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EDITORIAL

What So Special with Endometriosis Characteristics yet We Don't Know About?

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Nowadays, managing endometriosis in a woman with reproductive age still poses many problems especially in the mean of recurrence and its impact on infertility and morbidity consequence.[1-5] Although many strategies have already done in curing this disease, but there is no single therapeutic mean which is really effective in curing the disease without scarifying reproductive function.[6,7]

Endometriosis is defined as the growth of endometrium outside uterine cavity, or ectopic endometrium. Endometriosis is also considered as benign neoplasm but poses malignant characteristics. It grows and often recurs immediately as another malignant neoplasm, it spreads all over human body, that could be found in a far site of the human body, e.g. eye, lung and even on skin surface. It could be found in surgery as ovarian cyst or already infiltrated to another adjacent tissues and far tissues.[2,5,7]

Many doctors dealing with this disease do not realize the most important factor in endometriosis. It produces oxidative stress that differs it from another benign neoplasm in gynecology. Its oxidative stress is caused by the production of iron due to the lyse of red blood cells, in the endometriosis lesion following regular period. This iron is one of the strongest oxidative agents that could lead to a long chain of oxidative stress. The oxidative stress in the endometriosis lesion is also considered as one major factor that worsen local inflammation and stimulates its ectopic endometrial cells to behave aggressively even could transform into malignant cells. Many studies have already done regarding this "important" issue but still no promising result was get. Certainly there are so many genetic changes also found in endometriosis lesion, that don't help either.[4,8,9]

Medically fighting endometriosis often difficult, many things still we don't know about. Many efforts must be done to answer the big question behind its mystery, as every effort would be better then doing nothing.

References

1. Rogers PAW, D'Hooghe TM, Fazleabas A, et al. Defining Future Directions for Endometriosis Research. Reprod Sci. 2013; 20(5): 483-99.
2. Moradi M, Parker M, Sneddon A, et al. Impact of Endometriosis on Women's Lives: A Qualitative Study. BMC Womens Health. 2014; 14: 123.
3. Hirsch M, Duffy JMN, Kusznir JO, et al. Variation in Outcome Reporting in Endometriosis Trials: A Systematic Review. Am J Obstet Gynecol. 2016; 214(4): 452-64.
4. Wei JJ, William J, Bulun S. Endometriosis and Ovarian Cancer: A Review of Clinical, Pathologic, and Molecular Aspects. Int J Gynecol Pathol. 2011; 30(6): 553-68.
5. Bulletti C, Coccia ME, Battistoni S, et al. Endometriosis and Infertility. J Assist Reprod Genet. 2010; 27(8): 441-7.
6. Elnashar A. Emerging Treatment of Endometriosis. Middle East Fertil Soc J. 2015; 20: 61-9.
7. Signorile PG, Baldi A. New Evidence in Endometriosis. Int. J. Biochem. Cell Biol. 2015; 60: 19-22.
8. Turkyilmaz E, Yildirim M, Cendek BD, et al. Evaluation of Oxidative Stress Markers and Intra-Extracellular Antioxidant Activities in Patients with Endometriosis. Eur J Obstet Gynaecol Reprod Biol. 2016; 199: 164-8.
9. Kumar P, Ashok V. Role of Iron in The Oxidative Stress in the Pathophysiology of Endometriosis: A New Concept to Know The Potential Therapeutic Benefit. Fertil Sci Res. 2014; 1(1): 19-22.

Research Article

Assistance Influence on Labor Pain Level

Pengaruh Pendampingan terhadap Tingkat Nyeri Persalinan

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Abstract

Objective: To assess assistance influence on labor pain level.

Method: This study was a randomized-clinical, unmasked trial with concealment by measuring labor pain level in two patients group: with and without assistance during labor; each group consisted of 36 subjects. Pain intensity were measured using Faces Pain Rating Scale. Mann-Whitney analysis was done to assess significance of pain level between two groups.

Result: Majority of patient who were in non-assisted group had very painful score 50% with mean of VAS 7.38±2.12, meanwhile most of assisted group complained painful score 44.44%, with mean of VAS 6.11±1.90.

Conclusion: There was significance level of painful score between non-assisted and assisted subjects x($p<0.05$). Assistance had more impact in decreasing labor pain level in primigravida subjects.

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Keywords: assistance, labor pain, visual analog scale (VAS)

Abstrak

***Tujuan**: Untuk mengetahui pengaruh pendampingan terhadap tingkat nyeri persalinan.*

***Metode**: Menggunakan desain uji klinis acak tidak tersamar dengan metode penyembunyian dengan cara mengobservasi dan mengukur tingkat nyeri selama persalinan pada dua kelompok pasien, yaitu kelompok pasien dengan pendampingan dan kelompok pasien tanpa pendampingan; dengan jumlah pasien 36 orang tiap kelompok. Nyeri persalinan diukur dengan menggunakan metode Faces Pain Rating Scale. Analisis dilakukan dengan uji Mann-Whitney.*

***Hasil**: Tingkat nyeri pada ibu yang tidak didampingi lebih tinggi daripada ibu yang didampingi, di mana yang merasakan sangat nyeri pada ibu yang tidak didampingi sebesar 50%, dengan rata-rata VAS 7,38 ±2,12, sedangkan pada ibu yang didampingi merasakan nyeri 44,4%, dengan VAS 6,11±1,90.*

***Kesimpulan**: Terdapat perbedaan bermakna antara pendampingan dan tanpa pendampingan ($p<0,05$).*

[Maj Obstet Ginekol Indones 2016; 1: 3-7]

***Kata kunci**: nyeri persalinan, pendampingan, visual analog scale (VAS)*

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INTRODUCTION

Labor pain is affected by interaction of physical, psychological, environment and supportive factors which are complex and subjective.[1] Support and presence of family members during labor process were stated as factors which also affected labor pain.[2] However, in the past, husbands' assistance during labor was not allowed in order to avoid infection in delivery room.[3] Whereas, from previous studies, it was showed that mothers who were assisted during labor underwent less pain, shorter delivery time, and lower risk for any surgeries.[4] Presence of assistants now become a recommendation for normal delivery process. Supportive measures consist of continuous presences during active period of labor or giving touch and compliments which make comforts.

Objectively, labor pain was assessed by visual analog scale (VAS).[5] Besides assistance, many other factors were reported playing role in affecting VAS in laboring mothers, such as: fear, age, gravida, parity, and education level.[6,7] However, other study gave different results: there were no significances between several assessed variables (age, parity, duration of stage II delivery, babies birth weight) and labor pain intensity.[8]

This study have main focus in assessing specifically assistance influence on labor pain level. Similar studies were still limited. The results of this study were expected to become a helpful measures in making clinical decision about laboring assistances in the future.

