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EDITORIAL

Fertility Preservation: Save Our Hope for the Future It's Time to Re-think...

Budi Wiweko

Fertility preservation refers to every particular method in reproductive medicine performed in order to save reproductive function in special populations who are threatened of losing their reproductive capability.[1-4] This approach is becoming very popular within the last five years due to the increasing number of cancer survivors among young females of reproductive age.[4,5]

Both chemotherapy and radiotherapy are toxic to the ovaries. Therefore, they may reduce the number of follicles, as determined by the patients' age, dosages, and type of chemotherapeutic agents utilized. Women receiving alkylating agents have an increased risk for loss of follicles by 3.8 times, while platinum based chemotherapeutic agents will have an increased risk of 1.8-fold. Older women need only lower dosages of chemotherapy in order to cause follicle loss, leading to infertility or ovarian insufficiency.[6,7]

Before offering fertility preservation, doctors and patients should first determine the possibility of delaying cancer treatment.[1,2] If possible, oocyte or embryo freezing is a preferable option after patients have underwent ovarian stimulation and oocyte retrieval. When the cancer treatment must be performed immediately, ovarian tissue or immature oocyte freezing is the best method for fertility preservation.[1,8]

Today, ovarian tissue vitrification has been established with very promising results, which has been demonstrated as being comparable to slow freezing method.[4] This technique seems very simple, and is also easier and cheaper to be offered to patients. Indonesia has started ovarian tissue and pre-antral follicles vitrification since 4 years ago with satisfying results.[9] A pilot study was done on cervical and breast cancer patients as research model. Regarding ovarian tissue and isolated pre-antral follicles vitrification, the results were adequately satisfactory. Follicle morphology and in vitro culture is one of the methods used to assess the survival of warmed-vitrified follicles.

Today, trans-disciplinary collaboration is a really important factor in supporting the establishment of the Indonesian Fertility Preservation Center. A working group should at least consist of oncologists (gynecology, surgeon, pediatric, internal medicine), reproductive endocrinologists, counselors, nurses, and lawyers. Our challenge and opportunity is to set up a "one stop service" for young female cancer patients and survivors to have children, genetically from their own oocytes.

Are we ready to help our patients in saving hope for their future?

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Research Article

Maternal Serum Interleukin-6 Level in Correlation with Preterm Delivery

Hubungan Kadar Interleukin-6 Serum Maternal dengan Persalinan Preterm

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Abstract

Objective: To determine the correlation between maternal serum Interleukin-6 (IL-6) serum level with preterm delivery.

Methods: We performed an observational cross-sectional study of 30 pregnant women between 21 to 36 week gestational age with and wthout labour (delivery) in Prof. Dr. RD. Kandou Hospital Manado. Samples were collected with consecutive sampling method. Data was analyzed using Mann-Whitney test with significance level of $p<0.05$.

Results: We encountered 15 preterm pregnancy and 15 preterm delivery, 6 (40%) cases belonged to the age group <20 years old. Incidence of preterm delivery with history of preterm delivery in a previous pregnancy was 6 cases (40%). This study found that the mean maternal serum IL-6 level in preterm delivery was 12.9 pg/ml, which was higher than preterm pregnancy (2.14 pg/ml).

Conclusion: There is significant correlation between maternal IL-6 serum level with preterm delivery which was higher at 4.55-38.87 pg/ml than women with preterm pregnancy (1.5-3.07 pg/ml).

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Keywords: Interleukin-6 level, maternal serum, preterm delivery, preterm pregnancy

Abstrak

Tujuan: *Mengetahui hubungan antara kadar Interleukin-6 (IL-6) serum maternal dengan persalinan preterm.*

Metode: *Penelitian ini merupakan studi potong lintang observasi yang mengikutsertakan 15 perempuan hamil dengan persalinan preterm dan 15 perempuan dengan kehamilan preterm, dengan usia kehamilan 21-36 minggu yang datang ke bagian kebidanan dan kandungan RSUP Prof. Dr. RD. Kandou Manado. Sampel dikumpulkan melalui metode consecutive sampling. Seluruh pasien terlibat dalam penelitian ini secara sukarela yang dinyatakan dengan informed consent. Analisis data menggunakan uji Mann-Whitney test dengan tingkat kemaknaan p<0,05.*

Hasil: *Didapatkan persalinan preterm terbanyak pada kelompok usia <20 tahun dengan 6 kasus (40%), dan angka kejadian persalinan preterm pada riwayat persalinan preterm pada kehamilan sebelumnya didapatkan 6 kasus (40%). Pada penelitian ini didapatkan rata-rata kadar IL-6 serum maternal pada persalinan preterm (12,9 pg/ml) lebih tinggi jika dibandingkan dengan kehamilan preterm (2,14 pg/ml).*

Kesimpulan: *Terdapat hubungan bermakna antara kadar IL-6 serum maternal dengan persalinan preterm, di mana pada kelompok persalinan preterm lebih tinggi, yaitu 4,55 - 38,87 pg/ml, dibandingkan pada perempuan hamil dengan kehamilan preterm yaitu 1,5 - 3,07 pg/ml.*

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Kata kunci: *kadar Interleukin-6, kehamilan preterm, persalinan preterm, serum maternal*

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INTRODUCTION

Preterm delivery is still a serious problem until now, with preterm labor still occurring in >12% of pregnancies. In the United States, there is 1 incidence of preterm labor for 8 normal labors. Preterm delivery occurred approximately in 10% of all pregnancies per year worldwide and constitutes a major cause of morbidity and perinatal mortality. More than half of the infants who survive, experience long term morbidities.[1-3] The incidence of preterm labor in Prof. Dr. RD. Kandou Hospital, Manado in the period of January 1, 2012 until December 31, 2012 was 15.5%, accompanied by 44 cases of perinatal mortality, of which 57.39% was caused by respiratory distress syndrome. Based on the statistical data in 2011, the infant mortality rate in North Sulawesi amounted to approximately 33 per 1,000 live births. Most of the infant mortality rate (45-55%) was attributed to preterm labor.[4-6]

A simple, rapid, non-invasive, and safe marker examination related with the occurrence of preterm birth and neonatal respiratory morbidity may be useful both in the development of risk stratification strategies and morbidity prediction in pregnant women who will experience preterm labor; or in developing safe and efficient drugs that works selectively against uterine contraction to prevent preterm labor. This surely will provide significant

impact on early intervention, either in the prevention or treatment of preterm labor.

Cytokines may be a promising marker as the early phase mediators of an inflammatory response. IL-6 is a proinflammatory cytokine that is a useful marker in indicating the presence of intrauterine infection, preterm labor and neonatal morbidity.[7,8]

Several studies have consistently demonstrated an association between elevated levels of serum IL-6 in the fetal and/or neonatal 'compartment' (such as amniotic fluid, umbilical vein, fetal blood, neonatal blood) with preterm labor and/or neonatal morbidity.[9-12] However, data consistency is still lacking in the analysis of relationship between level of maternal serum IL-6 with the occurrence of preterm birth. IL-6 is a more promising examination than other methods of maternal serum screening. Several studies have shown that maternal serum IL-6 level is also elevated in intrauterine infection, either clinically evident or those only proven in histologic findings. Thus, the finding of increased IL-6 is not only observed in amniotic fluid, cervicovaginal secretions, umbilical vein, and fetal blood, but also in the serum of women with intrauterine infections, both clinically and subclinically.[8,13,17] On the other hand, other studies have shown an inconsistent relationship between maternal serum IL-6 level and preterm labor.

However, recent research conducted by Yoram Sorokin et al (2010) suggested a relationship between maternal serum IL-6 level with the occurrence of preterm birth and neonatal morbidity. In this sense, IL-6 can be a useful marker for intrauterine infection, preterm birth and neonatal morbidity. IL-6, a proinflammatory cytokine, is a major mediator of inflammatory response and infection; and an early marker of acute phase response.[14]

Based on the findings above, this study was conducted to determine whether there is relationship between level of maternal serum IL-6 with the occurrence of preterm birth.

METHODS

This study was an analytic cross-sectional study conducted in the department of Prof. Dr. RD. Kandou Hospital, Manado. It was conducted from December 2013 until the required sample size was met.

The study sample consisted of women with preterm labor and women with preterm pregnancies who presented to the delivery room of the Department of Obstetrics and Gynecology, Prof. Dr. RD. Kandou Hospital and RW. Monginsidi Hospital that met the inclusion and exclusion criteria. The sampling method used was consecutive random sampling with maternal serum IL-6 as the study variable. Examination of serum IL-6 was conducted in Prodia Laboratory. Three cc of peripheral venous blood samples were withdrawn and put into an SST test tube, which were then sent to Prodia Laboratory Manado to be centrifuged. The sample would then be sent to the Prodia in Jakarta for examination of serum IL-6 level using Quantikine ELISA KIT D6050.

Inclusion criteria in this study included pregnant women with a single live fetus, intrauterine, 21-36 weeks gestational age who experienced preterm labor with <2500gr baby as the case group; and pregnant women with a single fetus, live, intrauterine, 21-36 weeks gestational age that was not in labor with the estimated fetal weight <2500gr as the control group. Patients were recruited from the outpatient clinic and delivery room of the Obstetrics and Gynecology department of Malalayang Hospital, RW. Monginsidi Hospital. All the samples were willing to participate in the study voluntarily, which was expressed by informed consent. Exclusion criteria included women who had a history of diabetes, hypertension, heart, kidney and liver disease, pneumonia, tuberculosis, malaria, hepatitis, typhoid, multiple pregnancy, polyhydramnion, premature rupture of membranes, fetal congenital abnormalities as well as patients who were not willing to be recruited in the study.

RESULTS

From Table 1, we can observe the age group distribution of our samples in the study and control group.

Table 1. Characteristics of Sample by Age

Maternal age	Labor status			
	Preterm labor		Preterm Pregnancy	
	N	%	N	%
<20 years, ≥ 35 years	7	46.7	3	20
20 - 34 years	8	53.3	12	80
Total	15	100	15	100
x^2	p = 0.121			

As can be seen in Table 2, based on maternal parity, the highest number of preterm labor incidence occurred in primigravidae (60%).

Table 2. Characteristics of Sample by Parity

Parity	Labor status			
	Preterm labor		Preterm Pregnancy	
	N	%	N	%
Primigravida	9	60	5	33.3
Multigravida	6	40	10	66.7
Total	15	100	15	100
x^2	p = 0.143			

In Table 3, characteristic was based on history of preterm delivery. We can observe that the incidence of previous preterm labor in the preterm labor group was as much as 40%, whereas the incidence in preterm pregnancies (controls) only amounted to 7%.

Table 3. Characteristics of Sample based on History of Preterm Labor

History of preterm labor	Labor status			
	Preterm labor		Preterm Pregnancy	
	N	%	N	%
Yes	6	40	1	7
No	9	60	14	93
Total	15	100	15	100
x^2	p = 0.031			

In Table 4, we can observe the difference in maternal serum IL-6 level found in both groups was statistically significant (p = 0.001).

Table 4. Levels of Maternal Serum IL-6 in Preterm Labor Group and Preterm Pregnancy Group

IL-6 value	Labor status	
	Preterm labor	Preterm Pregnancy
Average IL-6 (pg/ml)	2.14	0.511
SD (pg/ml)	12.89	8.800
Mann-Whitney	p = 0.001	

DISCUSSION

Based on the age characteristics, the highest number of cases was observed in the <20 years age group, as much as 6 cases (40%). This was similar to the research conducted by Peacock et al (1995) and Creasy et al (2004), which stated that the incidence of preterm delivery were mostly obtained in women whose age were too young.[15,16] However, in this study, the statistical analysis showed no significant relationship between maternal age with the incidence of preterm labor. These results were in contrast to several studies that reported a significant association between young maternal age with preterm labor. This may be caused by the small sample size, causing random errors affecting the results of statistical tests on groups of very young maternal age.

In Table 2, based on the characteristics of parity group, the highest number was found in the primigravida group of preterm labor (60%). However, the results of statistical tests in this study showed no significant relationship between parity and the incidence of preterm labor (p=0.143).

In Table 3, based on the characteristics of previous history of preterm delivery, the incidence of preterm delivery in patients with a history of preterm labor in a past pregnancy was as much as 40%. These results were similar to the research conducted by Pennel et al (2007), which stated that women who had experienced preterm labor had a risk of experiencing preterm delivery in subsequent pregnancies by 15%.[16] The incidence of recurrent preterm labor can be caused by genetics, which is in line with research conducted by Hoffman and Ward (1999), which suggested that genetic factors play a role in the etiology of preterm

labor. Genes that regulate decidual relaxin is one of the cause. From the results of statistical tests, there was also a significant association between history of preterm labor with the incidence of preterm labor (p=0.031). Furthermore, research conducted by Goldenberg et al (2000) found a significant relationship between history of preterm labor with the occurrence of preterm delivery in subsequent pregnancies.[17]

This study found that serum maternal IL-6 level in preterm labor was 4.55 pg/ml to 38.87 pg/ml, whereas in preterm pregnancies the result obtained was 1.5 pg/ml to 3.07 pg/ml. In this study, it was found that the average level of serum maternal IL-6 was higher in preterm labor, which was 12.9 (SD=9.95) pg/ml compared with preterm gestation, which was 2.14 (SD=0.511) pg/ml. This result supported results of previous studies. Phillip et al (1997) found significant differences in the average level of serum maternal IL-6 between groups of preterm labor with preterm pregnancies (9.3 pg/ml vs. 1.9 pg/ml, p<0.001). This study found that improvement in maternal serum IL-6 may be a sign of impending preterm delivery in patients with and without subclinical intrauterine infection.[18] Moreover, Snjezana et al (2007) found that the level of maternal IL-6 serum was significantly higher in patients with preterm labor compared to patients with preterm pregnancies (6.8 pg/ml vs. 21.9 pg/ml, p <0.05).[19] Vogel et al (2007) evaluated levels of 17 inflammatory markers including IL-6 in 69 samples of preterm maternal serum, and obtained results that high level of maternal IL-6 serum was associated with increased risk of preterm labor at less than 35 weeks of gestational age.[20] Likewise, this study is similar with a study conducted by Yoram Sorokin et al (2010) at Wayne State University, who suggested a relationship between level of maternal serum IL-6 with the occurrence of preterm birth and neonatal morbidity.

IL-6 is a useful marker for intrauterine infection, preterm delivery, and neonatal morbidity. Increased level of maternal IL-6 serum is a risk factor for the occurrence of preterm labor. In the study by Yoram Sorokin, it was stated that the concentration of IL-6 of more than 5.15 pg/ml can lead to an increased risk of preterm labor by 38.30% (p<0.005).[14] Recent research conducted by Ramsey et al in 2011, obtained a significant association between IL-6 level with the onset of labor, where it was said that the average IL-6 level was significantly higher in pregnant women undergoing labor at term compared with full-term pregnant women who were not in labor yet (2.05 pg/ml vs. 0.95 pg/ml, p=0.03).[20]

CONCLUSIONS

In this study, level of maternal serum IL-6 in the preterm pregnancy group was 1.5 pg/ml to 3.07 pg/ml, and in the pretem labor group was 4.55 pg/ml to 38.87 pg/ml. Statistically, level of maternal serum IL-6 was significantly much higher in the preterm labor group (P=0.001). IL-6 level was correlated with the incidence of preterm labor. We suggest that a larger study with more samples be performed in order to determine the cut off point, so that level of maternal serum IL-6 can be used as a supporting tool to predict the onset of preterm labor.

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Research Article

Serum Zinc Level at Term Pregnancy and Newborn Anthropometry

Kadar Zink Serum Ibu Hamil Aterm dengan Antropometri Bayi Baru Lahir

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Abstract

Objective: To determine the relationship between serum zinc level at term pregnancy and newborn anthropometry.

Methods: This study is an observational study with cross-sectional design. Serum zinc level at term pregnancy was measured and then anthropometric measurement was done to the newborn, including birth weight, birth length and head circumference at birth. The data were statistically analyzed using regression correlation test.

Results: Mean serum zinc level at term pregnancy is 36.01 μg/dl (SD=18.34 μg/dl), the average birth weight is 3158 gr (SD=480.4 gr), the average birth length is 48.42 cm (SD=1.75 cm) and the average head circumference at birth is 33.13 cm (SD=1.14 cm). There was no statistically significant relationship between serum zinc levels at term pregnancy and birth weight (p-value=0.152). Meanwhile, there are statistically significant relationships between serum zinc level at term pregnancy with birth length and head circumference with p-value 0.026 and 0.012, respectively.

Conclusion: Serum zinc level at term pregnancy is correlated with birth length and head circumference, but is not correlated with birth weight.

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Keywords: birth length, birth weight, head circumference at birth, serum zinc level, term pregnancy

Abstrak

Tujuan: *Untuk mengetahui hubungan kadar zink serum ibu hamil aterm dengan antropometri bayi baru lahir.*

Metode: *Penelitian ini merupakan penelitian observasional dengan desain potong lintang. Dilakukan pengukuran kadar zink serum pada ibu hamil aterm, kemudian dilakukan pengukuran antropometri bayi baru lahir yang meliputi berat badan, panjang badan dan lingkar kepala lahir. Data dianalisis menggunakan uji korelasi regresi.*

Hasil: *Rerata kadar zink serum ibu hamil aterm adalah 36,01 μg/dl (SD=18,34 μg/dl), rerata berat badan lahir adalah 3158 gram (SD=480,4 gram), rerata panjang badan lahir adalah 48,42 cm (SD=1,75 cm) dan rerata lingkar kepala lahir adalah 33,13 cm (SD=1,14 cm). Tidak terdapat hubungan antara kadar zink serum ibu hamil aterm dengan berat badan lahir dengan nilai p=0,152 (p>0,05), namun kadar zink serum ibu hamil aterm berhubungan dengan panjang badan lahir dan lingkar kepala lahir dengan nilai p masing-masing 0,026 dan 0,012 (p<0,05).*

Kesimpulan: *Kadar zink serum ibu hamil aterm berhubungan dengan panjang badan dan lingkar kepala lahir, namun tidak berhubungan dengan berat badan lahir.*

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Kata kunci: *berat badan lahir, kadar zink serum, kehamilan aterm, lingkar kepala lahir, panjang badan lahir*

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INTRODUCTION

Millennium Development Goals (MDGs) set their goal to decrease infant mortality by two-thirds within 1990-2015.[1] But data from WHO recorded that the number of infant mortality and neonatal mortality in 2013 were 37 and 22 per 1000 births, and it was still considered high. Data from the Indonesian Health and Demographics Survey shows that the number of infant mortality in Indonesia is still considered to be high; around 32 from 1000 births. It was far from the MDGs 2015 target, with the expectation to decrease the number of infant mortality to 23 per 1000 birth.[2]

Low Birth Weight (LBW) was one of the major risk factors that contribute to 60 to 80% of neonatal death. The world prevalence of LBW is around 15.5%; 96.5% of them coming from developing countries.[3] Data from Basic Health Research (Riskesdas) in 2013 mentioned that the Indonesian prevalence of LBW in 2013 was 10.2%. As for West Sumatra, the prevalence of LBW was 7.5%, which was higher than 2010 (6%).[4]

In developing countries, intrauterine growth restriction (IUGR) mainly occurs because of poor nutrition in expectant mothers during their pregnancy.[5] The prevalence of IUGR in developing countries is 40% higher compared to that of modern countries that ranged around 10%. IUGR tends to cause short-term effects such as the escalation of mortality during the fetal life, neonatal period and infantry. It also causes growth, immune and intellectual disorder. As for the long-term effects, the infants tended to have chronic diseases when they reached adulthood, such as heart attack and diabetes type 2.[6]

Expectant nutrition is one of the determining factors during pregnancy; as it would help the infant to grow healthy inside their mother.[7] However, expectant mothers in developing countries consumes poor quantities of micro-nutrients during their gestation.[8] Around 82% of expecting mothers in developing countries have zinc deficiency;[9] and more than 80% of expecting mothers around the world consumes poor quantities of zincs.[10] In Indonesia, the prevalence of zinc deficiency in expecting mothers was found to be high. In East Nusa Tenggara (NTT), almost 71% of expecting mothers have zinc deficiency; and for Central Java it was around 70 until 90%.[11]

Zinc has been considered to be important throughout the gestation process, it is needed in the synthesis of both nucleic acid and proteins.[12] Zinc plays a significant role to support the function of several enzymes and growth hormones during pregnancy.[13] Zinc would regulate the growth hormones and Insulin-like Growth Factor-1 (IGF-1). This is the reason why zinc deficiency would lead to a decline in cell proliferation and protein synthesis that leads to infant growth disorder.[14]

METHODS

This research was conducted in the maternity section of RSUP Dr. M. Djamil Padang from February 6th until April 24th 2014. The samples for this research were taken from expecting mothers in RSUP Dr. M. Djamil Padang, West Sumatra who met the inclusion and exclusion criteria. Inclusion criteria involved expecting mothers with term singleton pregnancies, and living newborn. As for the exclusion criteria, it includes expecting mothers who were not willing to take part in the research, expecting mothers with infectious diseases during their gestation, expecting mothers with anemia, diabetes mellitus, hypertension, preeclampsia, and having a newborn with congenital disorder.

The level of serum zinc was measured using Zinc Colorimetric Assay Kit. The weight of the newborn baby was measured using GEA brand scale, and the length of the newborn baby was weighed using the measuring board; centimeter tape was used to measure the baby's head circumference.

Blood sample of the expecting mothers were taken before they delivered the baby. When the babies were born, anthropometric measurement was conducted to record the birth weight, birth length, and head circumference at birth on his/her first hour. The data were analyzed using regression correlation statistical tests.

RESULTS

From the observation, we included 38 expecting mothers with pregnancy at term, who fulfilled the inclusion and exclusion criteria.

Table 1. The Distribution of Maternal Serum Zinc Level, Birth Weight, Birth Length and Head Circumference at Birth of the Newborn Baby

	n	Mean	SD	%
Serum Zinc Level (μg/dl)	38	36.01	18.34	
≥ 56	4			10.5
< 56	34			89.5
Birth Weight (gram)	38	3158	480.4	
< 2500	2			5.3
2500-4000	35			92.1
> 4000	1			2.6
Birth Length (cm)	38	48.42	1.75	
48-52	28			73.7
< 48	10			26.3
Head Circumference at Birth (cm)	38	33.12	1.14	
33-37	27			71.1
< 33	11			28.9

Table 1 showed that maternal serum zinc level <56 μg/dl occurred in 34 term expecting mothers (89.5%), the average serum zinc level was 36.01 μg/dl (SD=18.34 μg/dl). From the anthropometric measurement, it was recorded that 39 babies (92.1%) weighed around 2500-4000 grams with average weight of 3158 grams (SD=480.4 gram). The length of 28 newborn babies (73.7%) was in the range of 48-52 cm with average length of 48.42

cm (SD=1.75 cm). In terms of the head circumference, 28 babies (71.1%) had their head circumference measured at the range of 33-37 cm, with the average head circumference at birth to be 33.13 cm (SD=1.14 cm).

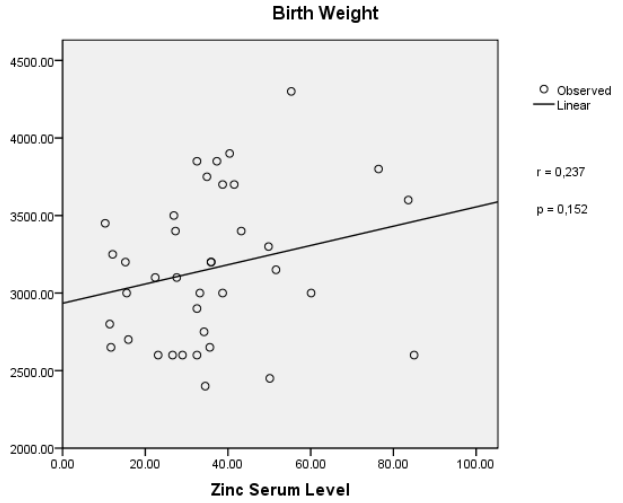

Figure 1. The Relationship between Serum Zinc Level in Term Pregnancy and Newborn Baby's Weight

Figure 1 showed that serum zinc level and newborn baby's weight had a positive correlation. But the correlation between those two factors was only weak (r=0.237), and it showed no statistical significance (p=0.152).

