questions that necessary to determine the bladder function. We took each 18 women who underwent radical and total hysterectomy. The data were processed and analyzed statistically.

### RESULTS

Of the study, the most respondents who had radical hysterectomy were at 41-45 years old (8/18 respondents); while 7/18 respondents were at 46-50 years old. Both radical and total hysterectomy respondents had the most parity of 2 (Table 1).

The bladder function consists of urinary incontinence disorders, emptying disorder, urinary pain, and total urinary disorder. Table 2 and 3 showed the comparison of bladder functions between radical and total hysterectomy.

Characteristics	Radical Hysterectomy N=18	Total Hysterectomy N=18
Age (years old) (n(%))		
31-35	1 (5.5)	0 (0)
36-40	2 (11.1)	3 (16.6)
41-45	8 (44.4)	4 (22.2)
46-50	6 (33.3)	7 (38.8)
51-55	4 (5.5)	4 (22.2)
Parity (n(%))		
0	0 (0)	3 (16.6)
1	4 (22.2)	4 (22.2)
2	7 (38.8)	6 (33.3)
3	4 (22.2)	5 (27.7)
>3	3 (16.6)	0 (0)

 Table 1.
 Characteristics of Participants.

Table 2. Comparison of Bladder Functions between Patients Performed Radical and Total Hysterectomy.

	Group	Mean	Mean (SD)		Median		
	aroup	Pre	Post	Pre	Post	P	
Universe In continuo os Discurdos	Radical Hysterectomy	14.9 (0.8)	14.2 (1.3)	15.0	14.0	0.002	
Urinary Inconunence Disorder	Total Hysterectomy	14.7 (0.7)	14.3 (1.0)	15.0	14.0	0.003	
Emptring Disordon	Radical Hysterectomy	3.7 (0.5)	3.4 (0.7)	4.0	3.5	0.009	
Emptying Disorder	Total Hysterectomy	3.7 (0.5)	3.4 (0.7)	4.0	4.0	0.008	
Uningwangin	Radical Hysterectomy	3.4 (0.5)	3.2 (0.6)	3.0	3.0	0.024	
offilary pain	Total Hysterectomy	3.4 (0.5)	3.3 (0.5)	3.0	3.0	0.034	
Total Uningry disorday	Radical Hysterectomy	22.1 (1.1)	20.7 (1.7)	22.0	21.0	0.001	
	Total Hysterectomy	21.8 (0.9)	21.1 (1.4)	22.0	21.0	0.001	

\*Wilcoxon statistical test

Table 3.	Comparison of Bladder Functions between Radi-
cal and To	otal Hysterectomy.

	Group	p*
Lucas Harris David	Radical Hysterectomy	0.418
Incontinence Pre-surgery	Total Hysterectomy	
	Radical Hysterectomy	0.973
Incontinence Post-surgery	Total Hysterectomy	
Emptying disorder	Radical Hysterectomy	0.721
Pre-surgery	Total Hysterectomy	
Emptying disorder	Radical Hysterectomy	0.778
Post-surgery	Total Hysterectomy	
	Radical Hysterectomy	0.739
Urinary pain Pre-surgery	Total Hysterectomy	
	Radical Hysterectomy	0.881
Urinary pain Post-surgery	Total Hysterectomy	
	Radical Hysterectomy	0.355
Total Pre-surgery	Total Hysterectomy	
	Radical Hysterectomy	0.584
Total Post-surgery	Total Hysterectomy	

\*Mann-Whitney statistical test

## DISCUSSION

In this study, we found that the most distribution age of radical hysterectomy was 41-45 years old and 46-50 years old for total hysterectomy. It was similar to study by Brooks, et al. which showed that the mean age of respondents who performed radical hysterectomy was 44 years old and 49 years old for total hysterectomy.<sup>6</sup> Study by Charoenkwan revealed the similar result where the mean age of the respondents who performed radical hysterectomy was 44.6 years old.<sup>7</sup>

This study showed that the most parity both in radical and total hysterectomy was two times. Brooks, et al. found that the mean parity of the cervical cancer patients who performed radical hysterectomy was 2.3 and 2 for total hysterectomy.<sup>6</sup> It was also similar to the result by Charoenkwan whereas patients' parity was 2.3 for radical hysterectomy.<sup>7</sup>

The bladder function consists of urinary incontinence, emptying, urinary pain, and total urinary disorder. In our study, respondents who performed radical hysterectomy got the mean result of urinary incontinence disorders before surgery 14.9 and after surgery 14.2. Meanwhile, in total hysterectomy respondents, the mean result of urinary incontinence disorders before and after surgery were 14.7 and 14.3. The Wilcoxon statistical test found that there was significant difference in urinary incontinence disorder before and after surgery (p=0003). Brooks, et al. in their study showed mild incontinence disorder in 50% of patients who performed radical hysterectomy and 43% for the control group. Severe incontinence disorder occurred in 33% of radical hysterectomy patients compared with 36% in control group. This occurrence was coming from sympathetic nerve disorder causing incompetence of the bladder neck.<sup>6</sup>

Our study stated that there was significant difference in urine emptying disorders before and after surgery (p=0.008). The result was similar to study conducted by Charoenkwan<sup>7</sup> and Brooks, et al.<sup>6</sup> Charoenkwan revealed 13.4 subjects suffering from urine emptying disorder<sup>7</sup>; while Brook, et al. found urine emptying disorder happened in 18.2% subjects after radical hysterectomy and 14.5% after total hysterectomy.<sup>6</sup> Otherwise, Rana, et al. stated the urinary retention incidence post radical hysterectomy was higher, namely 42.2%.<sup>8</sup>

Another indicator for bladder function test is the urinary pain. There was significant difference in urinary pain before and after surgery (p=0.034). Study by Charoenkwan presented subjects who experience pain during urination after radical hysterectomy were 6.4%.<sup>7</sup> Brooks found the result of the subjects who experienced urinary pain after hysterectomy radical was 21.2%, and 11.8% after total hysterectomy.<sup>6</sup>

There was significant difference in total urinary disorder before and after surgery. Similar to this study, Cheeveewat, et al. found that 64% patients experienced urinary problem after radical hysterectomy.<sup>9</sup>

Table 3 showed there was no statistically significant difference between subjects in bladder function who performed radical and total hysterectomy (p>0.05). This statistical test result was similar to study done by Brooks, et al. They found that there was no significant difference in bladder function after radical and total hysterectomy.<sup>6</sup>

### CONCLUSION

We can conclude that there is an association between radical hysterectomy and urination function. However, there is no association between the radical and total hysterectomy group. Before performing a radical hysterectomy, the gynecologist should do counseling and explain the operation procedure and complication so that patients can understand clearly for the bladder functions complication after radical hysterectomy. Apart from that, good counseling before surgery can support the psychology of the patients.

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**Research Article** 

## Epidemiology Data of Ovarian Cancer in Dr. Cipto Mangunkusumo Hospital, Jakarta

## Profil Data Epidemiologi Kanker Ovarium di RSUPN Dr. Cipto Mangunkusumo, Jakarta

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#### Abstract

**Objective**: To describe the incidence of ovarian cancer and its characteristic in Dr. Cipto Mangunkusumo Hospital in the last 5 years.

**Method**: This was cross sectional study design. The data was collected from Gynecology Oncology Division Cancer Registry and Dr. Cipto Mangunkusumo Hospital medical record from January 2009 to December 2013; follow up was performed to know the 4-years survival rate.

**Result**: There were 98 subjects in this study. The majority incidence of ovarian cancer was 45-54 years old (33.6%); the incidence of ovarian cancer decreased with the increased number of parity; the majority histotype was epithelial (76.5%); and most of them were diagnosed on advanced stage (55.1%). The 4-year survival rate for epithelial type was 77%; germinal type was 83.3%; and stromal type was 100%. Based on therapy, the 4-year survival rate was 84.1% for surgical only; 83.3% in adjuvant chemotherapy group; and 68.4% in neoadjuvant chemotherapy. In the group of adjuvant chemotherapy, the tresponse and 41.2% patients with complete response in neoadjuvant chemotherapy.

