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## EDITORIAL

## Is it time for "Universal Screening" of HIV Infection in Pregnant Women?

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One of the goals from Sustainable Development Goals (SDGs) by WHO is to decrease maternal and neonatal morbidity.<sup>1</sup> A significant part of these morbidities are attributed by Human Immunodeficiency Virus (HIV) infection in the pregnant women.<sup>1</sup> Our effort in controlling maternal to child transmission of HIV infection has been relatively effective with a significant decrease of transmission from 12 % in 1999 to approximately 2% in 2007.<sup>2</sup> The success has been mostly due to the use of highly effective anti-retroviral (ARV) therapy, primary pre-labor cesarean section, prophylaxis ARV for neonate, and avoidance of breastfeeding.<sup>1-3</sup>

Recent studies in the field has also improved our management to prevent maternal to child HIV transmission such as, better safety profile of ARVs that are now encouraged to be given in "ALL" HIV infected pregnancies as early as possible, even in the first trimester.<sup>3,4</sup> Improvement can also be seen in the management of specific resource settings, such as planned vaginal delivery in patients with undetectable viral load;<sup>5</sup> as well as, selective breastfeeding in these patients.<sup>5</sup>

Despite significant advances in prevention maternal to child HIV transmission, the problem remains elusive to eradicate. Screening of HIV infection in high risk population by voluntary counseling and testing (VCT) has helped a little difference. As recent studies showed that most HIV infections are found not only in high risk populations, but also in low risk individuals (ex. house wives) with high risk partners.<sup>6</sup>

How can we eradicate HIV transmission to the next generation? The best answer is whether we cannot find these infections anymore. The need for us to eradicate or to bring nil vertical transmission proposes a more effective approach in HIV screening on pregnant women. Universal screening may help in early detection and prompt ARV treatment of HIV infected pregnant women.<sup>7</sup> The Jakarta municipal government health services have proven that universal screening of HIV in pregnant women is feasible.<sup>8</sup> If we are truly committed to eradicate maternal to child HIV transmission, we may have to consider engagement of a nationwide program for a universal screening system of HIV in all pregnant women. At least in practice, we offer 80% of all pregnant women to be checked.

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Research Article

## Preterm Labor and the Associated Factors

### *Persalinan Preterm dan Faktor-faktor yang Terkait*

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#### Abstract

**Objective:** To determine the risk factors that affect preterm labor in Dr. Cipto Mangunkusumo Hospital.

**Methods:** This was an analytic descriptive study done in Emergency Unit of Dr. Cipto Mangunkusumo Hospital from July to December 2014. We recruited all women who gave birth in Emergency Unit of Dr. Cipto Mangunkusumo Hospital from July to December 2014 with gestational age less than 37 weeks. Of the total sampling method, we got 365 patients. We analyzed the data using chi square.

**Results:** The characteristic age of subjects were 14.0% of less than 20 years old, 69.0% of 21-35 years old, and the rest were more than 35 years old. Most subjects (93.1%) were employed, 94.5% were married once, 62.3% of subjects had cesarean section history, 73.7% were primiparous, only 4.4% had history more than 1 abortion, and most of them were at 33-36 weeks of gestation. Socio-demographic factor associated with preterm labor and Preterm Premature Rupture of Membrane (PPROM) was age ( $p=0.011$ ; OR 1.74; 95% CI 1.136-2.679). Obstetric history associated with preterm labor and PPRM was parity ( $p=0.017$ ; OR 1.78; 95% CI 1.132-2.878).

**Conclusion:** In this study, age and parity are associated with preterm labor and PPRM.

[Indones J Obstet Gynecol 2017; 5-1: 3-7]

**Keywords:** obstetric history, parity, PPRM, preterm labor, socio-demographic factors

#### Abstrak

**Tujuan:** Mengetahui faktor-faktor risiko yang mempengaruhi persalinan premature di RS Dr. Cipto Mangunkusumo.

**Metode:** Penelitian ini adalah studi deskriptif analitik yang dilakukan di IGD RS Dr. Cipto Mangunkusumo bulan Juli-Desember 2014. Populasi sampel penelitian ini adalah semua ibu yang melahirkan di IGD RSCM dengan usia kehamilan 20-36<sup>+</sup>6 minggu selama periode Juli-Desember 2014 yang memenuhi kriteria inklusi dan eksklusi. Pengambilan sampel dilakukan dengan total sampling. Jumlah sampel yang terkumpul 365 sampel. Analisis data menggunakan chi square.

**Hasil:** Dari hasil penelitian didapatkan 14,0% berusia kurang dari 20 tahun, 69,0% berusia 21-35 tahun, dan sisanya berusia lebih dari 35 tahun. Kebanyakan subjek (93,1%) bekerja; 94,5% memiliki riwayat menikah sekali; 62,3% memiliki riwayat sesar; 73,7% hamil anak pertama; hanya 4,4% yang pernah mengalami aborsi lebih dari 1 kali, dan sebagian besar dari mereka hamil usia kehamilan 33-36 minggu. Faktor sosiodemografi yang terkait dengan persalinan prematur dan ketuban pecah dini (KPD) prematur ialah usia ( $p=0,011$ ; OR 1,74; 95% IK 1,136-2,679). Faktor riwayat obstetri yang berhubungan persalinan prematur dan KPD prematur adalah paritas ( $p=0,017$ ; OR 1,78; 95% IK 1,132-2,878).

**Kesimpulan:** Pada penelitian ini, usia dan paritas memiliki hubungan dengan persalinan prematur dan KPD prematur.

[Maj Obstet Ginekol Indones 2017; 5-1: 3-7]

**Kata kunci:** faktor sosiodemografi, KPD prematur, paritas, persalinan prematur, riwayat obstetri

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## INTRODUCTION

Neonatal mortality is a health problem in the world. One of the major causes of neonatal mortality is preterm labor.<sup>1</sup> Preterm labor is a labor that occurs before 37 weeks of gestation.<sup>1</sup> World Health Organization (WHO) reported that about 15 million preterm labors occurred annually and more than 1 million newborns died every year because of the preterm labor complication.<sup>1</sup> Preterm newborn has certain health risks, such as cerebral palsy, intellectual impairment, chronic lung disease, and also vision and hearing impairment. The preterm labor does

not only impact to the health condition of the newborn, but also to the family and environment.

Spontaneous preterm labor can be classified into preterm premature rupture of membrane (PPROM) and spontaneous labor without rupture of membrane. Spontaneous preterm labor can be caused by infection (intrauterine and extrauterine), twin pregnancy, placenta solution, hormonal imbalance, and idiopathic. Socio-demographic factors including maternal age, education, work, marital status and obstetric history, such as parity, antenatal care, complication during pregnancy influence the occurrence of preterm labor.<sup>1-3</sup>

If the risk factor has been determined earlier, we can do the early management to decrease the number of preterm labor. In the end, we can suppress the morbidity and mortality as a consequence of preterm labor. Looking to the burden of preterm labor, this study aims to determine the risk factors that affect preterm labor in Dr. Cipto Mangunkusumo Hospital.

## METHODS

We used analytic descriptive study design to analyze the association between preterm labor and socio-demographic also obstetric factors in Emergency Unit of Dr. Cipto Mangunkusumo Hospital. The data was collected from medical records from July to December 2014. The subjects were collected using total sampling method. We got 365 subjects in this study. The subject population of this study was all women who gave birth in Emergency Unit Dr. Cipto Mangunkusumo Hospital with 20-36 weeks of gestation from July to December 2014 which were selected through using inclusion and exclusion criteria. The inclusion criteria of this study were pregnant women with 20-36 weeks of gestation and we excluded the intrauterine fetal death (IUFD) and women with medical complication. Socio-demographic factors analyzed in this study were age, work, and marital status. The obstetric history analyzed in this study included parity, abortion, antenatal care history, and low birth weight history. We analyzed using chi square in SPSS 21.0 version.

## RESULTS

**Table 1.** Characteristics of Preterm Labor in Dr. Cipto Mangunkusumo Hospital

| Characteristics        | n   | %    |
|------------------------|-----|------|
| Age                    |     |      |
| < 20                   | 51  | 14.0 |
| 21-35                  | 252 | 69.0 |
| > 35                   | 62  | 17.0 |
| Work                   |     |      |
| Employed               | 337 | 93.1 |
| Unemployed             | 28  | 6.9  |
| Marital Status         |     |      |
| Married once           | 345 | 94.5 |
| Married more than once | 20  | 5.5  |

|                          |     |      |
|--------------------------|-----|------|
| Labor method             |     |      |
| Vaginal birth            | 141 | 37.7 |
| Caesarean section        | 233 | 62.3 |
| Parity                   |     |      |
| Primiparous              | 269 | 73.7 |
| Multiparous              | 96  | 26.3 |
| History of pregnancy     |     |      |
| Primigravida             | 157 | 43.0 |
| Multigravida             | 208 | 57.0 |
| Abortion                 |     |      |
| 0-1                      | 349 | 95.6 |
| >1                       | 16  | 4.4  |
| Weeks of pregnancy       |     |      |
| 20-27 weeks              | 13  | 3.5  |
| 28-32 weeks              | 100 | 26.7 |
| 33-36+ weeks             | 261 | 69.8 |
| Antenatal care           |     |      |
| Regular                  | 235 | 64.4 |
| Irregular                | 130 | 35.6 |
| Low birth weight history |     |      |
| None                     | 337 | 90.1 |
| 1-2                      | 35  | 9.3  |
| >2                       | 2   | 0.6  |
| Preterm labor            |     |      |
| PPROM                    | 171 | 46.8 |
| Non PPRM                 | 194 | 53.2 |

In this study, we collected 365 subjects among women who gave birth within 20-36 weeks of gestation. The characteristics of preterm labor were 51 women (14.0%) whose age <20 years old, 252 women (69.0%) whose age 21-35 years old, and 62 women (17.0%) whose age >35 years old. The characteristic of occupation among subject population was 337 women (92.3%) employed and the rest of 28 women (1.6%) unemployed. The characteristic of marital status was 345 women (94.5%) having married once and 20 women (5.5%) marrying more than once. In our study, caesarean section (62.3%) had been performed on preterm labor. Around 73.7% of women were primiparous and 57.0% were multigravida. The characteristic of abortion was on 349 women (95.6%) having none or once history of abortion and 16 women (4.4%) having more than once history of abortion. More than 50% of subjects had regular antenatal care. About 90.1% women did not ever deliver low birth weight, 9.3% delivered once to twice of low birth weight, and the rest had history more than twice. There were 171 women (46.8%) having PPRM.

**Table 2.** Association between Preterm Labor and Socio-demographic Factors

| Socio-demographic Factors |                | Preterm Labor |      |           |      | <i>p</i> | OR   | 95% CI        |
|---------------------------|----------------|---------------|------|-----------|------|----------|------|---------------|
|                           |                | PPROM         |      | Non PPROM |      |          |      |               |
|                           |                | n             | %    | n         | %    |          |      |               |
| Age                       |                |               |      |           |      |          |      |               |
|                           | <25            | 75            | 43.9 | 60        | 30.9 | 0.011    | 1.74 | 1.136 - 2.679 |
|                           | >26            | 96            | 56.1 | 134       | 69.1 |          |      |               |
| Work                      |                |               |      |           |      |          |      |               |
|                           | Employed       | 10            | 5.8  | 18        | 9.3  | 0.219    | 0.6  | 0.738 - 3.672 |
|                           | Unemployed     | 161           | 94.2 | 176       | 90.7 |          |      |               |
| Marital status            |                |               |      |           |      |          |      |               |
|                           | Married once   | 163           | 95.3 | 182       | 93.8 | 0.536    | 1.34 | 0.536 - 3.368 |
|                           | More than once | 8             | 4.7  | 12        | 6.2  |          |      |               |

**Table 3.** Association between Preterm Labor and Obstetric Histories

| Obstetric Histories      |             | Preterm Labor |      |           |      | <i>p</i> | OR   | 95% CI        |
|--------------------------|-------------|---------------|------|-----------|------|----------|------|---------------|
|                          |             | PPROM         |      | Non PPROM |      |          |      |               |
|                          |             | n             | %    | n         | %    |          |      |               |
| Parity                   |             |               |      |           |      |          |      |               |
|                          | Primiparous | 136           | 79.5 | 133       | 68.6 | 0.017    | 1.78 | 1.132 - 2.878 |
|                          | Multiparous | 35            | 20.5 | 61        | 31.4 |          |      |               |
| Abortion status          |             |               |      |           |      |          |      |               |
|                          | 0-1         | 164           | 95.9 | 186       | 95.4 | 0.808    | 1.14 | 0.415 - 3.129 |
|                          | >1          | 7             | 4.1  | 9         | 4.6  |          |      |               |
| Antenatal care           |             |               |      |           |      |          |      |               |
|                          | Regular     | 115           | 67.3 | 120       | 61.9 | 0.283    | 1.26 | 0.823 - 1.949 |
|                          | Irregular   | 56            | 32.7 | 74        | 38.1 |          |      |               |
| Low birth weight history |             |               |      |           |      |          |      |               |
|                          | Yes         | 14            | 8.2  | 22        | 11.3 | 0.313    | 1.43 | 0.709 - 2.900 |
|                          | No          | 157           | 91.8 | 172       | 88.7 |          |      |               |

Analysis using chi square showed that socio-demographic factor in preterm labor which was associated with PPROM was age ( $p=0.011$ ; OR=1.74; 95%CI 1.136-2.679). Meanwhile, obstetric history in preterm labor which had association with PPROM was parity ( $p=0.017$ ; OR=1.78; 95% CI 1.132-2.278).

Table 2 showed that 75 women (43.9%) whose age  $\leq 25$  years old had PPROM and 60 women (30.9%) from the same age did not have PPROM. There were 96 women (56.1%) whose age  $>25$

years old having PPROM and 134 women (69.1%) not having PPROM. Age was statistically associated with PPROM in preterm labor ( $p=0.011$ ; OR=1.74; 95%CI 1.136-2.679). Women whose age  $\leq 25$  years old had the risk 1.74 times resulted into PPROM compared with women with more than 25 years old. In this study, women whose age  $\leq 25$  years old had 63% probability to be PPROM. The other factors including occupation and marital status were not statistically associated with PPROM in preterm labor.

Table 3 showed that parity was associated with PPRM in preterm labor. There were 136 primiparous women (79.5%) having PPRM. In the group of multiparous women, there were 35 women (20.5%) having PPRM and 61 women (31.4%) not having PPRM ( $p=0.017$ ;  $OR=1.78$ ;  $95\%CI$  1.132-2.878). Primiparous women took 1.78 times risk to undergo PPRM compared with multiparous women in preterm labor. Therefore, primiparous women had 64% probability undergoing PPRM compared with multiparous women.

## DISCUSSION

This study reported that preterm labor with PPRM occurred in 46.8% labor process; while, preterm labor without PPRM occurred in 53.2% of the labor process in Dr. Cipto Mangunkusumo Hospital. Hammond, et al. reported that the prevalence of preterm labor with PPRM in Australia during 1984-2006 were 37%; while, the prevalence of preterm labor without PPRM were 63%.<sup>2</sup> Singh, et al. reported that the incidence of PPRM differed each country which was influenced by economic, environmental status, and antenatal care percentage.<sup>4</sup> The most prevalent preterm labor in this study was found in women whose age between 21 to 35 years old (69.0%). Savits, et al. reported that the most prevalent preterm labor was in the group of women whose age between 25 and 34 years old (39.4%).<sup>5</sup> Study conducted by Okeke, et al. showed that the most prevalent preterm labor with PPRM was found in women whose age ranging from 26 to 30 years old, which reached 43%.<sup>6</sup> Sukhla, et al. showed that the most prevalent PPRM occurred in women whose age 21-25 years old (59%). The reason of this condition was due to early marriage and too close spacing pregnancy.<sup>7</sup>

Most women involved in this study were unemployed (92.3%) and had married only once (94.5%). Caesarean section was the most prevalent labor method reported in this study (62.3%). This report was contrasted to the study held by Noor, et al., which reported vaginal birth was the most prevalent labor method (66%). It was affected by local tradition to be preferred for the homebirth.<sup>8</sup> Primiparous women were the largest population having preterm labor in this study (73.7%). Okeke, et al. also reported that preterm labor was found as the most prevalent in primiparous women.<sup>6</sup>

Preterm labor was found as the most prevalent in multigravida group (57%) and in the group of women who had none or once history of abortion (95.6%). Preterm labor mostly occurred during 33-36<sup>+6</sup> weeks of gestation (69.8%) and in women who had regular antenatal care (64.4%). Most women involved in this study had no previous low birth weight history (90.1%).

Age was statistically related to PPRM in preterm labor ( $p=0.011$ ;  $OR=1.74$ ). This study also reported that women whose age  $\leq 25$  years old had 63% probability having PPRM compared with women whose age  $>25$  years old. Hammond, et al. reported that there was an increasing risk of preterm labor with PPRM in the group of women whose age  $>35$  years old ( $OR=1.92$ ;  $95\% CI$  1.76-2.08). Study in Sweden during 1973-2001 conducted by Morken, et al. reported that the older maternal age resulted in the higher risk of preterm labor.<sup>9</sup> Nevertheless, this result was different from Savitz, et al. which reported that age was not associated with PPRM ( $95\% CI$  0.9-1.4).<sup>5</sup>

Occupation and marital status were not associated with preterm labor. This result differed from study conducted by Hammond, et al., which stated that marital status was associated with PPRM ( $OR=1.41$ ;  $95\% CI$  1.3-1.51).<sup>2</sup> Zhang, et al. pointed out that among 1,391 women in Beijing, preterm labor was more frequent in the group of unmarried women.<sup>1</sup>

Parity was statistically associated with PPRM ( $p=0.017$ ;  $OR=1.78$ ;  $95\% CI$  1.132-2.878). In this study, primiparous women raised the risk of 1.78 times to have PPRM compared with multiparous women. Noor, et al. in Pakistan reported that PPRM was found more often in multiparous women (57.7%) than in primiparous women (42.3%). Hammond, et al. stated that primiparous women were the risk factor of PPRM; while, multiparous women were the protective factor against PPRM ( $OR=0.48$ ;  $95\% CI$  0.43-0.52).<sup>2</sup> Henderson, et al. through their study in Australian Hospital during 2004-2008 declared that PPRM was found more prevalent in primiparous women (50.4%) ( $p<0.001$ ), especially in 34-36 weeks of gestation.<sup>10</sup> This result was different from Morken, et al. which reported that parity was not associated with PPRM in preterm labor ( $95\% CI$  0.93-0.97).<sup>9</sup> Another study conducted by Savitz, et al. declared that PPRM was found more prevalent in multiparous women ( $OR=1.1$ ).<sup>5</sup>

In this study, abortion status and antenatal care were not associated with PPRM. Study in Beijing conducted by Zhang, et al. reported that antenatal care did not have association with preterm labor (OR=0.261; 95% CI 0.134-0.510). This result was different from Aragao, et al. which reported that antenatal care was associated with preterm labor.<sup>11</sup> The fact is that low socio-economic status has association with irregular antenatal care. Previous history of low birth weight was not associated with PPRM (OR=1.43; 95% CI 0.709-2.900).

### CONCLUSION

Most of preterm labor reported in Dr. Cipto Mangunkusumo Hospital occurs without the rupture of membrane. Several factors related to preterm labor in this study are age and parity.

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## Research Article

# Heme Oxygenases-1 (Hmox-1) and Serum Ferritin Level between Preeclampsia and Normal Pregnancy

## *Kadar Heme Oksigenase-1 (Hmox-1) dan Ferritin Serum pada Pasien Preeklamsia dan Kehamilan Normal*

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### Abstract

**Objective:** To determine the comparison of Hmox-1 to serum ferritin level between patients with preeclampsia and normal pregnancy.

**Methods:** This study used analytic observational with cross sectional design. We included 30 subjects with preeclampsia and the other 30 people with normal pregnancy in accordance with inclusion and exclusion criteria. Examination of Hmox-1 and ferritin level was performed through ELISA method. The data consisted of physical and laboratory examination and they would be continued to the calculation in the statistical analysis.

**Results:** The average of Hmox-1 level in normal pregnancy and preeclampsia was 1.2 (SD 1.6) ng/ml and 0.3 (SD 0.2) ng/ml ( $p < 0.05$ ). The average difference in ferritin level in normal pregnancy and preeclampsia was 32.9 (SD 56.0) ng/ml and 43.0 (SD 45.2) ng/ml ( $p < 0.05$ ). Meanwhile, there was no correlation between Hmox-1 and ferritin serum in normal ( $r = 0.131$ ) and preeclamptic ( $r = 0.174$ ) group ( $p > 0.05$ ).

**Conclusion:** The level of Hmox-1 in preeclamptic group is lower than normal pregnancy; while, the ferritin level in women with preeclampsia is higher than normal pregnancy. There is no significant correlation between Hmox-1 and ferritin serum level.

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**Keywords:** ferritin, Hmox-1, preeclampsia

### Abstrak

**Tujuan:** Untuk menentukan perbandingan kadar Hmox-1 terhadap ferritin serum pada pasien preeklamsia dan kehamilan normal.

**Metode:** Penelitian ini adalah analitik observasional dengan rancangan potong lintang. Sebanyak 30 orang masing-masing pada setiap kelompok digunakan sebagai subjek preeklamsi dan subjek kehamilan normal (kontrol) yang sesuai dengan kriteria inklusi dan eksklusi. Kami melakukan pemeriksaan kadar Hmox-1 dan kadar ferritin dengan ELISA Kit serta pemeriksaan fisik dan laboratorium, kemudian dilakukan perhitungan secara statistik.

**Hasil:** Dari penelitian, diperoleh rerata kadar Hmox-1 pada kehamilan normal dan preeklamsia adalah 1.2 (SB 1.6) ng/ml dan 0.3 (SB 0.2) ng/ml dengan menunjukkan perbedaan yang bermakna ( $p < 0.05$ ). Rerata kadar ferritin pada kehamilan normal dan preeklamsia adalah 32.9 (SB 56.0) ng/ml dan 43.0 (SB 45.2) ng/ml dengan perbedaan yang bermakna ( $p < 0.05$ ). Sementara itu, hasil korelasi antara kadar Hmox-1 dan ferritin serum untuk kehamilan normal dan preeklamsi adalah  $r = -0.131$  dan  $r = 0.174$  dengan perbedaan yang tidak bermakna ( $p > 0.05$ ).

**Kesimpulan:** Kadar Hmox-1 pada penderita preeklamsia lebih rendah dibandingkan dengan kehamilan normal, sedangkan kadar ferritin pada penderita preeklamsia lebih tinggi dibandingkan dengan kehamilan normal. Kadar Hmox-1 dan ferritin serum tidak berkorelasi secara bermakna.

[Maj Obstet Ginekol Indones 2017; 5-1: 8-11]

**Kata kunci:** ferritin, Hmox-1, preeklamsia

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## INTRODUCTION

Preeclampsia is one of pregnancy complication characterized by hypertension (systolic blood pressure more than 140 mmHg or diastolic blood pressure more than 90 mmHg) and proteinuria ( $\geq 300$  mg/24 hours) at the gestational age of more than 20 weeks.<sup>1</sup> Preeclampsia impacts to morbidity and mortality for the mother and fetus. Several effects influencing women include of complication of HELLP syndrome (hemolysis, elevated liver enzymes, low platelet level), pulmonary edema,

kidney disorder, bleeding, placental abruption or even death.<sup>1,2</sup> Meanwhile, in infant, it can lead to complication, such as premature birth, fetal distress, low birth weight (LBW) or intra uterine fetal death (IUFD).<sup>3</sup>

Preeclampsia is the leading cause of women's death contributing around 24%, below the bleeding cause as 28%.<sup>4</sup> Based on data from incidence of preeclampsia in Dr. Hasan Sadikin Hospital Bandung, the occurrence of bleeding was happened between 4.0 and 10.4%; while, the

incidence of preeclampsia was ranged from 2.3 to 4.3% in 2008 to 2010. In 2013, the incidence of preeclampsia in Dr. Hasan Sadikin hospital increased to 13.6% of total patients in obstetrics room with the average number of patients per month were as many as 38 people.<sup>5-8</sup>

The pathophysiology of preeclampsia is still unknown.<sup>9</sup> However, placental ischemic factor and angiogenic imbalance are considered as major causes of preeclampsia. Ischemic in placenta is caused by destruction of red blood cells, especially in the area of the placenta so that it results to the release of hemoglobin/heme and Fe in large quantities into the circulation. It can lead to the induction of ferritin system. Ferritin is the most important protein storing iron/Fe.<sup>10</sup>

Another factor of this cause is an angiogenic imbalance. According to study by Ahmed, they said that the rising level of soluble Fms-like tyrosine kinase receptor-1 (sFLT-1), zinc (soluble endoglin), and the decreasing level of placental growth factor (PlGF) had a major role for the incidence of preeclampsia. The increase of sFlt-1 caused endothelial damage and inhibited angiogenesis through binding and playing an antagonist role on the function of vascular endothelial growth factor (VEGF) and PlGF, also increasing oxidative stress and inflammation. The increase level of zinc caused limited signaling activity of transforming growth factor  $\beta$ 1 (TGF $\beta$ 1) and endothelial nitric oxide synthase (eNOS) that influenced to vessel damage and inhibited angiogenesis.<sup>9</sup>

The recovery of angiogenic balance becomes important and it is believed having effective result in the treatment of preeclampsia. Recent study showed pharmacological role of Heme oxygenase-1 (Hmox-1) and the metabolites (biliverdin, free Fe, and carbon monoxide) on preeclampsia by restoring the balance of angiogenic and reducing oxidative stress in the ischemic placenta.<sup>11,12</sup> Kawashima, et al. in his study stated that there was a relationship among the level of Hmox-1, ferritin, and bilirubin in patients suffering from anemia, growth inhibition, and hyperlipidemia.

Therefore, this study aims to determine the comparison of Hmox-1 to serum ferritin level between patients with preeclampsia and normal pregnancy.

## METHODS

### Research Subject

The subjects were 60 pregnant women coming to perform antenatal care in Dr. Hasan Sadikin Hospital and network hospitals, from December 1, 2014 to February 28, 2015. The study subjects had to meet the inclusion and exclusion criteria.