METHODS

This study was a randomized clinical unmasked trial with concealment by measuring labor pain level during period October 2012 - March 2013 in two patients groups: with and without assistance during labor; each groups consisted of 36 subjects.

Gestational age, and being in stage I of delivery. Those who were with any comorbidities or complications, cephalopelvic disproportion (CPD) suspect, got analgetic therapy, or planned to use sectio-caesarea method, were excluded from this study. Subjects allocation were randomized by third party and then concealed. All subjects were treated equally: underwent process of history taking, physical examination, laboratory examination, ultrasonography (USG) examination and cardiotocography (CTG) examination.

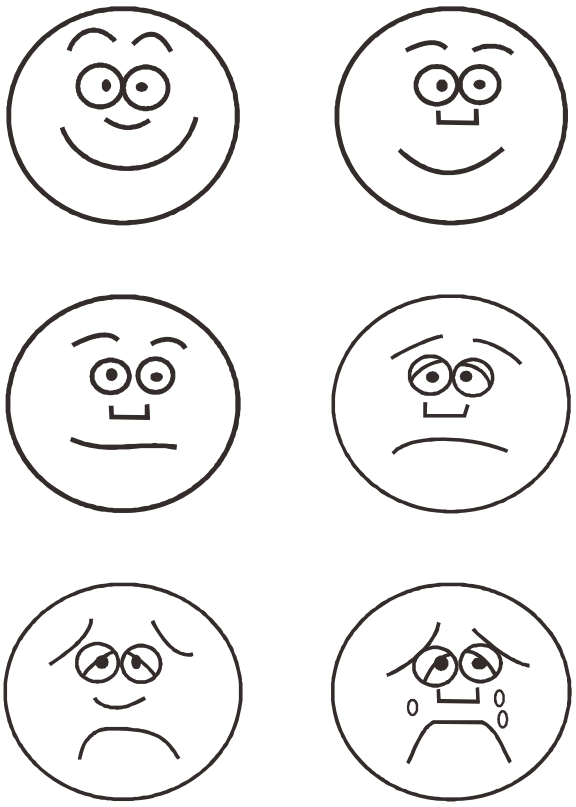
Patients Assistance

Assisted patients could choose their own assistants. However, the assistants needed to do special instructions of actions done in order to get uniform measures of assistances. Specified actions were : providing drinks between any contractions, assisting during micturition, giving support when patients groaned in pain, taking a walk with patients, massaging low back part of patients, consoling, giving hope and strenghtening patients mind. Besides, assistants needed to deliver patients' message to the health workers, help patients to be in proper position during labor, give courage during straining phase, and be in patients' side during labor until birth phase.

Pain Measurement and Analysis

Pain intensity during labor were measured using Faces Pain Rating Scale (Wong Baker® Visual Analogue Scale); categorically were as follows: 0 = not painful, 2 = quite painful, 4 = moderate painful, 6 = painful, 8 = very painful, and 10 = most painful (Figure 1). Descriptive data were presented in the form of frequency and percentage, consisted of age group, occupation, education level, gestational check-up frequency, and parity variables. VAS data were assessed using independent T-test when data distribution was normal. Otherwise, analysis was done.

The subjects chosen were laboring mother with spontaneous delivery method, aterm using Mann-Whitney test. However, it was found that data distribution was not normal, so that Mann-Whitney test was the one used. Correlation between independent variables and labor pain level was assessed using correlation test of Spearman. This study was approved by Medical Research and Ethichal Committee, Faculty of Medicine, University of Indonesia: 686/H2.F1/ETIK/2012.



RESULTS

Subjects Characteristics

Subjects age ranged from 17 to 45 years old, with median of 29 years. Most of the subjects were housewives (83.33% in non-assisted group; 77.78% in assisted group) and had gestation check-up frequency more than four times (75.00% in non-assisted group; 66.67% in assisted group). In assisted group, 66.67% of subjects were assisted by their own husbands (Table 1).

Table 1. Subjects Characteristics

Variables		Non-assisted n (%)	Assisted n (%)
Age group	a) <20 years	3 (8.33)	4 (11.11)
	b) 21- 30 years	20 (55.56)	19 (52.78)
	c) >30 years	13 (36.11)	13 (36.11)
Occupation	a) Labor	1 (2.78)	0 (0.00)
	b) Merchant	1 (2.78)	0 (0.00)
	c) Private employees	2 (5.56)	5 (13.89)
	d) Nurse/Government employees	1 (2.78)	2 (5.56)
	e) Housewives	30 (83.33)	28 (77.78)
	f) Enterpreneur	1 (2.78)	1 (2.78)
Education level	a) Elementary school	4 (11.11)	5 (13.89)
	b) Junior high school	3 (8,33)	4 (11.11)
	c) Senior high school	26(72.22)	23(63.89)
	d) Diploma	3 (8,33)	4 (11.11)
Gestation check-up Frequency	a) > 4x	27(75.00)	24 (66.67)
	b) 4x	5 (13.89)	5 (13.89)
	c) < 4x	4 (11.11)	7 (19.44)
Gravida	a) G1	14 (38.89)	15 (41.67)
	b) G2	8 (22.22)	8 (22.22)
	c) G3	10 (27.78)	9 (25.00)
	d) G4	4 (11.11)	3 (8.33)
	e) G5	0 (0.00)	1 (2.78)
Assistants	a) Husband	0 (0.00)	24 (66.67)
	b) Other than husband	0 (0.00)	12 (33.33)

Assistances and Pain Level

Differences of VAS score between non-assisted and assisted subjects were showed in Table 2. Majority of patients who were in non-assisted group had very painful score (50%), followed by most painful score (19.44%). Meanwhile, in assisted subjects, most of them complained painful score (44.44%), followed by very painful score (25.00%). Non-assisted subjects had mean of VAS 7.38±2.12, while assisted subjects had 6.11±1.90. There was significance of painful score between non-assisted and assisted subjects ($p < 0.05$) by Mann-Whitney test.

Table 2. Visual Analog Scale Comparison between Assisted and non-Assisted Subjects.

VAS	Non-assisted (n=36)		Assisted (n=36)	
	n	%	n	%
		5.5		5.5
2	2	6	2	6
		8.3		19
4	3	3	7	44
		16		44
6	6	67	16	44
		50		25
8	18	00	9	00
		19		5.5
10	7	44	2	6
Mean±SD	7.38 ± 2.12		6.11 ± 1.90	
Median (range)	8 (2-10)		6 (2-10)	

Correlation analysis was done using patients' characteristic as independent variables, consisting : age, gravida, education level and gestation check-up frequency. There were very low inverse correlations but there were no significances between mentioned variables and labor pain level using Spearman's correlation analysis (Table 3).