**Conclusion**: The highest incidence of ovarian cancer in Dr. Cipto Mangunkusumo Hospital belongs to the age of reproductive women ( $\leq$  55 years old) with the highest incidence occurs in nulliparity women. Most of the ovarian cancer cases are diagnosed in advanced stage (stage III-IV).

[Indones J Obstet Gynecol 2016; 4-2: 101-106]

**Keywords**: age, histotype, ovarian cancer, parity, response, stage, survival, treatment

Abstrak

**Tujuan**: Mengetahui gambaran umum kanker ovarium di RSCM 5 tahun terakhir beserta faktor-faktor yang berhubungan dengan kanker ovarium.

**Metode**: Penelitian ini menggunakan desain potong lintang. Penelitian ini mengambil data pasien kanker ovarium di Cancer Registry divisi Ginekologi Onkologi dan rekam medis di RSCM pada periode Januari 2009 - Desember 2013, dilakukan follow up untuk mengetahui kesintasan hidup selama 4 tahun.

Hasil: Terdapat 98 subyek penelitian. Pada penelitian ini didapatkan insidensi kanker ovarium terbanyak pada usia 45-54 tahun (33,6%), insidensi kanker ovarium menurun dengan bertambahnya jumlah anak, sebagian besar kanker ovarium merupakan tipe epitelial (76,5%) dan sebagian besar pasien didiagnosa pada stadium lanjut (55,1%). Kesintasan hidup 4 tahun pasien kanker ovarium tipe epitelial 77%; tipe germinal 83,3%; tipe stroma 100%. Kesintasan hidup 4 tahun dengan terapi pembedahan 84,1%; pembedahan disertai kemoterapi adjuvan 83,3%; kemoterapi neoadjuvan sebelum pembedahan 68,4%. Terdapat 63% respon komplit pada kelompok kemoterapi adjuvan; dan 41,2% pada kelompok kemoterapi neoadjuvan.

Kesimpulan: Insidensi kanker ovarium di RSCM lebih tinggi pada pasien usia reproduksi (≤ 55 tahun); insidensi kanker ovarium di RSCM lebih tinggi pada pasien nulipara; dan sebagian besar pasien kanker ovarium di RSCM didiagnosis pada stadium lanjut (stadium III dan IV).

[Maj Obstet Ginekol Indones 2016; 4-2: 101-106]

Kata kunci: histologi, kanker ovarium, kesintasan, paritas, respon, stadium, terapi, usia

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## INTRODUCTION

Ovarian cancer is the second most common gynecological cancer in Western countries with a high mortality rate. In Indonesia, ovarian cancer is the third most common gynecological cancer.<sup>1,2</sup> Unlike breast and cervical cancer, there is no effective screening method for ovarian cancer. The application of certain screening to high risk women is still controversial.<sup>3,4</sup> The incidence rate was varied worldwide according to geographic location, where as the rates in Asia were lower than Western countries. Highest incidence for more than 8 per 100,000 was found in developed countries.<sup>5,6</sup> Though Japan and Hongkong had the lowest incidence, there was an increase about 15-25% every 5 years.<sup>5</sup> Generally, ovarian cancer had been diagnosed in older women with the incidence of 61.3 per 100,000 in 75and 79-year-old women.<sup>7</sup> Moreover, the histopathology was varied according to geographic location. In Western countries, the rates of ovarian germ cell tumor were less than 5%; while in Asia and Africa-American, the incidence rates achieved 15%.<sup>8</sup>

The definitive therapy for ovarian cancer is surgery with or without chemotherapy. It can be given as neo-adjuvant or adjuvant therapy.<sup>9</sup> Several studies showed that chemotherapy as adjuvant therapy had improved overall survival rate and the recurrence of free survival.<sup>10</sup> However, the administration of chemotherapy as neo-adjuvant was still controversial.<sup>8</sup>

We conducted this study because there were differences of ovarian cancer risk factors in every region. This study aims to evaluate the incidence of ovarian cancer in Dr. Cipto Mangunkusumo Hospital based on age, menarche and number of parity. Due to the differences of ovarian cancer mortality rate in every region, we also evaluated the survival rate based on histopathology, therapy and response rate.

## **METHODS**

This was a cross sectional study design based on cancer registry. The inclusion data were ovarian cancer cases registered in cancer registry from 1<sup>st</sup> January 2009 to 31<sup>st</sup> December 2013 and had medical record. The exclusion criteria was borderline ovarian tumor. The initial step was to record the register number, medical record number, patient identity, parity, age of newly diagnosed cases, age of menarche, staging, histopathology result, cytology result, treatment regimen and response. The missing data from cancer registry were completed through medical record data. Subjects were followed-up until 30<sup>th</sup> November 2014.

All the data were executed using SPSS 21. The statistical data included descriptive categorical analysis for the incidence of ovarian cancer cases based on reproductive age status, menarche age, parity, cytology result, histopathology, treatment regimen and stage; while survival rate based on stage, histopathology, response rate from each treatment regimen would be analyzed using Kapplan Meier method. Survival analysis was subjects in the beginning period, subjects under censored and subjects under events from period. Ethical committee had approved the study with the register of 611/UN2.F1/ETIK/2014.

## RESULTS

There were 395 ovarian cancer subjects registered in cancer registry from 2009 to 2013. All data were tracked to hospital medical record; the incomplete data were excluded from the study. All of patients who had been diagnosed in 2009 were being excluded because of missing medical record. Moreover, most of the medical records were incomplete. There were only 98 patients fulfilled the complete data recorded in cancer registry and medical record. It consisted of 15 patients in 2010, 19 patients in 2011, 29 patients in 2012 and the rest 35 patients in 2013. The cytology documentation that was not recorded in cancer registry and medical record were tracked to Pathology Anatomy Department Dr. Cipto Mangunkusumo Hospital.

In order to evaluate the survival rate, the lost of follow-up subjects were updated based on family members' recall. However, this method was not eligible to complete the treatment response; thus it left empty response treatment. Therefore, response rate was switched to treatment response percentage.

renou or 2009-2013			
	Ν	%	95% CI
Year			
< 15 years	0	0	-
15 - 24 years	4	4.1	0 - 9.2
25 - 34 years	13	13.3	7.1 - 20.9
35 - 44 years	21	21.4	13.3 - 30.6
45 - 54 years	33	33.7	24.5 - 44.9
55 - 64 years	19	19.4	11.8 - 27.6
65 - 74 years	8	8.2	3.6 - 15.3
> 74 years	0	0	-
Parity			
Nulliparity	30	30.6	22.4 - 38.8
Primiparity	19	19.4	11.3 - 27.7
Multiparity	45	45.9	38.3 - 54.6
Grandemultiparity	4	4.1	1 - 9.2
Menarche			
< 12 years	12	14.3	6.6 - 22.6
12 - 16 years	72	85.7	77.4 - 93.4

Table 1.	Demographic Characteristic of Ovarian Cancer
Subjects in	n Dr. Cipto Mangunkusumo Hospital on the
Period of 2	2009-2013

<b>Table 2.</b> Chilled Characteristic of Ovarian Cancel	-
Subjects in Dr. Cipto Mangunkusumo Hospital	
on the Period of 2009 - 2013.	

	Ν	%	95% CI
Stage			
Ι	32	32.7	24.8 - 42.8
II	12	12.2	6.7 - 18.4
III	40	40.8	29.6 - 50.5
IV	14	14.3	7.1 - 20.4
Histotype			
Epithelial	75	76.5	67.9 - 86.2
Germinal	13	13.3	5.6 - 19.9
Stromal	10	10.2	4.1 - 16.8
Cytology			
Positive	29	38.7	21.3 - 49.4
Negative	32	42.7	32.0 - 53.5
Inconclusive	14	18.7	10.5 - 32.0
Therapy			
Surgery	35	35.7	27.1 - 46.9
Surgery followed by adju- vant chemotherapy	46	46.9	38.3 - 56.6
NeoAdjuvant Chemotherapy - Surgery - Adjuvant Chemoterapy	17	17.3	10.2 - 24.5

The distribution of demographic characteristic subjects were according to age, parity and age of menarche (Table 1). The distribution of clinical characteristics were according to staging of newly diagnosed cases, histopathology results, cytology results, survival rate based on histopathology, survival rate based on therapy and clinical response percentage based on therapy (Table 2).