### Clinical Examination Method

The study carried out a regular examination to determine the diagnosis of preeclampsia, such as blood pressure and proteinuria. The inclusion criteria of normal pregnancy group were single fetus of intrauterine pregnancy, gestational age  $\geq 20$  weeks from last menstrual period (LMP) or first sonography result, normal blood pressure and negative proteinuria. For preeclampsia group, it included single fetus of intrauterine pregnancy, gestational age  $\geq 20$  weeks from LMP or first sonography result, systole blood pressure  $\geq 140$  mmHg and diastole blood pressure 90 mmHg, also positive proteinuria. We excluded women with heart congenital fetus, infection, chronic diseases of women including kidney, heart, hypertension, hypothyroid and diabetic diseases, smoker and alcoholic, intrauterine growth retardation (IUGR). We had performed matching for both groups.

### Laboratory Examination Method

We examined the blood and protein level in the Laboratory of Clinical Pathology, Dr. Hasan Sadikin Hospital in accordance with national standard. Blood samples were obtained through phlebotomy conducted by health professional in the Department of Obstetrics and Gynecology, Dr. Hasan Sadikin Hospital on the cubital vein using a disposable 5 ml syringe. We put the blood sample in plain vacutainer and it was taken to the Laboratory of Clinical Immunology Department of Clinical Pathology, Dr. Hasan Sadikin hospital. Blood samples were let standing for around 30 minutes until they were freezing and then centrifuged at 3,000 rpm for 10 minutes. The result of serum was separated and put into plastic tubes and stored at  $-20^{\circ}\text{C}$ . Later, examination of Hmox-1 level was performed by using ELISA Kit. Meanwhile, ferritin level was measured by using the principle of Sandwich and analyzed through Ferritin Kit No. 03737551 COBAS.

## Data Analysis

Analysis was formerly performed with normality test. For categorical data analysis in both groups, we used the chi-square ( $\chi^2$ ) or the Fisher exact whether it was found the expected value of less than 5. To compare the average value of quantitative data, we analyzed through t-test for normally distributed data and Mann-Whitney test for not normally distributed data. To analyze the correlation between the four independent variables, it applied Pearson correlation analysis. All of them was operated using SPSS for Windows version 21.0.

## RESULTS

A total of 60 subjects met the criteria for inclusion, which consisted of 30 pregnant women with preeclampsia and 30 normal pregnant women as a control. We collected the characteristics demographic for both groups starting from blood pressure, Hmox-1, and ferritin level examination. Table 1 depicted the average level of Hmox-1 between normal and preeclamptic group.

**Table 1.** The Comparison of Hmox-1 Level between Normal and Preeclampsia Women

| Hmox-1 Level (ng/ml) | Group         |                     | Statistical Test |
|----------------------|---------------|---------------------|------------------|
|                      | Normal (n=30) | Preeclampsia (n=30) |                  |
| Average (SD)         | 1.2 (1.6)     | 0.3 (0.2)           | 0.011*           |
| Median               | 0.4           | 0.3                 |                  |
| Min-max              | 0.1-6.2       | 0.01-1.2            |                  |

\*Mann-Whitney test

Average result of Hmox-1 level in preeclampsia group showed lower values (0.3 (SD 0.2) ng/ml) compared with normal group ( $p=0.011$ ). Study by Farina, et al. stated that Hmox-1 mRNA level in women with preeclampsia was lower than normal pregnant women.<sup>13</sup> However, another study said that the average level of Hmox-1 in preeclampsia was higher than normal pregnancy group.<sup>14,15</sup>

**Table 2.** The Comparison of Ferritin Level between Normal and Preeclampsia Women

| Ferritin Level (ng/ml) | Group         |                     | Statistical Test |
|------------------------|---------------|---------------------|------------------|
|                        | Normal (n=30) | Preeclampsia (n=30) |                  |
| Average (SD)           | 32.9 (56.0)   | 43.0 (45.2)         | 0.028*           |
| Median                 | 14.4          | 22.5                |                  |
| Min-max                | 4.6-246.1     | 6.6-214.2           |                  |

\*Mann-Whitney test

Table 2 showed the result of average ferritin level between normal and preeclampsia group: It pointed out that the average ferritin level in preeclampsia group was higher (43 (SD 45.2) ng/ml) compared with normal pregnancy group (32.9 (SD 56.0) ng/ml). It was different significantly ( $p=0.028$ ). Fatima, et al. study indicated that the average value of serum ferritin in patients with preeclampsia was higher at 100.03 (SD 123.52)  $\mu\text{gm/l}$  and the value of control group was at 31.53 (SD 20.86)  $\mu\text{gm/l}$  ( $p<0.001$ ).<sup>10</sup> Some studies also showed similar result to Taheripanth and Farkush,<sup>16</sup> Zafar and Iqbal,<sup>17</sup> and Siddiqui, et al.<sup>18</sup>

**Table 3.** The Correlation of Hmox-1 and Ferritin Level between Normal and Preeclampsia Group

| Correlation               | Group                  |                       |
|---------------------------|------------------------|-----------------------|
|                           | Normal (n=30)          | Preeclampsia (n=30)   |
| Hm0x-1 and ferritin level | $r=-0.131$ $p = 0.494$ | $r=0.174$ $p = 0.357$ |

$r$  = pearson correlation coefficient

Based on Pearson correlation analysis, there was not correlation between Hmox-1 and ferritin level both in normal and preeclampsia group ( $p>0.05$ ) and the power of correlation was poor ( $r=0.131$  and  $r=0.174$ ) (Table 3).

## DISCUSSION

The result showed that the level of Hmox-1 in patients with preeclampsia was lower than normal pregnancy. Meanwhile, ferritin level in patients with preeclampsia was higher than normal pregnancy. High ferritin level indicated that there had been an increasing on the induction of ferritin system. The increasing induction of ferritin system

was caused by placental ischemia that occurred in patients with preeclampsia. In addition, the patients with preeclampsia, it was also characterized by an imbalance of angiogenic factors in their body that was indicated by the decreasing level of Hmox-1. Therefore, the result of this study described that patients with preeclampsia had low level of Hmox-1 and high ferritin level.

During pregnancy, serum ferritin level would reach the lowest point up to 20 ng/dl in the third trimester of pregnancy. An individual who had a serum ferritin value >400 mg/l could be said that these individuals experienced abnormalities of iron related to tumor. While, the raising of serum ferritin in pregnancy was influenced by hypertension and eclampsia.

There was no correlation between Hmox-1 and ferritin level in both preeclampsia and normal pregnant women. Temporary assumption was that higher ferritin level might be caused by ischemic placental. This condition was suffered from patients with preeclampsia.

## CONCLUSION

The level of Hmox-1 in preeclamptic group is lower than normal pregnancy; while, the ferritin level in women with preeclampsia is higher than normal pregnancy. There is no significant correlation between Hmox-1 and ferritin serum level.

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## Research Article

# High Sensitivity C-Reactive Protein (hs-CRP) Level on Premature Rupture of Membrane (PROM) at Term Pregnancy

## *Kadar High Sensitivity C Reactive Protein (hs-CRP) pada Ketuban Pecah Dini dengan Kehamilan Aterm*

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### Abstract

**Objective:** To compare the level of high sensitivity C-Reactive Protein (hs-CRP) on patients with premature rupture of membranes (PROM) at term pregnancy.

**Methods:** The study was cross-sectional design. The data collection included age, parity, gestational age, birth weight, and APGAR score on 28 pregnant women at term pregnancy consisting of 14 subjects of Premature Rupture of Membrane (PROM) less than 12 hours and the remaining one was equal or more than 12 hours. We took blood samples from the subjects for the examination of hs-CRP level. Data were analyzed using non-parametric statistical test and processed using Statistical Package for Social Sciences (SPSS) version 20.

**Results:** The mean hs-CRP level on PROM <12-hour and ≥12-hour group was 12.9 and 17.8 mg/l. There was not significant difference on hs-CRP level between both of group at term pregnancy ( $p=0.734$ ).

**Conclusion:** The level of hs-CRP does not have association with the incidence of PROM.

[Indones J Obstet Gynecol 2017; 5-1: 12-15]

**Keywords:** high sensitivity C-Reactive Protein, premature ruptures of membranes, term pregnancy

### Abstrak

**Tujuan:** Untuk membandingkan kadar high sensitivity C-Reactive Protein (hs-CRP) pada pasien ketuban pecah dini (KPD) dengan kehamilan aterm.

**Metode:** Penelitian ini merupakan studi dengan desain potong lintang. Pengumpulan data dilakukan pada 28 wanita hamil dengan kehamilan aterm yang meliputi usia, paritas, usia kehamilan, berat lahir, dan nilai APGAR. Subjek terbagi dalam 2 kelompok yaitu dengan KPD <12 jam dan ≥12 jam. Data dianalisis dengan menggunakan tes statistik non parametrik dan diproses melalui SPSS versi 20.

**Hasil:** Rerata nilai hs-CRP pada KPD <12 jam ialah 12,9 mg/l dan 17,8 pada kelompok KPD ≥12 jam. Tidak terdapat perbedaan bermakna pada kadar hs-CRP antara kedua kelompok pada kehamilan aterm. ( $p=0,734$ ).

**Kesimpulan:** Kadar hs-CRP tidak berhubungan dengan kejadian KPD.  
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**Kata kunci:** hamil aterm, high sensitivity C-Reactive Protein, ketuban pecah dini

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## INTRODUCTION

Premature rupture of membrane (PROM) at term pregnancy occurs before the onset of labor at term gestational age (between 37<sup>+0</sup> and 41<sup>+6</sup> weeks). Patients with PROM usually complain of vaginal bleeding, pelvic pressure, vaginal discharge, water going out of birth canal without uterine contraction.<sup>1,2</sup> The incident of PROM happened approximately 12% of all pregnancies; however, it was varied from 2 to 18% of pregnancies. About 70% PROM cases occur at term pregnancy. Recent studies showed that the incident of PROM was between 14 and 17% of all pregnancies. At term pregnancy, the incidence rate was approximately 5-18%. This breakage of amniotic sac was probably

influenced by several factors, such as cervico-vaginitis, incompetent cervix, maternal smoking, prenatal diagnostic procedures (amniocentesis, chorionic villus sampling), coitus, also mineral and vitamins deficiency.<sup>3-5</sup>

In term pregnancy, PROM is the beginning from 8 to 10% of all pregnancies and it will be followed by the onset of labor and birth. A random study found half of women with PROM delivered within 5 hours and 95% of them delivered in 28 hours after the rupture of membrane.<sup>6-8</sup>

Most PROM women would continue to deliver on the second or third day; therefore, patients and their fetal had to be fully observed. The expectative management for PROM can be considered on

patient at the first 12-24 hours. However, the risk of intrauterine infection increases during PROM. Some studies stated that the risk of chorioamnionitis increased up to 24% after the occurrence of prolonged PROM.<sup>8,9</sup>

C-Reactive Protein is an inflammation marker produced and released by the liver under the stimulation of cytokines interleukin 6 (IL-6), interleukin 1 (IL-1), and tumor necrosis factor  $\alpha$  (TNF-  $\alpha$ ).<sup>4,10</sup> High sensitive C-Reactive Protein (hs-CRP) is an acute phase reactant that is synthesized primarily in the liver cells as a response to pro-inflammatory cytokines. Furthermore, hs-CRP will stimulate the release of matrix metalloproteinase (MMP-1). The role of MMP plays in the process of remodeling/degradation of extracellular matrix causing rupture of membranes.<sup>4,5,10</sup>

A lot of studies has conducted to determine the impact of CRP on normal pregnancy, preeclampsia, premature labor. Some studies reported an elevation on the level of CRP in maternal serum above normal level which was common seen in non-pregnant women. It was also reported that high level of CRP in maternal serum at the beginning of delivery process would increase risk of fetal intrauterine infection. Wiser, et al. through their study reported increasing CRP to the level of more than 10 mg/l is indication of an infection.<sup>8,10,11</sup> Therefore, our study aims to compare the level of hs-CRP on patients with PROM at term pregnancy, whereas we differ into two-time limit for the PROM.

## METHODS

This study was a cross-sectional design conducted from December 2015 to February 2016 in Department of Obstetrics and Gynecology, Faculty of Medicine Universitas Sam Ratulangi/Prof. dr. R.D. Kandou Hospital, Manado and Kasih Manado Hospital. We recruited 14 women as study subjects at term pregnancy with PROM less than 12 hours and 14 women at term pregnancy with PROM equal or more than 12 hours. We took the distributive characteristics including age, weight, height, body mass index (BMI), and the women were examined the blood samples for hs-CRP level. The level of hs-CRP was measured by turbidimetry methods using Li heparin serum and ethylenediaminetetraacetic acid (EDTA).

We reviewed the medical records and then all patients who would be enrolled on the study got the counseling explanation about the purpose, benefit, and study procedures, as well as the risk could occur. If they agreed to be included in this study, they had to signed the informed consent. The collected data were analyzed using non-parametric statistical tests in SPSS.

## RESULTS

Table 1 showed that the most pregnant women with PROM were between 20 and 35 years old which the average of it was 29.4 years old. The gestational age on either PROM <12-hour or  $\geq$ 12-hour group was less than 40 weeks, with an average of 38.4 weeks of gestation. According to parity, most of them had not ever delivered; therefore, the average parity was only 0.7. Furthermore, the baby's birth weight on the PROM <12-hour and PROM  $\geq$ 12-hour group mostly had less than 3500 grams and the mean of it was 3103.6 grams. Meanwhile, APGAR score on PROM <12 hour and PROM  $\geq$ 12-hour group at first and fifth minute was >7 and >7; whereas, the average of 7.1 at minute 1 and 9.1 at minute 5.

The lowest and highest hs-CRP level on PROM <12 hour was 0.8 and 70.7 mg/l, with mean (standard of deviation/SD) of 12.9 (19.2) mg/l. While on PROM  $\geq$ 12-hour group, the lowest, highest, mean, and SD were 1.0, 158.1, 17.8, 40.7 mg/l; respectively.

Based on the data obtained, we conducted a normality test using Shapiro-Wilk test due to small sample size. The result on BMI, length of PROM, and hs-CRP pointed out that the p less than 0.05 so that it meant that the data distribution was not normal and we continued to non-parametric statistical test.

The Mann-Whitney non-parametric statistical test resulted that there was no significant difference in BMI between PROM <12-hour and PROM  $\geq$ 12-hour group ( $p=0.306$ ). It defined that the BMI did not have association with the incidence of PROM. Meanwhile, there was a significant difference on PROM length between groups ( $p<0.001$ ). The analysis of hs-CRP level described that there was not statistically different between groups ( $p=0.734$ ).

**Table 1.** Patient Characteristics on Premature Rupture of Membrane (PROM) group

| Characteristics         | PROM <12 hours<br>(n=14) | PROM ≥12 hours<br>(n=14) | Mean   | SD    |
|-------------------------|--------------------------|--------------------------|--------|-------|
| Age (years old)         |                          |                          |        |       |
| <20                     | 0                        | 1                        |        |       |
| 20-35                   | 12                       | 9                        | 29.4   | 6.4   |
| >35                     | 2                        | 4                        |        |       |
| Parity                  |                          |                          |        |       |
| 0                       | 8                        | 6                        |        |       |
| 1                       | 4                        | 5                        |        |       |
| 2                       | 1                        | 1                        | 0.7    | 1.0   |
| 3                       | 1                        | 2                        |        |       |
| >4                      | 0                        | 0                        |        |       |
| Gestational Age (weeks) |                          |                          |        |       |
| 37-<40                  | 13                       | 11                       |        |       |
| ≥40-<41                 | 1                        | 3                        | 38.4   | 1.0   |
| 41-<42                  | 0                        | 0                        |        |       |
| Birth Weight (grams)    |                          |                          |        |       |
| 2500-<3000              | 4                        | 7                        |        |       |
| 3000-<3500              | 8                        | 4                        | 3103.6 | 303.2 |
| 3500-<4000              | 2                        | 3                        |        |       |
| APGAR Score             |                          |                          |        |       |
| Minute 1                |                          |                          |        |       |
| 4-6                     | 3                        | 2                        |        |       |
| 7-10                    | 11                       | 12                       | 7.1    |       |
| Minute 5                |                          |                          |        | 0.8   |
| 4-6                     | 0                        | 0                        | 9.1    |       |
| 7-10                    | 14                       | 14                       |        |       |

**Table 2.** hs-CRP Level on Premature Rupture of Membranes (PROM)

| PROM Group            | hs-CRP Level  |                | Mean | SD   |
|-----------------------|---------------|----------------|------|------|
|                       | Lowest (mg/l) | Highest (mg/l) |      |      |
| PROM <12 hours (N=14) | 0.8           | 70.7           | 12.9 | 19.3 |
| PROM ≥12 hours (N=14) | 1.00          | 158.1          | 17.8 | 40.7 |

## DISCUSSION

A review held by Martinez, et al. in 2007 stated that there were eight main studies consisting of 610 cases that fulfilled the criteria; whereas, three of the studies concluded that hs-CRP was a diagnostic instrument for chorioamnionitis occurrence on PROM cases; although, one study suggested higher level of hs-CRP had more

diagnostic value. Meanwhile, the other five studies concluded that the hs-CRP as single indicator to diagnose chorioamnionitis incidence on PROM still lacked of power; therefore, it needed further study on that indicator.<sup>12</sup> Another study by Serkan Kahyaoglu, et al. on 86 patients concluded that hs-CRP as an inflammation marker, revealed less specific and sensitive to predict the labor with PROM.<sup>13</sup>

This study showed that the measurement of hs-CRP level on patients with PROM at term pregnancy was not a single indicator to predict the success of labor induction. The limitation of study included small sample size so that it would be difficult to be applied in general. We suggested to conduct another study covered bigger population in Indonesia to determine the use of hs-CRP on PROM at term pregnancy.

## CONCLUSION

The level of hs-CRP does not have association with the incidence of PROM.

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## Research Article

## Role of Glycated Albumin during Pregnancy

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## Abstract

**Objective:** To determine the glycated albumin profile during pregnancy with normal glycemic status.**Methods:** We recruited 60 pregnant women between 21 and 36 weeks of gestation. We conducted several laboratory tests, such as glycated albumin, blood glucose, and albumin. These parameters were compared among four groups of gestational age (21-24 weeks, 25-28 weeks, 29-32 weeks, and 33-36 weeks) using ANOVA or Kruskal-Wallis test continued by Post-hoc test.**Results:** Glycated albumin was not statistically different among the groups. Albumin level of 33-36 weeks of gestation women (3.6 (SD 0.2) g/dl) was lower than 21-24 weeks of gestation women (3.8 (SD 0.2) g/dl).**Conclusion:** Glycated albumin level is not affected by gestational age. Therefore, glycated albumin may be used as glycemic status indicator during pregnancy from 21 to 36 weeks.

[Indones J Obstet Gynecol 2017; 5-1: 16-18]

**Keywords:** HbA1c, glycated albumin, glycemic status, pregnancy

## Abstrak

**Tujuan:** Mengetahui karakteristik albumin glikat pada kehamilan dengan status glikemik normal.**Metode:** Enam puluh perempuan hamil 21-36 minggu. Dilakukan pemeriksaan albumin glikat, glukosa darah, dan albumin. Parameter-parameter tersebut dibandingkan antara empat kelompok usia kehamilan (21-24 minggu, 25-28 minggu, 29-32 minggu, dan 33-36 minggu) menggunakan uji ANOVA atau Kruskal-Wallis dan dilanjutkan dengan uji Post-hoc.**Hasil:** Kadar albumin glikat tidak berbeda antara keempat kelompok usia kehamilan. Kadar albumin pada kelompok kehamilan 33-36 minggu (3.6 (SB 0.2) g/dl) lebih rendah dibandingkan kelompok kehamilan 21-24 minggu (3.8 (SB 0.2) g/dl).**Kesimpulan:** Kadar albumin glikat tidak terpengaruh dengan usia kehamilan. Albumin glikat dapat menjadi penanda status glikemik pada usia kehamilan 21-36 minggu.

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**Kata kunci:** albumin glikat, HbA1c, kehamilan, status glikemik**Correspondence:** Thoeng Ronald; thoeng\_ronald@hotmail.com

## INTRODUCTION

In 2013, International Diabetes Federation (IDF) estimated that 21.4 million women in the world suffering from hyperglycemia in pregnancy. It was estimated that 16% of them suffered from gestational diabetes mellitus (GDM); thus, it required close monitoring during pregnancy and after childbirth. Southeast Asia had the highest prevalence of hyperglycemia in pregnancy at 25.0% compared with Europe at 12.6% (table 1).<sup>1</sup>

Untreated hyperglycemia can lead to several complications both to the mother and fetal, such as impaired invasion of cytotrophoblast that causes placental hypoxia releasing antiangiogenic factors, such as soluble fms-like tyrosine kinase-1 (sFlt-1)

resulting to preeclampsia, premature birth, fetal hyperinsulinemia that causes diabetic fetopathy including macrosomia, increasing the cesarean section rate, perinatal trauma, neonatal hypoglycemia, and fetal death. International Association of Diabetes and Pregnancy Study Groups (IADPSG) recommends screening for diagnosis of GDM using oral glucose tolerance test (OGTT) at 24-28 weeks of gestation. The procedure is through dissolving 75 grams of glucose to 200 ml water for all pregnant women.<sup>1-5</sup>

We need an indicator not only as a tool for monitoring the glycemic status during pregnancy, but also to predict about possible complication for mother and baby.

**Table 1.** Prevalence of Hyperglycemia in Pregnancy (20-49 years) in 2013<sup>1</sup>

| Region                          | Number of Cases per Live Births (Million) | Prevalence (%) |
|---------------------------------|-------------------------------------------|----------------|
| Africa                          | 4.6                                       | 14.4           |
| Europe                          | 1.7                                       | 12.6           |
| Middle East and North Africa    | 3.4                                       | 17.5           |
| North America and the Caribbean | 0.9                                       | 10.4           |
| Central and South America       | 0.9                                       | 11.4           |
| Southeast Asia                  | 6.3                                       | 25.0           |
| Western Pacific                 | 3.7                                       | 11.9           |

Glycated albumin is a new indicator for monitoring glycemic status which is not affected by the condition of anemia. Glycated albumin is formed through a process of non-enzymatic glycation, in which glucose is covalently bonded to the amino acid residues such as lysine, arginine, cysteine of albumin. Through Amadori reaction, it forms ketoamine stable form. Glycated albumin can indicate glycemic status for the previous 2 weeks because albumin half-life is only 15-20 days. Therefore, glycated albumin can be used to monitor short-term glycemic status. Study by Hashimoto, et al. on 47 pregnant women with gestational age of 21-36 weeks found glycated albumin level was not influenced by gestational age.<sup>6-9</sup> This study aims to determine the profile glycated albumin during pregnancy with normal glycemic status.

## METHODS

This was a cross-sectional study design which recruited 60 pregnant women with 21 to 36 weeks of gestation. The study was conducted from April to May 2016 and it was approved by the ethics committee of the Dr. Cipto Mangunkusumo Hospital/Faculty of Medicine, Universitas Indonesia with the approval number of 260/UN2.F1/ETHICS/2016.

We divided sixty pregnant women at gestational age 21-36 weeks into four groups: 17 subjects in group I (21-24 weeks of gestation), 11 subjects in group II (25-28 weeks of gestation), 16 subjects in group III (29-32 weeks of gestation), and 16

subjects in group IV (33-36 weeks of gestation). The inclusion criteria were all pregnant women with blood glucose levels less than 200 mg/dl and we excluded women with thyroid disease, cirrhosis, diabetes, proteinuria, and corticosteroid therapy. We took 4 ml serum for assessing glycated albumin, albumin, and blood glucose.

Glycated albumin level was measured using the reagent Lucica<sup>®</sup>GA-L (Asahi Kasei Pharma). Blood glucose was measured using a Cobas C 501 (Roche Holding AG).

Differences among groups were obtained through normality test of each group. The normally distributed data were shown in mean and standard of deviation; unless the data were described in median and minimum to maximum. After that, we held the one-way ANOVA and continued by analysis of Bonferroni or Tukey multiple comparison. If distribution of data was not normal and/or its variance was not homogeneous, we did the Kruskal-Wallis test followed by Mann Whitney U post-hoc analysis between group. We performed the statistical test using SPSS version 20.

## RESULTS

Glycated albumin level did not differ significantly among four groups ( $p=0.061$ ). Level of albumin in group of women with gestational age 33-36 weeks (3.6 (SD 0.2) g/dl) was significantly lower than 21-24 weeks of gestation group (3.8 (SD 0.2) g/dl) ( $p=0.006$ ). Table 2 depicted the characteristics of study subjects in each gestational age.

**Table 2.** Characteristics of Study Subjects in Each Gestational Age Group

| Variables             | Gestational Age     |                     |                     |                     |
|-----------------------|---------------------|---------------------|---------------------|---------------------|
|                       | 21-24 Weeks<br>N=17 | 25-28 Weeks<br>N=11 | 29-32 Weeks<br>N=16 | 33-36 Weeks<br>N=16 |
| Age (years old)       | 27.4 (5.4)          | 27.8 (6.1)          | 30.7 (5.6)          | 27.4 (4.7)          |
| Glycated albumin (%)  | 11.5 (0.9)          | 11.6 (1.0)          | 11.5 (10.8-14.6)    | 10.8 (1.2)          |
| Blood glucose (mg/dl) | 80.7 (67.0-149.4)   | 81.2 (16.2)         | 82.6 (67.1-137.2)   | 78.9 (67.9-123.6)   |
| Albumin (g/dl)        | 3.8 (0.2)           | 3.7 (0.2)           | 3.7 (0.2)           | 3.6 (0.2)           |

\* Mean (SD) if the variable was distributed normally

\*\* Median (min-max) if the variable was not distributed normally

## DISCUSSION

In this study, there was no significant difference in glycated albumin level between gestation groups. These results were similar to study by Hashimoto, et al. It was due to similar subjects of Asian population. This study found level of albumin in group IV (33-36 weeks of gestation) significantly was lower than the level of albumin in group I (21-24 weeks of gestation); this might be due to hemodilution. The hemodilution normally occurs because of the plasma volume increase in pregnancy.<sup>10</sup> Glycated albumin level was not influenced by hemodilution because the result in the form of glycated albumin level is a ratio of glycated albumin to albumin.<sup>11,12</sup>

## CONCLUSION

Glycated albumin level is not affected by gestational age. Therefore, glycated albumin may be used as glycemic status indicator during pregnancy from 21 to 36 weeks.

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Research Article

## Acceptor Comment of Post-Placental Copper T380A Intrauterine Device

### *Komentar Akseptor AKDR Copper T380A Pascaplasenta*

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#### Abstract

**Objective:** To describe the characteristics of subjective complaints reported by post-placental inserted intrauterine device (IUD) acceptor, especially those who used Copper T380A (CU T380A) type of IUD.