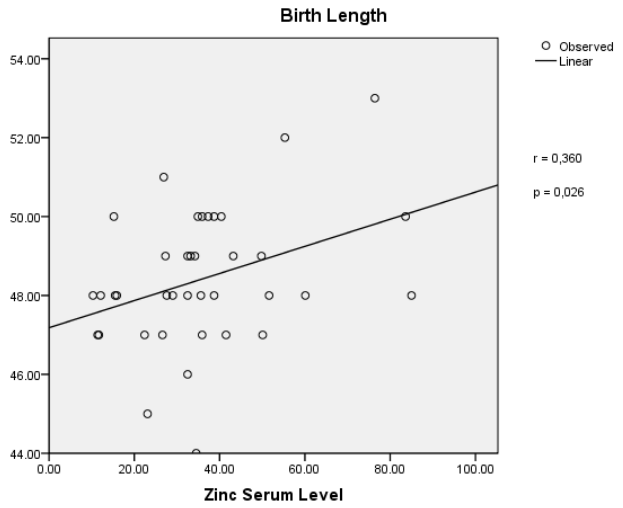

Figure 2. The Relationship between Serum Zinc in Term Pregnancy and the Newborn Baby's Length.

Figure 2 showed a positive correlation between serum zinc level at term pregnancy and the birth length of the newborn baby. However, both of them have a weak relation (r=0.360), but it was found to be statistically significant (p=0.026).

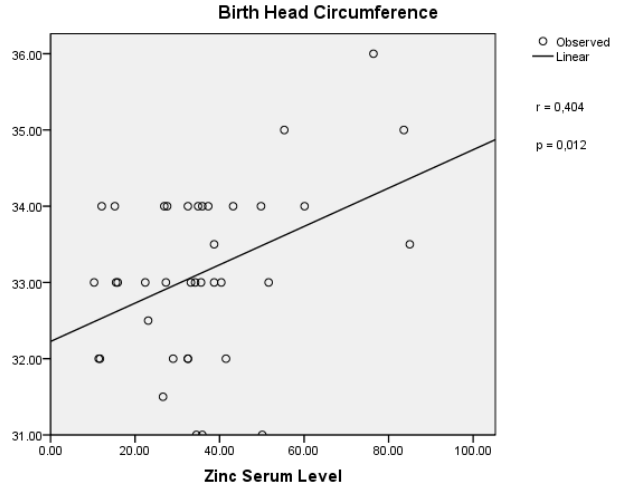

Figure 3. The Relationship between Serum Zinc Level in Term Pregnancy and Newborn Baby's Head Circumference

Figure 3 showed that serum zinc at term pregnancy and baby's head circumference at birth had a medium positive correlation (r=0.404), which was found to be statistically significant around (p=0.012).

DISCUSSION

From the observation during the research, 89.5% of expecting mothers with term pregnancy had zinc deficiency. The prevalence of zinc deficiency in expecting mothers during the research observation was higher than Widagdo's observation in 2006 in Jakarta that recorded 48% of expecting mothers had zinc deficiency.[15] In 1996, both in East Nusa Tenggara (NTT) and in Central Java; the prevalence of zinc deficiency has been previously observed. The percentages are high; both of them showing the prevalence to be around 71% and 70-90%.[11]

Countries around Asia also showed high prevalence of zinc deficiency in expecting mothers. As much as 45% of Chinese expecting mothers experienced zinc deficiency during their third trimester, Bangladesh recorded 55% expecting mothers had zinc deficiency during the gestation period, while India recorded 65% of this condition.[16]

From the observation, it was also seen that from 38 babies; 2 of them were born with birth weight less than 2500 gram. It was lower than that in Widagdo's observation in 2006 that recorded 9.3% of newborn baby weighing less than 2500 gram. Ten babies (26.3%) from 38 babies had body length less than 48 cm, 11 babies (28.9%) were born with head circumference less than 33 cm. However, the average for weight, length and head circumference were still within the normal range.

This research was quite similar to the previous research by Widagdo in 2006 in Jakarta. It recorded that the average measurement of newborn babies weighed around 3064 gram (SD=450 gram), length measuring 48 cm (SD=2 cm), and head circumference of 33 cm (SD=2 cm).[15] Meanwhile, Dehkordi's observation in India (2013) recorded that the average birth weight, length, and head circumference on babies from their sample of expectant mothers with normal zinc level were successively 3229 gram, 50.32 cm and 34.73 cm. The weight, length, and head circumference of the newborn babies from the expectant mothers with low zinc level is ranged around 3092 gram, 50.10 cm and 34.48 cm. It showed a significant difference in weight, length, and head circumference that occurred in babies from mothers with normal and low zinc level.[17]

The result from the correlation regression statistical test showed that zinc serum level on term expectant mothers was not corresponding to the weight of the baby ($p>0.05$). It showed that the increasing serum zinc level in expecting mothers would not increase the birthweight of the baby. Even if both of them had a positive correlation; but it carries no significance in increasing the baby's weight.

Osendarp's research result in 2000 noted that consuming zinc supplement around 30 mg/day during the last trimester would not be significant in increasing the baby's weight. The average baby's weight from expecting mothers that consumed zinc supplement and expecting mothers as comparator were 2513 (SD=390) and 2554 (SD=393) gram.[18] Norrozi's research in 2012 that took place in Iran also showed that consuming zinc supplement for 25 mg/day did not increase the baby's weight compared to expectant mothers who did not consume the supplement. The baby's weight on both arms were found to be around 3142 (SD=452) and 3230 (SD=527) gram.[19]

On the other side, India's researcher Dehkordi observed a significant relationship between zinc level in expecting mothers and the baby's weight ($p=0.007$). The average birth weight from expectant mothers with normal zinc level was higher than babies from expectant mothers with abnormal zinc level. Mojgan et al who conducted the research in Iran in 2012 also recorded that serum zinc level in expectant mothers were significant to the baby's weight. Expectant mothers with low zinc level tended to give birth to the baby with low average weight compared to expectant mothers with normal zinc level. Their risks to give birth to low weight baby were found to be 12 times higher.[20]

There are several factors that explained the inconsistency in the results of the research focusing on serum zinc level in correlation to the baby's weight. It could happen because of a low index of accuracy in the expectant mother's zinc level, small number of samples, the time and duration in consuming zinc supplements, expectant mother's weight, digestive illnesses, and dietary factors that have a great influence on zinc bioavailability.[21]

The result of regression statistic test showed a significant relationship between serum zinc level on term expectant mothers and the length of the newborn baby ($p<0.05$). The result showed that high serum zinc level on expectant mothers was usually followed by the increase of the newborn baby's length. It showed a positive relationship between both variables, so from the statistical point it could be said that the increasing zinc level on expectant mothers had a significant influence to the increasing baby's body length.

Merialdi's research in Peru (2004) observed a positive effect between prenatal zinc supplementation with a dose of 25 mg/days and the baby's femur length. Expectant mothers who consumed zinc supplements tended to have a baby with longer femur length compared to expectant mothers who did not consume zinc supplements. Prawirohartono et al in their research in Central Java, Indonesia (2013) also recorded that consuming zinc supplements yields a higher length in the newborn baby (48.8 cm) compared to the babies born from the control group (48.5 cm).[22]

Zinc plays an important role in bone metabolism, as shown on animal trials. Zinc stimulates bone metabolism, bone protein synthesis, and bone formation on tissue engineering, by increasing the main enzyme activities such as alkaline phos-

phatase. Zinc was also important to increase the anabolic effect of IGF-1 on osteoblasts. It is important to shape and mineralize bone's extracellular matrix during endochondral ossification. Zinc also plays an essential role in obstructing osteoclastic activities, which is responsible for bone resorption.[23]

From the result of correlation regression statistical test, there was a significant relationship between the level of serum zinc on expectant mothers and the baby's head circumference ($p<0.05$). Both aspects showed a significant relationship since the increase of zinc level in expectant mothers would be followed by the increase of the baby's head circumference.

Surkan's research in Nepal in 2012 was focused on the consumption of zinc supplement as micronutrient support and its relation to baby's head circumference. It recorded that consuming zinc supplements brought benefit to the baby's brain growth.[24] Tamura in his research that took place in USA in 2003 recorded that expectant mothers who consumed zinc supplements have a propensity to give birth to babies with a head circumference 0.4 cm larger than the babies from the comparator group. The escalation of baby's head circumference indicated a healthy brain growth.[25] Zinc is important for the baby's brain growth, and zinc deficiencies lead to a decrease of DNA synthesis on brain tissue that would result in declining brain tissue growth.[26] Zinc is an enzymatic cofactor that governed both protein and DNA biochemistry. Zinc deficiency would degrade the DNA, RNA, and brain protein system in infants. Zinc also controls both IGF-1 and the expression of gene receptor on baby's growth hormone that influence the infant's brain growth. Natural neurotropic factors had its own role in producing cell proliferation and differentiation during the normal brain growth and maturity.[27]

CONCLUSION

There is a relationship between zinc level in expectant mothers with newborn baby's body length and head circumference, but it showed no significant relationship to newborn baby's weight.

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Research Article

Effectiveness of Pelvic Organ Prolapse Surgery in Women with Depressive Symptoms and Decreased Quality of Life

Efektivitas Operasi pada Perempuan dengan Gejala Depresi dan Penurunan Kualitas Hidup yang Menjalani Operasi Prolaps Organ Panggul

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Abstract

Objective: To know the effectiveness of pelvic organ prolapse surgery in decreasing depressive symptoms (based on PHQ9) and improving quality of life in women with pelvic organ prolapse.

Methods: This experimental study without control is conducted at the Obstetrics and Gynecology Department of Dr. Mohammad Hoesin Hospital Palembang/Faculty of Medicine Sriwijaya University, from October 03 2012 until May 31, 2014. Data containing self-administrated questionnaire about depressive symptoms (PHQ9) and quality of life (PFIQ and PFDI) were recorded. Questionnaire was performed before and six months after surgery. Sample included 26 women with pelvic organ prolapse seeking pelvic organ prolapse surgery, which qualified the inclusion criteria. Data were analyzed using Chi Square and Fisher Exact test. Data analysis was done using SPSS 18.0.

Results: According to paired T test there is a significant difference between mean PHQ9 score before (6.69±3.80) and 6 months after surgery (1.96±1.75)(p=0.001). Total PFIQ score decreased from 17.15±9.39 to 2.88±4.01 with 14.27±5.38 reduction. PFDI score before surgery were 29.85±15.73 and decreased to 11.50±10.99, with a reduction of 18.35±4.74.

Conclusion: There was significant reduction in depressive symptoms and improved quality of life in women with prolapse after surgery, compared to before surgery.

[Indones J Obstet Gynecol 2015; 3-4: 196-199]

Keywords: depression, quality of life, uterine prolapse

Abstrak

Tujuan: *Mengetahui efektivitas operasi prolaps dalam mengurangi gejala depresi (berdasarkan PHQ9) dan meningkatkan kualitas hidup (PFIQ-7 dan PFDI) pada perempuan dengan prolaps organ panggul sebelum dan sesudah operasi prolaps organ panggul.*

Metode: *Penelitian uji eksperimental tanpa pembanding ini dilaksanakan di Bagian Kebidanan dan Penyakit Kandungan di RSMH/ Fakultas Kedokteran UNSRI Palembang. Waktu penelitian dalam rentang waktu antara tanggal 03 Oktober 2012 sampai dengan 31 Mei 2014. Data berupa kuesioner mengenai depresi (PHQ9) dan kualitas hidup (PFIQ-7 dan PFDI) yang diisi sendiri oleh sampel penelitian. Kuesioner dilakukan sebelum operasi dan diulangi 6 bulan pascaoperasi. Didapatkan 26 sampel yang didiagnosis prolaps uteri yang menjalani operasi dan memenuhi kriteria inklusi. Data dianalisis dengan menggunakan uji Fisher/Chi Square. Pengolahan data dibantu dengan program SPSS 18.0.*

Hasil: *Berdasarkan uji statistik T berpasangan, didapatkan perbedaan yang bermakna antara rerata skor PHQ-9 sebelum operasi (6,69±3,80) dan 6 bulan sesudah operasi (1,96±1,75)(p=0,001). Skor PFIQ secara keseluruhan mengalami penurunan yaitu 17,15±9,39 sebelum operasi, menjadi 2,88±4,01 sesudah operasi dengan besar penurunan 14,27±5,38. Skor PFDI sebelum operasi sebesar 29,85±15,73 dan turun 6 bulan sesudah operasi menjadi 11,50±10,99 dengan besar penurunan 18,35±4,74.*

Kesimpulan: *Terdapat penurunan yang bermakna pada gejala depresi dan perbaikan kualitas hidup pada perempuan dengan prolaps sebelum dibandingkan sesudah operasi prolaps.*

[Maj Obstet Ginekol Indones 2015; 3-4: 196-199]

Kata kunci: *depresi, kualitas hidup, prolaps uteri*

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INTRODUCTION

Pelvic organ prolapse is a condition in which the genital organs are protruding into the vagina. This occurs due to weakness of the muscle, fascia and supporting ligaments. Pelvic organ prolapse can be in the form of cystocele, rectocele, enterocele, cervical elongation, urethrocele, uterine prolapse, and vaginal prolapse. In Dr. Cipto Mangunkusumo Hospital, Indonesia, Junizaf et al reported that 50% of women who have given birth will suffer from pelvic organ prolapse, and nearly 20% of cases undergoing gynecological surgery were pelvic organ prolapse cases.[1]

Gregory WT reported that prolapse has behavioral and psychological implications, which affect the *Quality of Life* (QOL) and health-related QOL specific-condition. Prolapse can increase symptoms of depression and anxiety, while the symptoms of depression and anxiety can affect health behaviors, symptom burden, QOL, and functional impairment before and after surgery. Sze EH reported that the anterior vaginal wall prolapse compartment is a vaginal compartment prolapse that is often experienced, and have severe repercussions for the QOL of patients. Ghetti C reported that women with pelvic organ prolapse had a higher prevalence of depressive symptoms compared to controls without prolapse.[2-4]

Examination of depression and quality of life using a self-administered questionnaire consists of the Patient Health Questionnaire (PHQ-9), Pelvic Floor Distress Inventory (PFDI), and Pelvic Floor Impact Questionnaire (PFIQ).

PHQ-9 is a validated measurement of the severity of depression correlated closely with major depression diagnosis established by the Structured Clinical Interview for the Diagnostic and Statistical Manual of Mental Disorders (DSM). PHQ-9 is selected as a measure of general depression to control depression while investigating the effect of prolapse on body image in the parent study. PHQ-9 was also validated in an ambulatoric gynecologic population.

PFDI and PFIQ are validated self-administered questionnaires used to evaluate the effect of pelvic floor disorders on health-related QOL. Each questionnaire consists of three subcategories. PFDI investigates distress symptoms and contains three subscales, Urinary Distress Inventory (UDI), the Pelvic Organ Prolapse Distress Inventory (POPDI), and Colorectal-Anal Distress Inventory (CRADI) that assesses urinary, pelvic, and colorectal symptoms. PFIQ investigates the influence of the pelvic floor symptoms and contains three scales: Impact Questionnaire Incontinence, Pelvic Organ Prolapse Impact Questionnaire (POPIQ), and Colorectal-Anal Impact Questionnaire.

Depression is often found in women with urinary incontinence and is known as a major cause of disability in women around the world, the relationship between prolapse, major depression, anxiety disorders, and QOL is unknown. Information on the incidence of depression and QOL in women before and after prolapse surgery is still limited. Therefore, it is necessary to perform research on depressive symptoms and QOL in women before and after prolapse surgery.[3,4]

METHODS

This research is an experimental study without comparison. It was carried out in the Department of Obstetrics and Gynecology at RSMH/Faculty of Medicine UNSRI Palembang. Research period was between October 3, 2012 and May 31, 2014.

The study population included all women with prolapse who came and were treated in the Department of Obstetrics and Gynecology RSMH/ Faculty of Medicine UNSRI Palembang. Samples were women with stage II or higher prolapse, who were planned to undergo surgery and met the inclusion criteria. The inclusion criteria were women with pelvic organ prolapse stage II-IV, who were willing to have surgery, and signed the informed consent. Exclusion criteria were leukocyturia and bacteriuria from urinalysis prior to surgery, patients with neurological disorders (central and peripheral nervous system), suffering from systemic diseases such as diabetes mellitus, and patients who are undergoing estrogen therapy.

Before surgery, the patient underwent a guided assessment by self-administered questionnaires in the form of PFDI, PFIQ, and PHQ-9. Pelvic organ prolapse surgery was performed by urogynecology consultants. After 6 months post-surgery the tests by self-administered questionnaires (PFDI, PFIQ, and PHQ-9) were repeated. Data was analyzed using Fisher's exact test or Chi Square. Data processing was assisted by SPSS 18.0.

RESULTS AND DISCUSSION

The number of samples involved in this study was 30 samples, but after 6 months post-surgery only 26 subjects completed the study, whereas 4 other subjects dropped out due to changed addresses and any means of communication being inactive, causing loss of follow-up.

The general characteristics of the subject were analyzed, which included age, parity, education, and residence. Most of the research subjects belonged to the 51-60 years old age group, with as many as 13 subjects (50.0%), with a mean age of 60.58±8.89 years. The lowest age was 49, and the highest was 81 years old.

The most common parity status was 4, which was present in as many as 26.9% of samples, and the second largest group was those with parity 3 and 5 with 4 subjects in each group (15.4%). Only 1 subject belonged to the parity 0 and 1 group (3.8%).

The most common education level of the subjects was high school education for as many as 20 subjects (76.9%). While those whose education level was junior high school were 2 subjects (7.7%) and as many as 4 subjects (15.4%) had DIII/S1 education.

In terms of occupation, the subjects in this study were all housewives. Residence of participants in this study was divided evenly within the city and outside the city, each with 13 subjects (50%). Zeleke BM et al reported that patients with POP in Ethiopia who reside in rural areas amounted to 85.3% and the remaining 14.7% live in urban areas. While in this study, the proportion of the residential area were the same.[5]

Clinical Characteristics

Based on the degree of pelvic organ prolapse, we encountered 9 subjects (34.6%) in each of the pelvic organ prolapse degrees II and III groups. As many as 8 subjects (30,8%) had prolapse grade I. Research by ZY Yuan and Shen YH reported that mild (grade I) or moderate (grade II) prolapse may have no complaints. Usually there is a complaint or the complaint is felt by patients after stage III.[6]

Distribution of surgery that were most commonly done was total vaginal hysterectomy (TVH) with colpoperineorraphy + anterior colporraphy and TVH with anterior colporraphy with 7 subjects (26.9%) undergoing the procedure, respectively, whereas just as much as 6 subjects (23.1%) underwent TVH surgery only. TVH was chosen for cases of uterine prolapse because this technique provides more advantages than abdominal hysterectomy. Advantages include faster healing time, shorter hospitalization times and fewer infections.[7-10]

The mean PHQ-9 score before surgery was 6.69 ±3.80 and decreased after 6 months post-surgery to 1.96±1.75. Based on the paired t test, we found a significant difference in the mean PHQ-9 scores prior to and 6 months after surgery (p=0.001).

Table 1. Distribution of Subjects According to the Degree of Depression

Degree of Depression	Before Operation		6 months after operation	
	n	%	n	%
Not depressed	9	34.6	24	92.3
Mild depression	11	42.4	2	7.7
Medium depression	5	19.2	0	0
Severe depression	1	3.8	0	0
Total	26	100.0	26	100.0
	PHQ-9 scores			p
Prior to surgery	6.69±3.80			0.001
6 months after operation	1.96±1.75			

TVH in patients with pelvic organ prolapse can reduce the degree of depression. This was evident from the results of this study, which found the incidence of depression before surgery to be 61.4% which was reduced after surgery to 7.7%. TVH is effective in reducing depressive symptoms and improving quality of life in female pelvic organ prolapse.

PFDI overall score before surgery was 29.85±15.73 and dropped at 6 months after operation to 11.50±10.99, demonstrating an 18.35±4.74 decline. It has been proven statistically with paired t test that there were significant differences in *Pelvic Floor Distress Inventory* (PFDI) scores before and 6 months after surgery (p=0.001).

Table 2. Pelvic Floor Distress Inventory (PFDI) Scores

Parameter	Before Operation	6 months after operation	p
PFDI	29.85 ± 15.73	11.50 ± 10.99	0.001
UDI	8.35 ± 4.65	3.50 ± 3.31	0.001
POPDI	13.08 ± 5.01	3.15 ± 2.97	0.001
CRADI	8.19 ± 6.98	4.85 ± 4.84	0.001

Paired t test; p<0.05 is significant

The decreasing trend of PFDI scores in this study that assesses urinary (UDI), pelvis (POPDI), and colorectal (CRADI) symptoms before and after the operation was in line with the results of a research conducted by Ghetti C et al, which reported a mean UDI score before surgery by of (52-78) and after surgery dropped to 15 (10-21). They also obtained an average POPDI score before surgery of 76 (64-88) and fell after surgery to 22 (14-30),

while the mean score of CRADI obtained before surgery was 71 (59-84) and after surgery was 26 (17-34).

PFIQ overall scores decreased from before surgery to 6 months after surgery, with 17.15 ± 9.39 before surgery and declining by 14.27 ± 5.38 to 2.88 ± 4.01. Based on statistical tests performed using paired t test, there were significant differences in terms of PFIQ scores before and 6 months after surgery (p=0.001).

Table 3. Pelvic Floor Impact Questionnaire (PFIQ) Scores

Parameter	Before Operation	6 months after operation	p
PFDI	17.15 ± 9.39	2.88 ± 4.01	0.001
UIQ	5.92 ± 3.05	0.96 ± 1.11	0.001
POPIQ	6.46 ± 3.14	0.96 ± 1.45	0.001
CRAIQ	4.77 ± 3.62	0.96 ± 1.53	0.001

Paired t test; p < 0.05 is significant

The results showed that performing prolapse surgery improves the quality of life of patients with prolapse, where we can see a reduction of depressive symptoms.

CONCLUSION

There was significant reduction in depressive symptoms and improved quality of life in women with prolapse after surgery, compared to before surgery. Pelvic organ prolapse surgery is effective in reducing depressive symptoms and improving quality of life in female with pelvic organ prolapse.

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Research article

PPARy Expression in Eutopic and Ectopic Endometrium of Reproductive Age Women with Endometriosis

Ekspresi Reseptor PPARy Endometrium Eutopik dan Ektopik pada Penderita Endometriosis Usia Reproduksi

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Abstract

Objective: To evaluate the expression of PPARy receptor and to compare its expression in eutopic and ectopic endometrium in women with endometriosis.

Method: This is a cross sectional study. Ten female subjects with endometriosis that underwent laparoscopy or laparotomy who fulfilled the inclusion criteria were recruited by consecutive sampling. Two samples were taken, eutopic endometrium and ectopic endometrium from endometriosis cyst wall during surgery of each subject. PPARy expression was examined by two-step RT-qPCR. Our data was statistically examined using the paired t-test and Pearson's correlation test.

Result: PPARy was found to be expressed in eutopic and ectopic endometrium of women with endometriosis using the RT-qPCR method. The expression of PPARy was not statistically different in eutopic and ectopic endometrium (1.16 relative fold vs 1.25 relative fold; p=0.26). By Pearson's correlation there was a weak positive correlation between PPARy expression of eutopic and ectopic endometrium (r=0.16).

Conclusion: PPARy was detected by two-step RT-qPCR in eutopic and ectopic endometrium of women with endometriosis. Semiquantification of PPARy expression showed that there was no significant difference between PPARy expression in eutopic and ectopic endometrium of women with endometriosis. There was a weak positive correlation between PPARy expression in eutopic and ectopic endometrium of women with endometriosis.