Table 3. Correlation between Subjects' Characteristics and Labor Pain Level.

Variables	Spearman's Correlation	p
Age	-0.128	0.902 (ns)
Gravida	-0.024	0.845 (ns)
Education level	-0.182	0.126 (ns)
Gestation check-up frequency	-0.035	0.768 (ns)

Labor pain was further differentiated into category based on gravida: primigravida and multigravida. The mean of labor pain was higher in primigravida non-assisted group with mean 8.3 ±2.20 than in primigravida assisted group (6.1± 1.92). Meanwhile, multigravida group has quite similar labor pain, respectively for non-assisted and assisted: 6.8±1.92 and 6.1±1.95 (Table 4).

Table 4. Labor Pain Level and Parity Status.

Pain Level	Primigravida		Multigravida	
	Non-assisted	Assisted	Non-assisted	Assisted
2	1 (2.78)	1 (2.78)	1 (2.78)	1 (2.78)
4	0 (0.00)	3 (8.33)	3 (8.33)	4 (11.11)
6	1 (2.78)	5 (13.89)	5 (13.89)	11(30.56)
8	6 (16.67)	6 (16.67)	12 (33.33)	3 (8.33)
10	6 (16.67)	0 (0.00)	1 (2.78)	2 (5.56)
Total	14 (38.89)	15 (41.67)	22 (61.11)	21 (58.33)
Mean±SD	8.3±2.20	6.1±1.92	6.8±1.92	6.1±1.95

DISCUSSION

There was significant difference of labor pain intensity between subjects who were assisted and subjects who were not assisted during labor process ($p < 0.05$). This was possible due to their feeling of comfort, courage, and emotional support, all of which could strenghten subjects in their labor phase. Active attitude of the assistants, just as applied in this study, purposely gave support to decrease anxiety and pain level in mothers/patients. Psychology factor had a big role in affecting pain during labor, especially in the form of anxiety. This anxiety further caused fear and stress during delivery process. Stress could trigger production of excessive stress hormones, such as catecholamine and steroid. Those hormones induced smooth muscle tension and vascular vasoconstriction and led to decreased contraction of uterus, decreased uteroplacenta circulation, decreased consumption of oxygen to uterus, and generated ischemic condition of uterus, in which resulted in the increasing of pain impulse.[9-11]

In previous studies by Chunuuan et al and Henneborn et al were stated that family support could decrease anxiety and pain. Husbands active-role in assisting labor process could also increase mothers' self esteem, in example by reminding breathing and straining technique or by helping communicate with midwives.[12] Husbands' assistance role in decreasing anxiety was proven in several studies in United Kingdom, Finlandia and Hungaria.[13] Meanwhile study in Iran and China proved that husbands' presence could lower pain perception so that analgetic medicines administration during labor could be diminished.[14]

While husbands' role were proven significant, among assisted subjects during her labor, 12 subjects (33%) chose their trusted ones aside from their husbands (mother, mother in law, or sister). One of the reasons stated by subjects was their comfort when accompanied by fellow women. Cultures and beliefs played significant role in this comfort feeling; for example was Nepal. In Nepal, husbands' assistance was not something common because husbands were prohibited from touching blood products or vaginal fluid, which were believed as dirty things.[15] Quite similar reason was proposed by Russian women. They rejected their husbands' presence because they were anxious that their husbands would emotionally unable to see blood during labor process which could lead to loss of sexual desire after delivery.[15] Other than that reasons, there were guilty and shame feeling when their husbands saw and heard while they were screaming in pain, as well as discomfort feeling when their husband should take care all delivery needs.[12]

In this study, the labor assistant were given specific instruction about their actions and attitude. This intervention was given in order to get uniform and active attitude considering that every individual (assistant) came from different backgrounds, education level, and personality. Out of ten instructed attitudes, most of the assistants did as instructed. It showed that there were positive expectable supports which could help subjects in perceiving less pain. Therefore, the husbands or assistants should be given proper information about things to do during delivery psychologically and practically to help the mothers controlling pain perception.[16]

It is interesting to see the fact that in non-assisted primigravida subjects, there were higher pain level (8.3±2.20) compared to unaccompanied primigravida (6.1±1.92). It was also higher when compared to either non-assisted and assisted multigravida (6.8±1.92 and 6.1±1.95 respectively). It showed us that assistance had big impact in decreasing pain level, especially in primigravida subjects. This is possible due to positive psychological support from assistants so that the mother who experienced delivery process for the first time could feel calmer and be less anxious; led to decreased level of pain. Meanwhile, in multigravida, either non-assisted or assisted had quite similar pain level. It could root from psychological readiness from previous labor experience so that mothers had already adapted in the current labor process. In contrast with this study, previous study which assessed pain level when mother assisted by midwives, didn't show significance with pain level in primigravida and multigravida 8.31±0.99 and 8.37 ±1.17 respectively. Same insignificance were also obtained from different studies, in which 76.3% in primipara group and 73.3% in multipara group had pain level ≥ 8 ($p = 0.63$).[16]

CONCLUSION

This study discovered that there were significance level of labor pain between subjects who were assisted and not-assisted during delivery or labor process ($p < 0.05$). However, there were no correlation between subjects characteristics as independent variables (consisted of age, education level, parity, and gestation check-up frequency) and labor pain level. Assistance had more impact in decreasing labor pain level in primigravida subjects, while there were no difference of pain level between assisted and non-assisted multigravida in labour process.