In epithelial ovarian cancer group, the survival rate in the first year was 95.8%, second year 86.8%, while it was decreased on the third and fourth year becoming 77%. Under observation, there were 74 patients who had completed 1-year follow up, 55 patients for 2-year follow-up, 29 patients for 3-year follow-up, while only 13 patients had completed 4-year follow-up.

In germ-cell tumor group, the first year survival rate was 91.7% and second to fifth year survival rate was 83.3%. There were 12 patients completed 1-year observation, 12 patients completed for 2-year, 7 patients for 3 year and 3 patients for 4-year observation. Meanwhile, 5-year survival rate of stromal type group was 100%. The number of patients who completed 1, 2, 3 and 4 years of observation were 10, 9, 4, 2 patients, consecutively.

### Ovarian cancer data in Dr. Cipto Mangunkusumo 103

In surgical treatment group, first year survival rate was 97.1%, second year was 90.6%, third year was 84.1% and fourth year was 84.1%. There were 35 patients underwent first year, 28 continued to se-cond year, 13 patients completed the third year and 4 patients had completed fourth year of observation.

First year survival rate in surgical with chemotherapy as adjuvant therapy group achieved 97.7%, second year was 85.7%, third and fourth year was 83.3%. There were 45 patients had completed 1-year observation, then 34 patients left with 2-year survival, 19 patients completed for the 3-year and 12 patients completed through 4-year observation.

In neoadjuvant chemotherapy group, first year and second year of survival rate were 94.1%, 79.9%; respectively and the third and fourth year were 68.4%. There were 17 patients underwent 1year observation, 14 patients continued to 2-year, 8 patients on the 3-year, then 2 patients continued to 4-year observation.

In the group of surgery followed by adjuvant chemotherapy, the clinical response based on RE-CIST criteria showed 63% patients achieved complete response, 4.3% partial response, 4.3% showed progressive disease, 2.2% stable disease, while there were 26.1% patients were lost to follow up. Meanwhile, on the group chemotherapy as neoadjuvant, it showed 41.2% achieved complete response, 11.8% for partial response, 17.6% for progressive disease and 29.4% patients were lost to follow-up.

### DISCUSSION

National Cancer Institute (NCI) in 2007 had reported that 47.8% of the incidence for ovarian cancer occurred in 20-60 years old. In 2015, NCI stated the incidence had been increasing for above 65 years old.<sup>12,13</sup> John K Chan, et al. showed that most of ovarian cancer (78%) had been diagnosed above 50 years old during the last 14 years. Some studies stated that the incidence of ovarian cancer in USA had increased above 64 years old.<sup>3,6,7,11</sup> Meanwhile, in Indonesia, the highest incidence in 2002 was diagnosed in 45-54 years old and it was decreased after that.<sup>2</sup> This report described the incidence of ovarian cancer was varied in Indonesia.

Some studies concluded that age of menarche was a weak predictor for ovarian cancer. It also

showed by Whitemore study where they showed the weak relationship between risk of ovarian cancer and menarche age. However, the protective effect was not found in other studies, whereas Ernestoff et al. showed the decreased odd ratio for 11-15 years old was reported not significant.<sup>7,14-17</sup> In our study, most of the subjects (73.5%) had their menarche on 12-16 years old, 14.4% forgot about their first menstruation and 12.2% started the menstrual period before 12 years old. The average menarche age in Indonesia was 12-16 years old.<sup>18</sup>

Whittemore reported that multiparity women had lower risk to have ovarian cancer compared to nulliparity. Based on the population, the highest protective effect was found in the first term pregnancy and increased as the number of parity. It was also stated that women with history of more than five pregnancies would decrease the risk of pregnancy to 80%.<sup>7,15,16</sup> In our study, the most of ovarian cancer (45.9%) was diagnosed in multiparity. The percentage of ovarian cancer would decrease along with the number of childbirth and the lowest number was found in grande multiparity subjects. The declining incidence was 36.7% between nulliparity and primiparity and 86.7% between nulliparity and grande multiparity.

Another result from this study was most of the patients were diagnosed in stage III (40.8%). This result was different compared with previous study in 2007, whereas most of ovarian cancer cases (56.52%) were diagnosed in early stage.<sup>2</sup>

Most of the patients (41.8%) underwent surgical therapy; other 40.8% underwent surgery and adjuvant chemotherapy, while 17.3% received neoadjuvant chemotherapy, continued with combination of surgery and adjuvant chemotherapy. This was similar to staging distribution from newly diagnosed cases. Ovarian cancer stage  $\geq$  IC will need chemotherapy as adjuvant therapy. This study also showed most of the patients were diagnosed as advanced stage; therefore, combination of surgery and chemotherapy as neo-adjuvant/or adjuvant therapy would be an alternative therapy.<sup>19</sup>

National Cancer Institute stated 4-year relative survival rate of epithelial ovarian tumor in 2007 was 51.5%, 59.3%, 66.8% and 73.6%, respectively. Meanwhile, 5-year survival rate based on NCI data in 2011 was 58.7% for less than 65 years old and 28.4% for more than 65 years old. Baldwin, et al. reported the first, second and fifth year survival rate for this epithelial type was 65%, 44% and

36%, respectively for American women during 1995 - 2007. Furthermore, Fuh, et al. in 2015 showed Asian had higher survival rate than American women. These results added that ethnic was relative risk factor for survival rate. Several studies supported that Asian got higher survival rates compared with Western population.<sup>12,13,21,22</sup>

In this study, the result showed the survival rate for epithelial tumor in Dr. Cipto Mangunkusumo Hospital was higher than the previous studies. It was hypothesized that ethnic and majority of younger age compared to American women played role to the results. The type of ovarian cancer as well as differentiation was also contributed to survival results. The weakness of this study was majority of lost to follow up patients that might cause the accuracy of survival analysis.

Study performed by Chan, et al. stated 5-year survival rate of germ cell tumor achieved 94.7% in 2006 and NCI data had supported the similar results for 85% survival rates.<sup>3,12,13</sup> Meanwhile, this study showed the first, second and fourth year survival rate were 91.7%, 83.3% and 83.3%, respectively. This difference might be caused of limitation subjects (13 subjects) included to this group.

Other findings for stromal type had achieved 100% 5-year survival rate. Some studies showed 5-year survival rate of germ cell tumor in general was more than 85%. The weakness of this study was small number of subjects (10 subjects).<sup>4,12,19</sup>

On the surgery group, the results of 1-, 2-, 3-, 4year survival rates were 97.1%, 90.6%; 84.1% and 84.1%, respectively. On literature, surgery was performed to low risk ovarian cancer (stage IA-IB) with well differentiated. Surgical only had achieved 90% of curative stage and 5-year survival rate 78%. The success of therapy was decided if optimal surgery results achieved.<sup>11,20</sup> Trimbos, et al. stated that adequate surgical staging and degree of differentiation were the significant prognostic factors, instead of staging.<sup>20</sup>

The treatment response of ovarian cancer stage  $\geq$  IC after adjuvant chemotherapy was determined by amount and size of tumor implant after surgical staging.<sup>19,23</sup> Based on ACTION and ICON study, 5-year survival rate after chemotherapy as adjuvant therapy was 82%, and 74%.<sup>19</sup> In this study, survival rate of patient underwent surgery continued by adjuvant therapy was 97.7% in first year and 87.5% in the second year, also 83.3% from third

and fourth year. However, this study did not include the operation result.