[Indones J Obstet Gynecol 2015; 3-4: 200-205]

Keywords: endometriosis, PPARy, two-step RT-qPCR

Abstrak

Tujuan: *Menilai keberadaan reseptor PPARy serta membandingkan ekspresi reseptor PPARy pada endometrium eutopik dan ektopik pada penderita endometriosis.*

Metode: *Penelitian ini merupakan penelitian potong lintang (cross sectional). Sepuluh subjek penderita endometriosis yang menjalani laparoskopi atau laparotomi, yang masuk dalam kriteria penerimaan direkrut menggunakan consecutive sampling. Diambil dua sampel, yakni endometrium eutopik dan endometrium ektopik yang berasal dari dinding kista endometriosis saat dilakukan pembedahan, kemudian dilihat ekspresi reseptor PPARy dengan two-step RT-qPCR. Ekspresi masing-masing sampel diuji statistik dengan uji t berpasangan dan tes korelasi Pearson.*

Hasil: *Didapatkan ekspresi reseptor PPARy pada endometrium eutopik dan ektopik penderita endometriosis dengan metode RT-qPCR. Ekspresi reseptor PPARy endometrium eutopik dan ektopik didapatkan secara statistik tidak berbeda bermakna (1.16 lipatan relatif vs 1.25 lipatan relatif; p=0.26). Pada uji korelasi Pearson didapatkan korelasi positif lemah antara ekspresi PPARy pada endometrium eutopik dan ektopik (r=0.16).*

Kesimpulan: *Tampilan reseptor PPARy pada endometrium eutopik dan ektopik penderita endometriosis didapatkan dengan metode two-step RT-qPCR. Dengan semikuantifikasi ekspresi reseptor PPARy tidak didapatkan perbedaan antara ekspresi reseptor PPARy pada endometrium eutopik dan ektopik pada penderita endometriosis. Terdapat korelasi positif lemah antara ekspresi reseptor PPARy pada endometrium eutopik dan ektopik pada penderita endometriosis.*

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Kata kunci: *endometriosis, PPARy, two-step RT-qPCR*

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INTRODUCTION

Endometriosis is an endometrium-like lesion outside of the uterine cavity that is sensitive to the hormonal changes in the menstrual cycle.[1,2] The prevalence of endometriosis in women of reproductive age is around 10%.[3,4] This condition is more frequently encountered in women with pelvic pain and infertility, in which 35-50% have endometriosis.[2] Data from RS Dr. Cipto Mangunkusumo in 2000-2005 showed 111 new cases of endometriosis diagnoses by laparoscopy, 75% of which are found in women aged 25-39 years.[5,6]

Currently, most cases of endometriosis are treated with hormones, that may influence the menstrual cycle and fertility of the patients.[1,7] Non-hormonal treatments such as NSAID (non-steroidal

anti-inflammatory drugs) do not interfere with fertility, but long-time treatment with such agents may pose significant side effects.[1,2,7] A non-hormonal treatment that can be used for a long period without significant side effects and with no impact on fertility is urgently needed for endometriosis patients who wish to conceive. Understanding the pathogenesis of endometriosis may open doors to other treatment modalities, either by methods that are already available or under development.[1,3,7-9]

One agent that may be used as a modulator is the activation of PPARγ receptor, which is widely recognized in the treatment of diabetes.[3,10] The potential role of PPARγ in the pathogenesis of endometriosis and as an alternative non-hormonal treatment encouraged us to study the profile of PPARγ receptors in patients with endometriosis.

Researchers have widely known that activation of PPARγ influences the differentiation of adipocytes and insulin resistance.[11] PPARγ may also influence the production of proinflammatory cytokines by systemic immune cells and cells in peritoneal fluids, acting as an external factor to the development of endometrial tissue in the peritoneum.[12-16] Development in the last decade showed that PPARγ may effect the profile of certain genes in eutopic and ectopic endometrium.[17,18] These facts encouraged us to conduct a research, to find differences between the profile of PPARγ genes in eutopic and ectopic endometrium and to elaborate the role of PPARγ in the development of local endometriosis tissue.

Significant gene profile differences have been identified in eutopic and ectopic endometriosis tissue.[19-21] Several studies have revealed higher aromatase profiles in the nucleus of ectopic glandular endometrium compared to eutopic endometrium.[22-24] Factors enhancing neovascularisation, VEGF, and adhesion factors, CAM, are also more abundant in ectopic endometrium.[25,26] The profile of apoptosis-regulator genes, Bcl-2 and Bax, are also different in eutopic and ectopic endometrium.[27]

Administration of PPARγ ligands may inhibit adhesion of endometrial cells to the peritoneum by reducing the expression of ICAM-12.[28,29] The influence of PPARγ on implants and neovascularization is proven by reduced VEGF activity, thus inhibiting neovascularization.[19] Activation of PPARγ also reduces aromatase, thus reducing the production of estrogen in endometriosis lesions and reducing the proliferation of endometriosis lesions in vitro.[30] One study also reported reduction of anti-apoptotic genes in endometriosis lesions which had PPARγ ligands administered.[31]

Administration of PPARγ ligand agonists in animal with endometriosis also reduced the size and mass of ectopic endometrium.[32] In baboons, it reduced the size and area of pelvic endometriosis.[33] In humans, administration of PPARγ agonists reduced pain symptoms.[35] It has been reported that increased PPARγ expression (determined through immunohistochemistry) in peritoneal lesions reduced symtomps in endometriosis patients, especially pelvic pain, dysmenorrhea, and dyspareunia.[34,35]

The role of PPARγ is determined by both the availability of ligands and expression of its receptor.[32-35] Expression of PPARγ receptor in endometriosis tissue may be useful to study its role in the pathogenesis of endometriosis and as a predictor in determining the response to therapy with PPARγ ligands.[34,35] We conducted this study to evaluate the expression of the PPARγ and to compare its expression in eutopic and ectopic endometrium in reproductive age women with endometriosis.

METHODS

This a biomedical science study with cross sectional design, and with numerical comparison between two-paired group analysis. We collected data from February to April 2014. Subjects were recruited from general gynecology patients and patients from the fertility clinic who underwent surgery in the Department of Obstetrics and Gynecology, RS Cipto Mangunkusumo, Jakarta. Samples were analyzed in the Integrated Laboratory, Faculty of Medicine University of Indonesia.

Samples were obtained using endometrial biopsy when subjects were in the operating room. Samples were transported, then total DNA extraction was performed. After that, samples were stored in -80°C until analysis was performed. After all samples were collected, frozen endometrial tissues were thawed, and RT-PCR was conducted to assess the expression of PPARγ receptors in the endometrium.

RT-PCR examination of PPARγ receptors was initiated by thawing of samples stored in RNA ladder in room temperature, followed by RNA isolation.

The result of RNA isolation was total RNA of samples in elution buffer solution.

Development of cDNA from total RNA was started by assessment of concentration and purity of RNA by using spectrum analyzer in 260 nm and 280 nm wavelength. To assess the expression of PPARy with qPCR, we designed primers in silico (with computers) with IDT (Integrated DNA technologies) software, and we obtained the sequence of target and reference genes.

All qPCR were performed with Rotor Gene Q® machine. Quantification for each sample was calculated by using the CT (Cycle threshold) from target genes (PPARy) and the CT from reference genes (GAPDH). Semiquantification of gene expression was carried out with Livak method.[37]

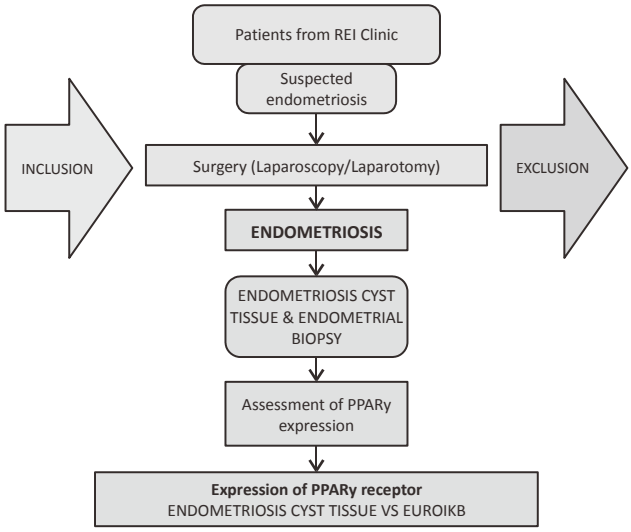

Figure 1. Research workflow.

RESULTS

We identified 49 patients who underwent surgery for endometriosis cysts from February to April 2014. Eighteen patients were excluded: 12 did not sign the consent form, 2 patients had tubo-ovarial abcess, 2 patients had obesity, and 2 patients were smokers. From the remaining 31 patients, we did not obtain adequate endometrial samples (nil or less than 0.5 ml). Seventeen patients were adequate for analysis, however to due to limited funds for reagens and material, only 10 patients (20 samples) were analyzed. The remaining 7 pair of samples were stored in -80°C for further studies.

This study included 10 subjects. All subjects underwent surgery for endometriosis cysts. The mean age of patients was 31.8±4.15 years, with BMI 22.21±1.38 kg/m^2, waist-hip ratio 0.81±0.52 cm, and mean leukocyte count 8,033±1,897. All subjects were stage III-IV endometriosis patients with endometriosis cysts; the mean size of the cysts was 6.8±2,1 cm and all subjects experienced menstrual pain with a mean visual analogue scale (VAS) 6.3±1.15. It could be concluded that the subjects belonged to a homogenoud group, which is women of reproductive age with menstrual pain and endometriosis cysts, with normal BMI and without other comorbidities.

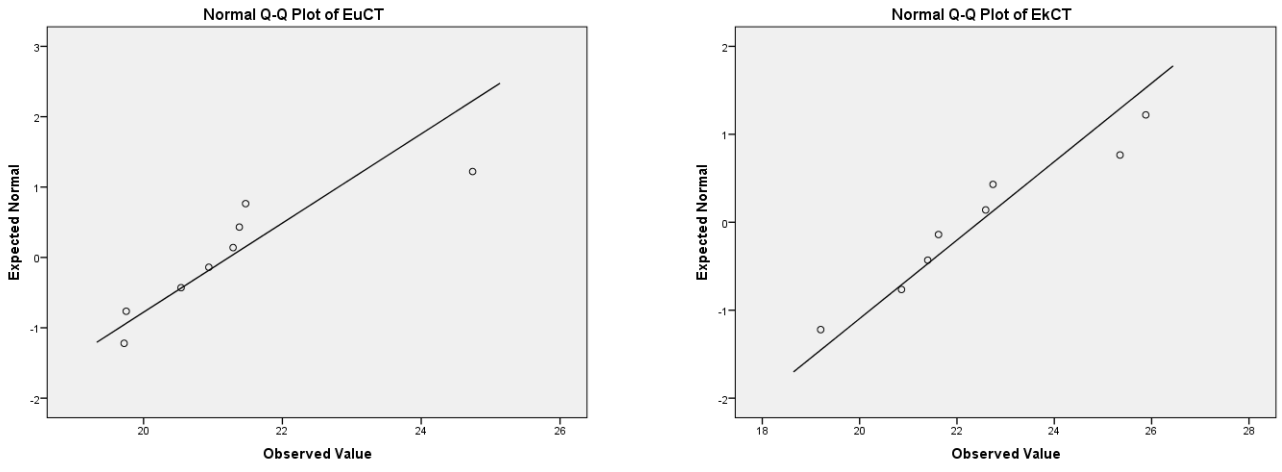

Figure 2. Normality plots of CT (Cycle Threshold) qPCR PPARy in Eutopic (left) and Ectopic Endometrium (right)

Quanitification of receptor expression was performed with Livak method by using the following formula: $2^{-\Delta\Delta CT}$ [$\Delta\Delta CT = \Delta CT$ - (median CT $_{PPARγ}$ - median CT $_{GAPDH}$)].[37] The result of this formula is a relative time-fold towards the reference gene (GAPDH), a housekeeping gene whose expression is constant in all tissues.

The mean expression of PPARγ in eutopic and ectopic endometrial tissue was 1.16u and 1.25u, respectively. Normality tests towards the CT (Cycle threshold) data using Kolmogorov-Smirnov and Shapiro-Wilks revealed normal distribution in both groups ($p>0.05$).

As the data was normally distributed and obtained from the same patient (eutopic and ectopic tissue), we conducted a paired t-test to find the difference between both groups. We found that the expression of PPARγ in ectopic endometrium was higher than in eutopic endometrium, but the difference was not significantly different (1.25 vs. 1.16, $p=0.26$) (Table 1).

Table 1. Comparison of PPARγ Receptor Expression

	Mean PPARγ receptor expression (Unit= relative-folds to GADPH, a reference gene)	p	Difference of mean
Eutopic tissue	1.16	0.26	0.09
Ectopic tissue	1.25		

On Pearson correlation test we found a weak correlation between the expression of PPARγ receptor in ectopic endometrium and eutopic endometrium ($r = 0.16$), as shown Table 2.

Table 2. Correlation between PPARγ Expression in Ectopic and Eutopic Endometrium in Endometriosis Patients

	Mean	r
PPARγ receptor expression in eutopic endometrium	1.16	0.16
PPARγ receptor expression in ectopic endometrium	1.25	

DISCUSSION

MacLaren et al found that PPARγ can be found in the reproductive system of mice and bovine.[37] Pritts et al reported that PPARγ was ultimately expressed in the nucleus of cultured endometrial tissue.[17] Peeters et al assessed the expression of PPARγ in cultured endometrial cells using immunohistochemistry and RT-PCR, and its effect on administration of PPARγ agonists and expression of VEGF.[18] Until now, no study have assessed the expression of PPARγ in ectopic and eutopic endometrial tissue in women with endometriosis and identifying the correlation between both groups.

In this study we obtained the expression of PPARγ receptor in eutopic and ectopic endometrium in patients with endometriosis, which indicates that the PPARγ ligand may directly influence endometrial tissue or locally, and may be the background for the administration of PPARγ agonists in patients with endometriosis. Ohama et al found that administration of PPARγ agonistsmay lower TNFγ and IL-8 in endometrial tissue.[38] Lebovic et al proved that PPARγ agonists significantly reduce the size of endometriosis lesions in mice and primates.[32,33] One of the possible mechanism for their findings are through the role of PPARγ receptors in target tissue (eutopic and ectopic endometrium), as we have found in this study.

This study also revealed that the expression of PPARγ receptors in eutopic and ectopic endometrium was not significantly different ($p>0.05$), which shows a correlation between the profile of eutopic and ectopic endometrial tissue. This is in line with other studies stating that endometriosis is a disease of eutopic endometrium.[19,20] Afshar et al found that gene expression abnormalities in eutopic endometrium may influence the behaviour of ectopic endometrium in primates.[19] Furthermore, Sha et al discovered that gene expression abnormalities identified by qPCR in eutopic endometrium of endometriosis patients compared to endometrium in patients without endometriosis may lead to the survival of endometrium undergoing retrograde menstruation.[20] Wren et al also concluded from 562 genes analyzed with microarray from several studies, that there are multiple correlations between expression of those genes in eutopic and ectopic endometrium.[21] Beside our study, until now there has been no study assessing the expression of PPARγ receptors in eutopic and ectopic endometrium.

We found a positive weak correlation between expression of PPARγ receptor in eutopic and ectopic endometrium of endometriosis patients ($r=0.16$). This shows that only 16% of PPARγ receptor expression in eutopic or ectopic endome-

trium can be predicted from the PPARy receptor expression in their counterparts in patients with endometriosis. This means that the expression of PPARy in eutopic endometrium samples did not entirely represent its expression in ectopic endometrium samples, and vice versa. A further study on PPARy receptor expression in endometrial samples from subjects without endometriosis is needed to evaluate the correlation between PPARy receptor expression in eutopic and ectopic endometrium of endometriosis patients and patients without endometriosis.

CONCLUSION

Expression of PPARy receptor in eutopic and ectopic endometrial tissue was not significantly different. Therefore, we can conclude that eutopic and ectopic endometrial tissue in endometriosis patients have several similarities. This also means that PPARy receptor may have a role in the pathophysiology of endometriosis in eutopic endometrium of endometriosis patients.

Our results also demonstrates the potential role of PPARy in the pathophysiology and pathogenesis of local endometriosis in endometriosis lesions and eutopic endometrium of endometriosis patients. Knowledge on pathophysiology of endometriosis is essential to understand the pathogenesis, diagnosis, and treatment of endometriosis. Each study trying to solve the puzzle of endometriosis may further the progress towards the understanding of endometriosis.

This study could be continued with samples from patients without endometriosis as the control group, if it is ethically approved. Further studies using samples from various endometriosis samples such as superficial lesions, deeply infiltrating endometriosis, and from endometriosis patients with various stages of endometriosis could also provide significant data on cracking the puzzle of endometriosis.

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Research Article

BRCA1 Gene Q356R (1186A→G) Polymorphism and Epithelial Ovarian Cancer Incidence

Polimorfisme Gen BRCA1 Q356R (1186A→G) dan Insidensi Kanker Ovarium Epitelial

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Abstract

Objective: To determine the association between BRCA1 gene Q356R (1186A→G) polymorphism and epithelial ovarian cancer incidence.

Methods: This study is an observational analytic study with case-control study design. All patients diagnosed with epithelial ovarian cancer that were treated in the outpatient clinic and inpatient ward of the Department of Obstetrics and Gynecology, Dr. Mohammad Hoesin Hospital, Palembang who met the inclusion criteria were included in this study. DNA extraction was performed on blood samples, followed by PCR-RFLP process.

Results: We obtained the genotype distribution of BRCA1 Q356R (1186A→G) polymorphisms to be QQ genotype (wild-type) on all 50 subjects in the case group (100%) and 50 control subjects (100%). Similarly, all BRCA1 alleles have the Q allele. The results of this study found no polymorphism of the BRCA1 Q356R (1186A→G) in the ovarian cancer and control groups.

Conclusion: Polymorphism of BRCA1 gene Q356R (1186A→G) was not significantly associated with epithelial ovarian cancer incidence.

[Indones J Obstet Gynecol 2015; 3-4: 206-211]

Keywords: BRCA1 gene, epithelial ovarian cancer, polymorphism

Abstrak

Tujuan: *Untuk mengetahui adanya hubungan polimorfisme gen BRCA1 Q356R (1186A→G) dengan kejadian karsinoma ovarium epitelial.*

Metode: *Penelitian ini merupakan studi observasional analitik dengan desain studi kasus-kontrol. Semua pasien yang didiagnosis karsinoma ovarium epitel yang datang ke Poliklinik Ginekologi dan yang dirawat di Instalasi Rawat Inap Obstetri dan Ginekologi RSMH Palembang yang memenuhi kriteria inklusi masuk ke dalam penelitian ini. Dilakukan ekstraksi DNA pada sampel darah dilanjutkan dengan proses PCR-RFLP.*

Hasil: *Dari hasil penelitian ini didapatkan distribusi genotip polimorfisme gen BRCA1 Q356R (1186A→G) yaitu genotip QQ (wild type) pada semua subjek dalam kelompok kasus (100%) dan 50 subjek kontrol (100%). Begitu pula dengan alel BRCA1 yang semuanya memiliki alel Q. Hasil penelitian ini tidak menemukan polimorfisme gen BRCA1 Q356R (1186A→G) pada kelompok karsinoma ovarium dan kontrol.*

Kesimpulan: *Polimorfisme gen BRCA1 Q356R (1186A→G) tidak berhubungan secara bermakna dengan kejadian karsinoma ovarium epitelial.*

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Kata kunci: *gen BRCA1, karsinoma ovarium jenis epitel, polimorfisme*

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INTRODUCTION

Ovarian cancer has the highest mortality rate among all the gynecologic cancers, being the 7th most common cancer in the United States, representing 3% of cancer malignancy to date, and contributing to 6% of the mortality rate due to cancer in women. Epithelial ovarian cancer represents 90-95% of all ovarian cancer. They occur in all ages, but have a higher incidence in older patients. Ovarian cancer incidence in women aged 40-44 years old is 15.7 every 100,000, inclining to 54 every 100,000 in women aged 75-79 years old.[1-3]

In South East Asia, including Indonesia, ovarian cancer incidence is 5.2%. Record of new cases of ovarian cancer for 3 years at the Histopathology Unit of Sriwijaya University at Dr. Mohammad Hoesin Hospital Palembang stated that ovarian cancer incidence in 2006 constitutes 12% of all cancers, with a decline to 7%, and rebounding to 10% in 2008, which represents a third of all malignancies.[2-5]

The important risk factor for ovarian cancer is family history of breast or ovarian cancer, where about 5-10% of patients have a predisposing genetic factor.[2] Hereditary Breast and Ovarian Cancer

Syndrome (HBOC) is an inherited cancer prone syndrome. The hallmark of this syndrome is more than one family member with breast or ovarian cancer, or both, one family member having both, and one family member having breast cancer in a very young age. Germline mutation of BRCA1 and BRCA2 is the culprit and main cause of most HBOC, even though an individual with one defective allele in BRCA1 or BRCA2 from maternal or paternal origin had a second functional allele, and even when this second allele becomes non-functional, cancer can still develop through accumulation of other mutations.[6]

Some studies have stated that polymorphism in other genes can change the penetration of BRCA1 and BRCA2 for ovarian cancer.[3] The risk of ovarian cancer is higher in BRCA1 carriers. Therefore, the functional polymorphism in BRCA1 is considered to be more likely to influence the occurrence of ovarian cancer compared to BRCA2. There are 10 polymorphisms in BRCA1 with allele frequency >5% in caucasians. Nevertheless, only 5 polymorphisms provoke the substitution of amino acid, which are Q356R (1186A➙G), P781L (2731T➙C), E1038G (3232A➙G), K1183R (3667A➙G) and S1613G (4956A➙G). These polymorphisms, with the exception of Q356R (1186A➙G), are in significant linkage disequilibrium, and the effects of all of these on ovarian cancer can be ascertained by considering only the Q356R (1186A➙G).[7,8]

Anton-Culver et al carried out a population based study about the variants of BRCA1 sequence, and concluded that there is a significant correlation between BRCA1 mutation with cancer patients having a family history of ovarian cancer. Janezic et al described the distribution of 91 polymorphism cases in Caucasians, a rare form of Q356R polymorphism (1186A➙G) where the rare 1186G allele of Q356R (1186A➙G) polymorphism has a higher frequency, and associated with family history of ovarian cancer (p=0.003). It shows that this polymorphism affects the occurrence of ovarian cancer. Wenham RM et al reported BRCA1 Q356R gene polymorphism provides the odd ratio of 1.9 to the occurence of ovarian cancer.[7-9]

Most studies on BRCA1 focused only on Caucasian populations. However, allele frequency from the gene with higher penetration to ovarian cancer in the Asian population is possibly higher than Caucasian population. Asian immigrant women, especially from South Asia who lived in western countries showed a higher incidence of ovarian and breast cancer. Risch et al reported a higher mutation frequency of ovarian cancer in Indo-Pakistani descent than North-West European or British descent who lived in Ontario, Canada.[10]

The contribution of BRCA1 mutation towards incidence of epithelial ovarian cancer in Asian population, especially in Indonesia has not been elucidated yet. Based on this reason, this study needs to be done. This study focuses on polymorphism of BRCA1 gene at the Q356R (1186➙G) position.

METHODS

This study is an observational-analytic study with case-control study design which was done from September 2013 until March 2014 at Dr. Mohammad Hoesin Hospital in Palembang.

The subjects were divided into two groups, a case group with patients who have been diagnosed with epithelial ovarian cancer based on surgical and histopathologic findings (n=50), and a control group with patients who have not been diagnosed with malignancy (n=50). Subjects who met the criteria was given explanation about the study procedures and the study benefit. Subjects who agreed, signed the informed consent and had blood samples drawn.