REFERENCES

1. Kamalifard M, Pirdel M, Bani S, et al,. Comparison and evaluation of labor pain and factors influencing pain perception in primiparous and multiparous woman referring to tabriz alzahra educational center in 2005-2006. Research J Med Sci 1. 2007; 5: 271-8.
2. Sheiner EK, Sheiner E, Vardi I, et al,. Ethnic Differences Influence Care Giver's Estimates Of Pain During Labour. Int Association for The Study of Pain. 1999; 81: 299-305.
3. Dagun SM. Calon Ayah dan Ketika Istri Hamil. Psikologi Keluarga (Peranan Ayah Dalam Keluarga). Jakarta: Rineka Cipta. 2002: 28-35.
4. Nolan M. Personel Pendukung Selama Persalinan. Kehamilan dan Melahirkan (Being Pregnant, Giving Birth). Jakarta: Arcan. 2003: 145-53.
5. Bergh I, Soudwerlund T, Maternsson LB. Reability and validity of the acceptance symptom assessment scale in assessing labor pain. Midwevery. 2012; 28: e684-8.
6. Saisto T, Kaaja R, Ylikorkala O, et al. Pain tolerance tested by cold pressure test in late pregnancy is lower among women fear of labor pain. Helsinki: University Hospital Helsinki; 2000.
7. Sheiner EK, Sheiner E, Vardi I. The Relationship between parity and labor pain. Int J Gynaecol Obstet. 1998; 63: 287-8.
8. Klostergaard KM, Terp MR, Poulsen C, et al. Labor pain in relation to fetal weight in primiparae. Eur J Obstet Gynecol Reprod Biol. 2001; 99(2): 195-8.
9. Ullman R, Dowswell T, Mori R. Parenteral opioid for maternal pain relief in labor. Cochrane Database Sys Rev. 2009; 3: 1-10.
10. Tournaire M, Theau-Yonneau A. Complementary and alternative approaches to pain relief during labor. Evid Based Complement Alternat Med. 2007; 4(4): 409-17.
11. May A, Elton C. The Effects of Pain and its management on mother and fetus. Bailliere's Clin Obstet Gynecol. 1998; 12(3): 423-39.
12. Nejad VM. Couples's attitudes to the husband's presence in delivery room during childbirth. La revue de Sante de la Mediterraee orintale. 2005; 22(4): 824-34.
13. Ip WY. Chinese husband's precence during labour: a preliminary study in Hong Kong. Int J Nursi Pract 2000; 6: 89-96.
14. Saisto T, Ylikorkala O. Factor associated with fear of delivery in second d pregnancies. Obstet Gynecol. 1999; 94(5): 679-82.
15. Sapkota S, Kobayashi T, Takase M. Women's experience of giving birth with their husband's support in Nepal. Bri J Midwifery. 2011; 19(7): 426-32.
16. Manizheh P, Leila P. Percieved environmental stressors and pain perception during labor among primiparous and multiparous woman. J Reprod Infertil 2009; 10(3): 217-23.

Research Article

Fetal Fibronectin (fFN) on the Imminent Premature Parturition in Correlation with Incidence of Preterm Labor

Fibronektin Fetus (fFN) pada Partus Prematurus Imminen dengan Kejadian Persalinan Prematur

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Abstract

Objective: To determine the relationship of fFn levels in cervicovaginal discharge of pregnant women who experience imminent premature parturition with the incidence of preterm labor.

Method: The study was carried out with Analytic Observational Prospective Cohort using cervicovaginal discharge of pregnant women that experienced imminent premature parturition taken from the delivery room of Obstetrics and Gynecology department dr. Saiful Anwar Hospital, Malang as well as Bangil Hospital and Ngudi Waluyo Wlingi Hospital. Statistical analysis was performed using the Shapiro-Wilk test and comparison test used independent samples t test for normal data, Mann-Whitney test if not. All analysis used SPSS for Windows 19.0 software.

Result: Thirty two patient samples was examined, 14 patients (43.75%) were primigravida and 18 patients (52.56%) is multigravida. 17 of these patients (53.13%) experienced aterm labor and 15 patients (46.87%) experienced preterm labor. Mann-Whitney test of the mean fFN levels between the aterm group (13.01 ± 7.57 ng/ml) and the preterm group (56.29 ± 27.77 ng/ml) showed a significant difference (p-value = 0.000 > 0.05). Moreover, Spearman's Rho correlation test also showed a strong correlation between fFN level and incidence of preterm labor (R = 0.797, p < 0.05).

Conclusion: fFN levels is significantly increase in cervicovaginal discharge from pregnant women with imminent premature parturition who experience preterm labor than pregnant women who experience aterm labor. Therefore, this result suggests that fFN has potential ability to become useful modality in preterm labor diagnosis.

[Indones J Obstet Gynecol 2016; 1: 8-14]

Keywords: cervicovaginal discharge, fFN, imminent premature parturition, preterm labor

Abstrak

Tujuan: *Mengetahui hubungan kadar fFN dalam sekret serviks dan vagina ibu hamil yang mengalami partus prematurus imminen dengan kejadian persalinan prematur.*

Metode: *Penelitian ini dilaksanakan secara Observasional Analitik dalam bentuk Kohort Prospektif menggunakan secret cervicovaginal wanita hamil yang mengalami partus prematurus imminen yang diambil dari kamar bersalin SMF Obstetri dan Ginekologi RSUD dr. Saiful Anwar Malang, RSUD Bangil serta RSUD Ngudi Waluyo Wlingi. Analisis statistik dilakukan dengan uji normalitas data sampel dengan uji Shapiro-Wilk, uji komparasi menggunakan uji t sampel bebas (independent sample t test) jika data terdistribusi normal, tetapi jika tidak maka digunakan uji Mann-Whitney. Semua penghitungan dilakukan dengan bantuan piranti lunak (software) SPSS for Windows 19.0.*

Hasil: *Dari jumlah total 32 sampel pasien yang diperiksa, 14 pasien (43,75%) merupakan primigravida dan 18 pasien (56,25%) merupakan multigravida. Dari pasien tersebut 17 diantaranya (53,13%) mengalami persalinan aterm dan 15 pasien (46,87%) mengalami persalinan prematur. Uji Mann-Whitney dari rerata kadar fFN antara kelompok aterm (13,01 ± 7,57 ng/ml) dan kelompok prematur (56,29 ± 27,77 ng/ml) menunjukkan perbedaan yang signifikan (p-value = 0,000<0,05). Hasil uji korelasi Spearman's Rho juga menunjukkan adanya hubungan yang kuat antara kadar fFN dengan kejadian persalinan prematur (R = 0,797, p < 0,05).*

Kesimpulan: *Kadar fFN mengalami peningkatan secara signifikan pada sekret serviks dan vaginal dari pasien dengan partus prematurus imminen yang mengalami persalinan prematur dibandingkan dengan yang mengalami persalinan aterm. Oleh karena itu, hasil ini menunjukkan fFN memiliki potensi untuk menjadi modalitas yang berguna dalam diagnosis persalinan prematur.*

[Maj Obstet Ginekol Indones 2016; 1: 8-14]

Kata kunci: *fFN, partus prematur imminen, persalinan prematur, sekret serviks dan vagina*