**Methods:** Seventy-two-married women whose age ranged from 19 to 44 years old and received post-placental IUD in Dr. Cipto Mangunkusumo Hospital, Jakarta, were included in this study. Subjective complaints regarding the use of CU T380A IUD were evaluated twice including during the puerperium and six months afterwards by a direct interview.

**Results:** Most respondents were 20-35 years old, 50% of whom were primiparous (n=36). There were 42% respondents reporting pain during insertion, 32% respondents reporting abdominal pain during the use, 22% respondents reporting menstrual disorder, 18% respondents reporting vaginal discharge, and 3% respondents complaining of having IUD repulsion.

**Conclusion:** There are variety of subjective complaints reported after post-placental IUD use. However, most of the respondents does not complain anything.

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**Keywords:** complication, contraception, intrauterine device (IUD) post-placental, postpartum

#### Abstrak

**Tujuan:** Untuk mendiskripsikan keluhan subjektif akseptor alat kontrasepsi dalam rahim (AKDR), khususnya jenis Copper T380A (CU T380A) dengan insersi pascaplasenta.

**Metode:** Kami meneliti 72 perempuan menikah, usia 19-44 tahun, yang menerima insersi AKDR pascaplasenta di RSUPN Dr. Cipto Mangunkusumo Jakarta. Kami mencari keluhan subjektif akseptor AKDR pascaplasenta selama pascasalin dan enam bulan setelahnya dengan wawancara langsung.

**Hasil:** Didapatkan rerata usia responden terbanyak adalah 20-35 tahun, dengan 50% responden adalah primipara (n=36). Sebanyak 42% mengeluhkan nyeri saat pemasangan, 32% mengeluh nyeri perut selama pemakaian, dan 22% mengeluhkan gangguan menstruasi, sebanyak 18% mengeluh keputihan, serta sebanyak 3% ekspulsi.

**Kesimpulan:** Terdapat beberapa keluhan subjektif pada akseptor AKDR pascaplasenta. Namun, mayoritas responden tidak mengalami keluhan.

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**Kata kunci:** alat kontrasepsi dalam rahim (AKDR) pascaplasenta, komplikasi, kontrasepsi, pascasalin

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## INTRODUCTION

Family planning program can reduce the maternal and infant mortality rate up to 10% if the couple join the program for a minimum of 2 years.<sup>1</sup> The short interval between two adjacent labors in a woman was associated with higher maternal and infant morbidity and mortality rate.<sup>2</sup> Post-placental contraception was an attempt to prevent unwanted pregnancy or short interval between labors in 12 months after delivery.<sup>1</sup> Postpartum women needed some effective but reversible contraceptive methods to prevent unplanned pregnancy.<sup>1,2</sup>

Among many available choices, Copper T380A (Cu T380A) type of intrauterine device (IUD) is one of the most effective and least expensive contraceptive method. It is a non-hormonal contraception that is very effective in preventing pregnancy and it can also be used by most women without interfering the production of breast milk. According to World Health Organization (WHO) 5<sup>th</sup> edition of Medical Eligibility Criteria, post-placental IUD can be inserted during 48-hour postpartum or 4 weeks afterwards.<sup>3-5</sup>

Intrauterine device is a long-term, reversible, and the most common contraception worldwide.

It was estimated that more than 160-million women, most of whom came from China and India, were using this contraceptive method. However, the use of Cu T380A IUD in Indonesia nowadays was decreasing. Different from in 1991, the coverage number of IUD had reached 13%, those coverage number decreased becoming only 5% in 2007.

In Indonesia, the use of post-placental IUD had been known for approximately 30 years.<sup>6</sup> Previous studies concluded that the 3-month compliance of post-placental multiload copper 250 (ML Cu250) type of IUD was 91.1%. The 12-month compliance of post-placental Cu T380A type of IUD was even higher than that of ML Cu250 (90.17% vs 87.54% respectively).<sup>7</sup> Factors influencing the number of compliance were the assumption of high expulsion rate as well as the existence of side effects, such as pain and bleeding.<sup>6,8,9</sup> Studies in many countries had shown that the average expulsion rate of post-placental IUD insertion was 11-15%. This number was smaller than those having the IUD inserted late (14-37%). In Indonesia, the expulsion rate was estimated between 6% and 10%.<sup>10</sup> Apart from timing of post-placental IUD insertion, expulsion event was also affected by the type of IUD and the technique of insertion.<sup>11,12</sup> Expulsion can be minimized by having someone professional to insert the IUD correctly and inserting the IUD at the level of uterine fundus.<sup>13</sup>

Nowadays, postpartum contraceptive implementation care, most post-placental Cu T380A IUD became concerned from all sections. Through good counseling and informed consent, we believed that it would result good compliance for the using of a contraceptive device.<sup>14</sup> Therefore, this study aims to describe the characteristics of subjective complaints reported by post-placental inserted IUD acceptor, especially those who used CU T380A type of IUD.

## METHODS

A secondary data using descriptive cross sectional study was carried out in the Emergency Room of Obstetrics and Gynecology Department, Dr. Cipto Mangunkusumo Hospital - Faculty of Medicine Universitas Indonesia from July to December 2014.

Women receiving a Cu T380A type of IUD right after giving birth either vaginally or surgically

(cesarean section), being mentally healthy, and having consented were included in this study, while the unavailability of contact number on the medical records was the exclusion criteria in this study.

After randomization, subjects were contacted via telephone to get explanation about the study and to be asked about the consent. After giving consent, subjects would be asked some questions according to the questionnaire. Subjects who cannot be contacted would be considered as drop out and replaced by randomizing other subjects after the target of 12 samples every month were reached.

## RESULTS

Seventy-two women fulfilling the inclusion and exclusion criteria and receiving post-placental IUD in the Emergency Room of Obstetrics and Gynecology Department, Dr. Cipto Mangunkusumo Hospital, were analyzed in this study. Those patients were then asked about their consent and given some questions based on the questionnaire via telephone. The demographics of those patients were displayed on Table 1.

**Table 1.** Demographic Characteristics of Subjects

| Variables                 | n  | %  |
|---------------------------|----|----|
| <b>Age (years old)</b>    |    |    |
| < 20                      | 4  | 5  |
| 20 - 35                   | 61 | 85 |
| > 35                      | 7  | 10 |
| <b>Educational status</b> |    |    |
| Junior high school        | 19 | 26 |
| Senior high school        | 34 | 47 |
| Diploma/Associate degree  | 9  | 13 |
| Bachelor degree           | 10 | 14 |
| <b>Parity status</b>      |    |    |
| Primiparity               | 36 | 50 |
| Multiparity               | 34 | 47 |
| Grand multiparity         | 2  | 3  |
| <b>Method of Labor</b>    |    |    |
| Vaginal delivery          | 34 | 47 |
| Cesarean section          | 35 | 49 |
| Vacuum extraction         | 4  | 4  |

|                                       |    |    |
|---------------------------------------|----|----|
| <b>Frequency of control (times)</b>   |    |    |
| 0                                     | 11 | 15 |
| 1                                     | 22 | 31 |
| 2                                     | 35 | 49 |
| 3                                     | 4  | 6  |
| <b>Duration of IUD usage (months)</b> |    |    |
| ≤ 6 month                             | 5  | 7  |
| ≥ 6 month                             | 67 | 93 |

Most patients were 20-35 years old (mean age 28.7 years old) and they had the last educational background of senior high school (47%). The comparison methods of labor (vaginal delivery and cesarean section) as well as parity status (primiparity and multiparity) were almost equal. While most patients had done follow-up about the IUD usage, 15% of them did not do the follow-up.

Counseling and decision making about IUD insertion, including factors associated with them, was shown on table 2. Most of the decision making about having IUD inserted was after discussing with her family (63%). Most patients were satisfied with the choice they made (81%). Counseling at the point of insertion was done in 68% of patients and most of them agreed with the idea of IUD insertion (61%).

There were only a few patients who reported severe pain during IUD insertion (2%). The most reported complaint was abdominal pain (32%), followed by menstrual disorder (22%) and vaginal discharge (18%). Other complaints, such as sexual-related disorder (10%) and IUD expulsion (3%) were relatively rare. There were 7% patients using IUD less than 6 months because of expulsion, having abdominal pain or vaginal discharge, and attempting to be pregnant.

**Table 2.** IUD Counseling and Decision Making

|                                                         | n  | %  |
|---------------------------------------------------------|----|----|
| <b>Counseling at the time of insertion</b>              |    |    |
| Yes                                                     | 49 | 68 |
| No                                                      | 23 | 32 |
| <b>Decision of having IUD inserted after counseling</b> |    |    |
| Yes                                                     | 44 | 61 |
| No                                                      | 28 | 39 |

|                                      |    |    |
|--------------------------------------|----|----|
| <b>Time of decision making</b>       |    |    |
| On antenatal care                    | 12 | 17 |
| Before labor                         | 28 | 39 |
| After labor                          | 32 | 44 |
| <b>Decision maker</b>                |    |    |
| Patient's own decision               | 0  | 0  |
| Patient and family                   | 45 | 63 |
| Husband                              | 9  | 12 |
| Parents                              | 0  | 0  |
| Others                               | 18 | 25 |
| <b>Satisfaction of IUD insertion</b> |    |    |
| Yes                                  | 58 | 81 |
| No                                   | 14 | 19 |

**Table 3.** Subjective Complaints after Insertion of Post-Placental IUD

| Complaints                            | n  | %  |
|---------------------------------------|----|----|
| <b>Pain during insertion</b>          |    |    |
| No                                    | 42 | 58 |
| Mild                                  | 24 | 33 |
| Moderate                              | 5  | 7  |
| Severe                                | 1  | 2  |
| <b>Expulsion</b>                      |    |    |
| Yes                                   | 2  | 3  |
| No                                    | 70 | 97 |
| <b>Abdominal pain</b>                 |    |    |
| Yes                                   | 23 | 32 |
| No                                    | 49 | 68 |
| <b>Menstrual disorder</b>             |    |    |
| Yes                                   | 16 | 22 |
| No                                    | 56 | 78 |
| <b>Sexual-related disorder</b>        |    |    |
| Yes                                   | 7  | 10 |
| No                                    | 65 | 90 |
| <b>Vaginal discharge</b>              |    |    |
| Yes                                   | 13 | 18 |
| No                                    | 59 | 82 |
| <b>Removal of IUD before 6 months</b> |    |    |
| Reason behind removal                 | 5  | 7  |
| Attempt to be pregnant                | 1  | 20 |
| Pain                                  | 3  | 60 |
| Discharge                             | 1  | 20 |

## DISCUSSION

Focusing on the use of post placental Cu T380A type of IUD at Dr. Cipto Mangunkusumo Hospital,

most patients were satisfied with their contraceptive decision of using that device; even though, many pronouncements were decided right after labor. The most common complaint after IUD insertion was pain and abdominal cramp (32%). Other IUD insertion complications, such as pain during insertion, menstrual disorder, vaginal discharge, sexual-related disorder, and expulsion of IUD could still be found in a small number of women compared with abdominal pain.

In spite of many subjective complaints reported, majority of patients (81%) were satisfied with their decision of choosing IUD as the contraceptive method. This result was similar with the one studied in India where more than 90% of patients were satisfied using IUD in six-month postpartum.<sup>12</sup> In this study, infection and perforation of the uterus were not found.

Regarding the attempt of contraceptive counseling, there were 32% of patients who did not get the counseling at the point of IUD insertion. Among 68% others receiving counseling, most of them were satisfied with the counseling given. Remembering the importance of IUD counseling as well as other general contraception, counseling about contraception must have been given since antenatal care. In counseling, explanation of the importance of follow-up after the insertion of IUD was also needed as there were 15% of patients who did not have the follow-up after IUD insertion.

Expulsion rate of IUD in this study was only 3%. This number was smaller than the previous study stating that the expulsion rate of IUD in the first year was about 6-10%.<sup>10</sup>

The limitation in this study was the unobserved subjective complaints after long-term IUD insertion. This limitation was due to the short time of study, which was 6-to-12-month follow-up after the insertion of IUD. Further study using a longer follow-up time was needed to know more about the long-term subjective complaints of IUD users. Beside the short time of study, this study also only focused on Dr. Cipto Mangunkusumo patients. Multicenter studies with larger sample size were needed to strengthen the accuracy of the result so that it can be generalized in the community in Indonesia. Expansion of post-placental IUD distribution and access accompanied by counseling, right method of insertion, and regular follow-up can be useful to support the successful of postpartum family planning program and

decrease both maternal and infant mortality rate.

## CONCLUSION

There are variety of subjective complaints reported after post-placental IUD use. However, most of the respondents does not complain anything.

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Research Article

## Accuracy of Preoperative Endometrial Sampling for the Detection of Endometrial Pathology: a Retrospective Study

### *Akurasi dari Pra-operatif Sampel Endometrium untuk Mendeteksi Patologi Endometrium: Studi Retrospektif*

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#### Abstract

**Objective:** To investigate the accuracy of endometrial sampling in the diagnosis of endometrial pathology and the need of intra-operative frozen section.

**Methods:** One hundred forty women who underwent endometrial sampling followed by hysterectomy between 2011 and 2014 were included in this study. Data were retrieved from patient files and pathology archives in Department of Obstetrics and Gynecology, Jose R. Reyes Memorial Medical Center, Manila, Philippines.

**Results:** There were 25 patients with malignancy but endometrial sampling detected only 22 of them. The endometrial sampling sensitivity and specificity for detecting cancer were 88% and 100%, respectively with negative and positive predictive values of 97.5% and 100%, respectively. In 3 patients, the endometrial sampling failed to detect malignancy; 1 patient had a preoperative diagnosis of complex hyperplasia with atypia, 1 patient had complex hyperplasia without atypia and 1 patient had adenofibroma. A total of eighty patients had benign findings. There were fifty-three cases with finding of proliferative endometrium and twenty-seven were secretory. Twenty-three (55.0%) and 11 (39.0%) cases were confirmed by the hysterectomy specimen, respectively. The sensitivity of endometrial sampling in detecting benign samples was 76.0% and the specificity reached up to 83.0%. The histopathology result of the other fourteen cases were reported of having atrophy, twelve cases were reported of having endometrial hyperplasia, four with basal endometrium, four with endometrial polyp and one with adenomyosis.

**Conclusion:** Outpatient endometrial biopsy has a high overall accuracy in diagnosing endometrial cancer when the specimen obtained is sufficient. A positive test result is more accurate for ruling in disease than a negative test result is for ruling it out. However, the diagnosis should be confirmed by frozen section in patients with complex hyperplasia and adenofibroma.

[Indones J Obstet Gynecol 2017; 5-1: 23-29]

**Keywords:** abnormal uterine bleeding, endometrial hyperplasia, endometrial sampling, frozen section, pipelle

#### Abstrak

**Tujuan:** Untuk menyelidiki keakuratan pengambilan sampel endometrium dalam mendiagnosis patologi endometrium dan kebutuhan yang memerlukan teknik potong beku selama operasi.

**Metode:** Seratus empat puluh perempuan yang menjalani pengambilan sampel endometrium diikuti oleh histerektomi antara 2011-2014 dilibatkan dalam penelitian ini. Data diambil dari file pasien dan arsip patologi.

**Hasil:** Terdapat 25 pasien dengan keganasan, namun endometrium sampel hanya mampu mendeteksi 22 dari mereka. Sensitivitas dan spesifisitas dari pengambilan sampel endometrium mendeteksi kanker adalah masing-masing 88% dan 100%, dengan nilai duga negatif dan positif masing-masing sebesar 97,5% dan 100%. Pada 3 pasien, pengambilan sampel endometrium gagal mendeteksi keganasan; 1 pasien memiliki diagnosis praoperatif kompleks hiperplasia dengan atipia, 1 pasien memiliki kompleks hiperplasia tanpa atipia dan 1 pasien memiliki adenofibroma. Sebanyak delapan puluh pasien memiliki temuan jinak. Ada 53 kasus dengan temuan proliferasi endometrium dan 27 yang sekretori. Masing-masing sebanyak 23 (55,0%) dan 11 (39,0%) dikonfirmasi oleh spesimen histerektomi. Sensitivitas sampling endometrium dalam mendeteksi sampel jinak adalah 76,0% dan spesifisitas adalah 83,0%. Hasil histopatologi dari 14 kasus lain dilaporkan memiliki atrofi, 12 kasus dilaporkan memiliki hiperplasia endometrium, 4 dengan basal endometrium, 4 memiliki polip endometrium dan 1 memiliki adenomyosis.

**Kesimpulan:** Rawat jalan biopsi endometrium memiliki akurasi keseluruhan tinggi dalam mendiagnosis kanker endometrium ketika spesimen yang diperoleh sudah cukup. Hasil tes positif lebih akurat untuk mendiagnosis suatu penyakit daripada suatu hasil tes negatif untuk mengesampingkan suatu penyakit. Namun, diagnosis harus dikonfirmasi oleh potong beku pada pasien dengan kompleks hiperplasia dan adenofibroma.

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**Kata kunci:** hiperplasia endometrium, perdarahan uterus abnormal, pipelle, potong beku, sampel endometrium

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## INTRODUCTION

Abnormal uterine bleeding (AUB) accounts for 20% of visits to the gynecologist. It is defined as any variation of bleeding outside from normal menstrual cycle that includes change in regularity,

frequency, duration of flow, and amount of blood loss. Several common pathological conditions causing abnormal vaginal bleeding are leiomyomas, endometrial polyps, and adenomyosis. Other less frequent conditions are endometritis and uterine cancers.<sup>1</sup>

Endometrial pathology has been identified and diagnosed using several methods. Endometrial sampling has gained popularity as an alternative diagnostic tool compared with more invasive procedures, such as fractional dilatation and curettage (D&C).<sup>2</sup>

The morphological appearance of atypical endometrial hyperplasia and well-differentiated endometrial carcinoma on biopsy specimen presents a major challenge to pathologists.<sup>3</sup> When a Pipelle endometrial biopsy or curettage specimen can be diagnosed as atypical hyperplasia, there is a risk of concomitant invasive carcinoma in the uterus. Seven to fifty percent (7-50%) of women with endometrial hyperplasia with cytological atypia were found to have concomitant carcinoma in subsequent hysterectomy specimens.<sup>4</sup> Exclusion of cancer in an atypical hyperplasia finding on biopsy is a difficult challenge for the gynecologist.

Hysterectomy is the most frequently performed surgery in gynecology. When it performed for benign indication, frozen section should still be kept doing if there is suspicion of malignancy in the gross specimen in spite of a negative preoperative biopsy.

Several studies had been performed to evaluate the accuracy of endometrial biopsy in identifying the cause of AUB and they showed variation of results.<sup>5-7</sup> According to this discrepancy, we conducted this study to compare the accuracy of preoperative endometrial sampling for the detection of endometrial pathology with postoperative hysterectomy specimen in local settings.

## METHODS

This was a retrospective cohort study design involving chart review of patients who underwent endometrial sampling and were treated with hysterectomy in Department of Obstetrics and Gynecology, Jose R. Reyes Memorial Medical Center, Manila, Philippines from January 2011 to December 2014. Clinical and pathological information were reviewed and obtained from patient charts to

complete the desired sample size for the study. All collected data were recorded, encoded, and presented in tables. We reviewed the charts of 140 patients who were qualified. Accuracy of endometrial sampling and hysterectomy specimen were determined by computing for the sensitivity, specificity, PPV, and NPV for each diagnostic modality by comparing the preoperative results with the final histopathology findings.

Inclusion criteria included all women with AUB and concomitant gynecological pathology who underwent endometrial sampling and were treated by hysterectomy within a year of the diagnosis. Exclusion criteria were all women who presented with AUB due to cervical pathology and patients who underwent hysterectomy more than a year of the diagnosis.

Review of charts, histopathological results of endometrial sampling, and final histopathological results were gathered from the medical records and Pathology Department from January 2011 to December 2014.

All the statistical tests were performed in SPSS version 20.0. We considered the result significantly if p-values less than 0.05.

## RESULTS

A total of 140 women were included in the study, with mean age of 47.9 years old (ranging from 28 to 70 years old) and mean gravidity of 2.8 (ranging from 0 to 11). Forty-two (30.0%) were post-menopausal women who were 50 years old or older.

Myoma was the most common indication for hysterectomy, as reported by 47 (33.6%) patients, followed by AUB (n=33, 23.6%), and endometrial carcinoma (n=22, 15.7%). Less common reasons were post-menopausal bleeding (n=8, 5.7%) and pelvic organ prolapse (n=1, 0.7%).

The preoperative results of endometrial sampling and the final pathological diagnosis were described on Table 1.

**Table 1.** Concordance Rate of Endometrial Biopsy and Hysterectomy Specimen Histopathology

| Finding                                | Pipelle Biopsy<br>N (%) | Hysterectomy Specimen<br>N (%) | Concordance<br>N (%) |
|----------------------------------------|-------------------------|--------------------------------|----------------------|
| <b>Malignant</b>                       |                         |                                |                      |
| Adenocarcinoma                         | 21 (15.0)               | 23 (16.4)                      | 21 (15.0)            |
| Malignant Mixed Müllerian Tumor (MMMT) | 0 (0)                   | 1 (0.7)                        | 0 (0)                |
| Serous Carcinoma                       | 1 (0.7)                 | 1 (0.7)                        | 1 (0.7)              |

| Finding                            | Pipelle Biopsy<br>N (%) | Hysterectomy Specimen<br>N (%) | Concordance<br>N (%) |
|------------------------------------|-------------------------|--------------------------------|----------------------|
| <b>Benign</b>                      |                         |                                |                      |
| Proliferative                      | 42 (30.0)               | 53 (37.9)                      | 23 (16.4)            |
| Secretory                          | 28 (20)                 | 27 (19.3)                      | 11 (7.9)             |
| <b>Endometrial hyperplasia</b>     |                         |                                |                      |
| Complex hyperplasia with atypia    | 2 (1.4)                 | 1 (0.7)                        | 0 (0)                |
| Complex hyperplasia without atypia | 2 (1.4)                 | 0 (0)                          | 0 (0)                |
| Simple hyperplasia with atypia     | 1 (0.7)                 | 1 (0.7)                        | 0 (0)                |
| Simple hyperplasia without atypia  | 15 (10.7)               | 10 (7.1)                       | 4 (2.9)              |
| <b>Adenofibroma</b>                | 1 (0.7)                 | 0 (0)                          | 0 (0)                |
| <b>Adenomyosis</b>                 | 1 (0.7)                 | 1 (0.7)                        | 1 (0.7)              |
| Atrophic                           | 12 (8.6)                | 14 (10)                        | 6 (4.3)              |
| Basal Endometrium                  | 1 (0.7)                 | 4 (2.9)                        | 1 (0.7)              |
| Endometrial polyp                  | 5 (3.6)                 | 4 (2.9)                        | 1 (0.7)              |
| Tissue Insufficiency               | 8 (5.7)                 | 0 (0)                          | 0 (0)                |

Of the twenty-three (16.4%) cases confirmed to have adenocarcinoma by hysterectomy specimen, 21 cases were correctly diagnosed by endometrial biopsy. One case of serous carcinoma was also correctly diagnosed by endometrial biopsy. One case of Malignant Mixed Müllerian Tumor (MMMT) was missed by endometrial biopsy.

For the benign cases, 53 (37.9%) cases were read as proliferative endometrium in the final specimen. Only 23 out of 42 cases were initially diagnosed by endometrial biopsy matched the result of hysterectomy specimen. Twenty-seven (19.3%) were confirmed secretory cases, but only 11 out of 28 cases were initially diagnosed secretory cases as confirmed by hysterectomy specimen. None of the proliferative and secretory reading by endometrial biopsy were interpreted as cancer on the final hysterectomy specimen.

Two cases of complex hyperplasia with atypia were diagnosed by endometrial biopsy, which on hysterectomy specimen was found to have proliferative endometrium and adenocarcinoma. Two cases of complex hyperplasia without atypia were diagnosed by endometrial biopsy; however, they were not confirmed at hysterectomy. One of them turned out to be simple hyperplasia on hysterectomy specimen and the other one was adenocarcinoma. Another case was accurately

diagnosed as simple hyperplasia both by sampling and hysterectomy specimen. One case of simple hyperplasia with atypia was initially diagnosed by endometrial biopsy; it was confirmed by hysterectomy section yet. Of the 15 cases of simple hyperplasia without atypia diagnosed by endometrial biopsy, four cases were confirmed by hysterectomy section. There were other six cases of simple hyperplasia without atypia as reported by hysterectomy specimen which endometrial biopsy failed to diagnose initially.

There were 71 patients with benign endometrial pathologies diagnosed by endometrial biopsy, but only 61 (85.9%) cases were confirmed by hysterectomy specimen results. Hysterectomy specimen showed that among those 10 patients, five had atrophy, two had simple hyperplasia without atypia, one had endometrial polyp, one had basal endometrium, and one had simple hyperplasia with atypia.

All the 22 patients with malignancy diagnosed by endometrial biopsy remained the same by final pathology. Endometrial biopsy failed to diagnose the other three cases of malignancy. Complex hyperplasia with and without atypia were the biopsy diagnoses of the two adenocarcinoma cases; while, adenofibroma was the initial diagnosis of the patient with MMMT.