The extraction method of DNA Chelex-100 used Phosphate Buffer Saline (PBS) pH 7.4; Safonin 0.5% on PBS; and Chelex 20% on dd H_2O pH 10.5. PCR was performed using the forward primer 5'-GGA CTC CCA GCA CAG AAA AA-3' and reverse primer 5'-TCC CCA TCA TGT GAG TCA TC-3'. The reaction was conducted in a final volume of 15 μl containing 0.5 ng/μl genomic DNA, 0.5 nmol/l forward primer, 0.5 nmol/l reverse primer, 0.2 mmol/l deoxynucleotide triphosphate (dNTP), 1.5 mmol/l MgCl2. PCR buffer, and 0.025 units/μl Taq DNA polymerase. PCR conditions consisted of an initial denaturation step at 95°C for 3 minutes, 30 cycles of 94°C for 45 seconds, 57°C for 45 seconds, and 72°C for 1 minute; an extension step at 72°C for 10 minutes, then at 4°C until digested. A digest of the amplicon was performed by combining 15 μl of the PCR amplification product, 2 μl Buffer and 10 units of AluNI enzyme in a final volume of 20 μl. Samples were incubated at 37°C for 4.5 hours, and analyzed immediately on a 2% agarose gel. The undigested arginine (R) allele can be seen as a band at 211 bp, whereas the glutamine (Q) allele

is represented by the digestion products at 134 and 77 bp.

Electrophoresis and visualisation was done on agarose gel made using 2 grams of agarose in Erlenmeyer glass with 40 ml buffer TAE added in. The mixture is mixed and heated until boiling point and then 4 μl of ethidium bromide was added. Solution was chilled in a mold for 30 minutes. Five μl PCR product was mixed with long buffer and was added to an electrophoresis device, which was set on 75 mV voltage, 350 A for 30 minutes. Visualization was performed using ultraviolet light with Gel-Doc made by BIO-RAD Laboratories USA, connected to a computer, and the visualisation result was analyzed using Quantity one software programme.

RESULTS

There were 50 women with epithelial ovarian cancer in the case group and 50 normal women or non-malignancy diagnosis in the control group. Subject characteristics can be seen in Table 1.

Table 1. Characteristics of Study Subjects

Characteristic	Case (n=50)		Control (n=50)		p
	n	%	n	%	
Age					
10 - 20	1	2.0	4	8.0	
21 - 30	5	10.0	23	46.0	
31 - 40	10	20.0	16	32.0	0.001
41 - 50	17	34.0	5	10.0	
51 - 60	16	32.0	1	2.0	
>60	1	2.0	1	2.0	
Ethnic group					
Sumateran	49	98.0	47	94.0	0.617
Javanese	1	2.0	3	6.0	
Education					
Elementary	13	26.0	7	14.0	
Junior High School	5	10.0	2	4.0	0.144
Senior High School	26	32.0	37	64.0	
Graduate	6	12.0	4	8.0	
Job					
Midwife	43	86.0	45	90.0	
Merchant	0	4.0	1	2.0	
Student	1	2.0	0	0	0.532
Farmer	1	2.0	0	0	
Civil servant	5	10.0	4	8.0	
History of contraception					
Hormonal	17	34.0	25	50.0	
Non-hormonal	0	0	1	2.0	0.139
None	33	66.0	24	48.0	
Family history of ovarian cancer					
Yes	17	34.0	9	18.0	0.068
No	33	66.0	41	82.0	

Table 1 demonstrated that there was no significant difference in terms of subject characteristics based on ethnic group, education, job, history of hormonal contraception, and family history of ovarian cancer between case group and control group, with p value of 0.617, 0.144, 0.532, 0.139 and 0.068, respectively. Only age showed a significant difference between the case and control group with p=0.001.

Based on surgical and histopathology result in the case group, the most common stage of epithelial ovarian cancer was stage IIIC and IIIB which constitutes 36% and 18% of the cases. For the remaining cases, the proportion were 2-10%. The complete result of epithelial ovarian cancer staging is presented in Table 2.

Table 2. Ephitelial Ovarian Cancer Staging Distribution in Case Group

Epithelial ovarian cancer staging	Case	
	n	%
Inadequate staging	6	12.0
stadium I A	4	8.0
stadium I C	5	10.0
stadium II A	2	4.0
stadium II C	1	2.0
stadium III A	3	6.0
stadium III B	9	18.0
stadium III C	18	36.0
stadium IV	2	4.0
Total	50	100.0

BRCA1 Q356R (1186A⇢G) Genotype

Examination result with PCR-RFLP (*Polymerase Chain Reaction - Restriction Fragment Length Polymorphism*) method showed all of the subjects in both groups have a wild type-normal genotype (QQ) of BRCA1 Q356R (1186A⇢G). There was no heterozygote genotype (QR) of BRCA1 Q356R (1186A⇢G) or mutant-homozygote (RR). Thus, there was no significant difference in the genotype of BRCA1 Q356R (1186A⇢G) between the case group and control group (p=0.999).

BRCA1 Q356R (1186A⇢G) allele on both case group and control group is Q. Thus, there was no significant difference in regards to BRCA1 Q356R (1186A⇢G) allele between case group and control group (p=0.999).

BRCA1 Polymorphism Relationship with Ephitelial Ovarian Cancer Incidence

To assess the polymorphism, we performed allele analysis with PCR-RFLP method. The results of this study showed no polymorphism discovery in the case group and the control group, indicating the absence of changes in codon 356 of glutamine (Q) amino acid to arginine (R) amino acid. In this study, the case or control groups amplicon digested on 211 bp, which means that the nucleotide was a wild type-normal or glutamine amino acid (Q) on codon 356. Polymorphism of BRCA1 (1186A⇢G) was not found in this study, which can be seen as that there was no digested amplicon on 134 bp and 77 bp or arginine amino acid (R) on codon 356. The PCR-RFLP result is shown on picture 1, for case sample number 1 to 4 (KS1 - KS4) and control number 1 to 4 (KT1 - KT4).

This proves that there was no variation of more than one phenotype that was genetically caused by the allele differences. Based on Fisher's exact test, there was no significant correlation between polymorphism of BRCA1 and ephitelial ovarian cancer incidence (p=0.999).

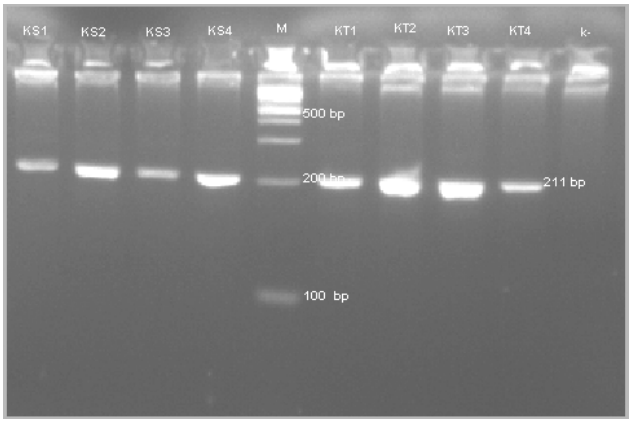

Figure 1. PCR-RFLP Result for Sample Case no 1-4 (KS1-KS4) and Control Case no 1-4 (KT1- KT4)

DISCUSSION

BRCA1 is a tumor supressor gene that will inhibit cellular function process and will lose its function when it is mutated. Growth factor causes cell growth, and if the growth noted by the body is adequate, then the growth suppression factor will be activated until homeostasis is achieved. If a muta-

tion occurred to this gene, the suppression process can not be done, and genomic instability will occur, which will ultimately lead to cancer.

The highest age distribution of ephitelial ovarian cancer patients in this study was 41-50 years old (34%) and the most common parity status was multipara (36%). This number was similar to a study by Wenham RM et al, which reported epithelial ovarian cancer to be encountered mostly in the age group of 20-50 years old (50%), and in the multiparous group (51%).[7] Several former studies have stated that ovarian cancer could occur in all age groups and that the incidence increases with age.[1-3]

Based on ethnicity, the Sumateran ethnic group makes up the largest proportion in both groups in our study, which is 98% in the case group and 94% in the control group. This is because the study was held in South Sumatera province, specifically in Palembang.

History of ovarian cancer in the family in the case group was 34%, which was higher than in the control group (18%). Menopause was more commonly found in the case group, which was 36%, compared to 4% in the control group. Schorge JO stated that risk factor of ovarian cancer that gave the most contribution to ovarian cancer incidence is family history of ovarian cancer, where 5-10% patients have an inherited genetic predisposition.[2] The most commonly used method of contraception was hormonal contraception in the case group (34%) and also in the control group (50%). Center of Disease Control (CDC) have stated that there is a decreased risk of ovarian cancer as much as 40% in women aged 20-24 years old who used oral contraceptives, with a relative risk of 0.6.[7] Other studies reported that oral contraceptive use for one year, decreases the risk by 11%. Meanwhile, the use of oral contraceptives for five years decreases the risk until 50%. The decreasing risk gets more significant with longer use.[11-13]

Ovarian cancer diagnosis in our study showed that many patients with epithelial ovarian cancer was diagnosed in the late stage, which is stage IIIC (36%) and IIIB (18%). This is caused by the fact that ovarian cancer is often asymptomatic and there is no effective screening method for ovarian cancer, so that 70% of cases are found in the late stage, and metastatic tumor have extended oustide the ovary.[13]

Our study showed that there was no significant relation of BRCA1 Q356 R (1186A→G) polymorphism with the incidence of epithelial ovarian cancer ($p>0.05$). The examination with PCR-RFLP showed that there was no variation of more than one phenotype that was genetically caused by the allele differences. This was proven by the absence of polymorphism at base pair 1186 from adenine nucleotide (A) into guanine (G); or glutamine (Q) on amino acid position 356 which is digested on 211 bp into arginine (R) which will be digested on 134 bp and 77 bp. Finally, there was no QR (heterozygote) genotype and RR (mutant-homozygote) genotype on BRCA1 Q356R (1186A→G) gene.

This result was concordant with a study by Wenham et al, who reported that BRCA1 Q356R gene has no correlation with risk of ovarian cancer. Their study stated that the frequency of heterozygote and homozygote from R allele is 9% and <1% in ovarian cancer cases, and in controls being 10% and <1%, respectively.[7]

Thus, the occurence of ovarian cancer in our study did not occur on DNA level, but it could occur due to epigenetic process. Epigenetic is a phenotype status change which is not based on the genotype changes, in other words there are gene expression changes that are not caused by changes in the DNA sequence. DNA mutations lead to changes in DNA sequence and irreversible gene expression. This epigenetic process is potentially reversible, but remains stable during cell differentiation so that this epigenetic changing can be inherited to children at cell differentiation.[14] According to Kwon, besides mutation the inactivation of BRCA1 as a tumor suppressor gene is related to hypermethylation of DNA on CpG islands. Transcriptional inactivation of tumor suppressor genes by DNA promoter hypermethylation on BRCA1 CpG island is one of the mechanism leading to development of tumor on ovarian cancer cells.[15]

Anton-Culver et al summarized that there was a significant correlation between the BRCA1 mutation with ovarian cancer cases with family history of ovarian cancer. Janezic et al found that a common polymorphism was determined in 91 Caucasian cancer cases. A rare form of polymorphism, Q356R (1186G), was significantly associated with a family history of ovarian cancer ($p=0.03$), suggesting that this polymorphism affects the occurence of ovarian cancer. In our study, there is no evidence of family history of ovarian

cancer being related to epithelial ovarian cancer incidence (p=0.068). This was thought to be caused by the difference of race, where in our study we involved the Indo-China race; meanwhile the Anton-Culver study was involving non-Hispanic white population, and Janezic et al involved Caucasians.

On this study, DNA sequencing was not done. It was based on PCR-RFLP result using *AluN1* enzyme, that showed no polymorphism on both case and control groups. *AluN1* enzyme specifically digests the nucleotide adenine (A) and guanine (G) on glutamine amino acid (Q) and arginine (R), allowing for nucleotides A or G to be identified precisely.

CONCLUSION

We can conclude that BRCA1 Q356R (1186A➙G) genotype on the epithelial ovarian cancer cases and controls was QQ (wild type-normal). Meanwhile, the allele of gene BRCA1 q356R (1186A➙G) in epithelial ovarian cancer patients as well as in controls was Q. There was no significant association between BRCA1 polymorphism with epithelial ovarian cancer incidence.

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Research Article

Treatment Response of Platinum-Based Chemoradiation on Locally Advanced Cervical Cancer

Respons Terapi dengan Kemoradiasi Berbasis-Platinum pada Kanker Serviks Stadium Lanjut Lokal

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Abstract

Objective: To evaluate the efficacy (treatment response), toxicity, and overall survival of concomitant chemoradiation (CRT) with three-weekly cisplatin-ifosfamide compared to CRT with weekly cisplatin in advanced stage cervical cancers (stage IIB-IIIB).

Method: This is a historical cohort between 32 patients receiving CRT with three-weekly cisplatin and ifosfamide and 29 patients receiving weekly cisplatin in Gynecologic Oncology division outpatient clinic and ward, Dr. Cipto Mangunkusumo Hospital.

Results: There was no significant difference in treatment response, overall and disease-free survival. There was more gastrointestinal toxicity in the cisplatin-ifosfamide arm compared to the other arm (p=0.014), but other toxicity effects were not different.

Conclusion: Platinum based-chemoradiation has the same efficacy in terms of treatment response for locally advanced cervical cancer.

[Indones J Obstet Gynecol 2015; 3-4: 212-221]

Keywords: cisplatin, concomitant chemoradiation, ifosfamide, locally advance stage cervical cancer

Abstrak

Tujuan: *Untuk menilai efektivitas (respons terapi), toksisitas, dan kesintasan keseluruhan dari kemoradiasi dengan cisplatin-Ifosfamide tiga mingguan dibandingkan dengan cisplatin mingguan pada kanker serviks stadium lanjut lokal (stadium IIB-IIIB).*

Metode: *Studi kohort retrospektif pada 32 pasien yang ditatalaksana dengan kemoradiasi cisplatin-ifosfamide tiga mingguan dan 29 pasien dengan cisplatin mingguan menjadi subjek penelitian di poliklinik dan ruangan perawatan divisi Onkologi Ginekologi RSUPN Dr. Cipto Mangunkusumo (RSCM).*

Hasil: *Tidak terdapat perbedaan bermakna pada efektivitas (respons terapi), kesintasan keseluruhan dan kesintasan bebas penyakit pada kedua kelompok tersebut. Toksisitas gastrointestinal lebih berat ditemukan pada kelompok cisplatin-ifosfamide tiga mingguan dibandingkan cisplatin mingguan (p=0,014). Sementara, tidak terdapat perbedaan bermakna pada toksisitas genitourinaria dan hematologi pada kedua kelompok.*

Kesimpulan: *Kemoradiasi berbasis platinum memberikan efektivitas yang sama terhadap penderita kanker serviks stadium lanjut.*

[Maj Obstet Ginekol Indones 2015; 3-4: 212-221]

Kata kunci: *cisplatin, ifosfamide, kanker serviks stadium lanjut lokal, kemoradiasi konkomitan*

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INTRODUCTION

Cervical cancer is the third most common malignancy in women, and the seventh in the world, with approximately 528.000 new cases in 2012. According to the data from GLOBOCAN 2008, the incidence of cervical cancer in Indonesia was 13,762 cases, with as many as 7,493 deaths.[1] This high mortality rate is because most patients present with locally advanced or terminal stage. Data in RSCM from 2006-2010, showed that there were 2,297 cases of cervical cancer, with as much as 76.7% locally advanced disease (IIB to IVB).[2]

According to the National Cancer Institute (NCI), the five-year survival rate for stage IIB-IIIB cervical cancer from 1996 to 2000 was 55%, while for stage IV was 14.6%.[3] The five-year survival rate for the same stage in 2002-2008 was 56.7%, and 16.2%.[4] Data from Dharmais Cancer Hospital, Jakarta in 1996, the survival rate of cervical cancer stage I, II, III, and IV are 56.6%, 56%, 23.7%, and 0% respectively.[5] Nuranna et al reported the five-year survival rate of cervical cancer in RSCM in 2005-2006 for stage I, II, III, and IV to be 73%, 52%, 36%, and 0%, respectively; or the survival-rate of early and advanced stage to be 67 % and 41%.[6]

This low survival rate for locally advanced stages of cervical cancer and treatment advances has triggered the shift of treatment from radiation to chemoradiation.[7-11] In 1999, based on five clinical trials, the National Cancer Institute (NCI) recommends the use of cisplatin-based chemoradiation as the standard of patient care with locally advanced cervical cancer in stage IIB to IVA.[12]

A meta-analysis by Lukka et al of eight randomized clinical trials have evaluated the role of cisplatin by itself or in combination with other chemotherapy agents, which was given concurrently with external radiation, in patients with locally advanced stage.[13] A systematic review from Green et al showed improvement of overall survival rate and progression-free survival, 10% and 13% respectively, favoring chemoradiation with cisplatin.[14]

The results of a meta-analysis of 18 randomized clinical trials by the American Society of Clinical Oncology, showed that there was a 6% (HR 0.81, p<0.001) increase in the overall survival rate, and an 8% increase in the Disease Free Survival (DFS) for 5 years. These advantages are also supported by other data demonstrating the improvement in local control and benefits in distant control are because of the systemic effects of chemotherapy.[15]

Available data shows that chemoradiation only increases the response rate by 20-30%[11] and the 5-year survival rate by 6%.[10] Efforts to improve the response to chemotherapy and survival rate in locally advanced cervical cancer are still continued. Attempts by using other chemotherapy or combined chemotherapy regimens with concomitant radiotherapy have been performed.

Geara, in a phase II study comparing chemoradiation with weekly cisplatin and paclitaxel in patients with locally advanced cervical cancer, found no significant clinical benefit.[16] Survival rate at two and five years in the paclitaxel group was 78% and 54%, while in the cisplatin group was 73% and 43%.[16]

Attempts to perform a combined chemotherapy regimen have been performed. Ranen Kanti, et al did not find significant differences in the use of cisplatin combination chemotherapy with weekly gemcitabine, with therapeutic response of only 67%.[17] Meanwhile, phase III clinical trials by Duen as-Gonzalez et al done in stage IIB and III cervical cancer comparing the standard cisplatin chemoradiation with cisplatin and gemcitabine, as well as two additional gemzitabin-cisplatin series found a significant increase in progression-free survival (PFS) in the third year (74.4% vs. 65.0%, p=0.029).[18]

GOG protocol 110 is a prospective, randomized; phase III study of 454 locally advanced cervical cancer patients. It found that combination of cisplatin-ifosfamide is superior to cisplatin alone (33% compared to 19%). Furthermore, these results showed superiority in terms of PFS (p=0.003), although there was no significant difference in the overall survival rate.[19] A phase II prospective study by Vrdoljak et al observed chemoradiation with cisplatin-ifosfamide regimen in 62 patients with locally advanced cervical cancer. Complete clinical response was achieved in 100% of patients, and both recurrence-free and overall survival rate was 88.7%.[20]

Due to GOG 110 study results, efforts in minimizing the effects of full-dose chemotherapy on locally advanced cervical cancer and improving therapeutic response and survival rate in locally advanced cervical cancer, the Gynecologic Oncology Division of Obstetrics and Gynecology Department, Dr. Cipto Mangunkusumo Hospital has been using chemoradiation with two chemotherapy regimens, which is weekly cisplatin and cisplatin-ifosfamide three weekly as the standard of treatment for locally advanced cervical cancer.

This study will evaluate the existing treatment regimens in terms of assessing better treatment response and survival rate, as well as toxicity profile as a part of protocol evaluation in the Gynecologic Oncology division.

METHODS

This is a historical cohort carried out in the Gynecologic Oncology outpatient clinic, radiotherapy department, and Gynecology Oncology division ward, Dr. Cipto Mangunkusumo Hospital (RSCM), from December 2013 until October 2014. The study subjects are patients who were treated using chemoradiation using cisplatin-ifosfamide and weekly cisplatin in RSCM from August 26th 2010 until June 28th 2014 who met the inclusion criteria. The total sample size in this study was 61 patients.

The inclusion criteria are stage IIB-IIIB cervical cancer patients who received chemoradiation with 3-weekly cisplatin-ifosfamide or weekly cisplatin,

with performance status based on the Eastern Cooperative Oncology Group (ECOG) criteria with score ≤2; having peripheral blood result of Hb ≥10g%, leukocyte ≥5000/mm^3, thrombocyte ≥150,000/mm^3; SGOT <27U/l, SGPT <36 U/l); and renal status of ureum <50 mg/dl, creatinin 0.60-1.20 mg/dl, CCT >68 ml/minute; had been given at least 3 series of chemotherapy; had the tumor size examined with transrectal USG; and underwent post-therapy follow up in the gynecologic oncology outpatient clinic of RSCM for at least 3 months post-therapy.

The exclusion criteria are cervical cancer patients with histopathologic findings other than squamous cell carcinoma, adenocarcinoma, and adenosquamous carcinoma; received any previous therapy including surgery, radiation, or chemoradiation; suffering other severe comorbidities (uncontrolled cardiovascular disease, uncontrolled diabetes mellitus, severe psychological impairment, active peptic ulcer) or immunodeficiency/ HIV; having primary cancer in other organs (synchronous tumor); and incomplete radiation therapy.

The steps of this study are, after receiving ethical clearance; the medical records of the locally advanced cervical cancer patients (IIB-IIIB) in the gynecologic oncology outpatient clinic who underwent one of the two chemoradiation therapies were collected. The medical data was taken from patients who were diagnosed from August 2010 until November 2013. Selection of medical records corresponded to the inclusion and exclusion criteria. The demographic data, clinicopathologic data in the medical record, and data added upon patients' admission were recorded. Radiation is divided into external radiation (2.0 Gray, 5 dose/ week, 25 times) and internal radiation/brachytherapy (2 x 8.50 Gray (850 rad) or 3 x 7 Gray at point A). Meanwhile, chemotherapy was divided into weekly cisplatin regimen (40 mg/m^2 dose in 6-8 hour prior to radiation, 1 dose/week, minimal 3 times), and cisplatin-ifosfamide regimen (cisplatin 50 mg/m^2 and ifosfamide 2 gr/m^2 given with uromitexan, for 3 weeks, 4 series). Chemoradiation response was evaluated by degree of tumor regression, defined by comparison between tumor size prior to and 3-months after therapy. The patients were then evaluated every month during therapy to observe the toxicity, until 3-months after the therapy was completed. The follow-up data included recurrence, data from the last visit, patient's latest condition, which were all documented from the medical records. Patient's latest condition was inquired through telephone to determine whether the patients last condition.

The statistical analysis included descriptive analysis, bivariate analysis, and survival rate analysis with Kaplan-Meier method. All the data analysis was performed using STATA ver 10 (Stata Corporation LP., Texas, USA).

Independent variable in this study is the type of cervical cancer therapy, whereas the dependent variables are the treatment response (complete response, partial response, stable tumor, and progressive tumor), survival rate (overall survival and disease free-survival), and toxicity (gastrointestinal, genitourinary, and hematological toxicities). However, the confounding variables included age, education, parity, cervical cancer staging based on FIGO (IIB, IIIA, IIIB), tumor size, performance status, histopathologic findings, tumor differentiation, cervical cancer therapy, and radiation overall treatment time (OTT).

RESULTS

There were 61 cases that fulfilled the selection criteria, with 32 cases receiving cisplatin-ifosfamide chemoradiation and 29 cases receiving weekly-cisplatin chemoradiation.

Assesment of treatment response between the two groups was performed at 3 months after completion of radiation therapy, and done through gynecological and ultrasound examination.

From the figure above, we obtained a hazard ratio (HR) of 1.4, but it was not found to be statistically significant (p=0.71). From the DFS rate there is intersection of the curve that did not fulfill the HR assumption. It showed no statistical significance (p=0.78).

Evaluation of toxicity between radiation group and chemoradiation group was performed based on RTOG and ECOG criteria. There were gastrointestinal toxocity, genitourinary toxicity, and hematologic toxicity, which were the most common toxicities encountered and mentioned in published references.