Chemotherapy as neo-adjuvant treatment is indicated to ovarian cancer with the lowest prediction to achieve optimal cyto-reduction after surgery. The other indication is ovarian cancer stage IIIC with ascites >500cc. One of the advantages from neo-adjuvant admission is low morbidity due to surgical procedure. Yet, the result to survival rate is unclear. Several studies showed no difference among survival rates, but the patients had better quality of life. Multicenter study in France showed survival improvement in neo-adjuvant group, while it showed no significant results in Japan. The most important prognostic factor to response of chemotherapy as neo-adjuvant therapy was if it was optimal or suboptimal-debulking. Bilici, et al. stated 50% patients would achieve 52.5 month length of life with optimal cyto-reduction.19,23

Furthermore, previous study reported 2-year survival rate in neo-adjuvant group was 90%. This study showed similar results, that first year of survival rate was 94.1% and 5-year survival rate of neo-adjuvant chemotherapy was only 68.4%. The reason was due to the limitation number of subjects.

Another result from combination of surgery and adjuvant chemotherapy group were 65.8% achieving complete response. Neo-adjuvant chemotherapy group showed 41.2% patients achieving complete response. Based on references, more than 50% in advanced stage patients whom received paclitaxel and carboplatin chemotherapy showed complete response, only 20-30% subjects could achieved partial response or progressive disease.<sup>24</sup>

## CONCLUSION

The highest incidence of ovarian cancer in Dr. Cipto Mangunkusumo Hospital belongs to the age of reproductive women ( $\leq$  55 years old) with the highest incidence occurs in nulliparity women. Most of the ovarian cancer cases are diagnosed in advanced stage (stage III-IV).

## CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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**Research Article** 

## Mesothelin versus Ca-125 in Screening the Ovarian Malignancy

### Mesothelin dan Ca-125 dalam Memprediksi Keganasan Ovarium

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### Abstract

lignancy.

**Objective**: To determine the sensitivity and specificity of mesothelin compared with Ca-125 as a tumor marker in predicting ovarian ma-

**Method**: The cross sectional study design with diagnostic tests was conducted in 30 samples of patients undergoing elective laparotomy due to ovarian tumor. We compared the sensitivity and specificity between mesothelin and Ca-125, then the data were analyzed using SPSS software version 22.0.

**Result**: According to the ROC curve analysis, optimal sensitivity and specificity value of mesothelin was 63.2% and 54.5% at a cut-off point of 0.45 pg/ml; or 42.1% and 72.7% at a cut-off point of 0.55 pg/ml. While the value of both optimal sensitivity and specificity of Ca-125 was 73.7% and 63.6% at a cut-off point of 46.63 U/ml.

**Conclusion**: Mesothelin and Ca-125 are not different significantly for the AUC value of 50%. Due to higher sensitivity and specificity of Ca-125 than mesothelin, Ca-125 is still used as tumor marker for screening the ovarian malignancy.

[Indones J Obstet Gynecol 2016; 4-2: 107-110]

Keywords: Ca-125, mesothelin, ovarian malignancy

### Abstrak

**Tujuan**: Untuk menentukan sensitivitas dan spesifisitas mesothelin dibandingkan dengan antigen Ca-125 sebagai penana tumor dalam memprediksi keganasan ovarium.

**Metode**: Studi potong lintang dengan uji diagnostic dilakukan pada 30 pasien yang akan menjalankan laparotomy elektif karena tumor ovarium. Kami akan membandingkan sensitivitas dan spesifisitas antara mesotelin dan Ca-125. Data yang diperoleh kemudian dianalisis dengan menggunakan SPSS versi 22.0 dan dilakukan pembahasan sesuai literatur yang ada.

**Hasil**: Berdasarkan analisis kurva ROC, sensitivitas dan spesifisitas yang optimal pada mesothelin adalah 63,2% dan 54,5% dengan nilai cut-off 0,45 pg/ml dan 42,1% dan 72,7% dengan nilai cut off 0,55 pg/ml. Sedangkan, nilai sensitivitas optimal dan spesifisitas Ca-125 adalah 73,7% dan 63,3% dengan nilai cut-off 46,6 U/ml.

**Kesimpulan**: Mesotelin dan Ca-125 tidak berbeda bermakna pada nilai kurva AUC 50%. Ca-125 memberikan nilai sensitivitas dan spesifisitas lebih tinggi dari mesotelin sehingga Ca-125 masih digu-nakan sebagai alat skrining keganasan ovarium.

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Kata kunci: Ca-125, keganasan ovarium, mesotelin

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### INTRODUCTION

Ovarian carcinoma as a malignant tumor in women after cervical and endometrial carcinoma has the prevalence rate of 2.4-5.6%. This carcinoma causes mortality higher than other malignant tumors. It is because 70% of the cases is diagnosed in advanced stage (between stage III and IV) so that it has spread far beyond the ovary. Therefore, there is a need to find a highly effective screening tools in ovarian malignant tumor to reduce the morbidity and mortality of this disease.<sup>1,2</sup> Excellent screening tools has high both sensitivity and specificity.<sup>1</sup> One of the antigens released from the malignancy of the epithelial ovarian cells is Ca-125.<sup>1</sup>

Approximately, 83% of patients with ovarian malignant tumor had a high level of Ca-125, which

showing the value more than 35 U/ml.<sup>1,3</sup> A study conducted in Sweden stated that the specificity of Ca-125 in ovarian malignant tumor was 97% and the positive predictive value (PPV) was 4.6%. The low level of PPV was caused by the elevation of Ca-125 happened in several condition such as other malignant tumors like breast, pancreas, bladder, lung and liver cancer; as well as in other benign abnormalities including endometriosis, myoma uteri, tuba-ovarian abscess, benign ovarian cyst, ectopic pregnancy, diverticulitis; even in physiological conditions for example pregnancy and menstruation. Meanwhile, other tumor markers that could be detected in ovarian malignancies including Ca-19-9, Ca 15-3, Ca 72-4, LSA (lipid-associated sialic acid), LPA (lysophosphatide acid), osteopontin, and mesothelin.<sup>2,3</sup>

Mesothelin as a 40-kDa glycoprotein is bound to glucosilphosphatydillinositol. In normal tissues, the expression of mesothelin can be found on the mesothelial cells (cells that border the pleura, peritoneum and pericardium) and also epithelial cells of the trachea, tonsils, fallopian tubes, and kidneys.<sup>4</sup> According to study by Hough, et al. in 2000, mesothelin was also expressed in ovarian carcinoma. Thus, it usually used as a screening tool in ovarian carcinoma.<sup>5</sup> Lowe, et al. in their study stated that increased levels of mesothelin were higher in patients who had undergone optimal debulking compared with those with advanced ovarian cancer.<sup>6,7</sup> In addition, 42% of patients who were at an early-stage of ovarian cancer also had elevated levels of mesothelin in the urine compared with only 12% of patients who had elevated in mesothelin serum. This proved that mesothelin had the potency as a screening tool to detect the ovarian carcinoma.8

Based on recent literatures, we would like to determine the relationship between mesothelin and Ca-125 as screening tools for ovarian malignant tumor.

#### METHODS

This cross sectional study to determine the diagnostic test was carried out in ovarian carcinoma patients who were registered at the gynecology out patient polyclinic in Prof. Dr. R. D. Kandou hospital. The inclusion criterias were the patients diagnosed as ovarian tumor, they would undergo the elective laparotomy, also they did not suffer from other malignancies. If they would like to participate, they had to sign the informed consent form. We excluded the patients who could not do the laparatomy. Besides, the result of biospy showed the tumor not original from the ovary was excluded. The patients were checked the blood samples to show the level of mesothelin. This examination was sent to Prodia laboratory, Manado. This study was conducted from June to August 2015.

By calculating using the formula, we had to fullfill minimally 30 samples of ovarian carcinoma. Data were analyzed by the ROC to calculate sensitivity and specificity levels of mesothelin to the histopathology samples of ovarian carcinoma. The data were processed using the software program of Statistical Product and Service Solutions (SPSS) for Windows version 22.0.