**Table 2.** Kappa, Sensitivity, Specificity, Positive Predictive Value, Negative Predictive Value of Endometrial Biopsy Compared with Hysterectomy Specimen

| Findings                               | N (%)     | Kappa  | Sensitivity   | Specificity      | PPV            | NPV              |
|----------------------------------------|-----------|--------|---------------|------------------|----------------|------------------|
| <b>Malignant</b>                       | 25 (17.9) | 0.923* | 22/25 (88.0%) | 115/115 (100.0%) | 22/22 (100.0%) | 115/118 (97.5%)  |
| Adenocarcinoma                         | 23 (16.4) | 0.946* | 21/23 (91.3%) | 117/117 (100.0%) | 21/21 (100.0%) | 117/119 (98.3%)  |
| Malignant Mixed Müllerian Tumor (MMMT) | 1 (0.7)   | 0.000  | 0/1 (0%)      | 139/139 (100.0%) | 0/0            | 139/140 (99.3%)  |
| Serous carcinoma                       | 1 (0.7)   | 1.000* | 1/1 (100.0%)  | 139/139 (100.0%) | 1/1 (100.0%)   | 139/139 (100.0%) |
| <b>Benign</b>                          | 80 (57.1) | 0.585* | 61/80 (76.3%) | 50/60 (83.3%)    | 61/71 (85.9%)  | 50/69 (72.5%)    |
| Proliferative                          | 53 (37.9) | 0.225* | 23/53 (43.4%) | 68/87 (78.2%)    | 23/42 (54.8%)  | 68/98 (69.4%)    |
| Secretory                              | 27 (19.3) | 0.253* | 11/27 (40.7%) | 96/113 (85%)     | 11/28 (39.3%)  | 96/112 (85.7%)   |
| <b>Others</b>                          | 35 (25.0) |        |               |                  |                |                  |
| <b>Endometrial hyperplasia</b>         | 12 (8.6)  | 0.370* | 7/12 (58.3%)  | 115/128 (89.8%)  | 7/20 (35.0%)   | 115/120 (95.8%)  |
| Complex hyperplasia with atypia        | 1 (0.7)   | -0.010 | 0/1 (0%)      | 137/139 (98.6%)  | 0/2 (0%)       | 137/138 (99.3%)  |
| Complex hyperplasia without atypia     | 0 (0)     | 0.000  | 0/0           | 138/140 (98.6%)  | 0/2 (0%)       | 138/138 (100.0%) |
| Simple hyperplasia with atypia         | 1 (0.7)   | -0.007 | 0/1 (0%)      | 138/139 (99.3%)  | 0/1 (0%)       | 138/139 (99.3%)  |
| Simple hyperplasia without atypia      | 10 (7.1)  | 0.256* | 4/10 (40.0%)  | 119/130 (91.5%)  | 4/15 (26.7%)   | 119/125 (95.2%)  |
| <b>Adenofibroma</b>                    | 0 (0)     | 0.000  | 0/0           | 139/140 (99.3%)  | 0/1 (0%)       | 139/139 (100.0%) |
| <b>Adenomyosis</b>                     | 1 (0.7)   | 1.000* | 1/1 (100.0%)  | 139/139 (100.0%) | 1/1 (100.0%)   | 139/139 (100.0%) |
| Atrophic                               | 14 (10)   | 0.407* | 6/14 (42.9%)  | 120/126 (95.2%)  | 6/12 (50.0%)   | 120/128 (93.8%)  |
| Basal endometrium                      | 4 (2.9)   | 0.393* | 1/4 (25.0%)   | 136/136 (100%)   | 1/1 (100.0%)   | 136/139 (97.8%)  |
| Endometrial polyp                      | 4 (2.9)   | 0.179* | 1/4 (25.0%)   | 132/136 (97.1%)  | 1/5 (20.0%)    | 132/135 (97.8%)  |
| Tissue insufficiency                   | 0 (0)     | 0.000  | 0/0           | 132/140 (94.3%)  | 0/8 (0%)       | 132/132 (100.0%) |

The endometrial sampling sensitivity and specificity of detecting malignant cancer were 88.0% [22/25] and 100.0% [115/115], with negative and positive predictive values of 100.0% [22/22], and 97.5% [115/118], respectively (Table 2).

On the remaining 47 patients that biopsy failed to detect as malignancy or benign pathologies, three cases were confirmed to be malignancy (two adenocarcinoma cases and one case of MMMT), 19 confirmed with benign cases (17 proliferative and two secretory), and 25 others (nine cases of atrophy, eight cases of simple hyperplasia without atypia, three cases of basal endometrium, three cases of endometrial polyp, one case of adenomyosis, and one case of complex hyperplasia with atypia).

Pipelle biopsy had a very good agreement [K=0.923,  $p<0.001$ ] in the diagnosis of malignant cancer. Specifically, it had very good agreement [K=0.946,  $p<0.001$ ] in diagnosis of adenocarcinoma with sensitivity and specificity of 91.3% and 100.0%, respectively, with positive predictive value of 100.0% and negative predictive value of 98.3%. It also had very good agreement [K=1.000,  $p<0.001$ ] in diagnosis of serous carcinoma with sensitivity, specificity, positive predictive value and negative predictive value of 100.0% for all results. However, it had poor agreement [K=0.000,  $p=1.000$ ] in the diagnosis of MMMT in 1 patient, which the endometrial sampling failed to detect malignancy of adenofibroma on a 64-year old woman with postmenopausal bleeding.

For benign samples, Pipelle biopsy had moderate agreement [ $K=0.585$ ,  $p<0.001$ ] with hysterectomy specimen results, having sensitivity and specificity of 76.3% and 83.3%, respectively. Specifically, proliferative [ $K=0.225$ ,  $p=0.007$ ] and secretory [ $K=0.253$ ,  $p=0.003$ ] of benign cases had fair agreement with hysterectomy specimen results.

Other findings showed that Pipelle biopsy had fair agreement in diagnosing atrophy [ $K=0.407$ ,  $p<0.001$ ], basal endometrium [ $K=0.393$ ,  $p<0.001$ ]; however, it described poor agreement in the diagnosis of endometrial polyp [ $K=0.179$ ,  $p<0.019$ ]. Among the endometrial hyperplasia, Pipelle biopsy showed significant agreement [ $K=0.256$ ,  $p=0.002$ ] with hysterectomy specimen results only in the diagnosis of simple hyperplasia without atypia. The Pipelle biopsy showed no significant agreement with hysterectomy specimen results in the diagnosis of other endometrial hyperplasia, such as

simple hyperplasia with atypia [ $K=-0.007$ ,  $p=0.932$ ], complex hyperplasia with atypia [ $K=-0.010$ ,  $p=0.904$ ] and complex hyperplasia without atypia [ $K=0.000$ ,  $p=1.000$ ]. Likewise, the Pipelle biopsy showed no significant agreement with hysterectomy specimen results in the diagnosis of adenofibroma [ $K=0.000$ ,  $p=1.000$ ] and tissue insufficiency [ $K=0.000$ ,  $p=1.000$ ].

Twenty patients diagnosed with endometrial hyperplasia through Pipelle biopsy were confirmed to have endometrial hyperplasia only in seven patients, leading to PPV of 35.0%. The remaining 13 patients were confirmed to have proliferative ( $n=8$ ), adenocarcinoma ( $n=2$ ), basal endometrium ( $n=1$ ) and secretory ( $n=2$ ). All descriptions were shown on Table 3.

Eight specimens were found to have tissue adequacy. Gravidity ( $p=0.127$ ) and menopausal status ( $p=0.299$ ) did not determine sample adequacy of Pipelle biopsy.

**Table 3.** Confirmation of Hysterectomy Specimen in Patients with Initial Endometrial Hyperplasia through Endometrial Biopsy

| Case | Age<br>(years old) | Indication              | Pipelle Biopsy                     | Hysterectomy specimen             |
|------|--------------------|-------------------------|------------------------------------|-----------------------------------|
| 1    | 57                 | Endometrial hyperplasia | Complex hyperplasia with atypia    | Adenocarcinoma                    |
| 2    | 47                 | Endometrial hyperplasia | Complex hyperplasia with atypia    | Proliferative                     |
| 3    | 51                 | Endometrial hyperplasia | Complex hyperplasia without atypia | Simple hyperplasia without atypia |
| 4    | 49                 | Endometrial hyperplasia | Complex hyperplasia without atypia | Adenocarcinoma                    |
| 5    | 47                 | Endometrial hyperplasia | Simple hyperplasia with atypia     | Simple hyperplasia without atypia |
| 6    | 44                 | Endometrial hyperplasia | Simple hyperplasia without atypia  | Secretory                         |
| 7    | 52                 | Endometrial hyperplasia | Simple hyperplasia without atypia  | Secretory                         |
| 8    | 43                 | AUB                     | Simple hyperplasia without atypia  | Proliferative                     |
| 9    | 44                 | AUB                     | Simple hyperplasia without atypia  | Proliferative                     |
| 10   | 47                 | Endometrial hyperplasia | Simple hyperplasia without atypia  | Proliferative                     |
| 11   | 65                 | Ovarian Cyst            | Simple hyperplasia without atypia  | Proliferative                     |
| 12   | 50                 | Endometrial hyperplasia | Simple hyperplasia without atypia  | Proliferative                     |
| 13   | 40                 | Myoma                   | Simple hyperplasia without atypia  | Proliferative                     |
| 14   | 40                 | Endometrial hyperplasia | Simple hyperplasia without atypia  | Proliferative                     |
| 15   | 55                 | Endometrial hyperplasia | Simple hyperplasia without atypia  | Complex hyperplasia with atypia   |
| 16   | 48                 | AUB                     | Simple hyperplasia without atypia  | Basal endometrium                 |
| 17   | 39                 | Endometrial hyperplasia | Simple hyperplasia without atypia  | Simple hyperplasia without atypia |
| 18   | 51                 | Endometrial hyperplasia | Simple hyperplasia without atypia  | Simple hyperplasia without atypia |
| 19   | 45                 | Endometrial hyperplasia | Simple hyperplasia without atypia  | Simple hyperplasia without atypia |
| 20   | 44                 | Endometrial hyperplasia | Simple hyperplasia without atypia  | Simple hyperplasia without atypia |

## DISCUSSION

Abnormal uterine bleeding has been the most common complaint of women during reproductive years and post-menopausal life. There are various benign reasons for AUB. However, uterine bleeding is the most common sign of endometrial cancer.

Endometrial sampling with Pipelle is cost effective and a safe procedure; it is widely used in the investigation of perimenopausal and post-menopausal women with AUB. We take only a few minutes to perform the procedure with Pipelle so that it is the most convenient and best tolerated. Besides, this procedure causes less pain.<sup>8</sup> In a local study by L. Co, et al. in 2000, Pipelle endometrial biopsy instrument was an effective office device for evaluating patients with AUB because it was associated with less pain, better specimen yield with similar histologic findings (100%) as the standard curettage.<sup>9</sup> Another study by Mochtar, et al. also had similar conclusion that the operating time and the percentage of patients who reported tachycardia and pain were significantly higher in Novak curette group compared with the Manual Vacuum Aspiration.<sup>10</sup>

Discrepancy was found between the histopathological result of endometrial samples and the hysterectomy specimens. In our study, patient who had discrepancy between the histopathological result of endometrial samples and the hysterectomy specimens, underwent intraoperative frozen section and were treated with complete surgical staging.

Pathologists are dealing with increasing number of endometrial specimens in which there is scant, or even absent endometrial tissue, especially when the endometrium is atrophic. These specimens may consist entirely of superficial strips or wisps of atrophic glands, with little or no stroma, admixed with cervical mucus, ectocervical or endocervical tissue, and tissue from the lower uterine segment. In published studies, inadequate rates of outpatient endometrial biopsies were ranged from 4.8 to 33%; although, in most of these studies, the criteria for adequacy was not clearly mentioned.<sup>11</sup>

Routine criteria for adequacy of endometrial biopsies was applied by the pathologist consultant in the routine biopsy. Adequacy of the preparation was assessed as satisfactory when sufficient endometrial material (endometrial glands and stroma) was presented to make a pathological

diagnosis or to exclude a pathological process. A specimen was assessed as inadequate if there was insufficient endometrial material in the cell block to exclude a pathological diagnosis. The pathologist recorded the quality of the biopsy sample and provided a diagnosis if the sample was sufficient.<sup>9</sup>

Our study result showed that outpatient endometrial biopsy was an accurate diagnostic procedure when adequate specimens were obtained, it had high overall accuracy in diagnosing endometrial cancer. As the diagnosis of endometrial cancer was very important, the likelihood ratio for a positive test should raise most pre-test probabilities over any threshold for advanced management. In contrast, the likelihood ratio for a negative test was not low enough to negate the need for further diagnostic testing.

Three endometrial cancers were missed among adequate biopsy specimens. Inadequate endometrial samples might come from poor biopsy technique, inherent problems with non-representative sampling, varied pathological interpretation or be consistent with the underlying atrophic endometrial state.

In our study, both inadequate samples on Pipelle were benign lesions and no case of endometrial carcinoma was missed. Our study had shown low sensitivity (76.3%) but high specificity (83.3%) for Pipelle in diagnosing benign diseases. However, malignant diseases had a sensitivity and specificity of 88.0% and 100.0%; respectively. This led to the conclusion that the Pipelle was superior for diagnosing malignant disease and hyperplasia as compared with benign diseases. This finding was reported in a study by Clark et al in 2002.<sup>5</sup> A finding which was also reported in a study by Festin MR, et al. in 2006, concluded that endometrial biopsy was a useful diagnostic procedure for the detection of endometrial abnormalities with accuracy of biopsy of 91.5% in patients underwent hysterectomy and was comparable to endometrial curettage with accuracy of 92.3%.<sup>12</sup>

## CONCLUSION

The endometrial biopsy is found to be accurate, easy, and safe. However, inconsistency is found between the histopathological results of specimens obtained by endometrial sampling and hysterectomy specimens.

The disadvantages of a Pipelle biopsy are that often only very scant tissue is obtained, especially in a postmenopausal woman with an atrophic endometrium, and focal lesions may be missed. Furthermore, the frequent association between atypical hyperplasia and carcinoma means that when a diagnosis of atypical hyperplasia is made, irrespective of the sampling method, the clinician must be concerned that endometrial carcinoma exists concomitantly within the uterus.

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## Research Article

**Anti Müllerian Hormone (AMH) Level as Ovarian Reserve Marker before and after Cystectomy Laparotomy*****Kadar Anti Müllerian Hormone (AMH) sebagai Penanda Cadangan Ovarium sebelum dan sesudah Tindakan Laparotomi Kistektomi*****Rosdiana Sahabuddin, Nusratuddin Abdullah, IMS Murah Manoe***Department of Obstetrics and Gynecology  
Faculty of Medicine Universitas Hasanuddin/  
Dr. Wahidin Sudiro Husodo Hospital  
Makassar***Abstract**

**Objective:** To investigate the comparison of the Anti-Mullerian Hormone (AMH) as the ovarian reserve marker before and after cystectomy.

**Methods:** This study used prospective cohort design which included all women with the ovarian cyst who underwent cystectomy laparotomy. The subjects were examined AMH level before and after the procedure. Data were analyzed using one-way Anova, T- paired test, and T-independent test with p value <0.05 considered significantly.

**Results:** We obtained 40 subjects consisting of 21 women (52.5%) suffering endometriosis cyst, 12 women (30.0%) suffering functional cyst, and 7 others (17.5%) suffering dermoid cyst. After the cystectomy, AMH level was different between endometriosis cyst and non-endometriosis cyst patients; whereas, the endometriosis cyst group showed greater decrease of AMH level ( $p < 0.05$ ). The mean AMH level in the cyst women before cystectomy laparotomy was 2.9 (SD 0.4) ng/ml and after the surgery, the mean value became 1.9 (SD 0.6) ng/ml.

**Conclusion:** The decrease of AMH level is significant in the endometrial ovarian cyst women who undergo the cystectomy.

[Indones J Obstet Gynecol 2017; 5-1: 30-34]

**Keywords:** Anti Müllerian Hormone, laparotomy, ovarian cystectomy, ovarian reserve

**Abstrak**

**Tujuan:** Untuk mengetahui perbandingan kadar Anti Mullerian Hormone (AMH) sebagai penanda cadangan ovarium sebelum dan sesudah kistektomi.

**Metode:** Penelitian ini menggunakan desain kohort prospektif. Semua perempuan dengan kista ovarium yang dilakukan kistektomi diperiksa untuk mengetahui perbedaan tingkat AMH sebelum dan setelah kistektomi. Data dianalisis dengan uji one-way Anova, T- paired test dan T independent dengan nilai  $p < 0,05$  adalah bermakna.

**Hasil:** Kami mendapatkan 40 sampel, yang terdiri dari 21 orang penderita kista endometriosis (52,5%), 12 penderita kista fungsional (30,0%), 7 penderita kista dermoid (17,5%). Setelah kistektomi, penderita kista endometriosis dan kista non endometriosis menunjukkan perbedaan tingkat AMH, yaitu pada pasien kista endometriosis menunjukkan penurunan tingkat AMH ( $p < 0,05$ ). Rata-rata kadar AMH penderita kista sebelum operasi kistektomi 2,9 (SB 0,4) ng/ml, dan setelah operasi dengan nilai rata-rata 1,9 (SB 0,6) ng/ml.

**Kesimpulan:** Penurunan kadar AMH terjadi secara bermakna pada pasien kista ovarium yang dilakukan tindakan kistektomi dengan penurunan yang lebih banyak pada kelompok kista endometriosis.

[Maj Obstet Ginekol Indones 2017; 5-1: 30-34]

**Kata kunci:** Anti Müllerian Hormone, cadangan ovarium, laparotomi kistektomi

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**INTRODUCTION**

The ovarian mass mostly has cystic form. The incidence of ovarian cyst was reported between 5 and 15 percent of all gynecological diseases.<sup>1</sup>

Generally, the most common ovarian cysts is functional cyst, followed by endometriosis and dermoid cysts. Each type of cyst has its own characteristics so that we have to be careful in performing cystectomy to avoid the damage of ovaries. In women with ovarian cysts who undergo cystectomy, ovarian tissue damage may occur. It

happens when normal tissue is removed due to the heat effect or cauterization process. Normal ovarian tissue should be maintained as much as possible because it is related to fertility condition signed by the ability of the ovaries to hold ovulation.<sup>2</sup>

Anti-Mullerian Hormone (AMH) is a homodimeric glycoprotein sulfide bond to TGF- $\beta$  group with molecular weight 140 kDa. It is a hormone that is produced by the granulosa cells of primary ovarian follicles and it plays a major role in the growth and differentiation of cells. The highest

expression is located in the follicular phase preantral and small antral follicles, and it will be no longer detectable when follicles undergo atresia. Level of serum AMH is strongly associated with the number of antral follicles. The AMH level describes the remaining of primordial follicles that reserved, which can be used as an illustration of ovulation induction success. The greater the ovarian tissue damage after cystectomy, the less number of normal follicles; thus, it will decrease AMH level after surgery.<sup>3</sup>

Ovarian reserve is a potential ovarian function that reflects the number and quality of remaining primordial follicles at such time. In the last two decades, serum AMH, Follicle Stimulating Hormone (FSH), Estradiol (E<sub>2</sub>), and inhibin B have been used as marker of ovarian reserve. However, inhibin B, FSH and E<sub>2</sub> level fluctuate during the menstrual cycle; while, the level of serum AMH is relatively stable throughout the menstrual cycle. Therefore, AMH is better marker for predicting ovarian response than age, FSH, or inhibin B. A lot of studies used AMH as a marker of the ovarian reserve damage rate due to ovarian cystectomy.<sup>2</sup>

Measurement of serum AMH level is more easily performed than hyperstimulation of ovarian to predict ovarian reserve. It is stated that the change of ovarian reserve can be measured through serial measurement of serum AMH level which reflects the number of primordial follicles after ovarian surgery.<sup>4</sup>

Based on these ideas, we conducted a study which included all women with kinds of ovarian

cysts undergo cystectomy surgery. The level of serum AMH on each type of cyst was measured before surgery, then repeated subsequently after surgery to determine the fluctuation of AMH level. Therefore, this study aims to investigate the comparison of the AMH as the ovarian reserve marker before and after cystectomy.

## METHODS

This study was a prospective cohort design which was conducted in several teaching hospitals of Obstetrics and Gynecology Department, Faculty of Medicine Universitas Hasanuddin, Makassar from Desember 2014 to July 2015. The population was women with ovarium cyst examining in hospital. Subjects who met the criteria offered to participate in this study and they had to sign the informed consent. All blood samples were taken and examined in Nehri Laboratory of Universitas Hasanuddin Hospital. We processed the data using SPSS through one-way Anova, T-independent test, and T-paired test. Hypothesis testing was considered significantly if  $p < 0.05$

## RESULTS

There were 40 subjects consisting of 21 patients with endometriosis cysts, 12 patients with functional cyst, and 7 patients with dermoid cyst. In this study, there was not difference in age, parity, body mass index, level of CA 125, and symmetry between endometriosis cysts, functional cysts, and dermoid cysts ( $p > 0.05$ ) (Table 1).

**Table 1.** Subjects' Characteristics

| Variable        | Endometriosis cysts<br>(n=21) |      | Functional cysts<br>(n=12) |      | Dermoid Cysts<br>(n=7) |      | p Value |
|-----------------|-------------------------------|------|----------------------------|------|------------------------|------|---------|
|                 | N                             | %    | N                          | %    | N                      | %    |         |
| Age (years old) |                               |      |                            |      |                        |      |         |
| 20-30           | 16                            | 64.0 | 7                          | 28.0 | 2                      | 8.0  | 0.074   |
| 31-40           | 5                             | 33.3 | 5                          | 33.3 | 5                      | 33.3 |         |
| Parity          |                               |      |                            |      |                        |      |         |
| 0               | 14                            | 60.9 | 7                          | 30.4 | 2                      | 8.7  | 0.198   |
| 1               | 7                             | 50.0 | 3                          | 22.4 | 4                      | 28.6 |         |
| 2               | 0                             | 0    | 2                          | 66.7 | 1                      | 33.3 |         |
| IMT             |                               |      |                            |      |                        |      |         |
| Abnormal        | 4                             | 40.0 | 4                          | 40.0 | 2                      | 20.0 | 0.641   |
| Normal          | 17                            | 56.7 | 8                          | 26.7 | 5                      | 16.7 |         |

| Variable     | Endometriosis cysts<br>(n=21) |      | Functional cysts<br>(n=12) |      | Dermoid Cysts<br>(n=7) |      | p Value |
|--------------|-------------------------------|------|----------------------------|------|------------------------|------|---------|
|              | N                             | %    | N                          | %    | N                      | %    |         |
| CA 125 Level |                               |      |                            |      |                        |      |         |
| Abnormal     | 12                            | 100  | 0                          | 0    | 0                      | 0    | <0.001  |
| Normal       | 9                             | 32.1 | 12                         | 42.9 | 7                      | 25.0 |         |
| Symmetry     |                               |      |                            |      |                        |      |         |
| Bilateral    | 11                            | 68.8 | 3                          | 18.8 | 2                      | 12.5 | 0.241   |
| Unilateral   | 10                            | 41.7 | 9                          | 37.5 | 5                      | 20.8 |         |

**Table 2.** AMH Level between Endometriosis and Non-Endometriosis Cyst

| AMH Level         | Endometriosis Cysts<br>(n= 21) |     | Non-Endometriosis<br>Cysts (n= 19) |     | p-value |
|-------------------|--------------------------------|-----|------------------------------------|-----|---------|
|                   | Mean                           | SD  | Mean                               | SD  |         |
| Before cystectomy | 2.7                            | 0.3 | 3.1                                | 0.4 | 0.001*  |
| After cystectomy  | 1.5                            | 0.4 | 2.4                                | 0.4 | <0.001* |

**Table 3.** Comparison Distribution AMH Level all Samples before Cystectomy and after Cystectomy

| Variable | Before Cystectomy<br>(n= 40) |     | After Cystectomy<br>(n= 40) |     | p-value |
|----------|------------------------------|-----|-----------------------------|-----|---------|
|          | Mean                         | SD  | Mean                        | SD  |         |
| AMH      | 2.9                          | 0.4 | 1.9                         | 0.6 | <0.001* |

Table 2 showed the average level of AMH before and after cystectomy on endometriosis also non-endometriosis cyst women. Before cystectomy through laparotomy, the mean of AMH level on endometriosis and non-endometriosis cyst group was 2.7 (SD 0.3) ng/ml and 3.1 (SD 0.4) ng/ml. The statistical test result using independent t-test showed there was difference in AMH level before cystectomy in patients with endometriosis and non-endometriosis cyst ( $p=0.001$ ). The mean of AMH level after cystectomy was greater on both types (1.5 (SD 0.4) ng/ml and 2.4 (SD 0.4) ng/ml). Apart from that, the statistical result showed significantly different between groups ( $p<0.001$ ).

Table 3 pointed out the mean level of AMH before and after cystectomy. There was a decrease trend of AMH level before and after cystectomy. The statistical test resulted the significant difference between this group ( $p<0.001$ ).

## DISCUSSION

The results of statistical test performed in this study showed the baseline characteristics of subjects were similar between groups so that these data would not influence the result of statistical test. Chang, et al. showed the decrease of AMH level one week after cystectomy and they found that AMH level would have recovery after 1 month and 3 months. The serum AMH level increased gradually after cystectomy and recovered about 65% after 3 months. There were several hypothesizes about the mechanism recovery of this AMH. Firstly, improvement of serum AMH level reflected the possibility of ovarian tissue reperfusion so that it released the AMH that normally produced by ovarian follicles group after forming the ovary blood vessel. Secondly, there was compensation in the form of hyperactivation on granulosa cells to produce the remaining follicle in ovary as the response of damage.

Although the number of follicles might decline after ovarian cystectomy, the amount of AMH secretion per follicle could be improved. Inflammation after surgery could stimulate ovarian follicle regeneration. Thirdly, some researchers speculated that the follicle could be saved from the follicle atresia.<sup>4</sup>

Study by Hirokawa and colleagues measured level of serum AMH before cystectomy and one month after laparoscopic cystectomy. They performed to 38 patients with endometrioma (20 unilateral and 18 bilateral). Serum AMH level significantly decreased after surgery. The decline in AMH level was found to correlate with bilateral cysts, not to diameter of cysts and age. Hirokawa, et al. also indicated the ovulation rate was significantly decreased in the ovary after cystectomy compared with before cystectomy.<sup>5</sup>

The measurement of AMH level after surgery dropped significantly after cystectomy and it occurred greater on endometriosis cyst. On cystectomy, a decline of AMH level might happen due to the removal of ovarian parenchyma during excision of the cyst wall. According to Roman, it was commonly occurred because as histologically, there was no barrier between the fields lining of the cyst and ovarian cortex. Therefore, the operator had to estimate the excision of cyst wall so that it increased the risk of ovarian cortex damage which finally contributed to the reduction of ovarian reserve.<sup>6</sup> In endometriosis cyst, there was no obvious barrier field due to fibrosis. This often led to accidentally remove number of ovarian cortex adjacent to the cyst wall and bleeding could occur in the hilum of the ovary; thus, the procedure required electrocoagulation that interfered with blood flow to the ovaries which were ended to reduce functional ovarian reserve.<sup>7</sup> The decrease of serum AMH level was caused by accidentally removing of normal ovarian cortex, damaging due to coagulation electro surgery during hemostasis, or injury due to inflammation. All of them would result to healthy follicle loss.<sup>4,8,9</sup>

Cystectomy in endometriosis cyst caused more damage than the ovarian cystectomy on functional cyst. Removal of benign ovarian cyst would takeaway the ovarian tissue; however, this adverse effect was lighter than cystectomy in endometriosis cyst.<sup>10</sup> Muzii, et al. hypothesized that this difference was caused by pseudocapsule

in endometriosis cyst compared with clear capsule in non-endometriosis cysts in order to make separate fields and clear dissection area. Muzii, et al. showed the non-endometriosis cyst which had clear cyst wall (dermoid or functional cyst) resulted to normal ovarian tissue only in 6% of cases; while, in excision of endometriosis cyst, the normal ovarian tissue was found higher to 54% of cases.<sup>11</sup>

Repair follicles may allow improvement of serum AMH level. It is because AMH is produced by the primary follicle, antral follicle, and small antral follicles. Developing follicles which are damaged after cystectomy will result to the lower level of serum AMH. Healthy primordial follicles are left behind because recruitment and growth of primordial follicle take time to produce AMH. Folliculogenesis from primordial follicles become preovulatory follicle about 85 days.<sup>12,13</sup>

## CONCLUSION

The decrease of AMH level is significant in the endometrial ovarian cyst women who undergo the cystectomy.