Table 1. Distribution of the Clinicopathologic Characteristics Based on Type of Chemoradiation

Clinicopathologic characteristics		Cisplatin-Ifosfamide (n=32)		Weekly Cisplatin (n=29)		Total		p-value
		n	%	n	%	n	%	
Performance status	0	19	59.4	21	72.4	40	65.6	0.487
	1	13	40.6	4	13.8	17	27.9	
	2	0	0,0	4	13.8	4	6.5	
Stage	IIB	9	28.1	8	27.6	17	27.9	0.863
	IIIA	1	3.1	1	3.4	2	3.3	
	IIIB	22	68.8	20	69.0	42	68.8	
Tumor size	<4 cm	10	31.25	12	41.4	22	36.1	0.370
	>4 cm	22	68.75	17	58.6	39	63.9	
Histopathology type	Squamous cell carcinoma	23	71.9	18	62.1	41	67.2	0.700
	Adenocarcinoma	7	21.9	9	31.0	16	26.2	
	Adenosquamous Carcinoma	2	6.2	2	6.9	4	6.6	
Degree of Differentiation	Well	7	21.9	8	27.6	15	24.6	0.831
	Moderate	18	56.2	16	55.2	34	55.7	
	Poor	7	21.9	5	17.2	12	19.7	
OTT*	<62 days	13	40.6	17	58.6	30	49.2	0.160
	>62 days	19	59.4	12	41.4	31	50.8	
Total		**32**	**100**	**29**	**100**	**61**	**100**	

[a] *Pearson chi-square test*
**OTT/Overall Treatment Time: Total period of radiation therapy from the first external radiation to the last internal radiation.*

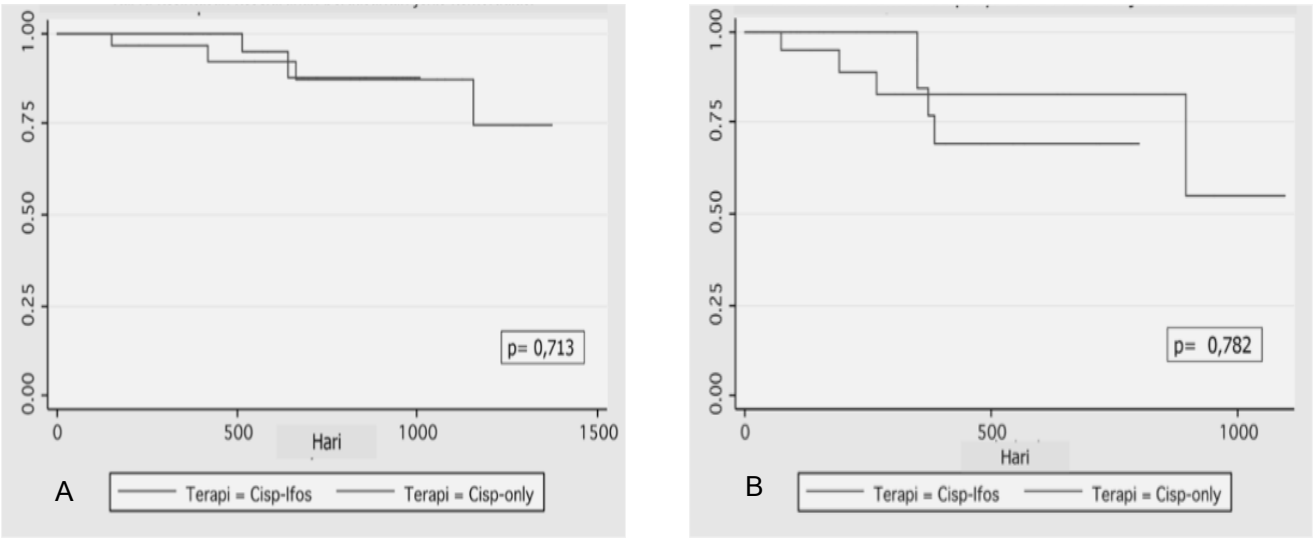

Figure 1. Overall Survival (A) and Disease-Free Survival (B) Rate based on Type of Chemoradiation.

Table 2. Comparison of Treatment Response According to Type of Chemotherapy

Treatment Response	Cisplatin-Ifosfamide n (%)	Weekly Cisplatin n(%)	Total n(%)
Complete response	30 (93.8)	26 (89.7)	56 (91.8)
Partial response	2 (6.2)	1 (3.4)	3 (4.9)
Stable tumor	0 (0)	0 (0)	0 (0)
Progressive tumor	0 (0)	2 (6.9)	2 (3.3)
Total	32 (100)	29 (100)	61 (100)

Pearson chi-square test; p = 0.290

Table 3. Distribution of Toxicity Based on Type of Chemoradiation

Toxicity		Therapy				Total n (%)	p-value
		Cisplatin-Ifosfamide		Weekly Cisplatin			
		n	%	n	%		
Gastrointestinal							
	Degree 0	-	-	-	-	-	
	Degree 1	10	31.3	11	37.9	21 (35)	
	Degree 2	22	68.7	18	62.1	40 (65)	0.014
	Degree 3	-	-	-	-		
	Total	32	100	29	100	61 (100)	
Genitourinary							
	Degree 0	-	-	-	-		
	Degree 1	29	90.6	26	89.7	55 (90.2)	
	Degree 2	3	9.4	3	10.3	6 (9.8)	0.337
	Degree 3	-	-	-	-		
	Total	32	100	29	100	61 (100)	
Hematologic							
	Degree 0	-	-	-	-	-	
	Degree 1	12	37.5	13	44.8	25 (41)	
	Degree 2	14	43.8	14	48.3	28 (45.9)	0.331
	Degree 3	6	18.7	2	6.9	8 (13.1)	
	Total	32	100	29	100	61 (100)	

DISCUSSION

This study is a historical cohort study on locally advanced cervical cancer (Stage IIB, IIIA, and IIIB) in the Division of Gynecologic Oncology, Department of Obstetrics and Gynecology, RSCM. In the period of December 2013 to October 2014, we obtained 61 samples that meet the inclusion criteria and completed follow-up for up to three months after completing treatment, which consisted of 32 cases who received chemoradiation therapy with three-weekly cisplatin-ifosfamide, and 29 cases who received chemoradiation therapy with weekly cisplatin. These patients received chemoradiation treatment between August 26, 2010 to June 28, 2014.

This study has limitations because the sample size is relatively small, and employed historical cohort as research design so that there was no randomization in sample collection. The advantages of this research is that the treatment response was assessed for three months after finishing radiation treatment and monitoring was continued afterward with minimal period of monitoring of up to three years.

Of the 61 subjects in the study, we obtained an age range of 35-66 years old, with a mean of 49 years old. Similar results were obtained in phase II multicenter clinical study conducted by Kato et al in 2009 in China, Philippines, and Vietnam; where the mean age was 48.5 years old.[18]

Cornain et al reported that the incidence of cervical cancer at age over 50 years old is two times higher (13.9/100,000) than at under 50 years (6.7/100,000), and the highest distribution is in the 45-49 years old group.[21] Research conducted by Aziz MF in RSCM in 2001 stated a risk of developing cervical cancer over the age of 50 years to be higher than that of those under the age of 50 years with an OR of 2.53 (95% CI 1.27 to 5.05).[22] Nuranna et al in 2011 have reported the distribution of characteristics of cervical cancer in the Division of Gynecologic Oncology RSCM, with the highest frequency being in the 35-64 year age group which constituttes 87.3% of cases.[2] Gunawan et al in 2012, found that more than 50% of patients with cervical cancer aged 46-68 years.[23] Moreover, Nuranna et al in 2014 in Dr. Cipto Mangunkusumo found 66.2% of cervical cancer patients were aged 30-49 years and 33.1% were aged >50 years.[6]

Range of parity of the sample in this study is 0 to 8, with a mean of 3.29 ± 1.7 children. The highest frequency is in the parity >2 group (60.1%), while in the parity 1-2 was 36.1%. MF Aziz in his research reported cervical cancer cases with parity ≥6 was up to 78 cases (75%), compared to the parity 0-1, which was 49 cases (25%).[22] In this study, the largest proportion of cervical cancer cases belonged to the 40-60 years old age group, with equal education level of elementary, junior high, or high school, which was 34.4%, 31.2%, and 34.4%.

The highest number of cervical cancer cases is in stage IIIB with 42 cases (68.9%), while stage IIB had 17 cases (27.9%) and there were only 2 cases of stage IIIA cancer (3.28%). Similar findings were observed on multicenter clinical investigations conducted by Kato et al.[18] Negi R et al supported this study with similar proportion of cases; 34 cases of stage IIB (38%), 54 cases of stage IIIB (60.7%), and only 1 case of stage IIIA (1.1%).[24] Gunawan et al in Dr. Cipto Mangunkusumo in 2012 obtained 16 cases of stage IIIB and 15 cases of stage IIB.[23]

Tumor size <4 cm was found in 22 cases (36.1%), 10 cases in the group of cisplatin-ifosfamide and 12 patients in weekly cisplatin, whereas tumor size >4 cm was found in 22 cases (68.8%) in the cisplatin-ifosfamide group, and 17 cases (58.6%) in the weekly cisplatin group. Rose PG et al also found a similar distribution of tumor diameter, tumors ≤40 mm with 76 cases (14.7%) and >40 mm with 440 cases (85.3%) in locally advanced cervical cancer.[10] Kong et al found 215 cases with tumor size >4 cm and 40 cases with size <4 cm.[25] Gunawan et al also observed that the more common tumor diameter is >4 cm for 28 cases, compared to the size of <4 cm with only 4 cases.[23] Another study conducted by Nuranna et al in 2014 found that 74.4% of cases had tumor size >4 cm, and only 25.3% had tumor size <4 cm. These study findings support a similar characteristic in terms of tumor size.[6]

Median ECOG performance status of the patient is 0 and 1. There were only four subjects with ECOG 2 (13.8%) in the group of weekly cisplatin. Similar distribution was found in the study conducted by Kato et al in the Philippines and Vietnam, where they found a score of 0 in 12 cases and 10 cases.[18] Restriction of ECOG score <2 was done in order to avoid bias in the results of treatment response due to patient's physical condition.

The most commonly encountered histopathologic type is squamous cell carcinoma with 41 cases (67.2%), followed by adenocarcinoma consisting of 16 cases (26.2%), and 4 cases (6.6%) of adenosquamous type. This finding is consistent with another study by Nuranna et al in 2011 in RSCM found that the most common histopathologic type of cervical cancer is squamous cell carcinoma with 1322 cases (70.2%) and 285 adenocarcinoma cases (15%).[2] Sakata et al in Japan (2008) also reported 231 cases of squamous cell carcinoma (94.2%) and 11 cases of adenocarcinoma (4.9%), while for adenosquamous was not encountered among the 226 cases.[26] Rose et al encountered the same results, with 472 cases of squamous cell carcinoma (89.7%), while there were only 18 cases of adenocarcinoma (3.4%).[10] Kanti et al obtained the distribution of squamous cell carcinoma to be 56 cases (86.5%), 4 cases of adenosquamous (5.9%), and 5 cases of adenocarcinoma (7.6%) of a total of 67 cases.[17] Kong et al showed similar results, namely squamous cell carcinoma for 238 cases, followed by adenocarcinoma and adenosquamous carcinoma at 9 cases.[25] Another study in 2014 by Nuranna et al, found 71.6% squamous cell carcinoma, followed by 11.9% adenocarcinoma and

13.6% adenosquamous, which was concordant with the other studies.[6]

The degree of differentiation holds a role in predicting the prognosis of cervical cancer. In general, a poorer differentiation may indicate a worse prognosis.[27] In this study, 34 cases (55.74%) were moderately differentiated, 15 cases (24.59%) were well differentiated, and 12 cases (19.67%) were poorly differentiated. Similar to our results, the study by Gunawan et al found that 23 cases (71.88%) were moderately differentiated, then 7 cases (21.87%) with well differentiation, and 2 cases (6.25%) with poor differentiation.[23] Nuranna et al in 2014 showed moderate differentiation made up 55.2%, 20.6% were well-differentiated, and 16.6% with poor-differentiation.[6]

Evaluation of the confounding demographic and clinicopathologic variables such as age, performance status, stage, tumor size, histopathologic type, degree of differentiation, and OTT radiation in both treatment groups showed no statistical significance. This result showed that there was an equal distribution of confounding variables in both groups of chemoradiation types. Thus, the effect of confounding variable can be eliminated.

In our study, treatment response was assessed three months after treatment in either groups with adjuvant concurrent chemoradiation of 40 mg/m^2/week cisplatin or chemoradiation with cisplatin-ifosfamide three-weekly. According to the operational definition, treatment response can be divided into complete response, partial response, stable tumors, and progressive tumors. In the group of cisplatin-ifosfamide, as many as 30 patients had a complete response (93.8%), and 2 patients had partial response (6.2%). This result is worse compared to the study by Vrdoljak et al, who achieved 100% complete response. Meanwhile in the group receiving cisplatin alone, as many as 26 patients achieved complete response (89.7%), 1 patient had partial response (3.4%), and 2 patients had progressive tumor (6.9%). There was a total of 5 patients (8.2%) who did not achieve complete response consisting of 3 cases (4.9%) who had partial response and 2 cases (3.3%) who had progressive tumor.

Two cases of partial response belonged to the cisplatin-ifosfamide group, and 1 case in the weekly cisplatin group. All cases with progressive tumors were from the weekly cisplatin group. Although no significant difference were found in the results of this response assessment, result of progressive tumor needs special attention because it represents an unresponsive condition.

Radiation protocols used in this study was 50 Gray external radiation and 2 x 8.50 Gray (850 rad) or 3 x 7 Gray for internal radiation. The duration of radiation was similar between both study groups. Similarly, Negi et al used a total dose of 81 Gray to point A with OTT anticipated to be 7 to 10 weeks.[24] Vrdoljak et al employed external radiation dose of 45 Gray plus 2x30 Gray internal radiation.[20] Kong et al employed 45 Gray external radiation in 25 fractions over 4-5 weeks with internal radiation of 30 Gray in 5 fractions at 1-week intervals.[25] This varying results may due to retrospective study. If any future prospective study is to be conducted, the type of radiation and radiation scheme employed should be determined in detail so that the radiation dose will be consistent.[20]

In this study, the median OTT was 63 days. In the group of cisplatin-ifosfamide, average OTT is 69 days, while the average for weekly cisplatin is 59 days. OTT for radiation is divided into two categories, OTT <62 days and >62 days. There were no significant differences in the distribution of radiation OTT in both study groups. Although radiation OTT variable in this study is a confounding variable, assessment of treatment response in both groups showed no significant difference (p=0.61). We should also consider that in the group with OTT <62 days, there were two cases that underwent progressive tumors, while in the group of more than 62 days there were three cases with partial response.

In a study conducted by GOG 85, GOG 120 and RTOG 90-01 the median OTT were 64, 63 and 58 days, respectively.[10,11,28] Unlike the GOG 85 and RTOG 90-01 study, in this trial the median OTT was similar with GOG 120, which was 63 days. Rose et al obtained results of median OTT being 63 days in the chemoradiation with cisplatin group, 65 days in the chemoradiation using cisplatin + fluorouracil + hydroxyurea group, and 62 days in the chemoradiation with hydroxyurea group.[10]

From the data obtained, survival analysis was performed to evaluate the OS and DFS. DFS was assessed from 8 cases that experience recurrence among the 56 cases. Recurrence was diagnosed on physical examination, histopathologic, and imaging data found in the medical record. There were 8 cases of recurrence, in both treatment groups.

There was no local recurrence in both groups. There were 4 cases of regional recurrence in both treatment groups, also 4 groups of recurrence with distant metastases to lungs and liver as target organs.

Two-years survival rate of cisplatin-ifosfamide was 89.4%, while for cisplatin alone was 86.5%. Vrdoljak et al reported DFS and OS of 88.7% at a median follow-up of 4 years.[20]

The survival curve showed that there was a hazard ratio of 1.4 in the sense that at any time there was a probability of death of 1.4 times in the cisplatin-ifosfamide compared to the cisplatin alone group. However, this difference was not proven to be statistically significant (p=0.71).

In terms of disease-free survival rate, in the cisplatin-ifosfamide group was 87.1% in the first year, while it was 82.7% in the cisplatin group. However, in the second year, cisplatin-ifosfamide DFS dropped to 68.8%, while cisplatin alone was maintained at 82.7%. (p=0.78). The presence of disease-free survival rate curve intersection occurred because of the design of retrospective study, causing no further monitoring protocol, instead relying solely on data contained in the medical record. Apart from the problem of data validity, this picture can be seen from the medical records of the two-year recurrence and after treatment monitoring.

Kong et al also found no significant difference in progression-free survival (PFS) rate and on the overall survival rate in the chemoradiation group compared with weekly chemoradiation to be 74.6% vs 64.3% and 78% vs. 73% (p=0.7105 and p=0.237).[25] While Roy found the 16-month DFS rate of 83% in the cisplatin chemoradiation with gemcitabine compared to 73% in weekly cisplatin.[29] Based on this DFS rate, other types of regimen were found to not be better than cisplatin-ifosfamide.

In this study, the incidence of degrees 0 acute toxicity was not found in terms of gastrointestinal, genitourinary, and hematology toxicities. Proven gastrointestinal toxicity was significantly different (p=0.014). Distribution shows that the most common degree of gastrointestinal toxicity was grade 2, complaining of nausea and vomiting which required antiemetic or abdominal pain which required analgesics, diarrhea that required treatment, rectal and abdominal pain requiring analgesics. There were 22 cases (68.7%) in the cisplatin-ifosfamide therapy group and 18 cases (62.1%) in the cisplatin group who experienced toxicity degree 2. A total of 21 cases (35%) were spread evenly in both treatment groups who experienced toxicity grade 1 in the form of nausea and abdominal discomfort which did not require any treatment, or increased frequency of bowel, or anal sore that did not require medication.

Kong et al also found that gastrointestinal toxicity is more common in monthly compared to weekly chemoradiation, 6 cases compared to 22 cases.[25] This toxicity included diarrhea (4 cases on monthly chemoradiation and 2 cases on weekly chemoradiation), nausea (17 cases in the monthly chemoradiation and 2 cases in weekly chemoradiation), and vomiting (11 cases on monthly chemoradiation and 7 cases on weekly chemoradiation).

In genitourinary toxicity, the highest degree of toxicity was grade 2 in the form of urinary frequency/nocturia less than every 1 hour, dysuria, urgency and bladder spasms that require treatment. There were three cases in each treatment group with grade 2 toxicity (9.4% and 10.3%). While 52 cases (90.2%) experienced grade 1 toxicity in the form of urination two times more often than usual, dysuria, or who did not require emergency treatment (90.6% in cisplatin-ifosfamide and 90.2% in cisplatin alone). There was no significant difference in terms of genitourinary toxicity (p=0.337).

Hematologic toxicity grade 3 in the form of anemia with hemoglobin level reaching 6.5-8 g/dl, or leukopenia (2000-3500 leukocytes/μl), or thrombocytopenia (platelet 50,000-100,000/μl) occurred in 18.7% of cases in the cisplatin-ifosfamide group and 6.9% of cases in the cisplatin alone group. While toxicity level 1 and 2 were distributed evenly in the two treatment groups. There were no significant differences between the two treatment groups in terms of hematologic toxicity (p=0.331).

Kong et al showed that the toxicity of monthly chemoradiation was greater than weekly chemoradiation, with 22 cases compared to 12 cases. A total of 7 cases on a monthly chemoradiation were anemic, more than that found in weekly chemoradiation, which were only 3 cases.[25] Likewise, leukopenia on monthly chemoradiation amounted to 11 cases, while weekly chemoradiation only had 7 cases. Thrombocytopenia in monthly chemoradiation consists of 4 cases, more than weekly chemoradiation with only 2 cases.

Major acute toxicity can be seen in hematologic and gastrointestinal toxicity. There were no treatment-related deaths. None of the patients stopped the chemotherapy by request or due to its toxicity. Overall, these three toxicity effects were treatable with appropriate therapy according to patient's complaints, thus preventing incomplete therapy.

CONCLUSION

Chemoradiation with three-weekly cisplatin-ifosfamide and weekly cisplatin have the same efficacy in patients with locally advanced cervical cancer, but weekly cisplatin chemoradiation is more tolerable. Our historical cohort design may bring about selection bias, that may affect the results of the study even though it had been minimized by performing confounding variables equality test. In addition, this study used a long period of monitoring time and closed data that may affect the validity of the results.

Nevertheless, the treatment of locally advanced stage cervical cancer in consideration of control of local recurrence, regional and distant recurrence remains an issue, thus allowing another potential therapy combination. It is suggested to conduct a multicenter randomized trial of prospective cohort to investigate new chemotherapy regimens assessing effects of particular radiosensitizer and the effects of chemotherapy in cervical cancer in order to improve the survival and quality of life of patients with locally advanced cervical cancer.

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Research Article

Fascin Expression as Prognostic Factor for Survival in Advanced Epithelial Ovarian Carcinoma

Ekspresi Fascin sebagai Faktor Prognostik Kesintasan pada Kanker Ovarium Jenis Epitelial Stadium Lanjut

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Abstract

Objective: To evaluate fascin expression as a prognostic factor and its correlation with survival and clinicopathologic factors (degree of differentiation and stage) in epithelial ovarian carcinoma.

Methods: This study is prognostic study with historical cohort design. Fascin was analyzed in paraffin block sections of 33 advanced stage ovarian carcinoma patients using immunohistochemistry. Fascin expression was tested for its correlation with overall survival as well as with grade and stage of the cancer.

Results: In this study, fascin expression has no correlation with survival. In the period of 17-22 months, samples with high fascin expression had a HR of 1.59 (95% CI=0.38-6.67, p=0.449), but in the period of 17-23 months, both groups had comparable HR. In the period of more than 23 months, samples with high expression of fascin had a better HR of 0.40 (95% CI=0.04-4.38, p=0.449). No significant correlation was found between fascin expression with grade (OR=2.08, 95% CI=0.44-9.84, p=0.442) and stage (OR=2.70, 95% CI=0.39-18.96, p=0.360).

Conclusion: In this study, there was no correlation between fascin expression and survival, and also no correlation between fascin, grade and stage. Further study with a larger, more homogenous sample, analyzing confounding factors is needed.