### RESULTS

Of 30 patients included in this study, most of them (18/50) were 40-59 years old and more than half of patients (18/30) were multi parity (Table 1). The histopathology characteristic of ovarian tumors showed that 2 samples were serous cystadenoma and 10 samples were mucinous cystadenoma as the benign ovary tumors. Meanwhile, ovarian serous cystadenocarcinoma was on 3 patients and the remaining was mucinous cystadenocarcinoma (15 samples) as malignant ovary tumors. This examination was done at the Laboratory of Pathology Anatomy Faculty of Medicine Universitas Sam Ratulangi, Manado.

 Table 1.
 Characteristics of Respondents

Characteristics		N (30)
Age	<20	1
	20-39	5
	40-59	18
	≥60	6
Parity	0	3
	1-2	9
	3-5	16
	>5	2
BMI	≤18	3
	18-24	14
	≥25	13

Based on the statistical group, Ca-125 levels had an average value of 412.0 U/dl with a standard deviation of 963.9. On the other hand, mesothelin levels showed an average value of 1.5 pg/ml with a standard deviation of 3.7 (figure 1). There was not correlation between mesothelin and ovarian malignant tumor (p=0.15; r=0.15).

From the ROC method above, we obtained 59.8% (95% CI 39.3-80.3%) for AUC values of mesothelin. It meant that mesothelin levels were not significantly different to the 50% value of AUC. While, the AUC values of Ca-125 was 60.5% (95% CI 38.3-80.3%) which meant that Ca-125 levels were not significantly different from AUC value of 50%.

From the table of coordinates, sensitivity and specificity for mesothelin levels with a "cut off point" of 0.45 pg/ml were 63.2% and 54.5%;



Figure 1. The Graphs of Mesothelin and Ca-125 Level.

meanwhile, if we set up the "cut off point" of mesothelin levels at 0.55 pg/ml, the sensitivity and specificity would be 42.1% and 72.7%. The interpretation was that 63.2% sensitivity values obtained from this study was valid if mesothelin levels of 0.45 pg/ml were used in 100 patients and 63 patients had mesothelin levels greater than or equal to 0.45 pg/ml, they had epithelial ovarian malignant tumor which confirmed through histopa-thology examination as a gold standard. This was implied vice versa for the specificity.

### DISCUSSION

Until 2010, ovarian cancer was still the third malignant diseases in women. According to study conducted by Jemal, et al. in the United States, there were an estimated of 21,880 new cases, whereas 13,850 patients passed away due to this disease. In Indonesia itself, the ovarian cancer was ranked the second after cervical cancer as the cause of gynecological cancer death. Nearly 70% of women with ovarian cancer were diagnosed in advanced stage so that the five-year survival rate was only 30%.<sup>2</sup>

In this study, the majority of patients were 40-59 years old and most of them had three to five children. According to another study, it was found that the population of patients with ovarian tumors were between 20 and 65 years old. This statement



was supported through study by Boy B et al and Diana HF et al where they said that the ovarian malignant tumor was rare under 40 years old.<sup>9,10</sup> A little bit different result by Martin Siregar, et al, they found that the distribution of patients with ovarian malignant tumor were mostly in the age group of 41 to 50 years old.<sup>11</sup>

Hala Abdel-Azeez, et al in their study reported that the cut-off value for mesothelin was 1.4nM, which meant that it had a lower sensitivity when compared with Ca-125 in detecting ovarian malignancies. Increased mesothelin levels in serum was ranged from 60% to 77% in women with ovarian cancer.<sup>12</sup> Several studies investigated the use of Ca-125 and mesothelin as single also combined markers in patients with ovarian malignancy. As a single marker, Ca-125 had higher sensitivity than mesothelin itself.<sup>14</sup> Although mesothelin could not be used as a single marker to detect ovarian cancer, when combined with Ca-125, it resulted high sensitivity. Serum mesothelin increased at a fraction of patients with normal Ca-125 serum. The combination of Ca-125 and mesothelin would rise the sensitivity compared with a single use of Ca-125 in detecting the ovarian malignancy. According to study by Badgwell, et al. in 2007, mesothelin in the urine acted more sensitive marker than mesothelin serum in the blood to differentiate patients with early-stage of ovarian malignancy.<sup>7</sup>

## CONCLUSION

We conclude that mesothelin and Ca-125 are not significantly different to the AUC value of 50%. The optimal sensitivity and specificity for mesothelin was lower than the results shown by Ca-125. Therefore, Ca-125 is still used as a tumor marker for screening the ovarian malignancy.

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**Research Article** 

## The Outcomes of Primary Debulking Surgery and Neoadjuvant Chemotherapy in Advanced Ovarian Cancer

Luaran Bedah Primer Debulking dengan Kemoterapi Neoajuvan pada Kanker Ovarium Stadium Lanjut

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### Abstract

**Objective**: To compare the outcomes and survival rate of primary debulking surgery with neoadjuvant chemotherapy.

**Method**: We selected advanced ovarian cancer patients from medical records. Subjects were allocated into groups of primary debulking surgery and neoajuvant chemotherapy by considering the inclusion and exclusion criteria. We analyzed the data using T test, Fisher's exact, and chi-square. The survival rate was presented in Kaplan Meier curve, whereas the significance was tested with Logrank. We managed the data using STRATA software version 12.

**Result**: We obtained 32 cases of primary debulking surgery group and 20 cases of the neoadjuvant chemotherapy group. Most of the subjects (44.2%) were 40-49 years old and 80.8% had delivered more than twice. The mean value of Ca-125 at admission was 3,594.8 u/ml (range 66.6 to 73,000 u/ml). Total of 31 subjects showed the serous histologic type (59.6%). There was no association between primary debulking surgery and neoadjuvant chemotherapy for the parameter of operative time, blood loss, organs injury, ICU stay, and hospital stay (p>0.05). Primary debulking surgery had a survival rate similar to neoadjuvant chemotherapy group (p=0.95).

**Conclusion**: The perioperative outcomes of advanced ovarian cancer patients has similar result between primary debulking surgery and neoadjuvant chemotherapy. Primary debulking surgery has a survival rate similar to neoadjuvant chemotherapy group.

[Indones J Obstet Gynecol 2016; 4-2: 111-115]

**Keywords**: advanced ovarian cancer, neoadjuvant chemotherapy, primary debulking surgery

#### Abstrak

**Tujuan**: Membandingkan luaran dan angka kebertahanan hidup antara bedah debulking primer dengan kemoterapi neoajuvan.

**Metode**: Peneliti mengambil pasien kanker ovarium stadium lanjut dari rekam medik. Subjek dialokasikan ke dalam kelompok bedah debulking primer dan kemoterapi neoajuvan dengan mempertimbangkan kriteria inklusi dan eksklusi. Data dianalisis dengan uji T, Fisher exact, dan chi-square. Angka kebertahanan hidup ditampilkan dalam kurva Kaplan Meier di mana kemaknaan diuji dengan Logrank. Kami mengolah data menggunakan software Strata versi 12.

**Hasil**: Kami mendapat 32 kasus bedah debulking primer dan 20 kasus dari kelompok kemoterapi neoajuvan. Kebanyakan subjek (44,2%) berusia 40-49 tahun dan 80,8% pernah melahirkan lebih dari 2 kali. Rerata nilai Ca-125 saat masuk ialah 3.594,8 U/ml (66,6 hingga 73.000 U/ml). Sebanyak 31 subjek memperihatkan tipe histologi serosa (59,6%). Tidak ada hubungan antara bedah debulking primer dengan kemoterapi neoajuvan untuk luaran waktu operasi, jumlah hilang darah, kerusakan organ, lama tinggal di ruang rawat intensif maupun RS (p>0,05). Bedah debulking primer memiliki angka kebertahanan hidup mirip dengan kelompok kemoterapi neoajuvan (p=0,95).

**Kesimpulan**: Luaran pasien kanker ovarium stadium lanjut yang dilakukan bedah debulking primer mirip dengan yang dilakukan kemoterapi neoajuvan. Bedah debulking primer memiliki angka kebertahanan hidup mirip dengan kelompok kemoterapi neoajuvan.