## RECOMMENDATION

Further study in a serial and longer follow-up is needed to look the fluctuation trend of AMH level after cystectomy at 1, 3, and 6 months after procedure.

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Research Article

## Incidence and Audit of Treatment on Third and Fourth Grade Perineal Tear

### *Insidensi dan Audit dari Tata Laksana Ruptur Perineum Derajat Tiga dan Empat*

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#### Abstract

**Objective:** To describe the incidence of 3<sup>rd</sup> and 4<sup>th</sup> grade perineal tear in Dr. Cipto Mangunkusumo hospital on the period of 2011 - 2014 and its compatibility of treatment based on RCOG guidelines.

**Methods:** Data were collected through medical records on women who had vaginal delivery in Dr. Cipto Mangunkusumo hospital from January 2011 to December 2014. This was a descriptive study with cross sectional design.

**Results:** The incidence of 3<sup>rd</sup> and 4<sup>th</sup> grade perineal tear each year were 3.55%, 4.35%, 3.95%, and 1.77% consecutively. About 100% treatment were accompanied by consultant; 3.5% performed in operating theatre; 90.7% were given postoperative antibiotic; 42.2% were given urinal catheter in 1x24 hour postoperative; 100% were given analgesic. About 61.4% of procedures were not compatible with RCOG guidelines.

**Conclusion:** The study showed that the incidence of 3<sup>rd</sup> and 4<sup>th</sup> grade of perineal tear was 3.66% for 4 years and the compliance to RCOG standard was 38.6%.

[Indones J Obstet Gynecol 2017; 5-1: 35-41]

**Keywords:** incidence, RCOG, third and fourth grade of perineal tear, treatment

#### Abstrak

**Tujuan:** Untuk mengetahui insidensi robekan perineum tingkat III dan IV di RSUPN Dr. Cipto Mangunkusumo pada tahun 2011 - 2014 dan kesesuaian tata laksana berdasarkan panduan RCOG.

**Metode:** Data diambil melalui rekam medis pada perempuan yang menjalani kelahiran di RSUPN Dr. Cipto Mangunkusumo pada bulan Januari 2011 - Desember 2014. Penelitian ini merupakan studi deskriptif dengan desain potong lintang.

**Hasil:** Insidensi robekan perineum tingkat III dan IV per tahun sebesar 3,55%; 4,35%; 3,95%; dan 1,77%. Sebanyak 100% penjahitan robekan perineum tingkat III dan IV dihadiri konsultan; 3,5% penjahitan di kamar operasi; 90,7% diberikan antibiotik pascaoperasi; 42,2% dipasang kateter 1x24 jam pascaoperasi; 100% diberikan analgetik. Sebanyak 61,4% subjek tidak ditata laksana sesuai standar RCOG tahun 2015.

**Kesimpulan:** Studi ini menunjukkan bahwa insidensi robekan perineum tingkat III dan IV sebesar 3,66% dalam 4 tahun dan kepatuhan mengikuti standar RCOG sebesar 38,6%.

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**Kata kunci:** insidensi, RCOG, robekan perineum tingkat III dan IV, tata laksana

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## INTRODUCTION

Third and fourth grade perineal tear involves the anal sphincter and epithelium which occurred from 0.1% to 10.2% of vaginal delivery. The incidence of 3<sup>rd</sup> and 4<sup>th</sup> grade perineal tear in United Kingdom was 2.9% (between 0-8%); whereas, 6.1% happened in primiparous and 1.7% in multiparous women.<sup>1,2</sup> Meanwhile, the incidence of 3<sup>rd</sup> and 4<sup>th</sup> grade of perineal tear in Austria were 1.5% and 0.1%, respectively.<sup>3</sup> The incidence of anal sphincter tear in mediolateral episiotomy population was 1.7% (2.9% on primiparous women) and 12% (19% on primiparous women) in mediana episiotomy population.<sup>1,2</sup>

The 3<sup>rd</sup> and 4<sup>th</sup> grade perineal tear often induces morbidity for women so that it influences the

quality of life. Serious complication on vaginal delivery caused by perineal tear consists of fecal incontinence, pelvic disorders, dyspareuni, chronic pain, and finally it inflicts to social and severe psychological problem.<sup>1,4,5</sup> An audit in national referral hospital in Jamaica showed that women suffered from high morbidity (43%) and fecal incontinence (23%) after the anal sphincter repair surgery.<sup>6</sup>

Based on high morbidity caused by perineal tear, several studies conducted an audit of appropriate treatment in 3<sup>rd</sup> and 4<sup>th</sup> grade perineal tear. The goal of this audit was to improve the service quality and decrease the morbidity referring to practical guideline. The audit in national referral hospital in Jamaica pointed out there were 26 women

suffering from anal sphincter tear; 9 cases (34.6%) reached the doctor compliance scores more than 6 and 17 cases (65.4%) got the scores 6 or lower. All perineal tears in this study was treated by experience doctor.<sup>6</sup> Nora G and Iram S in 2013 did the audit to assess the treatment of anal sphincter repairment. In general, most cases were done following the valid guidelines; however, the documentation about operator, suture technique, and suture material on some subjects were not recorded in medical records. For internal anal sphincter repair technique, the compliance rate reached 21.9%; meanwhile, the others were not recorded or stated in medical records.<sup>7</sup>

Royal College of Obstetricians and Gynaecologist (RCOG) released the guidelines of 3<sup>rd</sup> and 4<sup>th</sup> grade perineal tear in 2015.<sup>3</sup> The guidelines stated the recommendation of perineal tear pre, intra, and post-surgery. Therefore, this study is conducted to determine the incidence of 3<sup>rd</sup> and 4<sup>th</sup> grade perineal tear in Dr. Cipto Mangunkusumo hospital from 2011 to 2014 and the appropriate treatment to RCOG guideline in 2015.

## METHODS

This cross-sectional study was conducted in Dr. Cipto Mangunkusumo hospital by taking the secondary data from medical records on the period of January 2011 until December 2014. The sample was taken by consecutive sampling involving all subjects fulfilling inclusion criteria. Inclusion criteria on this study were all post vaginal delivery women which the data were completely recorded in medical records. By using formula, the minimal subjects were 270 women.

Third grade of perineal tear is defined by laceration starting from mucosal, perineal muscle, to anal sphincter which consists of IIIA grade: laceration less than 50% of external anal sphincter muscle; IIIB grade: laceration 50% or more of external anal sphincter muscle; and IIIC: laceration until internal anal sphincter muscle. Meanwhile, 4<sup>th</sup> grade of perineal tear is laceration from mucosal, perineal muscle, external and internal anal sphincter muscle, to rectal mucosal.

A proper treatment of 3<sup>rd</sup> and 4<sup>th</sup> grade perineal tear was assessed based on 9 components stated in RCOG guidelines, namely consultant attendance, repairment location, anesthesia application, suture technique, suture material, post-operative

antibiotic, urinal catheter insertion in 1x24 hour, sodium diclofenac analgesic, and laxatives using. The treatment was appropriate if fulfilling 7 of 9 criteria stated above.

The descriptive data was consisted of categorical variables (frequency and percentage of appropriate treatment to RCOG guidelines, consultant attendance, repairment location, anesthesia technique, anorectal mucosal suture technique, internal and external anal sphincter mucosal suture technique, suture material, post-operative antibiotic, urinal catheter insertion in 1x24 hour, analgesic and laxatives application, perineal tear classification, head fetal denominator, and induction of labor) and numerical variables (maternal age, number of parity, and time of phase II labor). The data was distributed by using SPSS 20 (IBM).

This study had got the ethical approval from *Komisi Etik Fakultas Kedokteran Universitas Indonesia (FK UI) RSUPN Dr. Cipto Mangunkusumo* number 1019/UN2.F1/ETIK/2015.

## RESULTS

From 6,095 deliveries recorded on this study, there were 223 subjects (3.66%) experienced 3<sup>rd</sup> and 4<sup>th</sup> grade perineal tear. From 2011 to 2014, the incidence of perineal tear was 3.55%, 4.35%, 3.95%, and 1.77%, respectively. There were 18 subjects which not completely recorded so that the data were excluded from this study. The mean of maternal age was 25.3 (SD 6.5) years old in 2011 and 26.6 (SD 5.2) years old in 2014. Between 2011 and 2014, the subject was dominated by primiparous women (64.7% to 87.5%). The subject undergoing normal delivery was more than the women doing induction of labor with misoprostol, oxytocin, and both. In 2011 and 2012, most women suffering from perineal tear delivered spontaneously (66.7% and 59.1%); however; in 2013 and 2014, the proportion of women experiencing perineal tear from vacuum extraction delivery reached 56.9% and 47.1%. The mean birth weight from 2011 to 2014 was 3,238; 3,234; 3,124; and 3,090 grams; consecutively. The IIIA grade perineal tear ranked the most from 2011 to 2014 which proportion was 45.2%; 45.2%, 70.8%, and 82.4%, respectively. (Table 1)

The incidence of 3<sup>rd</sup> and 4<sup>th</sup> grade perineal tear on primiparous women tended to decrease; namely 87.5% in 2011, 79.6% in 2012, 75.4% in 2013, and

**Table 1.** The Characteristics of Delivery in Dr. Cipto Mangunkusumo Hospital on the Period of 2011-2014

| Characteristics                                                            | 2011 (n=48)   | 2012 (n=93)   | 2013 (n=65)   | 2014 (n=17)   |
|----------------------------------------------------------------------------|---------------|---------------|---------------|---------------|
| Maternal age<br>(mean (SD)) (years old)                                    | 25.3 (6.5)    | 26.5 (5.6)    | 26.6 (5.2)    | 26.6 (5.2)    |
| Gestational age (mean (SD)) (weeks)                                        | 38.7 (1.3)    | 38.9 (1.3)    | 38.8 (1.4)    | 39.0 (1.6)    |
| Parity                                                                     |               |               |               |               |
| Primiparous                                                                | 42 (87.5%)    | 74 (79.6%)    | 49 (75.4%)    | 11 (64.7%)    |
| Multiparous                                                                | 6 (12.5%)     | 19 (20.4%)    | 16 (24.6%)    | 6 (35.3%)     |
| Induction of labor                                                         |               |               |               |               |
| Without induction                                                          | 26 (54.2%)    | 58 (62.4%)    | 32 (49.2%)    | 11 (64.7%)    |
| Misoprostol                                                                | 10 (20.8%)    | 13 (14.0%)    | 14 (21.5%)    | 2 (17.6%)     |
| Oxytocin                                                                   | 10 (20.8%)    | 17 (18.3%)    | 18 (27.7%)    | 2 (11.8%)     |
| Misoprostol + Oxytocin                                                     | 2 (4.2%)      | 5 (5.4%)      | 1 (1.5%)      | 1 (5.9%)      |
| Methods of delivery                                                        |               |               |               |               |
| Spontaneous                                                                | 32 (66.7%)    | 55 (59.1%)    | 11 (16.9%)    | 3 (17.6%)     |
| Vacuum                                                                     | 2 (8.3%)      | 22 (23.7%)    | 37 (56.9%)    | 8 (47.1%)     |
| Forceps                                                                    | 12 (25.0%)    | 16 (17.2%)    | 17 (26.2%)    | 6 (35.3%)     |
| Birth weight<br>(mean (SD)) (grams)                                        | 3,238 (459.8) | 3,234 (445.9) | 3,124 (427.8) | 3,090 (301.6) |
| Perineal tear                                                              |               |               |               |               |
| IIIA grade                                                                 | 28 (45.2%)    | 42 (45.2%)    | 46 (70.8%)    | 14 (82.4%)    |
| IIIB grade                                                                 | 10 (20.8%)    | 32 (34.4%)    | 11 (16.9%)    | 2 (11.8%)     |
| IIIC grade                                                                 | 5 (10.4%)     | 3 (3.2%)      | 5 (7.7%)      | 1 (5.8%)      |
| IV grade                                                                   | 5 (10.4%)     | 16 (17.2%)    | 3 (4.6%)      | 0 (0%)        |
| Incidence of 3 <sup>rd</sup> and 4 <sup>th</sup> grade<br>of perineal tear | 3.55%         | 4.35%         | 3.95%         | 1.77%         |
| Vaginal delivery                                                           | 1,354         | 2,138         | 1,645         | 958           |

64.7% in 2014. Meanwhile, on multiparous women, the incidence of it raised gradually from 12.5% in 2011 to 35.3% in 2014.

In 2014, the most subjects encountered 3<sup>rd</sup> and 4<sup>th</sup> grade perineal tear were due to vacuum extraction with episiotomy. Different from 2011, all subjects got this tear due to forceps extraction without episiotomy.

Table II showed the proportion of 3<sup>rd</sup> and 4<sup>th</sup> grade perineal tear patients which was treated correctly to RCOG guidelines in 2015. This study pointed out that almost half of subjects (42.2%) were not handled properly to RCOG guidelines. Discrepancy was found in surgery location, expert operator, and catheter insertion in 1x24 hour post-surgery. From 2011 to 2014, 96.5% from suturing of 3<sup>rd</sup> and 4<sup>th</sup> grade perineal tear was conducted in delivery room, not in operating theatre. The urine catheter insertion in 1x24 hour post-surgery was not applied in 57.8% women. All procedure in this study was done by obstetrics and gynecology residents.

All procedures were using anesthesia application; whereas, almost all of them (96.4%) were performed by local anesthesia. Starting from 2011 to 2014, 100% subjects were stitched by simple interrupted technique on anorectal mucosal. To stitch internal anal sphincter muscle, only 2 subjects (5.3%) got the simple interrupted methods; meanwhile, 36 subjects (94.7%) were stitched with horizontal mattress suture.

In this study, all subjects were reported for the external anal sphincter muscle stitches which using overlapping technique (41.7%) and end to end technique (58.3%). For external anal sphincter muscle, almost all subjects were stitch using PGA 2.0 (99.6%). All subjects got sodium diclofenac analgesic and laxatives in this study. Only 92 subjects (42.2%) got the insertion of urinal catheter in 1x24 hour post repairment. All subjects obtained amoxicillin-clavulanate; whereas, only 9.3% subjects also got metronidazole as an additional antibiotic.

**Table 2.** The Proportion of Appropriate Treatment on 3<sup>rd</sup> and 4<sup>th</sup> Grade Perineal Tear Patients in Dr. Cipto Mangunkusumo Hospital on the Period of 2011-2014 to RCOG Guidelines in 2015

| Parameter                                    |                              | 2011 n(%)  | 2012 n(%)  | 2013 n(%)  | 2014 n(%)  | Total       |
|----------------------------------------------|------------------------------|------------|------------|------------|------------|-------------|
| Operator                                     | resident T1-2                | 47 (97.9%) | 91 (97.9%) | 67 (98.5%) | 17 (100%)  | 219 (98.2%) |
|                                              | resident T3-4                | 1 (2.1%)   | 2 (2.1%)   | 1 (1.5%)   | 0 (0%)     | 4 (1.8%)    |
| Perineal tear documentation                  | Yes                          | 48 (100%)  | 93 (100%)  | 68 (100%)  | 17 (100%)  | 226 (100%)  |
|                                              | No                           | 0 (0%)     | 0 (0%)     | 0 (0%)     | 0 (0%)     | 0 (0%)      |
| Consultant attendance                        |                              | 48 (100%)  | 93 (100%)  | 68 (100%)  | 17 (100%)  | 226 (100%)  |
| Proof of consultant not attended             | Yes                          | 48 (100%)  | 93 (100%)  | 68 (100%)  | 17 (100%)  | 226 (100%)  |
|                                              | No                           | 0 (0%)     | 0 (0%)     | 0 (0%)     | 0 (0%)     | 0 (0%)      |
| Repairment location                          | Delivery room                | 47 (97.9%) | 87 (93.5%) | 67 (98.5%) | 17 (100%)  | 218 (96.5%) |
|                                              | Operating theatre            | 1 (2.1%)   | 6 (6.5%)   | 1 (1.5%)   | 0 (0%)     | 8 (3.5%)    |
| Anesthesia application                       | Local                        | 47 (97.9%) | 87 (93.5%) | 67 (98.5%) | 17 (100%)  | 218 (96.5%) |
|                                              | Spinal                       | 1 (2.1%)   | 6 (6.5%)   | 1 (1.5%)   | 0 (0%)     | 8 (3.6%)    |
| Anorectal mucosa stitch method               | Interrupted                  | 5 (100%)   | 16 (100%)  | 3 (100%)   | 0 (0%)     | 24 (100%)   |
| Internal anal sphincter muscle stitch method | Horizontal mattress          | 9 (90.0%)  | 18 (94.7%) | 8 (100%)   | 1 (100.0%) | 36 (94.7%)  |
|                                              | Interrupted                  | 1 (10.0%)  | 1 (5.3%)   | 0 (0%)     | 0 (0%)     | 2 (5.3%)    |
| External anal sphincter muscle stitch method | Overlapping                  | 20 (41.7%) | 51 (54.8%) | 19 (29.2%) | 3 (17.6%)  | 93 (41.7%)  |
|                                              | End to end                   | 28 (58.3%) | 42 (45.2%) | 46 (70.8%) | 14 (82.4%) | 130 (58.3%) |
| Suture material for anorectal mucosal        | PGA 2.0                      | 0 (0%)     | 13 (81.3%) | 0 (0%)     | 0 (0%)     | 13 (54.2%)  |
|                                              | PGA 3.0                      | 5 (100%)   | 3 (18.7%)  | 3 (100%)   | 0 (0%)     | 11 (45.8%)  |
| Suture material for internal anal sphincter  | PGA 2.0                      | 9 (90.0%)  | 19 (100%)  | 8 (100%)   | 1 (100%)   | 37 (97.4%)  |
|                                              | PGA 3.0                      | 1 (10.0%)  | 0 (0%)     | 0 (0%)     | 0 (0%)     | 1 (2.6%)    |
| Suture material for external anal sphincter  | PGA 2.0                      | 47 (97.9%) | 93 (100%)  | 65 (100%)  | 17 (100%)  | 222 (99.6%) |
|                                              | PGA 3.0                      | 1 (2.1%)   | 0 (0%)     | 0 (0%)     | 0 (0%)     | 1 (0.4%)    |
| <b>Post-operative treatment</b>              |                              |            |            |            |            |             |
| Antibiotic post-operative                    | Co-amoxiclav                 | 47 (97.9%) | 80 (86.0%) | 63 (92.6%) | 15 (88.2%) | 205 (90.7%) |
|                                              | Co-amoxiclav + Metronidazole | 1 (2.1%)   | 13 (14.0%) | 5 (7.4%)   | 2 (11.7%)  | 21 (9.3%)   |
| Urinal catheter insertion in 1x24 hour       | Yes                          | 19 (39.6%) | 42 (45.2%) | 25 (36.8%) | 8 (53.3%)  | 94 (42.2%)  |
|                                              | No                           | 29 (60.4%) | 51 (54.9%) | 40 (61.5%) | 9 (52.9%)  | 129 (57.8%) |
| Sodium diclofenac analgesic                  | Yes                          | 48 (100%)  | 93 (100%)  | 68 (100%)  | 17 (100%)  | 226 (100%)  |
|                                              | No                           | 0 (0%)     | 0 (0%)     | 0 (0%)     | 0 (0%)     | 0 (0%)      |
| Laxatives using                              | Yes                          | 48 (100%)  | 93 (100%)  | 68 (100%)  | 17 (100%)  | 226 (100%)  |
|                                              | No                           | 0 (0%)     | 0 (0%)     | 0 (0%)     | 0 (0%)     | 0 (0%)      |
| Appropriate scores                           | ≥ 7                          | 19 (39.6%) | 34 (36.6%) | 25 (38.5%) | 8 (47.1%)  | 86 (38.6%)  |
|                                              | < 7                          | 29 (60.4%) | 59 (63.4%) | 40 (61.5%) | 9 (52.9%)  | 137 (61.4%) |

## DISCUSSION

The incidence of anal sphincter tear in this study was 3.66%. For tertiary hospital, this incidence rate was higher than the rate published in RCOG guideline which was approximately 1%. In Sweden and Finland, the incidence of 3<sup>rd</sup> and 4<sup>th</sup> grade perineal tear was only 2.69% and 0.36%. Both of them were teaching hospital where the labor was done by midwife and perineal tear stitch was conducted by trainee.<sup>8</sup> Nevertheless, the incidence rate which published in systematic review from 451 studies was higher than this rate. Fowler<sup>9</sup> stated that in mediolateral episiotomy on delivery, the rate was ranged from 0.4% to 25%. Meanwhile, the rate could be reached 19% on population done medial episiotomy.

In 2014, the incidence of 3<sup>rd</sup> and 4<sup>th</sup> grade perineal tear was the lowest rate of all years before, namely 1.77% due to the lowest mean of birth weight. Jander, et al.<sup>10</sup> through their study said that the birth weight higher than 4,000 grams was the independent factor of anal sphincter tear. In another study, the birth weight higher than 3,325 grams was the most significant factors to increase anal levator muscle tear on three months after delivery. The proportional trend of primiparity and methods of delivery did not follow the trend of 3<sup>rd</sup> and 4<sup>th</sup> grade perineal tear although there was not analyzed statistically.<sup>11</sup> This result was different from Parnelli, et al.<sup>12</sup> which the decrease of incidence was influenced by the lower number of vacuum extraction and the improvement of delivery methods. O'Herlihy<sup>13</sup> stated that primiparity was the main factor of anal sphincter tear. A lot of researches studied about the risk factors of perineal tear<sup>14</sup>; therefore, we needed to conduct the advanced analytic study to know the most influencing risk factors.

In this study, 3<sup>rd</sup> and 4<sup>th</sup> grade perineal tear was higher in primiparous women in accordance with study by Pyykonen.<sup>15</sup> They stated that in primiparous women group with single life fetus in head presentation through spontaneous labor, the risk of 3<sup>rd</sup> and 4<sup>th</sup> grade of perineal tear was 1.4 times higher (OR 1.44; 95% CI 1.28-1.61) than multiparous group. Based on methods of delivery in 2014, the most subjects got 3<sup>rd</sup> and 4<sup>th</sup> grade perineal tear coming from vacuum extraction with episiotomy. In primiparous women, episiotomy decreased the incidence rate of anal sphincter tear (OR 0.83; 95% CI 0.72-0.92); however, episiotomy

increased the risk of perineal tear (OR 2.01 95% CI 1.67-2.44) in multiparous women.<sup>16</sup> In another study, mediolateral episiotomy raised the risk almost 5 times higher to be 3<sup>rd</sup> and 4<sup>th</sup> grade perineal tear. Vacuum extraction increased 2.64 times risk to get 3<sup>rd</sup> and 4<sup>th</sup> grade perineal tear (OR 2.64; 95% CI 1.25-5.54).<sup>17</sup> In 2011, all subjects who did delivery by forceps extraction without episiotomy got 3<sup>rd</sup> and 4<sup>th</sup> grade of perineal tear. Christianson, et al.<sup>18</sup> stated that forceps delivery and nulliparity were the risk factor of anal sphincter tear. In another study with large number of subjects, forceps extraction delivery was the strongest risk factor to be anal sphincter tear (OR 1.02; 95% CI 3.6-28.9).<sup>16</sup>

This study was the first research in Indonesia which analyzed the treatment of 3<sup>rd</sup> and 4<sup>th</sup> grade perineal tear. Sellars<sup>19</sup> said that to improve the clinical practice was through conducting an audit, collecting the data, analyzing it, and giving feedback. We could give feedback after analyzing the quality of service in an institution. Fernando, et al.<sup>20</sup> stated that to reduce the incidence rate of anal sphincter tear which impact to women's quality of life, they could choose vacuum extraction than forceps, limit the episiotomy procedure, and repair the anal sphincter tear by trained staff. These policies could be implemented after doing an audit to service and risk factors of perineal tear in each institution. The aim of this study is to give feedback so that it can improve the quality service of 3<sup>rd</sup> and 4<sup>th</sup> grade perineal tear.<sup>21</sup>

Although the consultant always attended the procedure, all perineal tear stitch on the subjects were done by residents, especially first and second grade of residents. Only 1.8% stitch was performed by third and fourth grade of residents. In Dr. Cipto Mangunkusumo hospital, in basic step, all residents got the training about 3<sup>rd</sup> and 4<sup>th</sup> grade perineal tear. Andrews<sup>22</sup> said that the outcome of anal sphincter tear stitch performed by doctor who had got the clinical practice was good. From 59 women, there were 6 subjects experiencing defect on ultrasonography; however, no one had the decrease symptom of fecal incontinence, urgency, or quality of life on one year ahead post-delivery. Fernando and Sultan<sup>20</sup> stated that perineal tear stitch conducted by expert could decrease the procedural complication. In a survey carried out by Fischer, et al.<sup>23</sup>, trainee or residents had better knowledge than consultant about the definition of anal sphincter tear. High rate of stitch in delivery room

was caused by higher rate of operating theatre for more emergency cases in Dr. Cipto Mangunkusumo hospital. There were still no written policies in Indonesia about a must to do 3<sup>rd</sup> and 4<sup>th</sup> grade of perineal tear stitch on operating theatre so that the place of procedure became the preference from operator and the availability of operating theatre.