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Keywords: advanced stage ovarian carcinoma, fascin, survival

Abstrak

Tujuan: *Melakukan penilaian terhadap hubungan ekspresi fascin sebagai faktor prognostik kesintasan kanker ovarium stadium lanjut secara umum, serta hubungan antara faktor klinikopatologis (derajat diferensiasi dan stadium) pada khususnya.*

Metode: *Penelitian ini merupakan studi prognostik dengan desain kohort retrospektif. Fascin dianalisa melalui 33 sampel blok paraffin dan dilakukan pemeriksaan imunohistokimia. Data diperoleh dari status medis, wawancara dengan pasien/keluarga atau melalui telepon.*

Hasil: *Pada periode 17-22 bulan, fascin dengan ekspresi tinggi memiliki HR=1,59 (IK 95%=0,38-6,67, p=0,449), tetapi pada periode 17-23 bulan, kedua kelompok memiliki hazard rasio yang sama. Pada periode lebih dari 23 bulan, fascin dengan ekspresi tinggi memiliki HR=0,40 (IK 95%=0,04-4,38, p=0,449). Tidak terdapat korelasi bermakna antara eksresi fascin dengan derajat diferensiasi dengan OR=2,08 (IK 95%=0,44-9,84, p=0,442) dan stadium (OR=2,70, IK 95%=0,39-18,96, p=0.360).*

Kesimpulan: *Didapatkan hasil yang tidak bermakna antara hasil ekspresi fascin yang tinggi dengan kesintasan, serta tidak terdapat korelasi bermakna antara ekspresi fascin dengan derajat diferensiasi. Diperlukan penelitian lebih lanjut dengan jumlah sampel yang lebih besar dan lebih homogen, dengan menganalisa faktor-faktor perancu yang dapat ditemui.*

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Kata kunci: *fascin, kanker ovarium stadium lanjut, kesintasan*

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INTRODUCTION

Epithelial ovarian cancer (EOC) is the most lethal of all gynecologic malignancies, and the fifth cause of mortality. Women with organ-confined tumors have an excellent prognosis, but the majority of early stage cancer is asymptomatic, and more than two-thirds (70-75%) of patients are diagnosed with advanced disease and has often spread as diffuse small-volume tumor deposits.[1]

A better understanding of ovarian cancer is urgently needed for patients who currently present with advanced disease requiring major surgery and adjuvant chemotherapy, with the great majority experiencing recurrence. Relapse occurs in the majority of advanced stage patients after complete response to initial treatments; and at least 70-90% of these patients eventually die with drug-resistant cancers, with only 10-30% showing long-term survival. Despite many therapeutic improvements, the

development of secondary metastatic tumors that are resistant to conventional treatment remains a major cause of morbidity and mortality.[1,2] Tumor invasion and metastasis are the result of highly coordinated processes involving multiple intracellular and extracellular factors. Understanding the early events that enable carcinoma cell migration and invasion is an important research goal that has the potential to improve early diagnosis of aggressive tumors and stimulate new approaches towards molecular adjuvant therapies. Carcinoma cell migration is facilitated by the altered differentiation status of the epithelial cells, including changes in cell and cell matrix adhesion properties and in the organization of the actin cytoskeleton. The cytoskeleton is a complex network that includes three types of protein: actin filaments, microtubules and intermediate filaments. Changes in cytoskeletal components or associated binding proteins may be implicated in the progression and metastasis of tumors.

Fascin is a globular actin cross-linking protein that has a major function in forming parallel actin bundles in cell protrusions that are key specializations of the plasma membrane for environmental guidance and cell migration. Fascin is a highly conserved actin-bundling protein, widely expressed in mesenchymal tissues and the nervous system, and is low or absent in adult epithelium. Recent data from a number of studies have highlighted that fascin is up-regulated in many human carcinomas and in individual tissues, correlating with the clinical aggressiveness of tumors and poor patient survival. In cell culture, over-expression or depletion of fascin modulates cell migration and alters cytoskeletal organization. The identification of biomarkers to provide more effective early diagnosis of potentially aggressive tumors, or identify tumors susceptible to targeted therapies, is an important goal in clinical research.[3-8]

There were many studies on fascin that showed correlation between fascin and malignancy. Daponte reported that strong fascin immunoreactivity was associated with poor prognosis; patients with low fascin expression had a median survival of 36.5 months versus 32 months for high fascin expression (p=0.041), and the median PFl was 24 versus 17.5 months, respectively (p=0.034). Fascin expression is an independent prognostic factor for survival of advanced ovarian serous carcinoma, and may represent a novel therapeutic target for patients with aggressive forms of ovarian cancer.[9] Eun et al reported that fascin expression was detected in the majority of borderline (100%, 32/32) and malignant tumors (90.5%, 67/74), but it was not seen in normal ovarian surface epithelial cells and benign tumors (p<0.001). Fascin expression was significantly correlated with the occurrence of peritoneal metastases in the carcinomas (p= 0.043).[10] Fascin overexpression has an important role in invasiveness and recurrence of uroephitelial malignancy. There were significant numbers of fascin-1-positive cells (50% of the neoplastic cells) in uroepithelial carcinomas in situ (n=10). These findings suggest an association between increased fascin-1 expression and increased invasiveness of carcinomas in the urinary bladder.[11]

The aim of this study is to evaluate the correlation between fascin expression, survival and clinicophatologic factors in advanced stage ovarian carcinoma. Fascin has emerged as a very interesting candidate for biomarker because its expression is low or absent in the majority of normal adult epithelium, yet up-regulation of the protein has been reported in many forms of human carcinoma. Irrespective of the tissue source of the tumor, high levels of fascin expression in primary carcinomas has been consistently correlated with a clinically aggressive phenotype and poor prognosis.[9]

METHODS

This study is prognostic study with historical cohort design. Thirty-three patients with a diagnosis of advanced stage epithelial ovarian carcinoma (91% were stage III and 9% were stage IV) who received postoperative chemotherapy (TC: 175 mg/m^2 paclitaxel and carboplatin after calculating the area under the concentration curve) were included in this study. Due to limited sample, only 23 patients had complete cycles (6 cycles). The patients who had complete medical records were selected for further analysis. Patients were examined, diagnosed and underwent therapy in the Gynecologic Oncology Division, Obstetrics and Gynecology Department, RSCM, Jakarta.

Pathological samples from paraffin-embedded tissue from each of the above patients were included in this study. Clinicopathologic information was obtained from medical records. Cancer patients were classified after a staging laparotomy; the most common initial surgical procedure consisted of abdominal hysterectomy, bilateral salphyngo-oophorectomy, omentectomy, and pelvic and

paraaortic lymph node dissection. The surgery was classified as complete resection when no macroscopic tumor remained. Surgical procedures were carried out in the Division of Gynecologic Oncology, University of Indonesia. All slides were reviewed by the pathologists.

Survival analysis was performed after the patients were dichotomized according to a fascin immunohistochemistry score of <3 and ≥3. The optimal cutoff point of fascin expression based on immunohistochemistry was calculated in consideration of sensitivity and specificity of ROC curve analysis for the survival. This research started at September 2013 until October 2014, with samples being taken from 2006. At the time of analysis, 11 of the 33 patients were deceased due to their disease.

Immunohistochemical staining of ovarian tissue was performed in a commercially available automated immunostainer. The samples were fixed in 10% buffered formalin solution, embedded in paraffin blocks and cut at 4 mm sections. For fascin expression, slides were incubated for 20 minutes at room temperature with clone IM20 (Novocastra, Newcastle upon Tyne, UK) diluted to 1:300.

All slides were initially evaluated by a pathologist. During a subsequent joint evaluation, a final consensus of immune reactivity score was obtained and used for statistical analysis. Cytoplasmic immune reactivity of tumor cells was assessed in comparison with tonsil specimen, which was used as positive controls. Two aspects of immune reactivity were semi-quantitatively evaluated: the extent and the intensity of staining. Intensity was considered as "weak to moderate" when it was less than that of tonsillar tissue and "intense" when it was similar to that of the tonsil. After preliminary analysis, the pathologists involved in the evaluation of immunohistochemical staining realized that the observed differences in immunoreactivity were best represented by counting only the cellular subpopulation showing intense immunohistochemical staining and expressing this as the HIES (highest immunohistochemical expression score). To calculate HIES, a value from 0 to 4 was assigned according to the percentage of cells showing intense staining (0: 0%, 1: <25%, 2: 25-50%, 3: 50-75%; 4: >75%).

Univariate analysis of categorical variable will be presented in percentages and frequencies. Overall survival was defined as the interval from the date of surgery to death from ovarian carcinoma or at the end of the study period on 27th October 2014. Correlation between fascin expression and survival will be tested using Kaplan Meier Method, Cox Regression and Life Table. Bivariate analysis of correlation between fascin expression and clinicopathologic characteristics, will be analysed using Fisher test. SPSS software v.22.0 for Mac was used for the statistical analysis, $p<0.05$ was considered to be statistically significant.

RESULTS

We included 33 tissue samples of patients with EOC. Our sample had an average age of 49.95 years, 39.4% in the age group of 40-49 years. Only 20 patients (60.0%) had optimal debulking surgery with complete resection (no macroscopic residual disease), and 39.4% had residual tumor more than 2 cm in diameter. In terms of histologic type, 17 patients (51.5%) had serous EOC, 9/33 had clear cell carcinoma, 4/33 had endometrioid, and 3/33 had mucinous type EOC, with 6/33 patients having well differentiated tumors, and 25/33 had moderate-poor differentiated tumors.

Following assessment of fascin immunoreactivity, 14 (42.4%) tumor samples were classified as having low fascin expression (immunohistochemistry score <3) and 19 (57.6%) having high fascin expression (immunohistochemistry score ≥3).

The cutoff point was determined from the sensitivity and specificity analysis and ROC curve analysis to survival. We found that the cutoff IRS

Table 1. Correlation between Fascin Expression and Survival

Time	Variable	Coefficient	SE	Wald	df	value p	HR	95% CI	
								Min	Max
0-17 months	Fascin ≥3	0.461	0.733	0.396	1	0.529	1.59	0.38	6.67
>23 months	Fascin ≥3	-0.929	1.229	0.572	1	0.449	0.39	0.04	4.39

score was 3 with sensitivity of 54.5% and specificity of 40.9% with AUC=0.450 (95% CI=0.240-0.661).

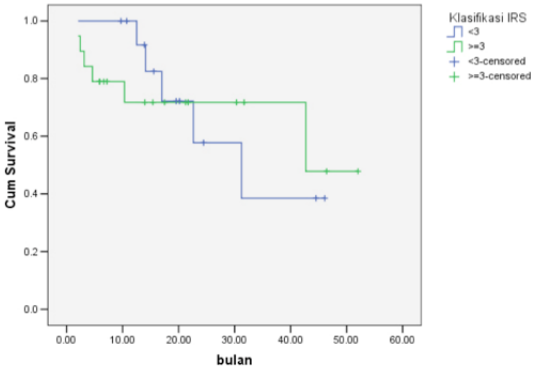

Figure 1. Kaplan Meier Curve Associated with Fascin Expression

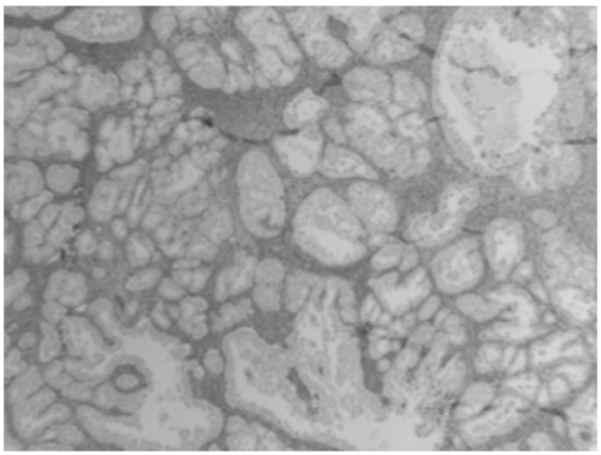

Figure 2A. Clear cell carcinoma with immunostaining for fascin. Note low cytoplasmic staining, negative intensity. IRS score : 0

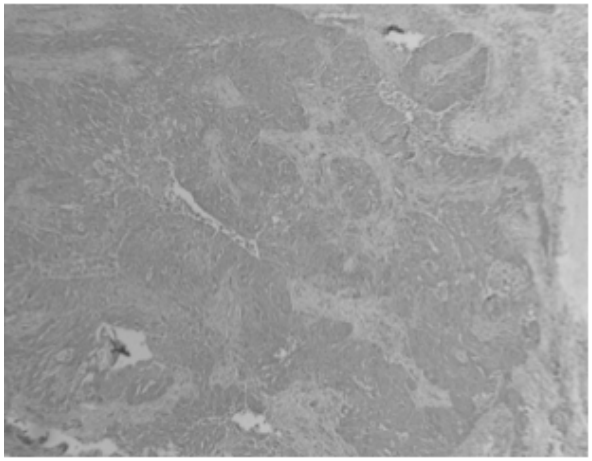

Figure 2B. Serous adenocarcinoma with immunostaining for fascin. Note moderate cytoplasmic staining, moderate intensity, IRS score : 8

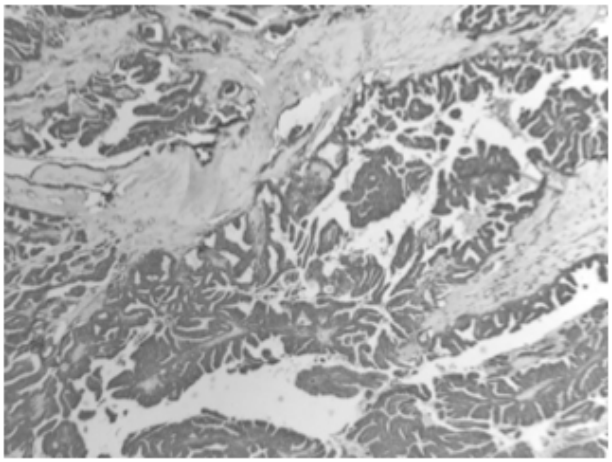

Figure 2C. Serous adenocarcinoma grade 1 with immunostaining for fascin. Note strong cytoplasmic staining, strong intensity, IRS score : 5.5

Table 2. Correlation between Fascin Expression and Grading

		Moderate-poor differentiation		Well differentiation		p-value	OR (95% CI)
		n	%	n	%		
IRS Score	Positive (≥3)	15	78.9	4	21.1	0.442	2.08 (0.44-9.84)
	Negative (<3)	9	64.3	5	35.7		

Table 3. Correlation between Fascin Expression and Stage

		Stage 4		Stage 3		p-value	OR (95% CI)
		n	%	n	%		
IRS Score	Positive (≥3.75)	3	23.1	10	76.9	0.360	2.70 (0.39-18.96)
	Negative (<3.75)	2	10.0	18	90.0		
IRS Score	Positive (≥3)	3	15.8	16	84.2	1.000	1.13 (0.16-7.82)
	Negative (<3)	2	14.3	12	85.7		

Among 33 samples, 11 patients were deceased due to their disease, and 22 (64.7%) were still alive at the end of the study. There were cutoff points in the period of 17 months and 22 months. In the period of month 17-22, samples with high fascin expression had a HR of 1.59 (95% CI=0.38-6.67, p=0.449), but in the period of month 17-23 both groups had comparable HR. In the period of more than 23 months, group with high fascin expression had a better HR of 0.40 (95% CI=0.04-4.38, p=0,449). This is presented in Figure 1 and Table 1.

The sensitivity and specificity analysis and ROC curve analysis for tumor grading showed an optimal cutoff point for fascin expression to be ≥3 with sensitivity of 62.5% and specificity of 55.6%, AUC= 0.525 (95% CI=0.307-0.744); p=0.824. The proportion of samples with high fascin expression and moderate-poor differentiation was 78.9%, compared with the well differentiated being only 21.1%. Meanwhile, proportion of samples with low fascin expression and moderate-poor differentiation was 64.3%, compared with those that are well differentiated being only 35.7%.

The ROC curve analysis for the staging, had an optimal cutoff point of 3.75 with sensitivity and specificity of 60% and 64.2%, with AUC of 0.607 (95% CI=0.329-0.885, p=0.451). High fascin expression in stage four EOC was 23.1%, compared with stage three EOC which was 76.9%. Meanwhile, low fascin expression in stage four EOC was only 10%, while in stage three EOC was 90%.

DISCUSSION

Ovarian cancer is the most common cause of gynecological cancer-related mortality. Patients with this disease generally undergo surgery followed by platinum-taxane chemotherapy (TC), with additional chemotherapy at occurrence of relapse. Although the prognosis for patients with advanced cancer is poor, with a five-year survival of only 30-40%, there is a wide range of outcomes for individual patients. Clinicopathological variables such as staging, grading, histological type, debulking status, and response to chemotherapy continue to provide the basis on which treatment decisions are made for individual patients. Additional biomarkers in cancer tissues need to be extensively evaluated to improve individualized therapy for patients.[9]

Fascin has emerged as a prognostic marker in some carcinomas. In this study, we examined ovarian neoplasms to confirm the presence of any correlation between fascin expression and established clinicopathologic parameters.[12]

Fascin is a 55 kDa globular protein that organizes F-actin into well-organizeded, tightly packed parallel bundles in vitro and in cells. Fascin have been well conserved in animal evolution: homologues are present in Drosophila, echinoderms and the platyhelminth *Schmidtea mediterranea*. Vertebrate genomes encode three forms of fascin: fascin-1, which is widely expressed by mesenchymal tissues and in the nervous system; fascin-2, which is expressed by retinal photoreceptor cells; and fascin-3, which is testis-specific. The focus of this article is on fascin-1 (also known as fascin), which contributes to the organization of two major forms of actin-based structures: cortical cell protrusions that mediate cell interactions and migration, and cytoplasmic microfilament bundles that contribute to cell architecture and to intracellular movements.[13]

Cell motility is one of the defining characteristics of invasive tumors, enabling tumor cells to migrate into adjacent tissues or through the basement membranes and extracellular matrices. The initial step in cell migration is the protrusion of the cell membrane. This is driven by localized actin polymerization. Reorganization of the actin cytoskeleton is the primary mechanism of cell motility and is essential for most types of cell migration. Invasive tumor cells have been demonstrated to present dysregulated cell motility in response to extracellular signals from growth factors and cytokines.[14]

A key structural requirement for cell protrusion is the need for a rigid cytoskeletal structure to support the localized extension of the plasma membrane with its characteristic morphology. This is achieved in finger-like protrusions by a central, unipolar bundle of filamentous actin (F-actin) and in lamellipodial protrusions by radial, rib-like actin bundles that are integrated with a dendritic meshwork of microfilaments. Fascin-1 is known to be the core actin bundling protein of dendrites, microspikes, filopodia, and lamellipodial ribs, and to be concentrated in cell protrusions during cell migration. Fascin-1 contributes to the formation of various actin-based cellular structures.[13,15,16] Among those, and critical in cancer cell biology, are the cellular surface protrusions that mediate cell

movement. In vitro studies, based on transfection experiments, have shown that elevated levels of fascin increased the speed of cell migration and emphasized the association between fascin expression and motility of transformed cells.[15]

Metastatic and invasive tumor cells often exhibit changes in cell morphology, disruption of cell-cell contacts, degradation of ECM and increase in cell migration, which result from rearrangements of the cytoskeletal microfilaments. Reorganization of the actin cytoskeleton is regulated by the action of actin crosslinking proteins. Fascin expression is either low or absent in adult epithelia and is often up-regulated in several types of epithelial cancers including breast, ovarian, skin, pancreatic, liver, esophageal squamous cell, urothelial carcinoma, glioblastoma and cervical cancer.[3,14,17-19]

A number of prior studies have shown that fascin up-regulation is associated with a more aggressive and metastatic phenotype in epithelial cancers. Several correlative studies have demonstrated the tumor promoting function of fascin, its role in tumor development and/or progression of ovarian cancer is yet to be comprehensively investigated. Meta-analyses demonstrate that there is strong evidence that fascin-1 protein is associated with an up to two-and-a-half-fold increased risk of mortality in breast, colorectal, and esophageal carcinomas. At present, there is little evidence that fascin-1 is associated with mortality for gastric and lung carcinomas. Fascin-1 is correlated with an increased risk of disease progression in breast and colorectal carcinomas, but not in lung carcinoma. Strong evidence for association of fascin-1 with increased risk of lymph node metastasis has been observed for colorectal and gastric carcinomas, but not for lung and esophageal carcinomas. Fascin-1 protein was also associated with a greater than 70% increased risk of distant metastasis in colorectal, gastric, and esophageal carcinomas, although the statistical evidence for association with esophageal carcinoma metastasis was weak. Pooled analysis of all carcinomas within our dataset provides strong evidence that fascin-1 may carry the potential as a novel biomarker for early identification of aggressive and metastatic tumors. These data will assist rational decision-making for focusing ongoing efforts investigating fascin-1 as a biomarker for the most relevant carcinoma.[20]

In general, fascin overexpression has been associated with invasive, high-grade tumors. Recently, however, Yamaguchi et al have reported overexpression of fascin and up-regulation of fascin mRNA in intraductal papillary mucinous neoplasms of pancreas that correlated with increasing histologic grade (adenoma, borderline neoplasm, and carcinoma with or without invasion) suggesting fascin up-regulation as an early event in the pathogenesis of pancreatic mucinous intraductal papillary neoplasms.[21] Down regulation of fascin has been shown to have inhibitory effects on the migration, proliferation, and invasiveness of esophageal squamous cell carcinoma cell lines, suggesting that fascin contributes to tumor progression and could possibly be a therapeutic molecular target.[22]

Kabukcuoglu et al studied fascin expression in ovarian tumors (serous, endometrioid, clear, mucinous, mixed, and transitional) and found various degrees of epithelial staining in 20% of cystadenoma, 62% of borderline tumors, and 64% of invasive epithelial ovarian tumors.[19,23] Hu et al have also shown increased expression of fascin in cell cultures derived from stage IV ovarian tumors versus cell cultures derived from stage II-III ovarian tumors. They also shown that the expression of fascin in ovarian tumor cell cultures is significantly associated with their ability to grow and spread intraperitoneally after intraperitoneal inoculation, supporting the role of fascin in ovarian tumor metastasis.[19] It was also observed that there was increased expression of fascin in paraffin-embedded sections from borderline ovarian tumors, whereas they did not see any expression of fascin in benign ovarian epithelium. Fascin up-regulation in human breast cancer cell lines has been associated with HER-2 overexpression, which is associated with poor prognosis in breast cancer. HER-2 is often positive in serous ovarian tumors as well, and its association with fascin up-regulation can be a subject for further investigation.[22]

In our study, we characterized fascin protein expression in a series of advanced stage epithelial ovarian carcinoma by immunohistochemistry. Figure 1 shows that survival in groups with fascin expression ≥3 and fascin expression <3 crossed in the periode of 17 months to 22 months. Between 0-17 months, subject with fascin expression ≥3 has worse HR of 1.59 (95% CI=0.38-6.67, p=0.449). However, between 17-23 months, both of the group have same hazard. After 23 months, higher fascin expression had a better hazard with HR of 0.40 (95% CI=0.04-4.38).

Previous studies have implicated fascin as a novel biomarker for human carcinomas and aggressive tumor behavior. In our study, statiscally these findings were not significant and it could be due to the heterogenicity and limited sample of this research.

In tissues with positive expression of fascin, up to 78.9% had moderate-poor differentiation, which was higher than those that are well differentiated, which was 21.1%. Statistically this is not significant, but the difference between positive and negative fascin expression is clinically significant. Positive expression of fascin in stage 4 EOC is 23.1%, compared with stage 4 in the negative expression group, which was only 10%. Nevertheless, this was not statistically significant.

The cadherin family of trans membrane glycoproteins is important for cellular adhesion in epithelial cells. It is known that E-cadherins mediate homotypic adhesions in epithelial tissues and serve to keep the epithelial cells together.[24] Ying et al suggested that fascin and cadherin binding sites within β-catenin overlaps; and that, in vitro, fascin and cadherins compete for binding to β-catenin in transformed epithelial cell systems.[25] Yamashiro et al observed that transfection of the fascin gene leads to cell-to-cell contact disorganization and increased cell motility by inducing the emission of microspikes on apical surfaces and on the extended lamelipodia on basolateral surfaces. Some of these changes are due to the downregulation and altered cytoplasmic distribution of the E-cadherin based adhesion complex induced by fascin over expression.[26] E-cadherin is the key functional component of adherent junctions between epithelial cells. Downregulation of E-cadherin in several types of human neoplasms usually correlates with poor tumor differentiation, more advanced disease stage, lymph node metastases, and poor survival rates. A relationship between fascin and E-cadherin has been documented, where cytoplasmic accumulation of fascin leads to a loss of cell-to-cell adhesion by disruption of the E-cadherin adhesion system.[24] This agrees with findings of Okada et al who showed that increased immunoreactivity for fascin had a tendency to disrupt membranous immunoreactivity for E-cadherin. Therefore, it may be postulated that the altered expression of E-cadherin is involved in fascin mediated cell motility.[27]

CONCLUSION

In conclusion, we have found an association between fascin expression and survival, as well as clinicopathologic properties (grade and stage) in epithelial ovarian cancer. However, the association was not statistically significant. Confounding bias (sample is heterogenous) and sample bias (small number of sample) could be the reason why these results were not significant. Further studies with a larger, more homogenous sample, with analysis of the confounding factors are required.