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Kata kunci: bedah debulking primer, kanker ovarium stadium lanjut, kemoterapi neoajuvan

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## INTRODUCTION

In 2009, 21,550 women were diagnosed with ovarian cancer and 14,600 of them died due to the disease. The ovarian cancer was the fifth most common causes of death from malignancy. In the United States, an estimated 1 of 72 women would develop the ovarian cancer in their lifetime and 1 of 100 women would die because of the disease.<sup>1</sup> In Europe, there were 61,000 new cases diagnosed and 39,000 deaths from ovarian cancer occurred annually.<sup>2</sup>

In Indonesia, cancer was the fifth causes of death. It was because the life expectancy rate is increased so that automatically, it will rise the degenerative disease; one of which is cancer. Life expectancy rate is related to the improvement of socioeconomic condition. More than 40% of women malignancies are gynecological cancers.<sup>3</sup>

Advanced epithelial ovarian cancer typically presents with widely disseminated intraabdominal disease. The standard treatment of advanced epithelial ovarian cancer includes primary cytoreduction or debulking surgery followed by adjuvant systemic chemotherapy.<sup>1</sup> Surgical cytoreduction of advanced stage ovarian cancer, also termed "tumor debulking", is defined as an attempt to maximally resect all visible and palpable disease.<sup>4</sup>

The ideally curative surgical approach to the cancer is through en-bloc resection of the tumor with wide margin of normal tissue. Unluckily, this method is not appropriate to most of ovarian cancer patients due to the existence of diffuse metastases to vital structures at the time of diagnosis. Therefore, the aim for these patients is to reduce the tumor burden as much as possible.<sup>5</sup>

The most effective surgical cytoreduction intends to reach the minimal risk of residual status. The microscopic residual disease has correlation with the overall survival rate in patients with advanced disease.<sup>1,6</sup>

In selected cases which it predicts that complete or optimal surgical cytoreduction will not be achieved at primary surgery, we should perform the neoadjuvant chemotherapy followed by interval debulking surgery (delayed primary surgery). Recent study showed that neoadjuvant chemotherapy followed by interval debulking surgery in the bulky stage III and stage IV disease was not inferior to primary surgery.<sup>7</sup>

Some gynecologist has suggested this approach, especially for the treatment of stage IV ovarian cancer or for patients with very high metastatic tumor load (for example the mass was more than 1,000 grams) or in patients with poor general condition.<sup>8</sup> Several advantages of neoadjuvant chemotherapy are such as a reduced risk of perioperative morbidity, a higher rate of optimal resection, and the contention that survival is not compromised by deferring the initial attempt at surgical debulking.<sup>9</sup>

Interval debulking surgery in patients with advanced stage of ovarian cancer offered the same chance of survival as primary debulking surgery; however, interval debulking surgery showed better toleration.<sup>10</sup> Therefore, this study aims to compare the outcomes of primary debulking surgery with neoadjuvant chemotherapy.

Table 1.	The Characteristics	of Patients.
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Variable	Primary Debulking Surgery (N=32)	Neoadjuvant Chemo- therapy (N=20)	p value
Mean of age - years old	49.1	48.7	0.55*
Parity - N (%)			
Nulliparity	1 (3.1)	2 (10.0)	0.55**
Primiparity	4 (12.5)	3 (15.0)	
Multiparity	27 (84.4)	15 (75.0)	
FIGO stage - N (%)			
IIIC	29 (90.6)	15 (75.0)	0.24***
IV	3 (9.4)	5 (25.0)	
Mean of serum Ca-125 at entry (U/ml)	1661.9	6687.36	0.05*
Histologic type - N (%)			
Serous	16 (50)	15 (75.0)	0.19**
Endometrioid	9 (28.1)	3 (15.0)	
Mucinous	3 (9.4)	2 (10.0)	
Clear cell	4 (12.5)	0	
Histologic grade - N (%)			
Well differentiated	3 (9.4)	5 (25.0)	
Moderately differentiated	15 (46.9)	8 (40.0)	
Poorly differentiated	14 (43.8)	7 (35.0)	
Degree of cytoreduction - N (%)			
Complete macroscopic resection	9 (28.1)	9 (45.0)	0.44**
Optimal cytoreduction	5 (15.6)	3 (15.0)	
Suboptimal cytoreduction	18 (56.3)	8 (40.0)	

\*t-test, \*\*chi-square, \*\*\*fisher exact

## METHODS

We obtained the data from stage IIIC and IV epithelial ovarian cancer patients' medical record. Each patient was classified into the group of primary debulking surgery and neoadjuvant chemotherapy. We needed 30 samples of each group. The perioperative outcomes were consisted of duration of surgery, intraoperative blood loss, intraoperative organs injury, length of stay in intensive care unit (ICU), and also the total hospitalization time.

We analyzed the data using T test for the numerical data and Fisher's exact also chi-square for the categorical data. The survival rate for both groups were calculated from the date of surgery to death (event). Survival rate was presented in Kaplan Meier curve, whereas the significance was tested with Logrank. We managed the data using STRATA software version 12.

## RESULTS

We could not fulfill the required samples due to the difficulty of searching the medical records. In our study, we obtained 32 cases of primary debulking surgery group and 20 cases of the neoadjuvant chemotherapy group. Most of the subjects (44.2%) were 40-49 years old and 80.8% had delivered more than twice. The mean value of Ca-125 at admission was 3,594.8 u/ml (range 66.6 to 73,000 u/ml). Total of 31 subjects showed the serous histologic type (59.6%). The characteristics of patients were distributed in Table 1.

In our study, there was no association between primary debulking surgery and neoadjuvant chemotherapy for the parameter of operative time, blood loss, organs injury, ICU stay, and hospital stay (Table 2). Figure 1 described the overall survival rates based on treatment options.

Table 2.	Perioperative	Morbidity in	Both Study	y Groups.
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Variable	Primary Debulking Surgery (N=32)	Neoadjuvant Chemo- therapy (N=20)	p value (t test)
Operative time (min)			
Mean	244.8	229.5	0.70
Range	105-510	165-375	
Blood loss rate (ml)			
Mean	1,704.7	1,152.5	0.66
Range	300-13,000	400-2,100	
Organs injury ICU stay (days)	3	1	0.57
Mean	0.3	0.3	0.57
Range	0-2	0-2	
Hospital stay (days)			
Mean	8.3	7.5	0.78
Range	5-17	5-14	

\*chi-square



Figure 1. Overall Survival Rate According to the Treatment Options.

## DISCUSSION

## Length of Surgery

Hou, et al. showed that the average length of surgery was significantly lower in the neoadjuvant chemotherapy compared with primary debulking surgery group (211 min vs. 276 min, p<0.001).<sup>11</sup> However, a study by Hegazy, et al. indicated the conflicting result where the average duration of the operation on neoadjuvant group was 150 minutes, while for primary surgery group was over 190 minutes. There was no significant difference between two groups in statistic.<sup>12</sup> Their result was similar to Kuhn, et al. study. Kuhn said that the average length of surgery for primary debulking surgery and neoadjuvant chemotherapy were 270 and 260 minutes. They found no significant relationship on length of surgery between two groups.<sup>13</sup> Our study had similar results with study by Hegazy et al. and Kuhn et al. The average length of surgery was 245 minutes versus 230 minutes, also we found no significant difference in duration of surgery between two groups (p=0.70).

## **Blood Loss Rates**

Hou, et al. studied 172 patients of advanced stage epithelial ovarian cancer consisting of 109 patients performing the primary debulking surgery and 63 patients undergoing the neoadjuvant chemotherapy from 1998 to 2005. Patients receiving neoadjuvant chemotherapy experienced significantly less blood loss during surgery (p<0.001).<sup>11</sup> The same result was also reported by Hegazy, et al. They reported that the group with neoadjuvant chemotherapy had significantly less blood loss during surgery (p=0.02).<sup>12</sup> Our study found the average blood loss in the neoadjuvant chemotherapy group was 1,152.5 ml, while the primary debulking surgery group was 1,704.7 ml. Clinically, the amount of bleeding was higher in the primary debulking surgery group; yet the statistical test had shown no difference between the two groups (p=0.34).