Almost all stitches were done in delivery room (96.5%). From 2011 to 2014, only 8 subjects (3.5%) conducted the stitch on operating theatre. Compared to study by Cawich, et al.<sup>1</sup>, about 38.5% subjects did the stitch on the operating theatre. About 96.4% subjects used local anesthesia and the other used spinal anesthesia. From 10 patients did in operating theatre, 7 subjects used general anesthesia and the remaining got regional anesthesia, and 16 subjects was performed in delivery room. Based on RCOG Green-top guidelines number 29, the 3<sup>rd</sup> and 4<sup>th</sup> grade perineal tear stitch should be conducted in operating theatre with regional or general anesthesia.<sup>3</sup>

For the suture technique of internal anal sphincter muscle and anorectal mucosal, it was appropriate to the standard of procedural which anorectal mucosal was stitched by interrupted technique; meanwhile, most of internal anal sphincter muscle was approximated by horizontal mattress. In this study, the technique used to stitch the external anal sphincter was end to end or overlapping depending on the grade perineal tear. Based on RCOG guidelines, both of techniques gave the same outcome.<sup>3</sup> Meanwhile, Jacobson<sup>24</sup> concluded the overlapping technique could decrease the incidence of fecal incontinence ( $p=0.009$ ; RR 0.07; 95% CI 0.00-1.21; NNT 4.2), urgency ( $p=0.02$ ; RR 0.12; 95% CI 0.02-0.86; NNT 3.6), and perineal pain ( $p=0.04$ ; RR 0.08; 95% CI 0.00-1.45; NNT 5) significantly in 12 months after procedure. Vicryl<sup>®</sup> was the yarn using in this study. As standard on RCOG in 2015, the stitch using this yarn could give the better outcome.<sup>23</sup>

All subjects got the amoxicillin-clavulanate. A study by Cawich, et al.<sup>1</sup> they gave the 2<sup>nd</sup> and 3<sup>rd</sup> generation of cephalosporin intra vein on single dose. Unfortunately, only 7.7% subjects got the antibiotic as standard guidelines; meanwhile, in another study, the antibiotic was administered more than 72 hours with different kinds. According to RCOG guidelines, the antibiotic should be

provided as local protocol with broad spectrum which the aim was to reduce the incidence of wound dehiscence after procedure.<sup>3</sup>

On this study, there were 9 criteria to assess the compatibility of treatment to RCOG guidelines. All criteria could be evaluated objectively in medical records. Ideally, every subject was able to fulfill all of the criteria. Due to not applicable in clinical service, we set 7 of 9 criteria should be fulfilled. This score was taken based on Cawich, et al.<sup>1</sup> study which more than 6 of 8 criteria were considered good according to the consensus from five experts who not involved in the study. On the consensus, they got the minimal score to be appropriate was 6.8 with SD 1.3. On the audit conducted in Leicester, United Kingdom, the appropriate score for operator, external anal sphincter stitch methods, suture location, suture material, and use of antibiotic were 100%, 92%, 98%, 90%, and 100%, respectively.<sup>25</sup> In This study, 86 subjects (38.6%) involved in the criteria appropriate to the RCOG standard. This rate was higher than number of subjects in study of Cawich, et al.<sup>1</sup> which only 9 of 26 cases (34.6%) was treated as RCOG guideline.

This study was the first study which conducted the audit to the treatment of 3<sup>rd</sup> and 4<sup>th</sup> grade perineal tear in tertiary hospital. High number of delivery in a year (more than 1,000 deliveries per year) gave the great description of the incidence rate of it in the real population. Almost all deliveries in our hospital was assisted by residents who had been trained to the same competence level as normal delivery care so that it could control the risk of anal sphincter tear from operator aspect. This data was taken for the last 4 years so that it could capture the whole service quality to be implemented in policy.

## CONCLUSION

The study shows that the incidence of 3<sup>rd</sup> and 4<sup>th</sup> grade of perineal tear is 3.66% for 4 years and the compliance to RCOG standard is 38.6%.

## 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

## Feasibility Profile of Endometrial Nodule Resection in the Uterosacral and Rectovaginal Ligament

### *Profil Kemungkinan Reseksi Nodul Endometriosis pada Ligamen Uterosakral dan Rektovagina*

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#### Abstract

**Objective:** To know the feasibility of uterosacral and rectovaginal nodule resection in endometriosis patients who underwent laparoscopy surgery in Fatmawati Hospital.

**Methods:** Observational study was done by involving trained and experienced laparoscopist who performed deep infiltrating endometriosis (DIE) nodule resection laparoscopy on uterosacral and rectovaginal ligament. We observed on 35 patients which were histologically proven of DIE. We recorded the total procedure time, surgical complications occurred intra-operative, postoperative, and length-of-stay. The data were described descriptively.

**Results:** Mean (SD) of total laparoscopic procedure time including nodule resection was 200 (SD 52) minutes. There were two procedures (5.7%) with intra-operative complications, one (2.9%) with bowel injury which was converted to laparotomy, and the other one (2.9%) with intra-operative bleeding so that the operator cancelled nodules resection. Mean (SD) on length of stay after the procedures was 2.5 (2.1) days. On follow up observation, there was not any postoperative complication.

**Conclusion:** Laparoscopic uterosacral and rectovaginal nodules resection in endometriosis patient is feasible to be done by trained and experienced laparoscopic surgeon.

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**Keywords:** endometriosis, laparoscopy, nodule resection

#### Abstrak

**Tujuan:** Untuk mengetahui tingkat kemungkinan prosedur reseksi nodul endometriosis pada ligamen uterosakral dan rektovagina pada pasien yang menjalani laparoskopi di RSUP Fatmawati.

**Metode:** Sebuah studi observasional prospektif dilakukan pada pasien endometriosis yang menjalani prosedur reseksi nodul via laparoskopi, nodul berlokasi di ligamen sakrouterina maupun rektovagina dan terbukti secara histologis. Lama tindakan, komplikasi selama operasi, pascaoperasi dan lama rawat inap dicatat. Data yang tersedia ditampilkan secara deskriptif.

**Hasil:** Rerata (Simpang Baku/ SB) lama tindakan 200 (SB 52) menit. Terdapat dua kasus (5,7%) dengan komplikasi intraoperasi yaitu cedera usus (2,9%) dan operasi dialihkan ke laparotomi dan pembatalan reseksi nodul (2,9%) dikarenakan perdarahan intra-operasi. Rerata (SB) lama rawat inap adalah 2,5 (SB 2,1) hari. Tidak ditemukan adanya komplikasi pascaoperasi.

**Kesimpulan:** Prosedur reseksi nodul endometriosis pada ligamen uterosakral dan rektovagina via laparoskopi dapat dilakukan oleh operator laparoskopi yang terlatih dan handal.

[Maj Obstet Ginekol Indones 2017; 5-1: 42-45]

**Kata kunci:** endometriosis, laparoskopi, reseksi nodul

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## INTRODUCTION

Deep Infiltrating Endometriosis (DIE) is a disease marked by the ectopic presence of endometrium deeper than 5 mm beneath the peritoneal surface and it infiltrates different pelvic location.<sup>1</sup> It is commonly found in the uterosacral ligament (USL), vagina; even, it can extend to intestine (bowel DIE), bladder, and ureter (urological DIE).<sup>1</sup> Endometriosis occurs in one-third women who undergo surgery for chronic pelvic pain.<sup>2</sup> Chronic pelvic pain often debilitates women with endometriosis for years; thus, it causes high risk of emergency department visit. This disease is associated with loss of productivity and physical also social weak-

ness.<sup>2</sup> Additional complaints in women with endometriosis-related pain can arise from comorbid pain, such as painful bladder syndrome (formerly called interstitial cystitis), migraine, and irritable bowel syndrome.<sup>2</sup> We could prescribe medicine although it was often ineffective or only temporarily effective in controlling associated symptoms.<sup>3,5</sup> Radical surgical resection of DIE lesion was the main treatment for this form of endometriosis.

Deep infiltrating endometriosis nodules surgery represents a real operative challenge due to common involvement of vital retroperitoneal structures including ureter, bowel, vessels, and nerves. Laparoscopy appears to be the most ideal

tool to perform surgery and it offers a lot of advantages, such as possibility of magnification, accurate hemostasis, precise dissection, and careful handling of delicate tissue.<sup>4</sup> Nevertheless, laparoscopic management of retroperitoneal endometriosis should not be undertaken by inexperienced surgeon. Good knowledge of pelvic retroperitoneal anatomy is a prerequisite for radical and uncomplicated removal of DIE nodules.<sup>4,5</sup> The nodules of DIE were commonly found at uterosacral ligament (USL) compared with rectovaginal location, followed by bowel and urological involvement.<sup>6</sup> If the nodules are located on uterosacral or rectovaginal ligament, nodules resection procedure can be performed more safely than other location of nodules. Meanwhile, if the nodules have bowel or urological involvement, endometriotic nodules resection requires more advanced technique with higher risk of organ injury. Therefore, in this study, we would like to determine the feasibility of uterosacral and rectovaginal nodule resection (without bowel and urological involvement) who underwent laparoscopy surgery in Jakarta.

## METHODS

All nodule resection procedures (involving uterosacral and rectovaginal) in endometriosis patients which had been proved by histological finding were recruited from Fatmawati General hospital as referral centre. The study period was from September 2015 to May 2016. All patients were followed-up and evaluated to create endometriosis nodule resection feasibility profiles which included total procedure time, intra-operative complications (bowel, bladder, ureteral, vascular injury, massive blood loss, cancelled nodule resection or converted to laparotomy), post-surgery complications (fever, transient urinary retention, incontinence, ureterovaginal or rectovaginal fistulas), and hospital's length of stay. All laparoscopic nodule resection procedures were performed by trained and experienced laparoscopic surgeons. In this study, all surgeons did the procedure through same technique described by Wattiez.<sup>6</sup> The technique consisted of general and specific strategy. First step in general strategy was to hold adhesiolysis so that it would describe better exposure to the pelvic anatomy, followed by ureter identification and pararectal space dissection. In specific strategy, the surgeon had to resect all visible nodules in uterosacral and

rectovaginal ligament. In case of any bladder, ureteral, or bowel involvement, we performed the procedure through multi-discipline approach.

## RESULTS

Between September 2015 and May 2016, we obtained 35 laparoscopic uterosacral and rectovaginal nodule resection procedure. It included 23 (65.7%) nodules located only at uterosacral ligament, 6 (17.1%) nodules located only at rectovaginal, and the other 6 (17.1%) women having nodule at both sites (Table 1). The mean (SD) of total laparoscopic procedure time which included nodule resection was 200.4 (SD 52.5) minutes (Table 2). There were two procedures (5.7%) with intra-operative complications, one (2.9%) with bowel injury which was converted to laparotomy; and the other one (2.9%) with intra-operative bleeding causing the cancellation of nodule resection (Table 3). The mean (SD) of length-of-stay in hospital after finishing procedures was 2.5 (2.2) days (Table 4). On follow-up observation, we did not find any postoperative complication.

**Table 1.** Location of Nodules

| Location of Nodules                | Responses |        |
|------------------------------------|-----------|--------|
|                                    | N         | %      |
| Uterosacral Ligament (USL)         | 23        | 65.7%  |
| Rectovaginal Ligament              | 6         | 17.1%  |
| Both USL and Rectovaginal Ligament | 6         | 17.1%  |
| Total                              | 35        | 100.0% |

**Table 2.** Time of Procedure

| Min (min) | Max (min) | Mean  | Std. Deviation |
|-----------|-----------|-------|----------------|
| 100       | 420       | 200.4 | 52.5           |

**Table 3.** Surgical Complication

| Complication                      | N (%)   |
|-----------------------------------|---------|
| <b>Intra-surgery Complication</b> |         |
| Bowel Injury                      | 1 (2.9) |
| Bladder Injury                    | 0 (0)   |
| Ureteral Injury                   | 0 (0)   |
| Vascular Injury                   | 0 (0)   |
| Massive Blood Loss                | 0 (0)   |
| Converted to Laparotomy           | 1 (2.9) |

**After Surgery Complication**

|                        |       |
|------------------------|-------|
| Fever                  | 0 (0) |
| Urinary Retention      | 0 (0) |
| Incontinence           | 0 (0) |
| Ureterovaginal Fistula | 0 (0) |
| Rectovaginal Fistula   | 0 (0) |

**Table 4.** Length of Stay in Hospital

| Min (min) | Max (min) | Mean | Std. Deviation |
|-----------|-----------|------|----------------|
| 2         | 14        | 2.5  | 2.2            |

**DISCUSSION**

The degree of pain related to severity of disease is varied among patients. Some cases of mild endometriosis can be associated with significant pain and other women with severe endometriosis may experience little or no pain. Meanwhile, 5% of patients with DIE were pain-free. Pain can derive from compression or infiltration of specific nerves by the ectopic endometrial growth. The anatomical position of the lesion on the organ is able to prevent or interfere with its function. Histological evaluation of DIE with severe pain is related to higher proportion of intraneural and perineural infiltration. Nerves have close relationship with the endometriotic nodules and the fibrotic tissue; thus, there is a relationship between endometriotic lesions and pain. The cytokines and growth factors (estradiol, prostaglandins, and nerve growth factor) associated with endometriosis have correlation with pain sensation. In addition to that, current studies proposed that endometriosis lesions could stimulate peripheral nerve fibers to sensitize the central nervous system and lead to phantom pains in the absence of lesion.

All patients in this study had already received hormonal therapy; however, it failed to reduce the symptoms. Radical surgical resection of DIE lesions was the main treatment for this form of endometriosis. Open access to posterior DIE nodule represents an operative challenge due to common involvement of vital retroperitoneal structures. Careful dissection is necessary to restore pelvic anatomy and preserve function.<sup>4</sup> In fact, there is possibility of unpredictable complexity intra procedure; therefore, we have to be prepared.

In this study, there was one case of bowel injury. This injury happened during the dissection of bowel from the rectovaginal adhesion. The surgeon failed to identify the correct plane during dissection. As bowel injury might happen anytime, we suggested bowel preparation before the procedure.<sup>7</sup> Other intra-operative complication found in this study was intra-operative bleeding obscuring the operative field. The surgeon decided not to resect the nodules because it would increase the risk of the surgery afterwards. Cancellation of nodules resection should not be done under any circumstances.

A multidisciplinary surgical team led by a experienced gynecologist has to work together in complex cases with urologist, gastrointestinal surgeons, and/or general surgeons. All of them may play an important role in providing adequate treatment and as well as increasing the likelihood of providing consistent, evidence-based, and cost efficient care.<sup>8,9</sup>

**CONCLUSION**

Laparoscopic uterosacral and rectovaginal nodules resection in endometriosis patient is feasible to be performed by trained and experienced laparoscopic surgeon although multidisciplinary team approach should be joined for severe cases.

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## Research Article

## Level of Retinol Deposit and Cervical Cancer

*Kadar Deposit Retinol dan Kanker Serviks*

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## Abstract

**Objective:** To analyze level of retinol deposit sufficiency in the natural history of cervical cancer.

**Methods:** Serum retinol level was measured by ELISA from peripheral blood of subjects with normal cervix, cleared and persistent high risk human papilloma virus (HR-HPV) subclinical infection, and cervical cancer who fulfilled the inclusion and exclusion criteria. The study was held in Dr. Cipto Mangunkusumo and Fatmawati Hospital, Jakarta, within 2 years (August 2013-2015). Blood was taken twice, consisting of post-8-hour fasting blood and 2 hours after 6000 IU retinyl palmitate oral administration.

**Results:** Of 47 total samples, sufficient level of retinol deposit in normal cervix, cleared and persistent HR-HPV subclinical infection, and cervical cancer group was 85.0% (reference), 75.0% (OR 1.89), 33.3% (OR 11.33), and 75% (OR 1.89); respectively. Statistically, there was no significant difference from sufficiency level of retinol deposit between normal cervix and clearance HR-HPV subclinical infection ( $p=0.628$ ), normal cervix and persistent HR-HPV subclinical infection ( $p=0.078$ ), normal cervix and cervical cancer ( $p=0.433$ ), cervical cancer and clearance HR-HPV subclinical infection ( $p=1.000$ ), cervical cancer and persistent HR-HPV subclinical infection ( $p=0.430$ ), persistent and clearance HR-HPV subclinical infection group ( $p=0.740$ ).

**Conclusion:** This study proves that normal cervix group has the highest level of retinol deposit sufficiency; however, it cannot be stated that cervical cancer group has less sufficiency level. Persistent HR-HPV subclinical infection group has the lowest level of retinol deposit (OR 11.33). There is no association between sufficient level of retinol deposit and clearance of HR-HPV.

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**Keywords:** cervical cancer, HR-HPV clearance, retinol deposit

## Abstrak

**Tujuan:** Untuk menganalisis tingkat kecukupan deposit retinol pada perjalanan alami kanker serviks.

**Metode:** Kadar retinol serum diperiksa dari darah perifer dengan metode ELISA pada kelompok serviks normal, infeksi subklinis human papilloma virus risiko tinggi (HPV-RT) klirens dan persisten, serta kanker serviks yang sesuai dengan kriteria inklusi dan eksklusi di Rumah Sakit Dr. Cipto Mangunkusumo dan Fatmawati, Jakarta, pada periode 2 tahun (Agustus 2013-2015). Sampel darah diambil dua kali yaitu setelah puasa 8 jam dan 2 jam setelah pemberian 6000 UI retinil palmitat peroral.

**Hasil:** Diperoleh 47 sampel total dari 4 kelompok yang diteliti. Deposit retinol yang cukup pada kelompok serviks normal, infeksi subklinis HPV-RT klirens, HPV-RT persisten, dan kanker serviks adalah berturut-turut 85%, 75% (OR 1,89), 33,3% (OR 11,33), dan 75% (OR 1,89). Secara statistik tidak terdapat perbedaan bermakna tingkat kecukupan deposit retinol antara kelompok serviks normal dengan infeksi subklinis HPV-RT klirens ( $p=0,628$ ), serviks normal dengan infeksi subklinis HPV-RT persisten ( $p=0,078$ ), serviks normal dengan kanker serviks ( $p=0,433$ ), kanker serviks dengan infeksi subklinis HPV-RT klirens ( $p=1,000$ ), kanker serviks dengan infeksi subklinis HPV-RT persisten ( $p=0,430$ ), infeksi subklinis HPV-RT persisten dengan klirens ( $p=0,740$ ).

**Kesimpulan:** Penelitian ini mampu membuktikan bahwa tingkat kecukupan deposit retinol tertinggi dijumpai pada kelompok serviks normal, namun tidak mampu membuktikan bahwa kanker serviks memiliki tingkat kecukupan deposit retinol yang kurang. Tingkat kecukupan deposit retinol terendah ditemukan pada kelompok HPV persisten (OR 11,33). Tidak terdapat hubungan antara deposit retinol yang cukup dengan klirens HPV-RT.

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**Kata kunci:** deposit retinol, HPV-RT klirens, kanker serviks

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## INTRODUCTION

Cervical cancer is one of the major leading causes of death among women due to cancer. It is the second most common cancer on women in the world, of which 83% of that occurs in developing countries.<sup>1,2</sup> In Indonesia, the incidence of cervical

cancer is estimated to be 100 per 100,000 populations among 79-million women at risk. The mortality rate of cervical cancer in Indonesia is very high because more than 70% of cases are diagnosed at advanced stage.<sup>3</sup>

This cancer is actually highly preventable due to known etiologic causes. The main factor

contributing to cervical cancer is persistent infection of high risk-human papillomaviruses (HR-HPVs), which is the precursor for malignancy. The hypothesis was a relationship between HPV infection and cervical neoplasia which was first introduced by Harold zur-Hausen as a German virologist.<sup>4-6</sup>

Although HPV has been identified as the cause of cervical cancer, most women infected with HPV do not always develop to be cervical cancer. Most of them, around 70-90% of cases, will experience a clearance. Therefore, this issue leads to the idea that there are other factors contributing to the induction of cancer other than HPV infection.<sup>7</sup> Local immune response is a determining factor that affects its susceptibility to HPV and progression into cervical cancer.<sup>8,9</sup> In individuals who have competent immune system, most HPV infections occur subclinically, and only small percentage will progress to pre-cancerous lesions and invasive cervical cancer. The mechanisms of HPV in keeping off the immune response are due to the modulation of cytokines to alter antigen presentation, interferon (IFN) regulatory pathways, and adhesion molecules. The avoidance of HPV to immune response is a critical point of the successful HPV infection to host cells.<sup>10</sup>

Nutritional cofactors, which are associated with immunity, have an important role in the defense against HPV infection. Various antioxidants have been known to boost the immune system against viruses and other microorganisms, as well as tumor cells. Retinol, as cofactor nutrient, is essential in cervical mucosal immunity. It is able to modulate the non-specific and specific immune system against HPV infection and tumor cells.<sup>11</sup> It also has central role in growth, development and differentiation of B and T lymphocytes, and as major regulator of cell activation on immune system.<sup>12</sup> The CD4<sup>+</sup>T and CD8<sup>+</sup>T cells can be modulated by retinol. T-cell is specific to the virus and protective factor against tumor cells. Meanwhile, CD8<sup>+</sup>T-lymphocytes or Cytotoxic T-Lymphocyte (CTL) assisted by molecular Major Histocompatibility Complex (MHC) class I acts to recognize and kill tumor cells. CD4<sup>+</sup>T-cells are generally not cytotoxic to the tumor, they may play an important role in anti-tumor response by producing cytokines which are necessary for the development of CTL cells into effector cells, yet

CD8<sup>+</sup>T-cells can eliminate viral infection by secreting IFN- $\gamma$ , and granzyme, to run cytolytic effect.<sup>13</sup>

Retinol is a potent HPV and carcinogenesis inhibitor. It acts mainly by three mechanisms, such as apoptosis, cessation of growth, and differentiation.<sup>14</sup> Retinol can inhibit cells immortalization by HPV; thus, retinol offers protective effect against the occurrence of cervical neoplasia.<sup>11</sup>

In the cervix, retinol can interact with HPV oncoprotein (E6 and E7) and increase the role of p53 and pRb (tumor suppression genes) to control cell cycle and proliferation. Retinol can inhibit not only early gene expression of HPV types 16 to 95% by lowering E2 and E5 mRNA, but also E6 and E7 proteins. It can lower the level of viral oncogenes transcription and inhibit neoplastic process. Retinol can protect the mucosa against viral infection and it has a cytostatic effect by inducing the cessation of cell cycle-dependent p53,<sup>14</sup> generating CD4<sup>+</sup>T-cells, inducing effective CD8<sup>+</sup>T-cells responses, suppressing inflammatory cells, and producing several cytokines, including tumor necrosis factor-alpha (TNF- $\alpha$ ) which are potent to control HPV infection.<sup>15-17</sup>

The transformation zone of cervix is a high-risk zone that can be altered by HPV infection. This zone is also the most sensitive zone to retinol. Retinol increases resistance to infectious micro-organism by maintaining the function, structure of epithelial cells, mucosal integrity, and stabilization of inter-cell linkage. When retinol is absent, goblet cells will disappear and mucosal epithelial atrophy will occur. Therefore, it will lead to irritation and infection.<sup>18</sup>

Until now, there has been no recommendations of retinol to increase the clearance of HPV infection as well as improve the response of cervical cancer therapy clinically. Apart from that, retinol is very cheap so that the cost problem will be minimally debated. This study aims to analyze the adequacy of retinol deposit on the natural history of cervical cancer, ranging from normal cervix, cleared and persistent subclinical HPV infection, to cervical cancer. To determine the adequacy status of retinol level in this study, we measured through level of deposit in the liver by the relative dose response (RDR) method in examination of retinol-binding protein 4 (RBP4) at fasting and 2 hours after

administration of retinylpalmitate. The RBP<sub>4</sub> is also popular as plasma retinol-binding protein which transports retinol in serum. Retinol is metabolized into retinaldehyde as some isomers of retinoic acid and retinyl esters. Retinaldehyde is important chromophore in rhodopsin photo-receptor; whereas, retinoic acid regulates many cellular differentiation and proliferation effects via intracellular receptors retinoic acid receptor (RAR) and retinoic X receptor (RXR). The RBP<sub>4</sub> adopts  $\beta$ -barrel structure with a central cavity that accommodates either retinol or retinaldehyde and it is synthesized primarily in hepatocytes and adipocytes as 21 kDa non-glycosylated molecules, non-phosphorylated, and non-sulfate.

According to aspect of nutrition, the lack of retinol is associated with cervical cancer; thus, it is that it can be used as a basic approach to primary prevention of cervical cancer. Giving retinol is expected to reduce the risk of persistent HR-HPV infection and progression towards pre-cancerous lesions and cervical cancer. Eventually, it can reduce the incidence, morbidity, and mortality of cervical cancer.

## METHODS

This study consisted of four groups representing the natural history of cervical cancer, such as the normal cervix, persistent subclinical HPV infection, HPV clearance, and cervical cancer. Normal cervix consisted of subjects with normal cervical cytology result and negative HPV DNA test. Cervix with subclinical HPV infection included those with negative cytology result and positive HR-HPV DNA test. Cervical cancer was expressed through the result of squamous cell carcinoma (SCC) type according to histopathology assessment. Normal group and cervical cancer data were taken cross sectionally; whereas, subclinical infection with HR-HPV clearance and persistent were coming from population with positive HR-HPV followed by continuous checking twice in 12-24 months. We recruited subjects from August 2013 to August 2015.

### Inclusion and Exclusion Criteria

The target population of this study was reproductive age women with negative cytology result or without positive HPV or cervical cancer. We included all women coming to the

Gynecologic Oncology Clinic, Department of Obstetrics and Gynecology, Dr. Cipto Mangunkusumo and Fatmawati Hospital during the study period who fulfilled the inclusion and exclusion criteria. Inclusion criteria for this study consisted of sexually active women aged 20-60 years old with negative cytology result, or SCC proven by histopathology assessment and they agreed to participate in the study. While, the exclusion criteria were married women under the age of 20 years old, having multiple sexual partners, having malnutrition, on pregnancy, having a history of intravenous narcotics use, promiscuity confession, using long term steroid agents or intrauterine device (IUD), and experiencing genitourinary tract infection.