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Case Report

CD4 Percentage and Absolute CD4 Accuracy not Different in Predicting Viral Load of HIV-Infected Mothers

Akurasi Persentase CD4 dan Absolut CD4 tidak Berbeda dalam Memprediksi Viral Load pada Ibu Hamil Terinfeksi HIV

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Abstract

Objective: To determine the accuracy difference between CD4 percentage and absolute CD4 in predicting the viral load of HIV-infected pregnant women.

Methods: This study is a diagnostic study involving 22 HIV-infected pregnant women who came for PMTCT at the Outpatient Clinic in Sanglah Hospital, from September 2011 until August 2012, who were randomly selected through consecutive sampling. Blood samples were collected to analyze the viral load, CD4, and complete blood count (CBC). Viral load examination was conducted using PCR in the molecular biology laboratory in the Faculty of Medicine University of Udayana. CD4 and CBC test was conducted in Sanglah Hospital Laboratory. Analysis was done with 2x2 table using SPSS for windows® version 17 to evaluate sensitivity, specificity and accuracy rate of CD4 percentage and absolute CD4 in predicting the viral load.

Results: Data analysis shows that CD4 percentage had 75.0% sensitivity, 88.9% specificity, and accuracy of 86.4% for predicting the viral load in HIV-infected pregnant women. Meanwhile, absolute CD4 had 50.0% sensitivity, 77.8% specificity, and 72.7% accuracy. Chi-square test shows that there was no significant difference in the accuracy of CD4 percentage and absolute CD4 (p=0.457).

Conclusion: CD4 percentage and absolute CD4 had high accuracy in predicting the viral load in HIV-infected pregnant women (86.4% and 72.7%). There was no significant difference of accuracy between the CD4 percentage and absolute CD4.

[Indones J Obstet Gynecol 2015; 3-4: 230-233]

Keywords: CD4, HIV-infected pregnant women, viral load

Abstrak

Tujuan: *Untuk mempelajari perbedaan akurasi antara persentase CD4 dibandingkan CD4 absolut dalam memprediksi viral load pada ibu hamil terinfeksi HIV.*

Metode: *Penelitian uji diagnostik ini melibatkan 22 ibu hamil terinfeksi HIV yang datang ke Poliklinik PMTCT RSUP Sanglah, dari bulan September 2011 sampai dengan Agustus 2012, yang diambil secara consecutive sampling. Darah diambil untuk pemeriksaan viral load, CD4, dan darah lengkap (DL). Pemeriksaan viral load dilakukan dengan PCR di laboratorium biologi molekuler FK Universitas Udayana. Pemeriksaan CD4 dan DL dilakukan di laboratorium RSUP Sanglah. Dilakukan analisis dengan tabel silang 2x2 menggunakan SPSS for Windows® version 17 untuk menilai sensitivitas, spesifisitas, dan tingkat akurasi antara persentase CD4 dan CD4 absolut untuk menilai viral load HIV.*

Hasil: *Hasil analisis menunjukkan persentase CD4 memiliki sensitivitas sebesar 75,0%, spesifisitas sebesar 88,9%, dan akurasi sebesar 86,4% dalam memprediksi viral load pada ibu hamil terinfeksi HIV. Sedangkan, CD4 absolut memiliki sensitivitas 50,0%, spesifisitas 77,8%, dan akurasi sebesar 72,7%. Hasil uji Chi-Square menunjukkan tidak ada perbedaan bermakna antara akurasi persentase CD4 dan CD4 absolut (p=0,457).*

Kesimpulan: *Persentase CD4 dan CD4 absolut memiliki akurasi yang tinggi dalam memprediksi viral load pada ibu hamil terinfeksi HIV (86,4% dan 72,7%). Tidak terdapat perbedaan akurasi yang bermakna antara persentase CD4 dan CD4 absolut.*

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Kata kunci: *CD4, ibu hamil terinfeksi HIV, viral load*

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INTRODUCTION

Pregnancy that is infected with HIV needs serious attention. Based on UNAIDS data in 2009, there are 33.3 million HIV cases throughout the world, with increasing number of cases being around 2.6 million per year. About 15.9 million (48%) among that number are women, and 2.5 million cases are found in young teenagers below 15 years old. More than 90% of cases in children are due to the transmission between mothers to the fetus. Gray and Mclntyre have previously shown that 8.5% among all HIV patients are pregnant women that deliver babies each year.[1,2]

High viral load (VL), defined as VL ≥10.000 copies/ml, is the main risk factor for HIV transmission from mother to the fetus throughout pregnancy, labor, and breastfeeding. If the VL is less than 1000 copies/ml, then the perinatal transmission risk is very low. VL is the main key in the prevention of HIV transmission from the mother to the fetus. VL

test is also important in analyzing body response towards ARV, knowing the perinatal transmission risk earlier, and choosing the appropriate delivery method in order to minimize the transmission risk. However, VL examination needs very high cost and can only be tested in a laboratory that is equipped with PCR. Laboratory with PCR facility is only available in a big city, especially in Java Island. Meanwhile, the number of HIV-infected pregnant women is very high especially in suburban areas, such as Papua.[3-5]

CD4 is used to determine the immunity status of an HIV patient by measuring the absolute CD4 number and percentage. The higher rate of HIV VL means that the CD4 is low in the patient. The examination of immune system with CD4 is economically reasonable, fast and available in many places in Indonesia. There is a correlation between a low immune system with the high HIV VL. As a result the percentage of CD4 and absolute CD4 can be used to predict HIV VL in the pregnant women's circulation.

This study was conducted to evaluate the accuracy of CD4 percentage and absolute CD4 in predicting VL in pregnant women circulation that are infected with HIV. If one or both of those examination have good sensitivity and specificity to predict HIV VL, then this examination can be used as an alternative in analyzing HIV VL during antenatal care (ANC) especially in areas with limited resources.

METHODS

This study was a diagnostic study (analytic observational cross-sectional). This study was conducted to determine the difference of accuracy between CD4 percentage compared to absolute CD4 in predicting the VL in HIV-infected pregnant women. We used Polymerase Chain Reaction (PCR) as the gold standard to evaluate VL in HIV-infected pregnant women. CD4 percentage was manually counted based on the comparison of absolute CD4 and total lymphocyte count in percentage.

This study was conducted in the outpatient clinic of the Department of Obstetrics and Gynecology, especially in PMTCT clinic of Sanglah Hospital, Denpasar, starting from September 1st 2011 until August 31st 2012. Samples were HIV-infected pregnant women who presented for PMTCT in Sanglah Hospital during the period of September 1st 2011 - August 31st 2012 who fulfilled the inclusion criteria and were willing to join this study that were selected with consecutive sampling. Inclusion criteria of this study were patients with a confirmed diagnosis of HIV/AIDS based on HIV antibody examination with the standard method in Sanglah Hospital, in their second and third trimester, willing to come to the PMTCT department in the morning, and willing to join the study. Exclusion criteria of this study were malnutrition (BMI < 18.5), had burn wound, and had undergone splenectomy.

Sample number was calculated using Lameshow formula and the result was 22 samples. Samples were obtained from the peripheral vein circulation of HIV-infected pregnant women as much as 6 cc that was inserted in EDTA tube (3 cc) for CD4 and CBC examination, and tube without EDTA (3 cc) for HIV viral load. Samples were sent to the Clinical Pathology Laboratory of Sanglah Hospital for CD4 and CBC examination, and Molecular Biology Laboratory Faculty of Medicine Udayana for PCR examination.

Data analysis was conducted using SPSS 17.0 for Windows program and data was presented using table and narration. Descriptive analysis was done to evaluate maternal characteristics such as age, education, occupation, parity, gestational age, and BMI. Data was inserted in a 2x2 table and evaluated for sensitivity, specificity, positive predictive value, negative predictive value, positive likelihood ratio, and negative likelihood ratio.

RESULTS AND DISCUSSION

Table 1. Characteristics Distribution of Age, Parity, Gestational Age, and BMI

Variable	Mean	SD	Range
Age (year)	24.73	3.87	19-34
Parity	1.73	0.88	1-4
Gestational age (week)	28.73	4.86	18-36
BMI	23.40	3.84	19.10-34.20

Based on the table above, the mean age of the study sample was 24.73±3.87 with a range of 19-34 years old. Mean parity was 1.73±0.88 with a range of 1-4. The mean of gestational age was 28.73±4.86 weeks, with a range of 18-36 weeks gestational age. Furthermore, the mean BMI was 23.40±3.84 kg/m^2 with a range between 19.10-34.20 kg/m^2.

Table 2. 2x2 Table of Absolute CD4 Towards HIV Viral Load

		Viral Load		Total
		≥ 10,000	< 10,000	
Absolute CD4	≤200	2	4	6
	>200	2	14	16
Total		4	8	12

In this study, the sensitivity of absolute CD4 towards HIV viral load was 50.0%, with specificity of 77.8%, positive predictive value of 50.0%, negative predictive value of 87.5%, positive likelihood ratio was 2.3, and negative likelihood ratio was 0.64. The cutoff point of absolute CD4 that was used in this study was 200 cells/ml. CD4 that was less than 200 cells/ml will increase the transmission of HIV significantly and having higher risk of opportunistic infection. The low CD4 and high VL number is the risk factor of transmission from mother to fetus.[6,7]

Table 3. 2x2 Table of CD4 Percentage Towards HIV Viral Load

		Viral Load		Total
		≥ 10,000	< 10,000	
CD4 Percentage	≤13	3	2	6
	>13	1	16	16
Total		4	18	22

In this study, the CD4 percentage sensitivity towards HIV viral load was 75.0%, specificity was 88.9%, positive predictive value was 60.0%, negative predictive value was 94.1%, positive likelihood value was 6.76, and negative likelihood value was 0.28. The cutoff point of VL was 10,000 copies/ml, based on the fact that HIV VL at that number or more than that will increase the risk of transmission from mother to fetus significantly. The cutoff point of CD4 percentage that was used in this study was 13%, since it was within the same range of absolute CD4 of 200 cells/ml. The high sensitivity and specificity showed the ability of CD4 percentage in predicting HIV VL in maternal circulation. It means, this study can be used to evaluate the effectiveness of ARV and transmission risk of HIV from mother to fetus.[7-9]

This study showed that absolute CD4 and CD4 percentage had high accuracy. Accuracy of absolute CD4 used to predict HIV VL was 72.7%, while the CD4 percentage had an accuracy of 86.4%. Based on the Chi-Square test, there was no significant difference between absolute CD4 and CD4 percentage accuracy (p=0.457).

Table 4. Comparison of Absolute CD4 and CD4 Percentage Accuracy Towards HIV Viral Load

Examination	Accuracy		Total	%	p
	Accurate	Not Accurate			
Absolute CD4	16	6	22	72.7	0.457
CD4 Percentage	19	3	22	86.4	

This study showed that CD4 percentage and absolute CD4 was accurate enough to describe the viral load condition in HIV-infected pregnant women. The lower CD4 count indicated a weaker immune system of the patient, and vice versa. This was probably caused by the fact that cells that contained CD4 was being the main target of HIV infection. Cells that have CD4 receptors, which are lymphocytes (T helper cell), monocytes/macrophages, dendritic/Langerhans cells, are the cells that are responsible in keeping human immunity, and these are the targets of HIV infection. HIV is related with cell target through CD4. Together with the viral replication, there was a destruction of the immune system, especially T Helper lymphocytes. The more immune cells are destroyed, will cause a higher viral load in the patient.[10]

Controlled variable in this study was malnutrition, diurnal variation, patients with burn wound, and history of splenectomy. Meanwhile, the variables that are not fully controlled are hemodilution and psychosocial stress. This study showed that hemodilution does not really affect the absolute CD4 count. Even though hemodilution could cause a variation of absolute CD4 number, but the range of variation does not reduce its ability to describe the immune condition of HIV patient. As the result, absolute CD4 still has a high rate of accuracy in predicting HIV VL.

CD4 percentage is known to be more stabile, and the result is not affected by other variables. This was probably due to CD4 percentage that consists of functional lymphocyte, which was CD4. CD4 percentage considers the total number of lymphocyte to evaluate the immune system. This study result also showed that CD4 percentage has better function in predicting VL in HIV-infected pregnant women with high accuracy, which was 86.4%. In some extreme situations, such as malnutrition,

burn wounds, and splenectomy history, CD4 percentage is more superior.[11]

CD4 percentage examination is not a difficult and expensive examination. CD4 percentage can be manually evaluated based on a ratio of absolute CD4 and total lymphocyte count, expressed in percentage unit (%). Total lymphocyte count is obtained from routine CBC examination during ANC. CD4 percentage could be obtained from all patients who went for ANC in the outpatient department. As the result, the HIV VL could also be evaluated during ANC based on the CD4 percentage. Using the 13% cutoff point, if the CD4 percentage is less than the predetermined cutoff point, it could be concluded that HIV VL in the maternal circulation could be more than 10,000 copies/ml with 86.4% accuracy.

CD4 percentage examination is planned to be one of the alternative examination beside PCR in evaluating viral load in HIV-infected pregnant women. This examination is simpler, less expensive and hopefully could be performed in all hospitals, which are appointed to manage HIV patients throughout Indonesia. Laboratory staff also does not need special training since this examination is simple.

The weakness of this study was that this study was an introductory study to assess HIV VL based on immune status during pregnancy. There was no previous or similar study in other places. As the result, this study could not be compared with other studies. Moreover, this study only involves HIV-infected pregnant women in the second and third trimester. Informative value would be higher if the study involved all trimesters of pregnancy.

The duration of ARV admission also could not be fully controlled in this study. Even though all samples within this study already received ARV, but it would be probably better if the samples were divided into two groups, which were patients that had not received ARV yet, and the patients who already received ARV previously. In the group with patients who received ARV could be further categorized into few groups based on the duration of ARV consumption.

CONCLUSION

CD4 percentage and absolute CD4 had high accuracy in predicting viral load in HIV-infected pregnant women (86.4% and 72.7%). There was no significant difference of accuracy between CD4 percentage and absolute CD4. Further studies are required involving samples from different centers (multicenter study) in order to provide better informative result.

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Case Report

A Case of Prenatal Diagnosis of Trisomy 18 with Ultrasound

Kasus Diagnosis Prenatal pada Trisomi 18 dengan Ultrasonografi

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Abstract

Objective: To report a case of trisomy 18 diagnosed in prenatal care.

Methods: Case report.

Case: A 24 years old primigravida woman was diagnosed with term pregnancy (37-38 weeks) with an intrauterine singleton live fetus with Edwards syndrome. In 15-16 weeks of pregnancy the omphalocele was discovered using ultrasound. Subsequently, amniocentesis was performed and the chromosome analysis result showed Edwards syndrome (47, XX +18). The patient chose to continue the pregnancy until term. In this patient, elective CS was performed at term pregnancy, involving teamwork between obstetrics and perinatology. A female baby was born weighing 1720 grams, 40 cm body length, and APGAR score of 5/7. The congenital anomalies discovered include umbilical hernia, rocker bottom feet, clenched hands, low set malformed ears, and a single umbilical artery. The baby was born with asphyxia, improved after resuscitation, and required treatment in the NICU. Pediatric surgeons planned umbilical hernia repair. Furthermore, because of the presence of suspected esophageal atresia, the baby was planned for gastrotomy, which was delayed because the baby was experiencing desaturation. Because of the unstable condition of the baby, echocardiography and gastrotomy were not done until the 18th day of treatment. At the 18th day, the baby's condition deteriorated and the baby died with metabolic acidosis.

Conclusion: Edwards syndrome can be diagnosed in the prenatal period by risk factors consideration, maternal serum markers, and ultrasonographic identification of organ abnormalities.

[Indones J Obstet Gynecol 2015; 3-4: 234-238]

Keywords: Edwards syndrome, prenatal diagnosis, trisomy 18, ultrasound

Abstrak

Tujuan: *Melaporkan kasus trisomi 18 yang didiagnosa pada masa prenatal.*

Metode: *Laporan kasus.*

Kasus: *Seorang perempuan $G_1P_0A_0H_0$, 24 tahun, didiagnosa dengan kehamilan aterm (37-38 minggu), janin tunggal hidup intrauterin dengan sindroma Edward. Pada usia kehamilan 15-16 minggu dijumpai omfalokel pada ultrasonografi sehingga dilakukan amniosintesis, dan dari hasil analisa kromosomnya didapatkan sindroma Edwards (47, XX +18). Pasien memilih untuk melanjutkan kehamilan hingga cukup bulan. Pada pasien ini dilakukan SC elektif pada kehamilan aterm dengan kerjasama tim kebidanan dan perinatologi. Lahir bayi perempuan dengan berat badan 1720 gram, panjang badan 40 cm, dan APGAR skor 5/7. Dijumpai kelainan kongenital berupa hernia umbilikalis, rocker bottom feet, clenched hands, telinga malformasi letak rendah, dan arteri umbilikalis tunggal. Bayi lahir dengan asfiksia, membaik setelah resusitasi, dan memerlukan perawatan NICU. Ahli bedah anak merencanakan reparasi hernia umbilikalis. Karena diduga adanya atresia esophagus, direncanakan gastrotomi, namun gastrotomi ditunda karena bayi mengalami desaturasi. Dikarenakan kondisi bayi tidak stabil maka echocardiography dan gastrotomi belum dilakukan hingga hari ke-18 perawatan. Pada hari ke-18 perawatan, bayi mengalami perburukan dan meninggal dengan asidosis metabolik.*

Kesimpulan: *Sindroma Edwards dapat didiagnosa pada masa prenatal dengan memperhatikan faktor risiko, marker serum pada maternal, dan temuan kelainan organ melalui ultrasonografi.*

[Maj Obstet Ginekol Indones 2015; 3-4: 234-238]

Kata kunci: *diagnosa prenatal, sindroma Edwards, trisomi 18, ultrasonografi*

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INTRODUCTION

Trisomy 18, also known as Edwards syndrome, is an autosomal chromosomal disorder due to an extra chromosome 18. The first fetal reports were described by Edwards et al and Smith et al in 1960. The prevalence in female infants is higher than in male infants (female-to-male percentage ratio 60:40), but this tendency is not identified if the sex ratio was calculated in infants born electively (female-to-male percentage ratio 48:51). The frequency of fetal death is higher in male than female fetuses. The female infants born alive showed a better survival than male infants.[1] Its overall incidence is 1 in 6,000 live births. The risk increases with increasing maternal age. In the UK, as many as 495 cases of trisomy 18 were diagnosed in 2008-2009, 92% of them were diagnosed prenatally, and only 35 cases (10.32%) were born alive.[2]

Edwards syndrome is genetically characterized by the presence of an extra chromosome 18, in whole or partially (by the process of translocation or mozaic-type). Ovum or sperm cells contain 23 chromosomes that will eventually form the 46 chromosomes. In the second meiotic division, if there is non-disjunction the resulting extra chromosome will lead to a total chromosome of 24. Fertilization of the ovum or insemination by sperm containing the extra chromosome would produce trisomy, or three copies of a chromosome. Trisomy 18 (47 XY, +18) is due to meiotic non-disjunction events. With non-disjunction, a gamete (sperm or ova) are produced with an extra chromosome 18, gametes then has 24 chromosomes. When joined with a normal gamete, the embryo has 47 chromosomes with an extra chromosome 18. On rare occasions, a portion of chromosome 18 is attached to another chromosome (translocated) before or after conception. Abnormalities in infants with partial trisomy of chromosome 18 is less severe when compared with Edwards syndrome.[2,3]

Babies with Edwards syndrome tend to have a low birthweight, microcephaly, small chin (micrognathia), malformations of the ear, heart and kidney malformations, omphalocele, esophageal atresia, mental retardation, clenched fists and foot malformations (clubfoot or rocker bottom feet), arthrogryposis, and undescended testes for male babies. In the mozaic type of Edwards syndrome or partial trisomy 18, most of the body's cells have trisomy 18, while the other part does not, in which case the clinical symptoms can vary from mild to complex.[2]

Edwards syndrome diagnosis may be suspected at the time of ultrasonographic examination, when the fetus' body structure looks abnormal. Early detection can help patients decide the continuation of pregnancy and childbirth preparation. Fetal clinical condition indicating the presence of Edwards syndrome include nuchal translucency that is more than the average, choroid plexus cysts, polyhydramnios, IUGR, microcephaly, non-immune hydrops fetalis, exomphalos (omphalocele or gastroschisis), palatoschisis, and clenched hand. By looking at the structure of fetal abnormalities with ultrasound, then amniocentesis examination can be done at 16-18 weeks of gestation for chromosome analysis. In omphalocele diagnosed by ultrasound in the first trimester or in the second trimester examination is needed to determine the genetic configuration.[4]

There is no genetic therapy for Edwards syndrome. Babies with Edwards syndrome frequently have major physical abnormalities which can be corrected with surgery based on the level of severity, but extreme invasive procedures are not performed on infants whose predicted survival is quantified in a matter of days or weeks. Medical therapy can be performed with the goal of palliation but not life extension. Edwards syndrome is a serious condition and can cause severe medical problems. Often these cases of trisomy 18 die before birth or during labor. In infants born alive, the average lifespan is 5-15 days, 50% survive to 1 month of age, and only <10% can survive more than 1 year.[2]

CASE

A 24 years old primigravida patient was treated in the obstetrics ward of Ibnu Sina Islamic Hospital, Padang on April 4th, 2014 with term pregnancy (37-38 weeks) of an intrauterine singleton living fetus with head presentation with omphalocele. The patient was not in labor and in good general condition.

Patient had antenatal care at the maternity clinic of Ibnu Sina Islamic Hospital, Padang. On the 4.5 months visit, there was the possibility of abnormalities in the fetal stomach, patients are encouraged to check the amniotic fluid and then the results of chromosome analysis of amniotic fluid identified Edwards syndrome (trisomy 18) without major structural abnormalities.

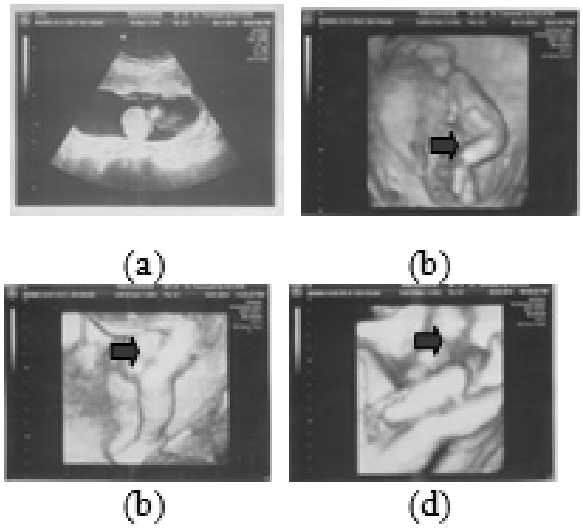

Figure 1. Ultrasound at 18-19 weeks (a and b), 26-27 weeks (c), and 31-32 weeks (d), showing the presence of omphalocele (red arrows)

At 37-38 weeks of pregnancy, ultrasound biometry obtained BPD: 9.25 cm, HL: 5.83 cm, FL: 6.68 cm, AC: 27.62 cm, with an estimated weight of 2269 grams and the fetal abdominal organs was found outside the abdominal cavity, which was diagnosed as omphalocele.

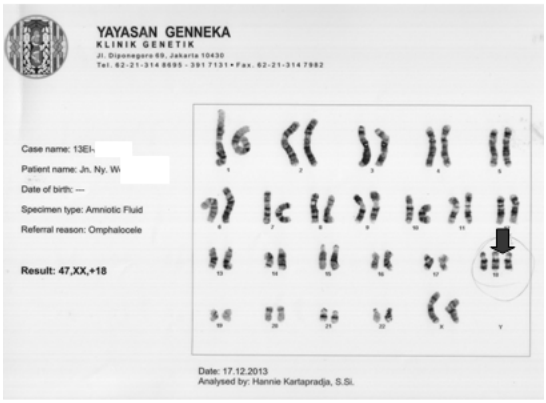

Figure 2. Result of chromosome analysis

Chromosome analysis was performed on samples derived from amniotic fluid obtained by amniocentesis. With G-Banding techniques, chromosomes of 20 cells from three different cell culture preparations were studied, and the number of chromosomes in each cell studied was found to be 47, XX, +18. This meant that the number of chromosomes was 47 with fetal sex chromosomes being XX, and an excess of 1 piece of chromosome 18 (trisomy 18). This disorder was called Edwards Syndrome. Major structural abnormalities were not identified. Abnormality of trisomy 18 usually occurs spontaneously/denovo due to non-disjunction in the process of cell division in meiosis 1 or 2. The number of repetition was very small and was unlikely to be derived from the patient's parents. Therefore, examination of the parents' chromosome in was not required. The impression from the examinations performed was Edwards syndrome without major structural abnormalities.