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INTRODUCTION

Preterm labor was defined as a delivery before 37 weeks' gestation. Preterm labor is still a major problem in obstetrics and 30% of cases occur with preterm premature rupture of membranes (PPROM).[1] Based on the health surveys of households conducted by the Ministry of Health said that the infant mortality rate as a result of premature birth in the Southeast Asia countries reached approximately 3 million cases annually, while in Indonesia the case of infant mortality is about 46 cases of 1000 live births in which preterm labor is

one of the main causes of the higher rate in prenatal mortality.[2] Although there is some intervention to prevent this, the incidence of preterm birth continues to rise. Premature parturition occurs in 7-12% of all pregnancies, in which three quarter of them associated with spontaneous labor.[3]

The incidence of preterm labor with or without rupture of membranes can be predicted through several ways including: measurement of IL-6 in the amniotic fluid which is increase in the incidence of preterm labor and collagen mRNA expression in amnion and chorion tissue which affects the threshold of ruptured membranes. Calculate the levels of MMP-8 in amniotic fluid, which is associated with increased amniochorionic infection that correlated with the occurrence of preterm labor. The predictors of preterm delivery above have good predictive value but very invasive, therefore it is necessary to find an alternative method in predict the incidence of preterm labor that are not invasive and has good predictive value as well. Fetal fibronectin (fFN) test and cervical scan has been investigated as a predictor for premature parturition.[4]

fFN is one of the most frequent biochemical parameters used to predict the risk of premature parturition and the success rate has been proven in a randomized controlled study in symptomatic and asymptomatic women. fFN test value lies in its high negative predictive value.[4]

Fibronectin is a glycoprotein with high molecular weight which can be found in body fluids such as plasma and extracellular matrix. This substance is derived from amniotic fluid, placental tissue and malignant cells which is responsible for managing a variety of biological functions, including coagulation and bacterial opsonization. fFN express an oncofetal domain.[3] fFN serve as "biological adhesive" that helps to attach the fetal chorionic membrane with maternal decidua.[5] In early pregnancy, when gestational saccus attached to the uterine wall, presence of fFN in cervicovaginal secretions are a normal condition. When choriodecidual surface has joined around 20-22 weeks, presence of fFN in this cervicovaginal discharge is not physiological. This may be caused by inflammation or disruption mechanism in the placenta or fetal membranes.[3] Measurement levels of fFN in cervicovaginal secretions of pregnant women who experience imminent premature parturition (IPP) is a non-invasive method to predict the incidence of preterm labor and the prediction of other pathological conditions.[6]

This research aim to investigate the relationship between fFN levels in cervicovaginal secretions of pregnant women who have IPP with the incidence of preterm birth in the delivery room and obstetric ward at Dr. Saiful Anwar Malang hospital, Bangil hospital as well as Ngudi Waluyo Wlingi hospital.

METHODS

Subjects Selection

The inclusion criteria for this study were: pregnant women with gestational age of 30 weeks to 34 weeks experienced an imminent premature parturition, not accompanied by intrauterine infection according to Gibbs criteria which require termination of pregnancy, fetal congenital abnormalities, deformities of the uterus, antepartum hemorrhage, preeclampsia, eclampsia, gemeli and polyhydramnios, willing to follow the study. Exclusion criteria for this study were: pregnant women with imminent premature parturition but rejected to receive standard therapies, with cervical manipulation or have sexual intercourse within 24 hours before admission, have ruptured membranes, accompanied by non-obstetric medical complications.

Measurement of Fibronectin Fetus

Terms of specimen retrieval

Intact amniotic membrane, minimum cervical dilatation (<3 cm) and sampling was not performed at the gestational age < 30 weeks and > 34 weeks.

Taking specimen

Specimens taken from the posterior vaginal fornix while performed sterile speculum examination (before performed another procedure such as digital cervical examination, vaginal ultrasound, etc.) Specimens taken by Adeza Biomedical Specimen Collection Kit in the form of polyester tipped swab (Dacron swab). Swab is inserted into the vagina and turn lightly along the posterior fornix for about 10 seconds to absorb the cervicovaginal secretions. After that, remove the swab from the vagina carefully and place it in a tube that has been filled with fluid buffer (packet Specimen Collection Kit) until the tip of Dacron submerged. Break the swab on the sign holder stems available. Make sure the rest of the rod just about the tube and close the tube

tightly. Give label Specimen Transport Tube. If the sample is not immediately checked, immediately put in the refrigerator.

Measurement Levels of fFN (Fetal Fibronectin Enzyme Immunoassay)

The principle is to use the ELISA examination. Prepare all reagents and samples. Put 100 µl sample and 100 µl standard liquid into a micro titer well. Cover with the adhesive strip provided. Samples were incubated in a micro titer well were plated FDC-6 (monoclonal antibody) for 2 h in the temperature of 37°C. Dispose of water from each well. Add 100 µL Biotin antibody (1x) in each well. Cover with a new adhesive strip. Incubate for 1 hour in a 37°C temperature. Warm to room temperature and mix gently until solution appears homogeneous. Aspirate from each well and then wash, reprocess a sample of 2x and 3x leached standards. Washing is by filling each well with Wash Buffer (200 µL) using a squirt bottle, multi-channel pipette, manifold dispenser, or auto washer and let stand for 2 minutes. After the waste until completely clean. After the last wash, wash all of the wash buffer by aspirating ordecanting. Turn well and stick to clean blotting paper. Add 100 µL avid in HRP (1x) in each well. Close microtiter new adhesive strip. Then Incubate for 1 hour at a temperature of 37°C. Repeat the process of washing and aspiration as much as 5 times. Add 90 µL of TMB substrate to each well. Incubasition for 15-30 minutes at a temperature of 37°C. Keep it away from light. Add 50 µL Stop Solution to each well, and tap-tap the plate gently to mix the solution. After that the samples were examined with a spectrophotometer at a 550 nm wavelength to determine the concentration of fetal fibronectin.

Data Analysis

In this study, the data analysis techniques used namely normality test sample data with the Shapiro-Wilk test, comparison test used independent samples t test (independent sample t test) if the data were normally distributed, but if not then used Mann-Whitney test. All calculations performed in software SPSS for Windows 19.0.

RESULTS

Characteristics of study subjects based on parity/ pregnancy status can be seen in Table 1.

Table 1. Pregnancy Status Frequency Distribution

Pregnancy status	Frequency	Percentage
Primigravida	14	43.75 (%)
Multigravida	18	56.25 (%)
Total	32	100 (%)

From the table above, it appears that the subjects are scattered by parity/pregnancy status among primigravida group consisted of 14 people (43.75%) and multigravida group consisted of 18 (56.25%).