### Patient Enrollment

During the period of August 2013 until August 2015, we obtained 71 samples among 538 potential subjects for subclinical HR-HPV infection clearance and persistent group. Subject with normal cervix (control), clearance, and persistent group taken from Women's Health Clinic (WHC), Dr. Cipto Mangunkusumo Hospital in Kencana Cluster. While, we got cervical cancer group from WHC outpatient clinic, Gynecologic Oncology outpatient and inpatient, Department of Obstetrics and Gynecology, Dr. Cipto Mangunkusumo and Fatmawati hospital. Due to several reasons related to geographical problem of their living area, we successfully recruited 7 women (9.9%) for subclinical HR-HPV infection clearance and persistent group. Initially, we took 22 samples for control group; however, two samples were excluded because of sample lysis and HPV-positive result after re-checking. There were four subjects for subclinical HR-HPV infection clearance group and three subjects for persistent group. There were 27 samples for cervical cancer group, seven samples were excluded due to lysis, yet.

### Laboratory Protocol

We examined the samples through sandwich ELISA. The manufacturer kit R&D system instructed this method. To determine the status of the retinol adequacy, we measured the level of retinol deposit in the liver by Relative Dose Response (RDR) method through examination of serum retinol-binding protein 4 (RBP<sub>4</sub>) at fasting

and 2 hours after administration of 6,000 IU of retinylpalmitate orally. Retinol deposit is defined to be sufficient if there is an increase of RBP<sub>4</sub> of less than 20%, insufficiency if there is an increase of more than or equal to 20%.

Blood sampling was performed using EDTA tubes after signing the informed consent. The ELISA plates coated with antibodies of RBP<sub>4</sub> were used. The blood samples were centrifuged to separate serum from red blood cell at a speed of 3,500 rpm for 10 minutes. Serum was collected and placed in a threaded tube and stored at -80°C. The serum, standard and control, was put in each plate as much as 20 µl with previously added diluent 200 µl. After that, the plate was incubated in an orbital shaker for one hour at room temperature. After an hour, the plate was washed three times using 400 µl buffer solution. The next step was to provide as much as 200 µl conjugate RBP<sub>4</sub>, then incubated for one hour and continued by washing the plate. The final step was the addition of 200 µl substrate solution and incubated for 30 minutes. To stop the reaction, fifty microliters stop solution were given. At this time, the color of the solution would turn into yellow. We utilized a microplate reader Glo-Max at a wave length of 450 nm to read and interpret the result.

## Statistical Analysis

We used SPSS version 20.0 to analyze the data. The association between groups with sufficiency level of retinol deposit was analyzed as unpaired

categorical comparative table. We used chi-square analysis resulting in the odds ratio (OR) and confidence interval (CI) 95%. To avoid multiplicity, chi-square and odds ratio were calculated with logistic regression procedure.

## RESULTS

Over 2 years of period, from August 2013 to August 2015, forty-seven samples were obtained. The characteristics of the subjects were presented in Table 1.

According to the table, it could be seen that the average age of subjects with cervical cancer, cleared and persistent subclinical HR-HPV infection, and normal cervix (control) were 48.5, 40.0, 46.0, and 41.0 years old, respectively. The mean age of first marriage was regarded as the first sexual contact when relatively young, which all results showed for more than 20 years old. Most of the subjects and their partners were married only once; and most of them had low parity. Almost all subjects did not smoke. Based on these demographic characteristics, the subjects in this study had lower risk factors for HPV infection and cervical cancer.

Meanwhile, the deposit adequacy level of retinol was shown in Table 2. Of 47 total samples, retinol deposit sufficiency level in normal cervix, subclinical HPV infection clearance, persistent, and cervical cancer group was 85.0% (reference), 75.0% (OR 1.89), 33.3% (OR 11.33), and 75% (OR 1.89), respectively.

**Table 1.** Demographic Characteristics

| Variables                             | Cervical cancer<br>(n = 20) | Persistent HPV<br>(n = 3) | HPV clearance<br>(n = 4) | Control<br>(n = 20) |
|---------------------------------------|-----------------------------|---------------------------|--------------------------|---------------------|
| Age (years old)                       | 48.5 ± 9.5                  | 40.0 ± 10.0               | 46.0 ± 5.0               | 41.0 ± 16.0         |
| The age of first marriage (years old) | 21.5 ± 1.5                  | 21.5 ± 0.5                | 23.0 ± 3.0               | 24.0 ± 3.0          |
| Marital status                        |                             |                           |                          |                     |
| • Married once                        | 17 (85.0%)                  | 3 (100.0%)                | 3 (75.0%)                | 19 (95.0%)          |
| • Married more than once              | 3 (15.0%)                   | 0 (0)                     | 1 (25.0%)                | 1 (5.0%)            |
| Partner's marital status              |                             |                           |                          |                     |
| • Married once                        | 15 (75.0%)                  | 2 (66.7%)                 | 3 (75.0%)                | 17 (85.0%)          |
| • Married more than once              | 5 (25.0%)                   | 1 (33.3%)                 | 1 (25.0%)                | 3 (15.0%)           |
| Parity                                | 3.0 ± 2.0                   | 3.5 ± 0.5                 | 4.0 ± 1.0                | 2.0 ± 2.0           |
| Smoking status                        |                             |                           |                          |                     |
| • Smoking                             | 3 (15.0%)                   | 0 (0)                     | 0 (0)                    | 0 (0%)              |
| • Not smoking                         | 17 (85.0)                   | 3 (100.0%)                | 4 (100.0)                | 20 (100.0%)         |

**Table 2.** Sufficiency Rate of Retinol Deposit

| Groups            | Deposit adequacy level of retinol |        | OR    | IK (95%)       | p-value |
|-------------------|-----------------------------------|--------|-------|----------------|---------|
|                   | Less                              | Normal |       |                |         |
| Cervical Cancer   | 5                                 | 15     | 1.89  | 0.39 to 9.27   | 0.433   |
| Persistent HR-HPV | 2                                 | 1      | 11.33 | 0.76 to 167.97 | 0.078   |
| Cleared HR-HPV    | 1                                 | 3      | 1.89  | 0.14 to 24.79  | 0.628   |
| Control           | 3                                 | 17     | Ref   |                |         |

*Chi-Square test***Table 3.** Relationship between the Deposit Adequacy Level of Retinol Group

| Group (Sufficiency Rate Deposit of Retinol) | OR   | IK (95%)       | p-value |
|---------------------------------------------|------|----------------|---------|
| Cervical Cancer vs Cleared HR-HPV           | 1.00 | 0.08-11.93     | 1.000   |
| Cervical Cancer vs Persistent HR-HPV        | 0.17 | 0.01 to 2.26   | 0.430   |
| Persistent HR-HPV vs Cleared HR-HPV         | 6.00 | 0.22 to 162.54 | 0.740   |

*Chi-Square Test*

Statistically, there was no significant difference of retinol deposit sufficiency level between normal cervix and cleared subclinical HPV infection ( $p=0.628$ ), normal cervix and persistent subclinical HPV infection ( $p=0.078$ ), normal cervix and cervical cancer ( $p=0.433$ ), cervical cancer and cleared subclinical HPV infection ( $p=1.000$ ), cervical cancer and persistent subclinical HPV infection ( $p=0.430$ ), persistent subclinical HPV infection and clearance ( $p=0.740$ ).

As demonstrated on Table 2 and 3, there were not significant differences in the level of retinol adequacy deposits in all groups studied.

## DISCUSSION

Statistically, there were no significant differences in the level of retinol deposit sufficiency level in all groups studied, such as between the normal cervix and cervical cancer, normal cervix and persistent subclinical HR-HPV infection, normal cervix and cleared subclinical HR-HPV infection ( $p=0.433$ ;  $0.078$ ;  $0.628$ ); cervical cancer and clearance subclinical HR-HPV infection, cervical cancer and persistent HR-HPV subclinical infection ( $p=1.000$ ;  $0.430$ ), and subclinical HR-HPV infection persistence and clearance ( $p=0.740$ ).

In this study, we obtained the difference in the sufficiency level of retinol deposit in the group of subclinical infection with HR-HPV persistence, clearance, and normal cervix (control). This result was in accordance with predetermined clinical

justification. However, the result was far below clinical justification for cervical cancer group.

Until now, the study of retinol in the immune system related to the natural history of cervical cancer was inconsistent, especially when applied to population. There were many studies of endogenous retinoid in plasma level on the natural history of cervical cancer, there was still lack of observation on the adequacy of retinol deposit. This study showed that there was no statistically significant difference of sufficiency retinol deposit level in the group of normal cervix, cervical subclinical HR-HPV infection clearance, persistent, and cervical cancer. The results of this study were relatively consistent with study conducted by Siegel, et al.<sup>19</sup> and Palan, et al.<sup>20</sup> Siegel, et al. stated that there was no significant association between endogenous retinoic acid and a clearance of HPV infection also cervical lesion regression. These results indicated that the role of retinoic acid as an HPV and carcinogenesis inhibitor in vitro could not be demonstrated in epidemiological studies on the setting of clinical population.<sup>19</sup> Palan, et al. pointed out that there were no significant differences in the mean level of plasma retinol on 235 subjects with normal cervix, cervical intraepithelial neoplasia (CIN), and cervical cancer. The study also found lower level of carotenoid and alfa-tocopherol in the plasma of CIN and cervical cancer patients. There was also significant linear trend in level of carotenoid and alfa-tocopherol to degree of cervical histopathological abnormalities.<sup>20</sup> Another

study by Giuliano, et al.<sup>21</sup> stated that persistent HPV infection could be inhibited by several antioxidant micronutrients. Consumption of papaya regularly at least once a week was associated with significant barrier to persistence of infection. Sedjo, et al.<sup>22</sup> concluded that high consumption of vegetables and fruits was related to 54% decreased risk of HPV persistence. Goodman, et al.<sup>23</sup> also found a significant correlation between antioxidant level and the risk of CIN. Siegel, et al. from another study also revealed contrary result that retinol was not considered as a protective agent against persistent infection with oncogenic HPV on female population in Brazil.<sup>19</sup> This finding was also supported by Alvarez, et al.,<sup>24</sup> who showed that there was no significant difference in the rate of cervical lesion regression in placebo, retinoid low dose, and high dose group. Retinol regulates kind of essential cells in the body. Retinoid plays an important role in the process of growth, cleavage, tissues maintenance, reproductive function, metabolism, differentiation, haematopoietic, bone formation, spermatogenesis, and embryogenesis. Deficiency of vitamin A will impact to unwanted effects.<sup>25,23</sup> Some factors significantly affecting the level of endogenous retinol include age, race, use of oral contraceptives, and number of pregnancies.<sup>26</sup> The concentration of retinoid did not differ significantly between fasting and non-fasting population.<sup>27</sup>

Dragnev, et al. stated that retinol had apoptosis effect, anti-proliferative, and regulator of cell differentiation, a chemo-preventive agent,<sup>28</sup> thus, it could be used in anti-cancer therapy. Retinol acts via nuclear receptor to activate target genes containing responsive element resulting to biological effect. Anti-cancer activity of retinoid is the result of three main mechanisms, such as cytodifferentiation, growth cessation, and apoptosis.<sup>28,29</sup> Some retinoids are clinically effective as chemo-preventive and anti-cancer therapy in promyelocytic acute leukemia, but, it shows less effective against most solid tumors and results unwanted side effects.<sup>23,29,30</sup> Headache is the most frequent adverse events after retinoid treatment on approximately 74% of high doses of retinoid.<sup>24</sup> In combination with IFN, retinoid has potential effect in cervical SCC. Retinoic acid has the action as a radiosensitizer that does not require the function of p53 in vitro.<sup>31</sup> Narayanan, et al. showed an increased

expression of p53 and inhibition of E6/E7 transcription after retinoid administration. This finding suggested an important role of retinoic acid as a cell cycle regulator, chemo-preventive, and anti-viral agents.<sup>23,32,33</sup>

Our results had differences compared with previous epidemiological studies, in which the study showed the opposite relationship between the development of cancer and vitamin A-containing diet. Systemic administration of retinol can reduce the thickening of arterial intima layer significantly after endothelial injury in vivo. In vitro and in vivo, retinol has a pro-inflammatory effect and it can enhance the expression of TNF- $\alpha$  as a cytokine that has an important role in acute inflammation.<sup>31-33</sup> Immunomodulating pharmacological concentration of vitamin A can lower the incidence of tumor in experimental biochemistry. Some studies proved that natural and synthetic retinoids could inhibit the growth and development of various tumor types.<sup>31</sup>

Apoptotic process includes a series of action that is associated with retinoid. Geissmann, et al. showed that retinoid could induce apoptosis through its heterodimer receptor in spite of no signal inflammation. The existence of cross communication with inflammatory cytokines allows retinoid to activate DNA-binding-factor- $\kappa$ B in the core of dendritic cells, triggers MHC class II, induces differentiation and maturation of dendritic cells, as well as improves specific T-cell response to antigens.<sup>34</sup> Khan, et al. reported that human keratinocytes (HKC), which had been immortalized due to transfection of HPV-16, was more sensitive to inhibition of retinoic acid compared with normal HKC. Retinoic acid also can inhibit mRNA expression of E6/ E7 and E2/ E5 HPV-16. Retinoic acid became barrier to immortalization due to transfection of HPV-16 and it reached up to 95%.<sup>35</sup>

Retinoic acid is a fraction of vitamin A in the blood that is active in the process of cell differentiation and growth. When the body requires vitamin A, it will be mobilized from the liver in the form of retinol by retinol binding plasma (RBP). The uptake of retinol by various cells of the body relies on receptor on the membrane surface specifically to RBP. Later on, retinol is transported through the cell membrane and then it is tied to cellular retinol-binding protein (CRBP) and RBP; finally, it will be released.<sup>36</sup>

Retinol and vitamin A derivatives affect cell differentiation, proliferation, and apoptosis, and play an important physiological role in various biological processes. Retinol is primarily obtained from animal products. Its intracellular bioavailability is regulated by specific and CRBP. The CRBP-1 as the most common CRBP isoform is a small 15 kDa cytosolic protein that is highly expressed in various tissues. It acts to regulate absorption, esterification, and bioavailability of retinol, also plays a major role in wound healing and remodeling process of arteries. In recent years, the role of retinoid signalling CRBP-1 during the development of cancer became the aim of several studies.<sup>37</sup> Expression of CRBP-1 is associated with cervical epithelial cell differentiation. High amount of CRBP-1 could be found in columnar and epithelial cells.<sup>38</sup> The drop of CRBP-1 was coincided with the disappearance of retinol response in rat cervical epithelial cells.<sup>36</sup>

There are two types of retinol metabolism in the smooth muscle cell. Increased production of retinoic acid was found in the intima cell.<sup>37,39,40</sup> Studies in vitro showed that the retinoid, in particular 9-cis-RA, could inhibit the growth of estrogen receptor through blocking the cell cycle.<sup>41</sup> Synthetic retinoid is generally quite promising for the treatment of cancer and several clinical trials are also running, but only a few synthetic retinoid have been approved by Food and Drug Administration (FDA). Preclinical studies indicated that synthetic retinoid inhibited the growth of human cancer. Fenretinide (4-HPR) is one of the most promising retinoid clinically. It demonstrated significant cytotoxic activity of tumor cell through induction of apoptosis and non-apoptotic routes.<sup>37,42</sup>

The pattern of CRBP-1 on human epithelial endocervical is identical to those reported in mice.<sup>43</sup> In addition, in humans, CRBP-1 was sufficient in myometrium of non-pregnant women along with protein CRABP; thus, they showed the role of ATRA in proliferation control of the myometrium in vivo. The level of CRBP-1 was down regulated on the upper and lower segment of uterus during the first and second trimesters of pregnancy.<sup>44</sup> The CRBP-1 gene function in controlling the bioavailability of vitamin A suggested that it might have particular relevance in the inhibition of cancer transformation. However, in human cancer, the presence and role of protein that specifically binds to retinol and retinoic acid had not been widely

investigated. The dysregulation of CRBP-1 occurred in some tumors, such as breast tumors, ovarian, endometrial, prostate, renal, cervical, larynx, nasopharynx, lymphoma, and gastrointestinal cancer. Furthermore, hypermethylation of CRBP-1 was responsible for the inactivation of some cancer cells. Thus, epigenetic disruption of CRBP-1 was a common event in human cancer and it might have important implication for cancer prevention and therapy of retinoid.<sup>37</sup>

Retinoid has long been used for the treatment of psoriasis and acne. Retinoid is effective in some pre-cancerous lesions, such as oral leukoplakia, actinic keratosis, and cervical dysplasia. It is able to delay the development of skin cancer in individual with xerodermapigmentosum; therefore, it shows chemopreventive potency. Moreover, several malignancies have been treated with retinoid-based therapy, as sole agent for pathological promyelocytic including acute leukemia, Kaposi's sarcoma, cutaneous T-cell lymphoma, leukemia myelogenous juvenile chronic, SCC, and kidney cancer.<sup>45,46</sup>

## CONCLUSION

This study has proved that the normal cervix has sufficient level of retinol deposit. However, this study can not prove that cervical cancer and persistent HPV infection have less retinol deposit. These results provide important data on the contribution of some previous studies on the role of vitamin A that is still inconsistent as a chemopreventive measure in the natural history of cervical cancer. Theoretically, retinol administration is expected to reduce the risk of HR-HPV persistence and progression towards pre-cancerous lesion on the natural history of cervical cancer. However, result of this study would like to describe that there is no association between adequacy of retinol deposit and history of cancer. Therefore, based on the result of this study, supplementation of vitamin A can not become basic approach to primary prevention of cervical cancer in terms of nutrition.

## RECOMMENDATION

Further studies with other isomers of retinoid, such as retinoic acid should be conducted in

relation to its sensitivity towards stimulation of retinylpalmitate to activate mechanisms through RAR. Retinol stimulation in vitro to activate several parameters of local immune response in cervical tissue is a promising study in the future.

## Conflict of Interest

The authors hereby affirm that there is no conflict of interest in this study.

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Research Article

## Neutrophil and Platelet to Lymphocyte Ratio in Ovarian Malignancy

### *Rasio Netrofil dan Trombosit terhadap Limfosit pada Keganasan Ovarium*

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#### Abstract

**Objective:** To determine the sensitivity and specificity of neutrophil - lymphocyte ratio compared with platelet - lymphocyte ratio in predicting ovarian malignancies.

**Methods:** This was an observational analytic study with diagnostic test design on 37 ovarian tumor patients who were planned to perform laparotomy in Prof. Dr. R.D. Kandou Hospital Manado and network hospitals in Manado from March to May 2016. We took blood samples to assess the complete blood count.

**Results:** During the period, we obtained the mean age of 28.1 years old (range 18-71 years old). A total of 13 samples showed malignancy which the cystadenocarcinoma mucinosum (27.0%) as the most common histopathological types. Sensitivity and specificity value of neutrophil - lymphocyte ratio were 92.3% and 95.8% with 2.47 as the cut-off point. Meanwhile, the sensitivity and specificity of platelet - lymphocyte ratio were 61.5% and 75.0% with 152.86 as cut-off point.

**Conclusion:** Both neutrophil - lymphocyte ratio and platelet - lymphocyte ratio are associated with ovarian malignancy. The sensitivity and specificity of neutrophil - platelet ratio show better prediction for ovarian malignancies.

[Indones J Obstet Gynecol 2017; 5-1: 55-59]

**Keywords:** neutrophil - lymphocyte ratio, ovarian cancer, platelet - lymphocyte ratio

#### Abstrak

**Tujuan:** Mengetahui sensitivitas dan spesifisitas rasio netrofil - limfosit dibandingkan rasio trombosit - limfosit dalam memprediksi keganasan ovarium.

**Metode:** Penelitian ini merupakan penelitian observasional analitik dengan rancang uji diagnostik pada 37 penderita tumor ovarium yang direncanakan laparotomi di RSUP Prof. Dr. R.D. Kandou Manado dan rumah sakit jejaring di Manado pada bulan Maret sampai Mei 2016. Sampel diambil melalui darah vena untuk dilakukan pemeriksaan darah lengkap.

**Hasil:** Didapatkan rerata usia pasien adalah 28,1 tahun (rentang 18-71 tahun). Sebanyak 13 sampel mengalami keganasan dengan jenis histopatologi terbanyak adalah kistadenokarsinoma musinosum (27,0%). Nilai sensitivitas dan spesifisitas rasio netrofil - limfosit adalah 92,3% dan 95,8% dengan titik potong 2,47 dan rasio trombosit - limfosit adalah 61,5% dan 75,0% dengan titik potong 152,86.

**Kesimpulan:** Rasio netrofil - limfosit dan rasio trombosit - limfosit berhubungan dengan keganasan ovarium. Sensitivitas dan spesifisitas rasio netrofil - trombosit menunjukkan hasil yang lebih baik dalam memprediksi keganasan ovarium.

[Maj Obstet Ginekolog Indones 2017; 5-1: 55-59]

**Kata kunci:** kanker ovarium, rasio netrofil - limfosit, rasio trombosit - limfosit

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## INTRODUCTION

Incidence of epithelial ovarian cancer is around 3% of all cancers in women worldwide and it ranks the third among all gynecological cancers. In 2002, epithelial ovarian cancer contributed to more than 125,000 deaths each year of gynecological cancer.<sup>1</sup> Although there has been improvement in the technique of surgery and chemotherapy, the prognosis of epithelial ovarian cancer patients was still poor with 5-year survival rate of 40%. It was because most of patients generally came at advanced stage.<sup>2,3</sup>

Recent studies stated that there was correlation between genetic transformation causing

malignancy and activation of cancer inflammation reaction.<sup>3</sup> There were many factors that involved in angiogenesis process correlating to ovarian cancer, such as inflammatory marker or types of blood cell. Increased absolute neutrophil, platelet, and lymphocyte count or neutrophil - lymphocyte and platelet - lymphocyte ratio had been reported as one of prognostic factors in ovarian cancer. The inflammatory response effects on tumor progression by activating the inflammatory cytokines that induce cellular DNA damage, inhibit apoptosis, and trigger the process of angiogenesis around tumor. This response causes the abnormal growth, progression, and metastases of tumor.<sup>4,5</sup> Therefore, this study aims to determine the

sensitivity and specificity of neutrophil - lymphocyte ratio compared with platelet - lymphocyte ratio in predicting ovarian malignancies

## METHODS

This was an observational analytic study with diagnostic test design. This study was conducted in the Prof. Dr. R.D. Kandou Hospital Manado and network hospitals in Manado from March to May 2016. The inclusion criteria were all patients with ovarian tumors planned to perform laparotomy in Prof. Dr. R.D. Kandou Hospital Manado and network hospitals in Manado. Apart from that, the patients did not show any other malignancies. The exclusion criteria were patients with other disease that influenced significant amount of neutrophil, platelet, lymphocyte and or leukocyte, such as cardiovascular disease, viral infection, autoimmune disease, diabetes mellitus, blood disease. We excluded the patients who did not show the ovarium tumor as the primary source. We took intravenous blood sample to assess the complete blood count so that we could analyze neutrophil - lymphocyte ratio and platelet - lymphocyte ratio. Variable of neutrophil - lymphocyte and platelet - lymphocyte ratio was described in the cross tabulated table paired with histopathological result. The analysis of Receiver Operating Characteristic (ROC) curve was used to calculate the sensitivity and specificity of neutrophil - lymphocyte and platelet - lymphocyte ratio to histopathology examination as the gold standard of diagnosis. We analyzed through SPSS and p value of 0.05 was considered statistically significant.

## RESULTS

We got 37 ovarian tumor patients who underwent surgery in Prof. Dr. R.D. Kandou Hospital Manado and network hospitals in Manado. Table 1 showed the characteristics of subjects. The mean age of patients was 40.4 (range 18-71) years old. We found 20-39 years old as the most prevalent of ovarian tumor patients in this study (40.5%). Most of subjects had ever delivery once to twice (43.2%) and they had normal body mass index (BMI) (64.8%).

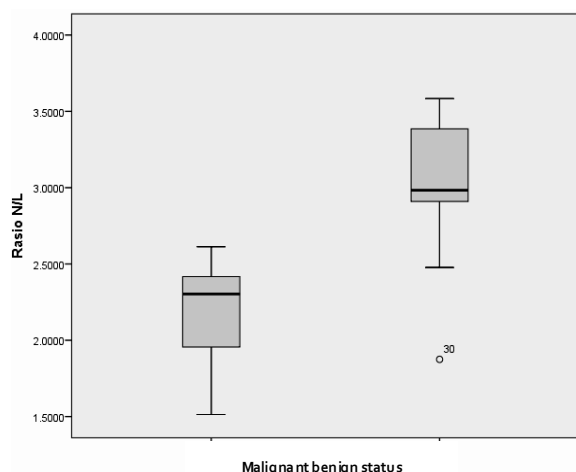
**Table 2.** Characteristics of Histopathology on Ovarian Tumor

| Histopathology Profile       | N  | %    |
|------------------------------|----|------|
| <b>Benign</b>                |    |      |
| Cystadenoma Serosum          | 5  | 13.5 |
| Cystadenoma Mucinosum        | 9  | 24.3 |
| Dermoid cyst                 | 3  | 8.1  |
| Hemorrhagic Cyst             | 7  | 18.9 |
| <b>Malignant</b>             |    |      |
| Cystadenocarcinoma Serosum   | 3  | 8.1  |
| Cystadenocarcinoma Mucinosum | 10 | 27.0 |

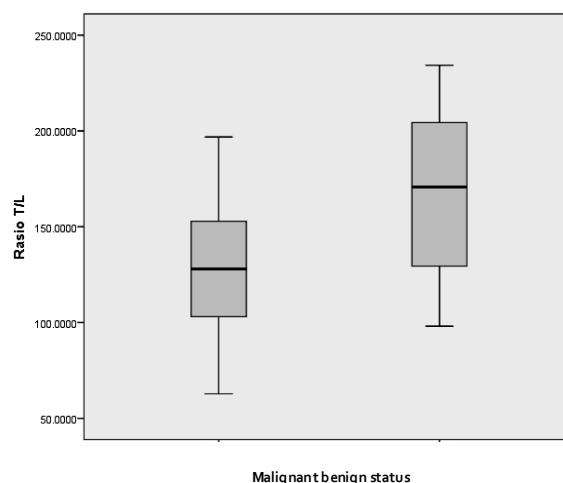
Of the 37 cases, we found the malignant cyst adenocarcinoma mucinosum as many as 10 patients (27.0%) and cystadenocarcinoma serosum as many as 3 patients (8.1%). While most were benign ovarian tumor of cystadenoma mucinosum (24.3%), cystadenoma serosum (13.5%), hemorrhagic ovarian cysts (18.9%), and the least number of dermoid cyst (8.1%).