## **Organs Injury**

Kuhn, et al. in a retrospective study involving stage IIIC ovarian cancer patients including 31 patients received neoadjuvant chemotherapy and 32 patients were allocated to the primary debulking surgery group. The result showed that 11 and 9 women were found injured at the primary debulking surgery and neoadjuvant chemotherapy group. They did not find the difference in intraoperative organs injury.<sup>13</sup> Similar result was reported by Hegazy, et al. where they did not find a significant association between therapy modality and intraoperative organs injury.<sup>12</sup> Our study released the similar result to both study above.

## **Hospital and ICU Stay**

Hegazy, et al in their study showed that neoadjuvant chemotherapy group was shorter in length of hospital (p=0.05) and ICU stay (p=0.03) significantly.<sup>12</sup> Contrary to the result stated by Hegazy, et al., Hou, et al., in their study involving 172 patients, they concluded that there was no significant association between administration of neoadjuvant chemotherapy and length of stay in hospital also ICU.<sup>11</sup> Of all the perioperative outcome variables, neoadjuvant chemotherapy group experienced shorter operative time, less blood loss, and shorter treatment duration. Neoadjuvant chemotherapy group offered better clinical outcome compared with primary debulking surgery despite lack of statistical evidence.

## **Overall Survival Rate**

Vergote, et al. in a multicenter study involving 718 patients, reported that the median overall survival rate of primary debulking surgery group was 29 months, whereas in the neoadjuvant chemotherapy group was 30 months. There was no statistical difference in survival rate between the two groups (p=0.98).<sup>14</sup> Hegazy, et al. also declared similar result to study stated above. Primary debulking surgery group had median overall survival rate of 28 months, whereas in the chemotherapy group was 25 months; however, it was not statistically different (p=0.5).<sup>12</sup> Loizzi, et al. in a case-control study examined the outcomes of primary debulking surgery and neoadjuvant chemotherapy in advanced ovarian cancer. The result did not differ statistically. (p=0.66).<sup>15</sup> Steed, et al. also reported that there was no significant difference in terms of progression-free survival (PFS) (HR=1.61; p=0.04; 95% CI=1.03-2.53) and overall survival (OS) (HR=1.85; p=0.03, 95% CI=1.06 - 3.23) rate for both groups. It was the only one study that stating both groups had the same survival rate.<sup>16</sup> Our study also confirmed that both groups had the same overall survival rate.

#### The outcomes of primary debulking surgery 115

## CONCLUSION

The perioperative outcomes of advanced ovarian cancer patients has similar result between primary debulking surgery and neoadjuvant chemotherapy. However, through clinical judgement, neoadjuvant chemotherapy group has better perioperative outcomes compared with primary surgery group. Primary debulking surgery has a survival rate similar with neoadjuvant chemotherapy group.

## CONFLICT OF INTEREST

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article. We received no financial support for the research, authorship, and/or publication of this article.

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**Research Article** 

## Female Sexual Function after Vaginal Delivery with Episiotomy and Cesarean Section

Fungsi Seksual Perempuan Pascapersalinan Pervaginam dengan Episiotomi dan Seksio Sesarea

## I Made W Jembawan, I Made Darmayasa

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#### Abstract

**Objective**: To determine the difference of sexual function after vaginal delivery with episiotomy and cesarean section in Sanglah Hospital, Denpasar.

**Method**: This research was conducted using cross sectional method. Sample was collected using consecutive sampling, starting from October 2011-September 2012. Our sample consists of 86 women, 43 post-episiotomy and 43 post-cesarean section. Sexual function was assessed using FSFI (Female Sexual Function Index). Total score was analyzed using independent t-test and difference of sexual function was tested using Chi-square, with significance level p<0.05.

**Result**: Subject characteristics in both groups did not differ significantly. The average time to first sexual intercourse in both groups was 3 months after delivery (p>0.05). There was no significant difference between the two groups in term of sexual arousal and lubrication, with p-value 0.160 and 0.67, respectively. However, we found significant difference in other domains, namely desire (p=0.014), orgasm (p=0.045), satisfaction (p=0.018), pain (p=0.02), and total FSFI score (p=0.006). Sexual dysfunction was found in 18.60% of the episiotomy group and 2.33% of the cesarean section group, with p=0.030.

**Conclusion**: Female sexual dysfunction was found to be significantly different between women post vaginal delivery with episiotomy and women who had cesarean section.

[Indones J Obstet Gynecol 2014; 4: 199-203]

Keywords: cesarean section, episiotomy, female sexual function

#### Abstrak

**Tujuan**: Untuk mengetahui perbedaan fungsi seksual pascapersalinan pervaginam dengan episiotomi dan pascaseksio sesarea di Rumah Sakit Sanglah, Denpasar.

**Metode**: Penelitian ini menggunakan rancangan potong lintang. Rekrutmen sampel dilakukan secara consecutive sampling dari bulan Oktober 2011 sampai dengan September 2012. Didapatkan 86 sampel yang memenuhi kriteria inklusi dan kriteria eksklusi, yang terdiri dari 43 pasien pascaepisiotomi dan 43 pasien pascaseksio sesarea. Selanjutnya fungsi seksual dinilai dengan pengisian kuesioner FSFI (Female Sexual Function Index). Skor total kuesioner dianalisis dengan uji t independen, dan perbedaan fungsi seksual diuji menggunakan uji Chi-Square, dengan tingkat kemaknaan p<0,05.

**Hasil**: Karakteristik dasar subjek tidak menunjukkan perbedaan yang bermakna antara kedua kelompok. Rata-rata saat memulai hubungan seksual pada kedua kelompok adalah tiga bulan pascamelahirkan, dengan p>0,05. Tidak terdapat perbedaan yang bermakna antara kedua kelompok pada domain rangsangan dan lubrikasi masing-masing dengan p=0,160, dan p=0,067. Sedangkan domain lain yang menunjukkan perbedaan bermakna yaitu hasrat (p=0,014), orgasme (p=0,045), kepuasan (p=0,018), nyeri (p=0,02), dan skor total FSFI (p=0,006). Ditemukan disfungsi seksual pada 18,60% dari kelompok pascaepisiotomi dan 2,33% pada pascaseksio sesarea, dengan nilai p=0,030.

**Kesimpulan**: Ditemukan perbedaan yang signifikan antara perempuan pascapersalinan pervaginam dengan episiotomi dan perempuan yang menjalani seksio sesarea.

[Maj Obstet Ginekol Indones 2014; 4: 199-203]

Kata kunci: episiotomi, fungsi seksual perempuan, seksio sesarea

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#### INTRODUCTION

Most women will face pregnancy and the delivery process during the course of her life. The delivery process has an important influence on female sexual dysfunction. In daily practice, clinicians often forget about this aspect. However, the problem of female sexual dysfunction can affect a woman's quality of life, which also affect her partner and marriage life.<sup>1</sup> To determine female sexual function, we used Female Sexual Function Index (FSFI) with total score of 26.55 categorized as sexual dysfunction.<sup>2</sup>

The delivery process can be spontaneous vaginal delivery and cesarean section. Although not employed as routine practice, mediolateral episiotomy is performed during most spontaneous vaginal deliveries (especially in primiparous women) as prophylaxis from futher injury caused by fetal head passage. Conversely, episiotomy itself can cause pelvic floor dysfunction, pudendal nerve lesions, and other complications possibly affecting sexual function in the future. One of the benefit of cesarian section is less involvement of pelvic floor muscles, therefore preserving the sexual function, especially in elective cesarean sections. In emergency cesarean sections, trauma to the pelvic floor has already occurred. Moreover, numerous complications may result from the surgery that can also affect sexual dysfunction in the future.<sup>3,4</sup>

## **METHODS**

This study utilized a cross-sectional method. Eighty six primiparous women were enrolled in this study, consisting of 43 women who underwent spontaneous vaginal delivery with episiotomy and 43 women who underwent cesarean delivery. We did FSFI questionnaire in the respondent's house.