Characteristics of study subjects based on age can be seen in Table 2 below.

Table 2. Age Frequency Distribution of Subjects

Age (yo)	Frequency	Percentage
< 20	10	31.25 (%)
20-35	17	53.12 (%)
> 35	5	15.63 (%)
Total	32	100 (%)

From the table above it is known that the study subjects scattered in the age group < 20 years old were 10 people (31.25%), age group 20-35 years were 17 (53.12%) and the age group > 35 years were 5 people (15.63%). Characteristics of study subjects based on the incidence of preterm and aterm delivery presented in Table 3 below.

Table 3. Occurrence of Labor Frequency Distribution

Occurrence of labor	Frequency	Percentage
Aterm	17	53.13 (%)
Preterm	15	46.87 (%)
Total	32	100 (%)

From the table above it appears that the study subjects are scattered among the groups based on the occurrence of aterm labor consisted of 17 people (53.13%) and preterm group consisted of 15 people (46.87%).

Parametric Test

In this study, the results of data analysis on the normality test performed using the Shapiro-Wilk test. The decision criteria, i.e when the Sig or p-value is greater than $\alpha = 0.05$ thus the data is nor-

mally distributed, while the Sig or the p-value is smaller than $\alpha = 0.05$, the data were not normally distributed. If the data were normally distributed, the comparison test used independent samples t test. Meanwhile, when the data were not normally distributed, the comparison test used Mann-Whitney test. Shapiro-Wilk test analysis showed that subjects age data (years old) in the aterm group (p-value = 0.114>0.05), and the preterm group (p-value = 0.783>0.05), p-value indicates the value greater than the significance level $\alpha = 0.05$. So the subject age data has proved to be normally distributed, therefore it were analyzed using independent samples t test. While fFN levels (ng/ml) in aterm group (p-value = 0.005<0.05) and preterm group (p-value = 0.031<0.05), p-value indicates the value is smaller than the significance level $\alpha = 0.05$. So fFN levels data were not normally distributed and therefore it were analyzed using the Mann-Whitney test to prove the research hypothesis.

Comparison Test Results of Subjects Age

In the comparison test results for age (years) based on the occurrence of labor between aterm group (n = 17) and preterm group (n = 15) using independent samples t test briefly described and shown in the table below. Based on the results of independent samples t-test showed that there was no significant difference (p-value = 0.445>0.05), the mean age of the study subjects, between aterm group (25.24 ± 7.97 years) and the preterm group (7.27 ± 27.33 years). It is explain that age of subjects spread evenly based on the incidence of labor between aterm group and preterm group.

Comparison Test Results of fFN Levels

In the comparison test results of fFN levels (ng/ml) based on the occurrence of labor between aterm group (n = 17) and preterm group (n = 15) using the Mann-Whitney test briefly described and shown in the table above. Based on the results of the Mann-Whitney test showed that there was a highly significant difference (p-value = 0.000<0.05) between the mean levels of fFN (ng/ml) in aterm group (13.01 ± 7.57 ng/ml) and preterm group (56.29 ± 27.77 ng/ml). It is showed that subjects with incidence of preterm birth showed higher levels of fFN compared to subjects with aterm labor.

Comparison test results of fFN levels (ng/ml) based on pregnancy parity/status between primigravida group (n = 14) and multigravida group (n = 18) using the Mann-Whitney test briefly described and shown in the table below. Based on the results of the Mann-Whitney test, it is showed that there was no significant difference of subjects fFN levels (ng/ml) (p-value = 0.000 <0.05) among primigravida group (28.46 ± 25.45 ng/ml) and multigravida group (37.06 ± 32.21 ng/ml). However, based on the average value of the fFN levels, subjects with multigravida tend to be higher compared with subjects in the primigravida, although this difference was not statistically significant.

Correlation Test Results of fFN Levels with Incidence of Preterm labor

Based on the data analysis from Spearman's rho correlation test, occurance of labor with fFN levels in the study sample (n = 32) briefly described and shown in the table below. There was a highly significant relationship between the occurrence of labor with fFN level (p-value = < 0.000) on research subjects (n = 32) with strong relationship (correlation coefficient = 0.797). Positive value (0.797) indicates there is a very strong relationship between the two variables, i.e increase in the fFN levels was related to increase of preterm labor, and so did the other hand, when there is a reduction in the incidence of preterm labor, fFN would also decreased.

DISCUSSION

Fibronectin (FN) is a glycoprotein dimer with a molecular weight of 440 k Da. There are two types of FN, i.e soluble FN (soluble plasma fibronectin) which is the main protein in plasma (300 pg/ml) produced by hepatocytes in the liver cells and insoluble FN (insoluble cellular fibronectin), which is the main component of the extracellular matrix produced by various cells, especially fibroblasts and other cells such as chorion, membrane basalis and some cancer cells.[7] Fetal fibronectin (fFN) included in the large glycoprotein with a molecular weight produced by chorionic membranes during pregnancy and at desidua basalis near intervilli space. fFN is a major component of the extracellular matrix amniotic membrane.[3,8] fFN is important in embryogenesis, especially in the process of cell adhesion and migration during embryo development.[9] fFN can be detected in the connective tissue of the fetus, placental tissue and amniotic tissue. fFN generally can also be found in cervicovaginal

discharge until 20 weeks gestational age but are no longer found in gestational age from 24 to 34 weeks because of a consolidation uteroplacental junction and the formation of amniotic membrane is already perfect. Occurrences of fFN in 24-34 weeks showed the presence of leakage or disintegration fFN from uteroplacental junction that indicating a pathologic separation condition between the fetal's membrane with maternal decidua or fetal membrane rupture that exposed fFN from amniotic fluid to cervicovaginal discharge. Besides, the fFN in cervicovaginal discharge in symptomatic women with gestational age of 24-34 weeks, indicating high risk of imminent premature parturition.[10,11]

In this study, the fFN level of mother with aterm labor was 13.01 ± 7.57 ng/ml and in preterm labor was 56.29 ± 27.77 ng/ml. Measurement using the Mann - Whitney test showed that there were significant differences between the 2 groups (p = 0.000 p < 0.05). These findings correspond with the results of previous studies by Peaceman (2007), which showed that the detectable fFN in a significant level (> 50 ng/ml) showed an increased risk of preterm labor. The results also showed that the increase in the fFN level in cervicovaginal discharge caused by the fibronectin secretion during acute perturbation phase of the intrauterine area.[3] Another study by Schmitz (2006) showed that in pregnant women with fFN level > 50 ng/ml, there was a risk of getting labor before 35 weeks gestational age or in 7-14 days after fFN rate was detected.[11] This is also supported by research from Bolt (2011), which indicated that the mother with a short cervix size (<15 mm) and high fibronectin level (> 50 ng/ml) has a high risk factor for experiencing delivery before age 35 week.[12]