**Table 1.** The Characteristics of Subjects

| Characteristics | N     | %  | Range (min-max) | Mean |
|-----------------|-------|----|-----------------|------|
| <b>Age</b>      | <20   | 3  |                 | 18.3 |
|                 | 20-39 | 15 | 18-71           | 28.1 |
|                 | 40-59 | 12 |                 | 47.4 |
|                 | ≥ 60  | 7  |                 | 64.3 |
| <b>Parity</b>   | 0     | 10 |                 | 0    |
|                 | 1-2   | 16 | 0-5             | 1.6  |
|                 | 3-5   | 11 |                 | 3.4  |
| <b>BMI</b>      | ≤ 18  | 3  |                 | 15.8 |
|                 | 18-24 | 24 | 16.4-34.5       | 21.4 |
|                 | ≥25   | 10 |                 | 30.6 |

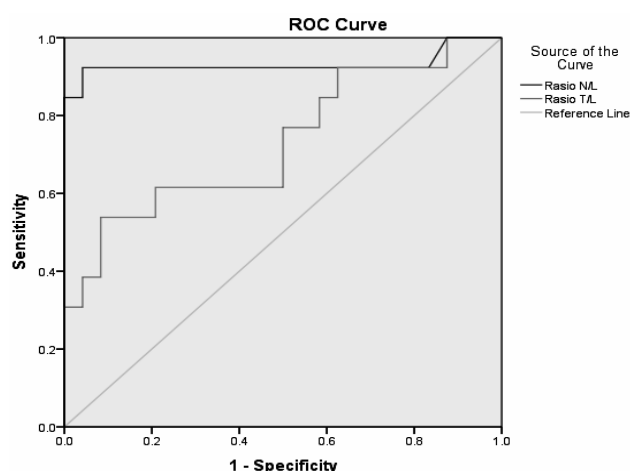


**Figure 1.** Distribution of NLR to Tumor Stage



**Figure 2.** Distribution of PLR to Tumor Stage

In the Shapiro-Wilk normality test due to small sample size, we indicated that the data of both neutrophil - lymphocyte ratio and platelet - lymphocyte ratio in benign and malignant ovarian tumors were normally distributed ( $p > 0.05$ ). Thus, we processed the test into parametric test. The result of T test showed that there were significant differences in neutrophil - lymphocyte also platelet - lymphocyte ratio between patients with benign and malignant ovarian tumors ( $p < 0.05$ ). It meant that the ratio of them was associated with ovarian malignancy.



**Figure 3.** ROC Curver

Of the ROC curve, we obtained area under the curve (AUC) value of neutrophil - lymphocyte ratio was 93.1% ( $p < 0.05$ ; 95% CI 80.6-100.0%). Meanwhile, AUC value of platelet - lymphocyte ratio was

60.5% ( $p = 0.022$ ; 95% CI 55.0-91.1%). The analysis of ROC stated that the optimal sensitivity and specificity of neutrophil - lymphocyte ratio was 92.3% and 95.8% with a cut-off point on the value of 2.47. While, the value of optimal sensitivity and specificity of platelet - lymphocyte ratio was 61.5% and 75.0% with 152.86 as the cut-off point. Both ratios were confirmed with histopathology results.

## DISCUSSION

Progression and metastases of cancer consist of cascade steps that involve interaction among tumor microenvironment, including factors that contribute to angiogenesis and inflammation. The capacity of tumor cells to invade, get vascularization and metastasis is initiated by a signal from the primary tumor of micro-environment, blood vessel, and secondary location of tumor.<sup>6,7</sup> The inflammatory response has correlation with tumor progression, such as angiogenesis and tumor invasion through upregulation of cytokines. In response to the various forms of inflammation, tumor microenvironment containing innate immune cells (macrophage, neutrophil, mast cell, dendritic cell, and natural killer cell) and adaptive immune cells (lymphocyte T and B) has associated with the production of cytokines and chemokines and they act in autocrine and paracrine communication to organize and establish the growth and progression tumor.<sup>8,9</sup> Systemic inflammation associated with the release of several pro-inflammatory mediators, such as IL-1, IL-3 and IL-6 is believed to be able to stimulate proliferation of megacaryocyte and

platelet activation that will produce proangiogenic factor. The proangiogenic factor is essential for tumor growth. In addition, a number of immunological mediators, such as IL-10 and TGF- $\beta$  are released; they will cause significant immunosuppressive effects in order to disrupt lymphocyte function and reduce the number of lymphocyte.<sup>7,8,10-12</sup> Ovarian cancer is associated with inflammation that triggers activation of transcription factors, such as hypoxic-inducing factor (HIF), signal transducer, activator of transcription 3 (Stat3), and nuclear factor B (NFB). These transcription factors result in the production of chemokines, cytokines and prostaglandins, which not only produce inflammatory cells, but also stimulate angiogenesis and cell proliferation. Anti-inflammatory systemic mediator releases inhibitors, including IL-10 and TGF- $\beta$ , which will suppress the immune system and reduce the function of lymphocytes.<sup>13-15</sup> Lymphopenia process has prognostic factor which is associated with poor outcome of different kind of cancers, such as renal, pancreatic, and ovarium.<sup>7,15-18</sup>

In this study, most subjects were 20-39 years old (40.5%). Aziz MF, et al. obtained the ovarian tumor patients were 20-65 years old; whereas, this range of age in population was the most common for ovarian tumor.<sup>19</sup>

The result of T test showed that both neutrophil-lymphocyte and platelet - lymphocyte ratio had association with malignant ovarian tumor ( $p < 0.05$ ). Yildirim M, et al. stated that there was strong correlation between neutrophil - lymphocyte ratio and the process of cancer, including cancer staging, prognosis and response to therapy. The ratio of neutrophil - lymphocyte and neutrophil - lymphocyte is a marker of inflammation that is effective to predict pre-operative ovarian cancer. Both of these markers are cheap, effective, and generally accepted in predicting malignancy of ovarian tumor.<sup>6</sup> The inflammatory tumor microenvironment plays a role in cancer growth. Inflammation can increase the mutation rate and improve the proliferation of mutated cell. Inflammation is related to cancer each other. The damage of DNA in cancer cells can cause more severe inflammation and promote cancer. Some pro-inflammatory gene products have been identified of having an important role in the suppression of apoptosis, proliferation, angiogenesis, invasion, and metastases.

Raunkaewmanee S, et al. in 2012 conducted study on platelet - lymphocyte ratio as a prognostic factor in epithelial ovarian cancer. Several previous studies reported an increase in absolute neutrophil and platelet, the decrease of lymphocyte count in the incidence of cancer.<sup>4</sup> Furthermore, in a study conducted by Asher V, et al. in 2011, cancer patients increasing platelet - lymphocyte ratio had worse prognosis. The inflammatory response affects tumor progression by activating the inflammatory cytokines that induce cellular DNA damage, inhibiting apoptosis, and triggering the process of angiogenesis around tumor. This causes the abnormal growth of tumor, progression, and metastases.<sup>5</sup>

In this study, the ROC curve showed that the optimal sensitivity and specificity of neutrophil - lymphocyte ratio was 92.3% and 95.8% with a cut-off point on the value of 2.47. While, the optimal sensitivity and specificity of platelet - lymphocyte ratio was 61.5% and 75.0% with a cut-off point on the value 152.86. This result had been compared to histopathology results. We could conclude that the ratio of neutrophil - lymphocyte and platelet - lymphocyte had good value for predicting ovarian malignancies. Neutrophil - lymphocyte ratio had better marker compared with platelet - lymphocytes ratio.

According to Yidrim, et al. in 2014, neutrophil - lymphocyte and platelet - lymphocyte ratio might be used in combination with Ca-125 because Ca-125 was believed to have high sensitivity in differentiating benign and malignant ovarian tumor. The Ca-125 as screening method remains the most important; however, the neutrophil - lymphocyte ratio and the number of neutrophil can be used in single or combination with Ca-125 to detect early stage of ovarian tumor.<sup>6</sup>

## CONCLUSION

Both neutrophil - lymphocyte ratio and platelet - lymphocyte ratio are associated with ovarian malignancy. The sensitivity and specificity of neutrophil - platelet ratio shower better prediction for ovarian malignancies. Both of these markers are cheap, effective, and generally accepted in predicting malignancy of ovarian tumor pre-operatively.

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## Research Article

Single vs Multiple Dose of Cefazolin Prophylaxis  
in Elective Cesarean Section*Perbandingan Cefazolin Dosis Tunggal dengan Dosis Multipel  
pada Seksio Sesarea Berencana*

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## Abstract

**Introduction:** Caesarean section (CS) is one of port d'entrée from infection in women and it is related to maternal morbidity during puerperal period. Until now, there is still lack of consensus regarding prophylactic antibiotic protocol before CS procedure. This study aims to determine the comparative efficacy between single dose and multiple doses of cefazolin prior incision toward the incidence of maternal infection.

**Methods:** This was a single-blind, randomized, clinical trial study with two methods of intervention including 2-gram single dose cefazolin at 30 minutes' prior incision and 2-gram single dose cefazolin at 30 minutes' prior incision continued 1-gram cefazolin after 8 hours of procedure. We recruited women undergone elective CS at Fatmawati and Anna Hospital, Jakarta from January to March 2016. The primary outcomes were surgical site infection, urinary tract infection, and endometritis based on clinical findings during 30 days of follow-up period.

**Results:** A total of 46 subjects were recruited which 23 of them were in single dose cefazolin group and the other 23 subjects were in multiple dose of cefazolin group. There were 9 subjects having infection (19.6%). There was no statistical difference in the incidence of infection between two groups ( $p=1.00$ ; relative risk 0.80, 95% CI 0.25-2.61).

**Conclusion:** Single dose of cefazolin shows similar rates of infection incidence to multiple dose. Therefore, single dose of cefazolin can be a protocol in CS based on its efficacy and efficiency.

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**Keywords:** cefazolin, maternal infection, multiple dose, single dose

## Abstrak

**Tujuan:** Operasi seksio sesarea (SC) merupakan salah satu pintu masuk infeksi yang berkaitan dengan morbiditas maternal pasca-persalinan. Hingga saat ini, belum ada protokol tetap mengenai dosis antibiotik profilaksis sebelum prosedur SC. Penelitian ini bertujuan mengetahui adakah perbedaan kejadian infeksi pascapersalinan dengan penggunaan cefazolin profilaksis dosis tunggal dan multipel.

**Metode:** Penelitian ini merupakan penelitian uji klinis acak tersamar tunggal dengan dua kelompok perlakuan yaitu cefazolin dosis tunggal 2 gram pada 30 menit sebelum insisi dan cefazolin dosis multipel (cefazolin dosis tunggal 2 gram pada 30 menit sebelum insisi dan 1 gram pada delapan jam setelah dosis awal). Penelitian dilakukan pada wanita yang menjalani operasi SC berencana di RS Fatmawati dan RS Anna, Jakarta pada Januari - Maret 2016. Luaran utama yang dinilai adalah infeksi selama 30 hari setelah prosedur meliputi infeksi luka operasi, infeksi saluran kemih dan endometritis berdasarkan temuan klinis.

**Hasil:** Didapatkan 46 subjek dengan 23 subjek pada kelompok cefazolin dosis tunggal dan 23 subjek pada cefazolin dosis multipel. Didapatkan 9 dari seluruh subjek mengalami infeksi (19,6%). Tidak ditemukan perbedaan kejadian infeksi pada kedua kelompok perlakuan ( $p=1,00$ ; risiko relatif 0,8 95% IK 0,25- 2,61).

**Kesimpulan:** Dosis tunggal cefazolin memperlihatkan angka kejadian infeksi yang serupa dengan dosis ganda. Oleh karena itu, pemberian dosis tunggal dapat dijadikan petunjuk dalam prosedur SC terkait efikasi dan efisiensi.

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**Kata kunci:** cefazolin, dosis multipel, dosis tunggal, infeksi pasca-operasi**Correspondence:** Muhammad Fadli, fadliamran@hotmail.com

## INTRODUCTION

In Indonesia, there is unavailable data of cesarean section (CS) rate. Meanwhile, there were around 1,341 CS procedure per year in Dr. Cipto Mangunkusumo hospital as the center of referral hospital in Indonesia. It meant that CS procedure covered 38% of all delivery cases. In 1999-2000, 13.9% CS was performed without medical indication.<sup>1</sup> Cesarean section often leads to

complications, including surgical site infection (SSI), endometritis, and urinary tract infection.<sup>2</sup> These complications can increase the length of stay in hospital which impacts to the health expense. According to data in United States, complications after CS procedure resulted 10 days of extra care in hospital and it spent about USD 2,000 per case.<sup>3</sup> Meanwhile, the risk of complications after CS was different from 0.3% to almost 25.3% in Turkey.<sup>4</sup> Committee of Prevention and Infection Control at

Dr. Cipto Mangunkusumo Hospital stated that in Department of Obstetrics and Gynecology starting from January to December 2011, the incidence of infection was varied between 0.8% and 37.5%.

Currently, standardization of procedures for antibiotic prophylaxis, the type and mode of administration to patients undergoing CS are still unclear and they are varied among operators; however, this procedure is commonly used. Mini pilot study in Dr. Cipto Mangunkusumo hospital showed that there were different antibiotics for CS procedure consisting of Clavomax 1 g intravenous route at 60 minutes prior to incision, Cefazolin 2 g intravenous route at 30-60 minutes prior to incision, and Ceftriaxone 2 g intravenous route at 30 minutes before incision or after CS surgery.<sup>5</sup> Another study in the United States stated that 95.5% operators used a class of antibiotics in the first generation of cephalosporine; whereas, 84.4% of them used cefazolin as prophylactic in CS.<sup>6</sup>

Of the various types on infection prevention in CS, one of the major cost component is the use of antibiotics. Antibiotics are given 30 minutes before incision to reach the highest concentration on the tissue to prevent wound infection effectively. Sometimes, the complications are worsened by general condition and low nutritional status.<sup>7</sup> Antibiotics are usually given before performing surgery; nevertheless, several narrow spectrum antibiotics sometimes were injected after cord clamping for the baby's interest. On the other studies, broad spectrum antibiotics had proof to be able to lower the number of maternal morbidity without affecting the baby.<sup>2</sup> Looking to the differences in infection prevention procedures in CS procedure, we consider that we have to conduct the study about the administration of antibiotic prophylaxis in CS procedure. Therefore, this study aims to determine the comparative efficacy between single dose and multiple doses of cefazolin prior incision toward the incidence of maternal infection.

## METHODS

This study was a single blinded randomized clinical trial study between single dose of cefazolin prophylaxis as control group and multiple doses of cefazolin as intervention group in patients undergoing elective CS surgery. The study was conducted at Fatmawati and Anna Hospital from January to March 2016.

Subjects in this study were pregnant women who delivered by elective CS at Fatmawati and Anna Hospital from January to March 2016 fulfilling the inclusion criteria. The inclusion criteria were pregnant women that planned to perform the elective CS and they were willing to participate in this study. Meanwhile, the exclusion criteria were women with history of allergy to cephalosporin generation, signs and symptoms of infection prior to surgery, immunosuppressive disease, and having an auto-immune disease.

We recruited samples by consecutive sampling. We took all pregnant women who gave birth by elective CS in the Fatmawati and Anna Hospital according to inclusion and exclusion criteria.

We examined patients' history and performed physical examination. Healthy patients would go into routine blood test to rule out the infection. Patients who matched the criteria for inclusion obtained explanation and information about this study. Those who agreed to be subject were asked to sign the informed consent. We told subjects to complete the characteristics' demography for this study. Subjects were asked about their full name, complete address, and phone number. Subjects got the serial number and to enroll in this study, they opened the envelope containing of randomization of intervention. Trained personnel determined the treatment given to the subject. Subjects were divided into 2 groups namely group 1 which got 2-gram cefazolin antibiotic once at 30 minutes before incision and group 2 obtaining 2-gram cefazolin at 30 minutes before incision and continued by 1-gram cefazolin at 8 hours after CS procedure.

Patients then underwent CS surgery. During the surgery, we monitored the duration of surgery and the amount of blood lost. Subjects who experienced surgery beyond 3 hours and the amount of blood >1500 cc were excluded and considered as drop out. We did not count the drop out subjects into analysis which meant that we did not do the intention to treat analysis. The subject of study was prohibited taking antibiotics or traditional medicine after surgery.

Observation was held on days-1, 2, 3, 7, 14, and 30. We observed them through examination of vital signs including blood pressure, heart rate, respiratory rate, temperature and also other clinical complaints. Signs of infection in SSI and urinalysis were also assessed at 24-hour post-

surgery. Urinalysis was performed to check the bacteria in the urine. Any patients with positive bacteria in urine considered as infected. Patients who were lost to follow-up counted as drop out.

Data were analyzed statistically. Researchers analyzed normality of numerical data using Shapiro-Wilk test due to small sample size. The data then were presented in mean (standard deviation) for normal distribution or median (minimum-maximum) for abnormal distribution. Data on the number of infection frequency between 2 groups were analyzed using Fisher-exact test. The relative risk (RR) were calculated with 95% confidence interval at each output. We did not do the intention to threat analysis. We used SPSS version 22 for Windows.

## RESULTS

Total subjects obtained in this study were 58 women; which 46 of them were followed until the end of study. They consisted of 23 women in each study group (single dose and multiple doses of cefazolin administration). There were 12 women

dropped out (5 women from single dose group and 7 women from multiple dose group). The mean age of the women was 31.5 (SD 5.7) years old and the median of parity was 1 (min-max 0-4) time (s).

The characteristics of women based on laboratory parameters were assessed through blood test and urinalysis. Based on the results of blood test, the mean of hemoglobin value was 11.4 (SD 1.4) g/dl; median of leukocyte was 10,696/ $\mu$ l with the value from 4,500 to 25,300/ $\mu$ l. All patients' urinalysis showed negative result of nitrite and bacteria; however, the majority of results described +1 of epithelial cells.

Of the total 46 women, we found 9 women (19.6%) having an infection during follow-up period. Infection occurred in the form of asymptomatic bacteriuria. Four incidences of infection were happened in subject obtaining a single dose of cefazolin (8.7%,  $p=1.00$ ), and five infections were occurred in subjects in multiple doses of cefazolin (10.9%,  $p=1.00$ ). We did not found subject with wound infection and endometritis.

**Table 1.** Age and Parity Characteristics of Women in This Study

| Characteristics  | Total      | Single dose of Cefazolin (N= 23) | Multiple dose of Cefazolin (N= 23) |
|------------------|------------|----------------------------------|------------------------------------|
| Age (years old)  |            |                                  |                                    |
| Mean (SD)        | 31.5 (5.7) | 32.5 (5.7)                       | 30.5 (5.7)                         |
| Parity           |            |                                  |                                    |
| Median (min-max) | 1 (0-4)    | 1 (0-4)                          | 1 (0-2)                            |
| Mode             | 1          | 1                                | 1                                  |

*\* Normally distributed numerical data were presented as mean (standard deviation); Abnormally distributed data were presented in median (minimum-maximum)*

**Table 2.** Characteristics of Women based on Laboratorium Examination before Cesarean Section (CS) Surgery

| Variables                                | Single dose of cefazolin (N=23) | Multiple dose of cefazolin (N=23) |
|------------------------------------------|---------------------------------|-----------------------------------|
| Blood analysis                           |                                 |                                   |
| Hemoglobin (g/dl) (mean (SD))            | 11.1 (1.2)                      | 11.7 (1.6)                        |
| Leukocyte (/ $\mu$ l) (median (min-max)) | 10,980<br>(5,980-25,300)        | 88,400<br>(4,500-17,720)          |

| Variables                    | Single dose of cefazolin<br>(N=23) | Multiple dose of cefazolin<br>(N=23) |
|------------------------------|------------------------------------|--------------------------------------|
| <b>Urinalysis</b>            |                                    |                                      |
| <b>Color</b>                 |                                    |                                      |
| Pale yellow                  | 22                                 | 22                                   |
| Yellow                       | 1                                  | 1                                    |
| <b>Clarity</b>               |                                    |                                      |
| Clear                        | 11                                 | 12                                   |
| Slightly turbid              | 10                                 | 10                                   |
| Turbid                       | 2                                  | 1                                    |
| <b>pH</b> (median (min-max)) | 6.5 (5-7)                          | 6.5 (6-7)                            |
| <b>Nitrite</b>               |                                    |                                      |
| Positive                     | 0                                  | 0                                    |
| Negative                     | 23                                 | 23                                   |
| <b>Leukocyte esterase</b>    |                                    |                                      |
| Positive                     | 18                                 | 18                                   |
| Negative                     | 5                                  | 5                                    |
| <b>Epithelial cells</b>      |                                    |                                      |
| Positive 2                   | 1                                  | 1                                    |
| Positive 1                   | 18                                 | 22                                   |
| Negative                     | 4                                  | 0                                    |
| <b>Bacteria</b>              |                                    |                                      |
| Positive                     | 0                                  | 0                                    |
| Negative                     | 23                                 | 23                                   |

\* Normally distributed numerical data were presented as mean (standard deviation); Abnormally distributed data were presented in median (minimum-maximum); Categorical data were presented as frequency.

**Table 3.** Infection Incidence After Cesarean Section (CS) Surgery

| Variables                | Single dose of cefazolin<br>N=23 (%) | Multiple dose of cefazolin<br>N=23 (%) | p     | RR (95% CI)      |
|--------------------------|--------------------------------------|----------------------------------------|-------|------------------|
| Post surgery infection   | 4 (8.7%)                             | 5 (10.9%)                              | 1.00* | 0.80 (0.25-2.61) |
| Surgical wound infection | 0 (0%)                               | 0 (0%)                                 | -     | -                |
| Urinary tract infection  | 4 (8.7%)                             | 5 (10.9%)                              | 1.00* | 0.80 (0.25-2.61) |
| Endometritis             | 0 (0%)                               | 0 (0%)                                 | -     | -                |
| Fever morbidity          | 0 (0%)                               | 0 (0%)                                 | -     | -                |

RR= relative risk of single dose group to multiple dose group; \*Fisher-exact Test

## DISCUSSION

This study was a single blinded randomized clinical trial that compared the effectiveness of single vs multiple doses of cefazolin in preventing maternal infection after elective surgery of CS. The main finding of this study was similar incidence of infection between two groups. Therefore, we propose the use of a single dose of cefazolin as prophylactic antibiotic in elective CS to be applied in our practice.

In this study, the primary outcome was the incidence of post-operative infection including SSI, urinary tract infection, and endometritis. These three infections are the highest risk of infection up to 5-20 folds compared with women with vaginal delivery.<sup>8</sup> These three infections also contribute to the morbidity causing high cost of hospital treatment to post labour women.<sup>9</sup>

There were limitations to this study. The small sample size including 46 women lowered power of this study. In the determination of urinary tract infection, we confirmed the diagnosis only through urinalysis without culture examination due to limited resources; thus, it might be impacted to the false positive results.

Subjects of this study focused on women with elective CS which actually had a relatively low risk of infection. In the last decade, there was a trend of increasing demand for elective CS related to patients' request so that we considered that this study became essential to determine the effectiveness of prophylactic antibiotic.<sup>10,11</sup>

The result found that the incidence of infection happened in 9 of 46 women (19.6%) where all infections were asymptomatic bacteriuria. The incidence of infection was slightly higher than previous report which stated that the incidence of postoperative infection in the study by Witt, et al. comparing cefazolin before incision, after cord clamping, and placebo. Administering cefazolin before the incision pointed out 4.9% of postoperative infection incidence.<sup>12</sup> Kalaranjini, et al. also conducted a similar study and found that no subjects with endometritis found by administration of cefazolin as a prophylactic antibiotic. The incidence of fever and urinary tract infection was also low below 2.5%.<sup>13</sup> The incidence was higher in this study due to differences in the operational definition and determination of the urinary tract infection status compared with previous studies. On the other hand, we did not reveal the cases of endometritis and SSI. This study was consistent with previous study confirming the importance of antibiotic prophylaxis; however, subjects with placebo had postoperative incidence of infection up to 12.1%.<sup>13</sup> In another study, Tita, et al. concluded that the administration of prophylactic antibiotic decreased the incidence of infection by 50%.<sup>11</sup> Smaill, et al. also mentioned that prophylaxis in elective CS or non-elective CS could decrease the risk of infection by 70%.<sup>14</sup>

In this study, we performed single dose of cefazolin before incision. This is in line with the conclusion of systematic review that also supported the provision of cefazolin as a prophylactic antibiotic regimen of choice before incision.<sup>10</sup> Through the development of study related to prophylactic antibiotic regimen and the

right time of administration in CS during last decade, systematic review by Mackeen, et al. stated that of 10 recent studies concluded that prophylactic antibiotic before incision was superior compared with administration after cord clamping. (RR 0.57 (95% CI 0.45-0.72)).<sup>15</sup>

Differences in dosage of prophylactic antibiotic also had been studied previously. The study compared the effectiveness of prophylactic antibiotic of multiple doses and a single dose. The antibiotic was ampicillin/amoxicillin and metronidazole. The study indicated that there were no difference of maternal infection risks in these two treatments. Thus, it was important to reduce the burden of labor cost in women undergoing CS surgery. Moreover, there has not been consensus in general and consistently applied so that the finding of this study could form the basis of daily clinical practice guideline.<sup>16</sup> Trials in emergency CS without the risk of infection by using regiment of gentamicin and metronidazole concluded that a single dose was sufficient to reduce the risk of SSI and there was not different result between multiple dose and a single dose.<sup>17</sup>

We obtained study results' difference in the incidence of infection was between 4/23 and 5/23 so that it was interpreted as similar clinical efficacy. Apart from that, Fischer exact test revealed  $p > 0.05$ , which meant no statistically significant.<sup>18</sup>

Based on the result of this study, it showed that there were not significant differences in giving cefazolin either single or multiple doses to the incidence of infection. This might imply that the effectiveness of a single dose of cefazolin was as effective as the provision of multiple doses of cefazolin to prevent postoperative maternal infection after elective CS.

One variable that had not been investigated in this study was the morbidity in neonates. However, administration of cefazolin as prophylaxis indicated low incidence of neonatal infection ( $\leq 13\%$ ) in women who underwent CS surgery with a low risk of infection. However, systematic review on the administration of prophylactic antibiotic stated that we lacked number of studies focussing on side effects and incidence of infection in neonates so that we could not conclude the effectiveness of prophylactic antibiotic to the neonatal outcome.<sup>14</sup>

## CONCLUSION

Cefazolin effectively prevents maternal infection in postoperative elective CS and there is no difference in the effectiveness between a single dose and multiple doses of cefazolin in preventing maternal infection.

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