The inclusion criteria was married women, primiparous, who underwent spontaneous vaginal delivery with mediolateral episiotomy or cesarean delivery and delivered a living baby at the time of study. Subjects were also required to live with the husband who could have sexual intercourse in the last month and signed the informed consent. The exclusion criteria was previous history of medical problems, multiparity, delivery assisted by vacuum or forceps, perineal rupture grade III or IV, multiple gestation, history of misscariage, history of hormonal contraception use, and not having sexual intercourse in the last month.

## RESULTS

The demographic characteristics of our study subjects are presented in Table 1 below.

Variables	Post Episiotomy (n=43)	Post Cesarean Sec- tion (n=43)	р
Age (year)	24.53 4.23	23.72 4.23	0.375
Education			0.250
Uneducated	0 (0%)	1 (2.33%)	
Primary school	0 (0%)	4 (9.30%)	
Secondary school	6 (13.95%)	6 (13.95%)	
High school	35 (81.40%)	30 (69.77%)	
Graduate	2 (4.65%)	2 (4.65%)	
Post-graduate	0 (0%)	0 (0%)	
Occupation			0.371
Housewife	20 (46.51%)	18 (41.86%)	
Employee	17 (39.53%)	23 (53.49%)	
Private worker	4 (9.30%)	1 (2.33%)	
Civil worker	2 (4.65%)	1 (2.33%)	
Pensioner	0 (0%)	0 (0%)	
Payment			1000
Insurance	23 (53.49%)	23 (53.49%)	
Private	20 (46.51%)	20 (46.51%)	

### **Table 1.**Demographic Characteristics

Variables	Post Episiotomy (n=43)	Post Cesarean Sec- tion (n=43)	р
Age (year)	24.53 4.23	23.72 4.23	0.375
Birth weight			0.621
< 2500 g	12 (27.91%)	10 (23.26%)	
2500-4000 g	31 (72.09%)	33 (76.74%)	
4000 g	0 (0%)	0 (0%)	
Breastfeeding			0.270
Yes	39 (90.70%)	36 (83.72%)	
No	4 (9.30%)	7 (16.28%)	
Use of childcare support (babysitter)			0.279
Yes	26 (60.47%)	21 (48.84%)	
No	17 (39.53%)	22 (51.16%)	
Presence of family problems			0.596
Yes	8 (18.60%)	10 (23.26%)	
No	35 (81.40%)	33 (76.74%)	

Based on independent t-test, there was no significant difference in demographic characteristics (p>0.05). The finding showed that demographic characteristic had no effect on the results. Time to first sexual intercourse after delivery in each group is shown in Table 2. There was no difference in the time to first sexual intercourse (p>0.05). The majority of subjects initiated sexual intercourse 3 months after delivery, both in post episiotomy group (60.47%) and post cesarean group (46.51%). We performed independent t-test to determine the difference of sexual function in both groups and the result is presented in Table 3.

**Table 2.** Time to First Sexual Intercourse after Delivery

Time to first sexual intercourse (months)	Post episiotomy (n=43)	Post cesarean section (n=43)	р
1	0 (0%)	0 (0%)	0.215
2	11 (25.58%)	17 (39.53%)	
3	26 (60.47%)	20 (46.51%)	
4	6 (13.95%)	4 (9.30%)	
5	0 (0%)	2 (4.65%)	
6	0 (0%)	0 (0%)	

Variable	Post episiotomy (n=43)	Post cesarean section (n=43)	р
FSFI total score	13.53 3.02	14.90 0.96	0.006
Desire	4.70 1.15	5.21 0.62	0.014
Arousal	4.90 1.45	5.23 0.55	0.160
Lubrication	5.46 1.21	5.83 0.50	0.067
Orgasm	5.43 1.27	5.85 0.45	0.045
Satisfaction	5.16 1.49	5.75 0.56	0.018
Pain	5.06 1.62	5.89 0.44	0.002
Sexual dysfunction (Score 26.55)			
Yes	8 (18.60%)	1 (2.33%)	0.030
No	35 (81.40%)	42 (97.67%)	

Table 3. Mean FSFI Score in Post Episiotomy and Post Cesarean Section

Analysis of sexual function in both groups showed no significant difference (p>0.05) in arousal and lubrication, whereas in terms of desire, orgasm, satisfaction, pain, FSFI total score, and sexual dysfunction there was a significant difference (p<0.05).

### DISCUSSION

Decrease of sexual desire can be caused by trauma from spontaneous vaginal delivery, including pain and prolonged delivery process, therefore affecting sexual function. Moreover, sexual orientation in women after delivery also tend to change due to family priority where most women prioritize taking care of the baby, breastfeeding and using contraception.<sup>5</sup> Incidence of loss of sexual desire after delivery is 53% in the first three months and 37% in the first six months postpartum, compared with 9% in previous pregnancy.<sup>6</sup>

Although sexual desire after delivery tend to decrease, when a women can focus on arousal from herself or her partner, it is not impossible for women to get through arousal phase.<sup>7</sup> In "Sexual response circle" theory, arousal phase in women does not always follow desire. Sexual desire can be achieved after sexual arousal.<sup>8</sup>

Lubrication happens when women are stimulated both physically or mentally.<sup>9</sup> If no problem occurs in the arousal phase, sexual desire can be achieved, as characterized by warm and ticklish feeling, lubrication, and vaginal muscle contraction. Moreover, if the woman was multiparous and during the delivery episiotomy was done properly, no further rupture occurred, and there was no delayed healing, it will not affect Bartholin gland's function in sexual reaction, so then lubrication process can start normally.<sup>10</sup>

In previous literatures, incidence of failure to achieve orgasm was 33% in the the first three months and 23% in the first six months, compared to 14% who had this problem one year before pregnancy. This may be caused by perineal trauma, delayed wound healing or asymmetric wound, because the pain will make sexual intercourse uncomfortable.<sup>11</sup> Failure to achieve orgasm usually leads to no sexual satisfaction, although there are other factors such as emotional bonding that can affect sexual satisfaction.<sup>8</sup>

Pain during sexual intercourse was found to be significantly different between the two groups in this study (p=0.02). This is concurrent with the findings of Baksu et al, who concluded that pain and satisfaction had the biggest effect towards FSFI score.<sup>1</sup> According to Signorello et al, incidence of perineal pain was 42% in women after delivery and was significantly decreased to 22% and 10% at eight and twelve weeks after delivery.<sup>12</sup>

In general, this study showed that there is a significant difference in FSFI total score (p=0.006), and incidence of sexual dysfunction in both groups (p=0.030). The result was similar with the study from Baksu et al who reported a significant decrease in total score in every domain of sexual function (desire, arousal, lubrication, orgasm, satisfaction, and pain) six months after vaginal delivery with episiotomy compared with before pregnancy.<sup>1</sup>

It is not easy to analyze female sexual function, we realized that there is time and sample limitation in this cross sectional study, so from this study we can only conclude that the sexual function in both groups are different. Although we could describe the sexual function in both groups, the effect of episiotomy and cesarean section towards sexual function after delivery has not been described vet. Our study also did not analyze sexual function before pregnancy and delivery in each groups with the assumption that the pregnancy itself shows the presence of good sexual function. There are numerous other factors also affecting sexual function after delivery that are not discussed here, such as the length of the 2<sup>nd</sup> stage of labor, indication of cesarean section whether it is emergency or elective, postpartum depression, quality of breastfeeding, and other cultural factors. Therefore, further research is needed to complete the result of this study.

## CONCLUSION

Based on our results, we can conclude that there is a significant difference in women sexual function between women who underwent vaginal delivery with episiotomy and women who underwent cesarean section in terms of desire, orgasm, satisfaction, pain, FSFI total score, and incidence of sexual dysfunction.

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