In this patient, an elective CS was planned. At the time of delivery, which was attended by a perinatologist, a female baby was born weighing 1720 gram, body length measuring at 40 cm, Apgar score 5/7. The placenta was born with light traction on the cord, and was found to be complete. The size of the placenta was 16x17x2.5 cm, 350 grams in weight, 30 cm in cord length, paracentral insertion. Congenital abnormalities encountered include herniated umbilical cord, rocker bottom feet, clenched hand, low set malformed ear, and one umbilical artery. The baby was born with asphyxia, which improved after 5 minutes of resuscitation. Clinically, no heart-lung abnormality was found, and the baby required care in the NICU. Perinatologist recommended an echocardiography to ensure that cardiac abnormalities that are usually found in Edwards syndrome were not present. The baby received ampicillin sulbactam and gentamicin. The pediatric surgeons planned to repair the umbilical hernia. Furthermore, because of the presence of suspected esophageal atresia, the baby was planned for gastrotomy, but the gastrotomy was delayed because the baby suffered desaturation. Due to the unstable condition of the baby, the echocardiography and gastrotomy cannot be done until the 18th day of treatment. On the 18th day of treatment the baby experienced a deterioration, desaturation, and metabolic acidosis; and at 12 pm the baby was pronounced dead.

DISCUSSION

In this case, a 24 years old primigravid patient was diagnosed with term pergnancy (37-38 weeks) and omphalocele and Edwards syndrome, who was treated in the obstetrics ward and planned for elective cesarean section managed by obstetricians and perinatologist. Although the patient had known the possibility of congenital abnormalities in the fetus, the patient decided to continue the pregnancy and wanted comprehensive management of labor and for her baby. Prior to elective cesarean section, this case was consulted to perinatology for management of the possible congenital anomalies encountered at birth.

The baby was born with asphyxia, but improved after resuscitation, the perinatologist decides treatment of the infant in the NICU. Congenital abnormalities encountered in the infant include a herniated umbilical cord, rocker bottom feet, clenched hand, low set malformed ear, and singular umbilical artery. Birth weight of the baby indicates the presence of IUGR, with the baby weighing 1720 grams. There were suspected abnormalities in the baby's heart and lungs, thus the perinatologist recommended echocardiography examination. The pediatric surgeon planned to repair the umbilical hernia. Furthermore there was suspected esophageal atresia, and thus a gastrotomy was planned. The infant had been with stable condition, but worsened on the 18th day of treatment, and finally died after 18 days of intensive care.

This patient was diagnosed with fetal omphalocele in pregnancy through an ultrasound examination. Ultrasound plays an important role in the determination of prenatal diagnosis, providing better and more accurate prenatal diagnosis.[5] This patient was diagnosed in the second trimester of pregnancy. The obstetrician who handled the pregnancy diagnosed the presence of omphalocele. The diagnosis was based on the presence of abdominal organs outside the abdominal cavity visualized on ultrasound examination. The diagnosis of omphalocele can be confirmed when the lateral ectomesodermal folds fails to meet at the midline, leaving the stomach contents only covered by the two plated amniotic sac and peritoneum. Prenatal diagnosis of omphalocele should be immediately followed by karyotyping from the amniotic fluid, and ultrasonographic scanning of the fetal heart and intestines. In this patient, after the diagnosis of omphalocele, amniocentesis was performed with the result of Edwards syndrome without major structural abnormalities (trisomy 18). This is in accordance to previous literature, stating that fetal omphalocele can be associated with a genetic disorder of trisomy 18.[5-7]

Babies with omphalocele need to be delivered in a hospital with perinatologists and pediatric surgeons. Infants with omphalocele require care in the neonatal intensive care unit after they are born. Labor in fetus with omphalocele is controversial, considering the possibility of vaginal delivery with birth-related vaginal trauma and rupture of the fetal omphalocele. Therefore, abdominal delivery is the right choice. Avoiding vaginal delivery and choosing cesarean section before the onset of labor improves neonatal outcomes. Complications of vaginal delivery include ruptured omphalocele pockets, compression of intestines caused by uterine contractions, and visceral exposure to the existing bacterial flora in the lower genital tract.[8,9] However Eyal et al concluded in adequate data exists to support cesarean section as being more beneficial for the fetus compared to vaginal delivery, and there was no difference in morbidity, mortality, and incidence of visceral injury in neonates.[10]

There are three approaches to treat the omphalocele; a primary repair, repair at a later time, and non-surgical approach. A primary repair surgery usually occurs within 1-2 days after the baby is born. This is done if the baby has a small omphalocele, and the stomach contents can be easily placed back in the baby's abdomen, with the omphalocele contained in a special bag. During 5-7 days the surgeon will push little by little, until the viscera can be returned to the baby's stomach, until it can be closed with surgery. Prior to infants recovering from intestinal surgery, the baby will not be able to eat, and should be treated intravenously. Babies can still be fed, just not directly.[11]

Non-operative approach is usually the only option available for a large omphalocele. This is because there are several abdominal organs outside the body (usually the liver, stomach, and intestines) and these babies often do not have the stomach big enough to hold it all back. In this case, omphalocele babies will be covered with a special cream to help the growth of new skin on top of the membrane. Finally, when the baby is older, surgery is performed to put the organs back into the abdominal cavity.[12] If normal chromosomes and no birth defects is present in addition to omphalocele, the severity of disability depends entirely on the size of the omphalocele.[12,13]

Babies with Edwards syndrome have a low life expectancy, with an average lifespan of 5-15 days, with the cause of death being heavy malformations of the heart or other organs. At 18 days of intensive care, the baby in our case experienced dyspnea, and later became apneic, and after resuscitation finally died. This condition was due to desaturation and metabolic acidosis in the infant.

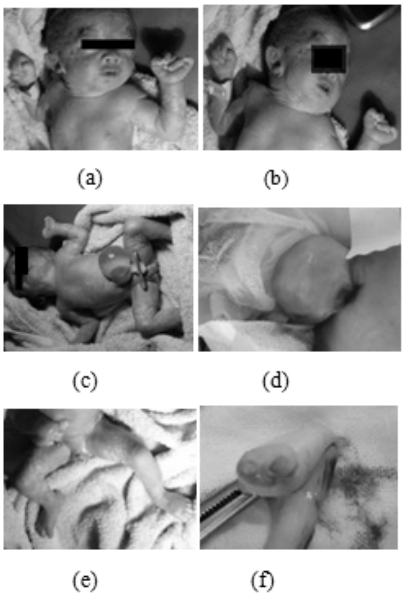

Figure 3. Baby W with Edwards syndrome, (a) clenched hand, (b) low set malformed ear, (c and d) umbilical cord hernia, (e) rocker bottom feet, and (f) single umbilical artery.

CONCLUSION

Edwards syndrome can be diagnosed in the prenatal period by taking into account risk factors including maternal age, screening of maternal serum markers, and detection of organ abnormalities by ultrasound. There is no cure for Edwards syndrome. Palliative procedures are performed for those who choose to continue the pregnancy. Most infants with Edward syndrome die intrauterine. After delivery, 20-30% will die within one month, and 10% of babies born alive will last up to one year.

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Literature Review

Promising Male Hormonal Contraceptive are Well Established - Soon a Reality

Perkembangan Kontrasepsi Hormonal pada Lelaki - Segera Menjadi Kenyataan

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Abstract

Objective: To understand the modalities used for male hormonal contraception.

Methods: Literature review

Conclusion: Male contraceptive methods are still limited but hormonal contraceptive methods are being developed. The basic mechanism of male hormonal contraception is to inhibit spermatogenesis by suppression of the hypothalamic-pituitary-testicular axis function. Administration of testosterone or androgen derivative that is given in combination with progestin or GnRH antagonist shows that male hormonal contraceptive is reversible, effective, and acceptable as a male contraceptive method. However, no method of male hormonal contraceptive is ready for clinical use and marketed due to limited studies.

[Indones J Obstet Gynecol 2015; 3-4: 239-243]

Keywords: GnRH antagonist, male hormonal contraceptive, progestin, testosterone

Abstrak

Tujuan: *Untuk mengetahui modalitas yang dapat digunakan untuk kontrasepsi hormonal pria.*

Metode: *Kajian pustaka*

Kesimpulan: *Metode kontrasepsi pria masih terbatas, namun metode kontrasepsi hormonal masih terus dikembangkan hingga saat ini. Mekanisme dasar kontrasepsi hormonal pria adalah menghambat spermatogenesis dengan cara menekan fungsi dari aksis hipotalamus-hipofisis-testis. Pemberian kombinasi antara testosteron atau turunan androgen dikombinasikan dengan progestin atau antagonis GnRH menunjukkan bahwa metode kontrasepsi hormonal pria efektif, reversibel dan dapat diterima. Namun, saat ini belum ada kontrasepsi hormonal pria yang digunakan secara klinis dan dipasarkan oleh karena keterbatasan penelitian.*

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Kata kunci: *antagonis GnRH, kontrasepsi hormonal pria, progestin, testosteron*

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INTRODUCTION

Historically, family planning programs and studies have focused on women. Studies on male-involvement in family planning has increased contraception use.[1] Male contraceptive methods are still limited, with condoms and vasectomy being the most widely used method. Condom use in men is ranked fourth globally but is often used incorrectly and inconsistently. Vasectomy is an effective and safe method but not favorable because it is not easily reversible.[2,3] Therefore, there is a need for a new contraceptive method is effective, reversible, and acceptable.

Male hormonal contraceptive methods are constantly being developed. In theory, the male hormonal contraception works by providing negative feedback to suppress spermatogenesis. Gonadotropins from the pituitary will be suppressed by administration of testosterone or androgen derivative that is often given in combination with anti-gonadotrophic agent, such as progestin or GnRH antagonists. Methods of male hormonal contraception are one of the reversible contraceptive options with high effectiveness and will be discussed further below.[4]

The Physiological Basis of Male Fertility

There are 4 aspects that play a role in physiological basis of male fertility, which are:

The hypothalamic-pituitary axis: control of gonadotropin secretion[3]

The hypothalamic-pituitary-testicular axis with negative feedback mechanism of the downstream products played the pivotal regulatory role in maintaining homeostasis. Hypothalamus will control pulsatile GnRH secretion, which regulates pituitary glands to

produce gonadotropins (follicle stimulating hormone/FSH and luteinizing hormone/LH), whereas gonadotropins regulates testicular production of both hormones and sperm. Steroid and peptide hormone produced in the testes will provide a negative feedback signal to the pituitary and hypothalamus. Therefore, disruption of GnRH secretion is a potential target for contraceptive methods.

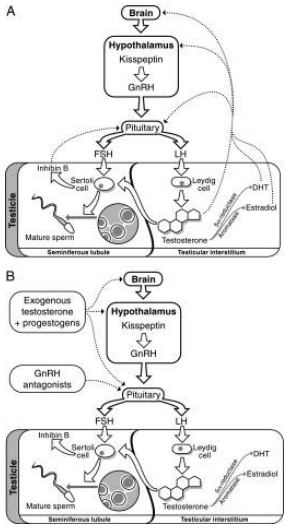

Figure 1. (A) Spermatogenesis in normal circumstances; (B) Effects of hormonal contraceptive intervention in the reproductive axis. Source: Page ST, Amory JK, Bremner WJ. Advances in male contraception. Endocrine reviews. 2008; 29(4): 465-93. PubMed PMID: 18436704. Pubmed Central PMCID: 2528850

Researchers found that the G protein receptor GPR 54 plays an important role in the secretion of gonadotropin and 54-amino acid peptide kisspeptin-54 (metastin) is the ligand for GPR 54. Subsequent investigations have demonstrated that kisspeptin administration in the brain results in GnRH and gonadotropin secretion in animal models and gonadotropin secretion can be blocked by administration of GnRH antagonists. Moreover, the negative feedback of testosterone and estradiol on GnRH secretion is mediated via inhibition of kisspeptin production in the arcuate nucleus of the hypothalamus. Inhibitors of kisspeptin, GPR 54 agonists and antagonists, in combination with testosterone in theory may be a strategy of contraception.

Testosterone is a steroid hormone produced primarly in the testes, but significant aromatase activity in the testicle and peripheral tissues result in production of estradiol as well. Testosterone works by inhibiting transcription of kisspeptin and GnRH and gonadotropin secretion. Estradiol also plays an important role in steroid negative feedback in men, particularly by decreasing LH production. It is also known that FSH regulation is more dependent on estradiol compared to testosterone.

The precise role of progesterone in normal male physiology is unknown, but progesterone receptors have been demonstrated in the male hypothalamus, pituitary, and reproductive tract. Additionally progestin administration enhance male hormonal contraceptive efficacy when combined with androgens. The effect is attributed to increased hypothalamic-pituitary suppression of gonadotropin secretion either directly or through the androgen receptor.

Finally, the nonsteroidal testicular product inhibin B contributes to hormonal feedback in males. Inhibin B is predominantly produced by Sertoli cells and will give negative feedback on the production of FSH. Theoretically, exogenous inhibin B in conjunction with androgens might further supress FSH, and can be used as a hormonal male contraceptive regimen.

Testosterone production and androgen action[3]

Testosterone is produced by the Leydig cells in the testes as a response to LH stimulation. However, studies in mice have shown evidence that testosterone production can also be non-LH dependent. Therefore, the regulation of testosterone production in non-dependent gonadotropin is a critical aspect in developing an effective male hormonal contraceptive method because hormonal contraception relies on blockade of gonadotropin secretion for efficacy.

In experiments, suppression of LH and FSH levels profoundly decreases testosterone production and reduces spermatogenesis in men. However, research on mice with LH-receptor-deficiency show that even with low androgen levels and further blockade of androgen receptor (flutamide) for complete suppression of residual androgen action, spermatogenesis can still occur, which will lead to contraceptive failure. Further investigation into the precise regulation and role of intratesticular androgens in supporting spermatogenesis in men is still needed, because targeted disruption of residual androgen production and action might be an adjunt to increase effectiveness of current hormonal methods.

Using a hormonal approach of contraception, testosterone is given to block gonadotropin secretion while maintaining nongonadal androgen-dependent functions such as sexual drive and muscle mass. Significant supraphysiological dosing of testosterone can

be associated with side effects, which includes high-density lipoprotein (HDL) suppression, acne, and increased hemoglobin concentrations that would not be desirable in a regimen designed or long-term use.

Spermatogenesis[3]

During spermatogenesis, spermatogonia will undergo 4 stages to develop into mature spermatozoa. First, type A spermatogonia undergo mitosis, resulting in renewal of germline stem cells as well as type B spermatogonia that continue to undergo differentiation. Secondly, type B spermatogonia undergo meiosis to become spermatids. Thirdly, there is spermiogenesis, where mature spermatids become motile spermatozoa. Fourth, the interaction with Sertoli cells mediate the spermiation process, where cytoplasmic material from the spermatid is removed and the mature sperm will be released into the lumen of the seminiferous tubules. This whole process requires regulation of endocrine hormones. FSH and LH are required for the above processes.

Epididymis[3]

Once spermatogenesis has been completed, sperm are released from the Sertoli cells into the lumen of the seminiferous tubules, and move through the epididymis before ejaculation. FSH and testosterone are thought to play a role in spermiation and the release of spermatid into the tubule lumen. Failure of spermiation will lead to sperm retention and phagocytosis by the Sertoli cell. Within the epididymis, sperm undergo further maturation steps, allowing for maximal motility and fertilization capacity.

Male Hormonal Contraceptive Mechanism

The mechanism of male hormonal contraceptives is to inhibit spermatogenesis by suppression of the hypothalamic-pituitary-testicular axis function. A reversible azoospermic condition is targeted by this inhibition.

Hypothalamus secretes gonadotropin-releasing hormones (GnRH) which stimulates the secretion of LH and FSH in the pituitary gland. LH binds to Leydig cells in the testicular interstitium and stimulates the production of testosterone. The resultant testosterone diffuses into the seminiferous tubules, and induces spermatogenesis, mediated by FSH stimulation from Sertoli cells. This Testosterone also enters the bloodstream and, in combination with estradiol, serves to regulate its own production by negative feedback at the level of the hypothalamus and pituitary gland.[5]

This regulation is used as a basic concept of male hormonal contraception. High level of exogenous testosterone will lead to inhibition of negative feedback regulation. It is accepted as a positive feedback in the hypothalamus and pituitary glands, so that the secretion of LH and FSH will be suppressed, as well as spermatogenesis. This regulation is expected to be reversible.

However, in several men, administration of testosterone alone fails to completely suppress sperm production. Therefore, ongoing study for combination male hormonal contraceptive still continues.

Male Hormonal Contraceptive Studies

Studies on male hormonal contraception have started since 1939. Testosterone administration is used to suppress secretion of LH and FSH from the pituitary. Low concentrations of these hormones will inhibit spermatogenesis and results in decreased sperm concentrations. First studies revealed reversible azoospermia in some patients with daily use of testosterone alone regimen administered in the duration of 60 days. Improvement of delivery frequency and dosage are still undergoing studies until now. However, some data have shown that administration of testosterone alone regimens fail to completely suppress spermatogenesis in many groups, due to ethnic differences, body fat content, baseline testosterone levels, different susceptibility of reductase levels for each groups and other reasons. The fact is, 91% of Asian and 60% of Caucasian patients become azoospermic due to differences in endocrine response.[6]

Recent studies still compared between administration of a testosterone-only regimen and testosterone combined with exogenous progestin.

Androgen Only

Testosterone-only administration has many side effects; including mood changes, weight gain, increase in hemoglobin and hematocrit, skin problems (oily skin and acne), decrease in testicular volume, gynecomastia, sleep apnea, and possible effects on cholesterol levels.[6]

Testosterone Buciclate

Testosterone Buciclate is a long acting testosterone derivative and have shown moderate effect of suppression of spermatogenesis at a dose of 1200 mg per month given through intramuscular injection. The first study was conducted by World Health Organization (WHO) in 1995, and revealed minimal side effects. This trial helped in the initiation of another study combining long acting testosterone with progesterone derivatives.

Testosterone Enanthate (TE)[6,7]

The first study conducted by WHO applied a regimen of weekly intramuscular injections of 200 mg Testosterone Enanthate (TE) for a year. As a result, 70% of the 271 patients became azoospermic within 6 months. The mean time needed for the process in the development of azoospermia was 3 months, and the mean time to recovery was 3-4 months. There was only one pregnancy occurring among the azoospermic patients who followed a 12-month efficacy phase. However, 12% of the men discontinued from the study because of the discomfort caused by the weekly intramuscular injections and also administration of testosterone-only as a contraceptive purpose failed to suppress spermatogenesis.

Similar with the first testosterone study conducted by WHO, the second study found some difficulties with the administration of 200 mg intramuscular TE for induction phase, and 100 mg weekly intramuscular injections in 399 volunteers. Although 98% patient experienced suppressed spermatogenesis to below 3 million sperms per ml, but 25% of patients discontinued this study due to several reasons, which include weekly injection discomforts.

Testosterone undecanoate (TU)[6,7]

A more recent multicenter study used a very-long-acting formulation of testosterone undecanoate (TU), which can be administered once every 3-4 months for testosterone replacement in men with hypogonadism. In two large studies in China, 500 mg TU was used as a single contraceptive agent. The first study enrolled 308 healthy men, and their partners administered TU once a month for 12 months. During the study, 299 men showed oligospermia with sperm concentrations below 3-million sperm/ml ejaculate, whereas nine men did not show suppression to this extent. As many as 296 of the subjects entered an efficacy phase, and six subsequently found it necessary to restart other forms of contraception because of their rising sperm counts. An overall efficacy rate of 95% was obtained from this study, with one pregnancy occurring in the study group. During the recovery period, hormonal levels and semen parameters returned to baseline.

A more recent study also carried out in China enrolled 1,045 men and their partners for a 1.5-year period using 500 mg TU monthly dosage. As a result, this group showed a 95% rate of spermatogenesis suppression and 95% contraceptive efficacy rate. From both studies, TU seems to be a highly acceptable, effective, safe, and reversible method for male contraception in healthy fertile Chinese men.

Synthetic Androgens

7α-methyl-19-norestrosterone (MENT)

MENT is synthetic androgen with potent gonadotropin inhibitory activity and prostate sparing effects.[8] This synthetic androgen is more potent than testosterone, it is resistant to 5 α-reduction, and has diffusion characteristics that make it easy to be administered in the form of depot implant. However, it has a similar side effect profile with testosterone and only provides partial dose-dependent suppression of spermatogenesis. Other sources have shown that MENT has a short half life, making it unsuitable for long-term injection; and that it does not show any significant FSH suppression.[6]

Dimenthandrolone undecanoate

Dimenthandrolone undecanoate is a potent synthetic androgen that has some progestational activity, which is currently in development for therapeutic uses in men. It appears to suppress LH secretion in vitro and in rabbit models. It works without stimulating prostate growth and is resistant to 5 α-reduction.[9]

Combined with Progestin

Combination of progestin and androgen replacement therapy as a contraceptive has been found to be well tolerated, safe, and effective. This method was known to be more effective in producing azoospermia at lower doses.

TU + Progestine

Norethistrone enanthate 200 mg, a long-acting injectable progestin with weak androgenic and estrogenic activity, shows great promise as an adjunct agent in combination with TU (1000 mg) for male hormonal contraception.[7] During a 6-months study in 40 men, comparing TU alone with TU + norethistrone enanthate, combination therapy was found to be more consistent in producing suppression of spermatogenesis and serum gonadotropins.[10]

In phase 2b, WHO conducted a contraceptive trial on 400 couples using TU and norethistrone enanthate injected once every 8 weeks for 48 weeks. This trial will provide additional data regarding the efficacy, safety, and tolerability of an injectable contraceptive regimen for men.

TE + Progestin

An RCT of levonorgestrel and TE combination has shown that this combination was superior to TE-only in achieving azoospermia. Levonogestrel implants (160 μg daily) combined with TE injection, was known to be more effective than Levonogestrel (125 μg daily) combined with testosterone patches.[7] Injection of depot medroxyprogesterone acetate (DMPA) and TE also resulted in azoospermia in 98% of subjects.

Combination with GnRH Antagonist[3,4]

GnRH antagonist regimens have been shown to be more acceptable in suppressing spermatogenesis than GnRH agonist, when combined with androgen agents. GnRH *antagonists* can suppress FSH and LH production within hours of administration, and inhibit gonadotropin secretion more completely than agonists.

There have only been a few studies investigating GnRH antagonist Nal-Glu, administered by daily subcutaneous injections, combined with intramuscular TE resulting in an azoospermia rate of between 67% and 93% at 6 to 16 weeks of use. A more recent study reported that daily cetrorelix combined with 19-nortestosterone-hexyloxyphe-nylpropionate achieved azoospermia in 100% of subjects by 12 weeks. However, GnRH antagonists have the problem of needing daily subcutaneous administrations and being expensive.

Recently, a new long-acting GnRH antagonist (acyline) given at twice weekly intervals has shown promise in a small number of subjects producing an azoospermia rate of 67% at 8 weeks. However, there are problems with local injection site reactions including erythema and induration.

CONCLUSION

The development of an effective, reversible, and acceptable male hormonal contraceptive is possible. Until now, combination therapy of androgen and progestin has been shown to be more effective than the use of androgen-only regimens or GnRH antagonists. However, no male hormonal contraceptive is currently ready for clinical use, due to limited available studies.

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