From the findings above, it was known that most (53%) of pregnant women with IPP have preterm labor and almost half (47%) of pregnant women with IPP were not have preterm labor (aterm). Theoretically, it can be mentioned that there were many cause of imminent premature parturition including infection, inflammation, hemorrhage, preeclampsia, eclampsia, trauma and other causes. Previous study showed that fFN not act directly as the cause of preterm labor in mothers with IPP. However, in several studies have shown that some mechanism that caused preterm labor like bleeding, eclampsia, preeclampsia, infection can trigger the occurrence of inflammation, which can cause increased expression of fFN in cervicovaginal discharge after a gestational age > 24 weeks. Research showed that pro-inflammatory cytokines such as TNF-α, IL-6, PAF (platelet activating factor), CRP (C-reactive protein) and nitric oxide can trigger softening and dilatation of the cervix, uterine contractions and begins the process of breaking the locker liquor, through activation of cyclooxygenase band in amniotic, chorionic, desidual and myometrium, increased infiltration of leukocytes, and stimulating the production of matrix metalloproteinase. Such a condition would induce the release of fetal tissue from decidua maternal and cervical thinning that caused leakage of fFN from uteroplacental junction and amniotic fluid into the cervicovaginal discharge. This reflects the separation of the chorionic layer of the uterus and detachment of decidual chorionic components in cervicovaginal discharge. This leakage will be followed by preterm delivery a few weeks afterwards.[2,6,13-15]

The results of this study indicated that the mother who experienced preterm labor had fFN level (56.29 ± 27.77) that significantly higher compared to mothers who had aterm labor (13.01 ± 7.57). This study was similar to several studies that showed whether mothers who experienced preterm labor had a high fFN level in fFN examination at 24-34 weeks gestational age.[11] This indicated that the fFN can play a role in predicting the occurrence of preterm labor in pregnant women who have an IPP.

In this study the findings indicated that there was a strong relationship between the fFN level in cervicovaginal discharge with occurrence of preterm labor. This was proved by an increase in the fFN level significantly (p = 0.000 p <0.05) in mothers with preterm labor compared to mothers with aterm labor. The Spearmen's rho correlation test also showed that the value of R = 0.797 (p = 0.000, p <0.05), which indicated a very strong relationship between the two variables. These results are in accordance with several previous studies that showed there was a strong relationship between the two variables. Research by Chandiramani (2011) showed that the fFN level and the length of the cervix could be used as a predictor of spontaneous preterm birth. This is also supported by Skoll study (2006) which indicated that the increase of fFN level in symptomatic women can be used to predict the likelihood of preterm labor.[16,17]

Various studies have been conducted to determine the role of fFN in the pathophysiology of pre-

term labor. Based on the majority of the research results, it is showed that the fFN had no direct role in causing the occurrence of preterm labor. Research by Yamani and Soliman in 2002 showed that an increase in Interleukin-1B and Interleukin-8 was believed to play a role in increasing the fFN level in mother with preterm labor. Another study by Jacobsson (2003) showed that other pro inflammatory cytokines such as Interleukin-6 and Tumor Necrosis Factor-α (TNF-α) also play a role in increasing fFN level. This could happen because of an inflammatory process in mothers with preterm labor is caused by various mechanisms, particularly as a result of infection, allergic phenomena or excessive uterine distention.[18,19]

Important role of fFN as a good predictor for preterm labor has been shown in several previous study but its role as direct cause of preterm labor is remain controversial. Interestingly, recent study by Mogami (2012) showed that it has the potential to not only act as a predictor of the occurrence of preterm delivery, but could have a direct role in the process. Administration of fFN in vitro on amniotic cells can induce a rise in expression of MMP-1 and MMP-9 and increased synthesis of COX-2 and Prostraglandin-E2. It is believed to play an important role in inducing the occurrence of Preterm Premature Rupture of Membranes (PPROM) and excessive contractions of the uterus that can cause the occurrence of preterm labor. This study is strengthened by the occurrence of preterm labor in pregnant mice after being injected with fFN. These results provide a new picture of fFN role in the pathogenesis of preterm labor and the process is likely to happen given the significant increase of fFN level in the mother with imminent premature parturition that experienced preterm labor, as shown in the results of this study. However, further research was needed to prove the role of fFN in induced preterm labor in humans.[20,21]

CONCLUSION

fFN levels is significantly increase in cervicovaginal discharge from pregnant women with imminent premature parturition who experience preterm labor than pregnant women who experience aterm labor. Therefore, this result suggest that fFN has potential ability to become useful modality in preterm labor diagnosis.

REFERENCES

1. Wardhani Isna. Correlation between premature rupture of membrane with preterm labor at Dr. Moewardi Surakarta Hospital in 2007-2008. J 500070093 Faculty of Medicine Univ. Muhammadiyah Surakarta. 2008.
2. Morgan RP. Immunology of Term and Preterm Labor. Reprod Biol Endocrinol, 2003; 1: 122.
3. Matsuura H and Hakomori S. The oncofetal structure of human fibronectin defined by monoclonal antibody FDC-6. J Biol Chem. 2008; 262: 3314-22.
4. Peaceman AM, Andrews WW, Thorp JM et al. Fetal fibronectin as a predictor of preterm birth in patients with symptoms: a multicenter trial. Am J Obstet Gynecol. 2007; 177: 13-8.
5. Plaut MM, Smith W, Kennedy K. Fetal fibronectin: The impact of a rapid test on the treatment of women with preterm labor symptoms. Am J Obstet Gynecol, 2003; 188: 1588-95.
6. Main DM, Gabbe SG, Richardson D, Strong S. Can preterm deliveries be prevented? Am J Obstet Gynecol. 2005; 151: 892-8.
7. Pankov R and Yamada KM. Fibronectin at a Glance. J Cell Science, 2002; 115(Pt 20): 3861-3.
8. Dewi, Juliani, Ati Rastini. Fetal fibronectin as premature parturition predictor. CDK, 2007; 34(5).
9. Kurosaka S, Kashina A. Cell Biology of Embryonic Migration. Birth Defects Res C Embryo Today, 2009; 84(2): 102-22.
10. Lockwood, Charles J. Fetal Fibronectin in Cervical and Vaginal Secretions as a Predictor of Preterm Delivery. N Engl J Med. 2011; 312: 82-90.
11. Schmitz T, Maillard F, Bacquaert SB et al. Selective use of Fetal bronectin Detection After Cervical Length Measurement to Predict Spontaneous Preterm Delivery in Women With Preterm Labor. Am J Obstet Gynecol. 2006; 194: 138-43.
12. Bolt LA, Chandiramani M, De Greeff A et al. The value of combined cervical length measurement and fetal fibronectin testing to predict spontaneous preterm birth in asymptomatic high-risk women. J Maternal Fetal Neonatal Med. 2011; 24(7): 928-32.
13. Klein, Laura L and Ronald S Gibbs. Infection and Preterm Birth. Obstet Gynecol Clin N Am. 2005; 32(2): 397-410.
14. Malgorzata, Szcepanska. The influence of viral infection on fetal fibronectin and IL-6 expression in cervical mucus among women with fetal defects. Archives Perinatal Med. 2007; 13(3): 40-3.
15. Bastek J, Gomez LM, Elovitz MA. The Role of Inflammation and Infection in Preterm Birth. Clin Perinatol. 2011; 38 (3): 385-406.
16. Chadiramani M, Di Renzo G, Gottschalk E et al. Fetal? bronectin as a predictor of spontaneous preterm birth: a European perspective. J Matern Fetal Neonatal Med, 2011; 24(2): 330-6.
17. Skoll A, Louis S, Amiri N et al. The Evaluation of the Fetal Fibronectin Test for Prediction of Preterm Delivery in Symptomatic Patients. J Obstet Gynaecol Can, 2006; 28(3): 206-13.

18. Yamani RH, Soliman MG. Interleukins and Fetal Fibronectin Levels In Preterm Delivery. Egy J Hospital Med, 2002; 8: 37-49.
19. Koucky M, Germanova A, Hajek Z et al. Pathophysiology of Preterm Labor. Prague Med Report, 2009; 110 (1): 13-24.
20. Li W, Unlugedik E, Bocking AD. The role of prostaglandins in the mechanism of lipopolysaccharide-induced pro MMP9 secretion from human placenta and fetal membrane cells. Biol Reprod, 2007; 76: 654-9.
21. Mogami H, Kishore AH, Shi H et al. Fetal Fibronectin Signaling Induces Matrix Metalloproteases and COX-2 in Amnion Cells and Preterm Birth in Mice. J Biol Chemist, 2012; 12(2): 115-41.

Research Article

Glutathione Peroxidase-1 Gene Polymorphism (GPx-1 Pro198Leu) in Association with Blighted Ovum

Hubungan Polimorfisme Gen Glutation Peroksidase-1 (GPx-1 Pro198Leu) dengan Kehamilan Kosong

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Abstract

Objective: To evaluate salivary GPx-1 gene polymorphism in pregnant women suffering from blighted ovum.

Method: In this case-control study, 34 blighted ovum patients and 34 healthy controls were studied. Genomic DNA was extracted from the saliva. The genotypes were determined by restriction fragment length polymorphism (RFLP-PCR) technique. Mad Calc (version 12.1) was used for statistical analysis.

Result: The frequency of CC, CT, and TT genotypes of GPx-1 gene were 41%, 44% and 14%, respectively in blighted ovum patients and in healthy volunteers were 44%, 47%, and 8.82-9%, respectively. After statistical analysis, the study showed no significant association between this polymorphism and blighted ovum (with p = 0.63).

Conclusion: These results indicated no significant association between GPx-1 (Pro198Leu) polymorphism and blighted ovum. However, further research is required to clarify the role of gene polymorphism in blighted ovum.

[Indones J Obstet Gynecol 2016; 1: 15-18]

Keywords: abortion, blighted ovum, glutathione peroxidase-1, GPx-1, RFLP-PCR

Abstrak

***Tujuan**: Untuk mengevaluasi polimorfisme gen GPx-1 dari ludah perempuan hamil yang menderita kehamilan kosong (blighted ovum).*

***Metode**: Dalam studi kasus - kontrol ini, 34 pasien dengan kehamilan kosong dan 34 kontrol yang sehat dipelajari. Genomik DNA diekstraksi dari air liur pasien tersebut. Genotipe ditentukan dengan teknik RFLP-PCR. Mad Calc (versi 12.1) digunakan untuk analisis statistik.*

***Hasil**: Frekuensi genotype CC, CT dan TT dari gen GPx-1 berturut-turut adalah 41%, 44% dan 14% pada pasien kehamilan kosong dan pada pasien yang sehat: 44%, 47% dan 8,82-9%. Setelah dianalisis secara statistik, tidak terdapat hubungan yang signifikan antara polimorfisme gen tersebut dengan kehamilan kosong (p = 0,63).*

***Kesimpulan**: Tidak terdapat hubungan yang signifikan antara polimorfisme gen GPx-1 (Pro198Leu) dengan kehamilan kosong. Bagaimanapun, penelitian lebih lanjut diperlukan untuk mengklarifikasi pengaruh dari polimorfisme ini pada kehamilan kosong.*

[Maj Obstet Ginekol Indones 2016; 1: 15-18]

***Kata kunci**: aborsi, GPx-1, glutation peroksidase-1, kehamilan kosong, RFLP-PCR*

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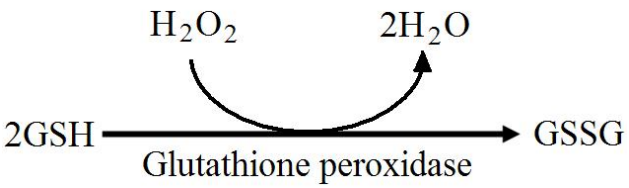
INTRODUCTION

One of the most common causes of abortion in the first trimester of pregnancy is blighted ovum. Once the egg is fertilized by sperm, the fertilized egg is implanted in the uterus and cell division begins. Cell divisions in succession to the formation of the placenta and the pregnancy sac continue, but the embryonic division is likely to be stopped and the pregnancy sac is formed, the embryo is not observed.

This condition is also called blighted ovum or anembrionic pregnancy. The cause is unknown, but the researchers generally consider the cause of disease to be genetic and chromosomal abnormalities. The cause of 50% of first trimester abortion is due to pregnancy without an embryo.[1,2] The symptoms of this disorder are mild retardation, menstrual or abdominal pain, swelling in the breasts, brown vaginal discharge, a positive pregnancy test and a subjective impression of a natural pregnancy. Although there is no fetus, the placenta continues to grow and pregnancy hormones from the placenta secrete into the mother's blood. This disease can be diagnosed before the second month of pregnancy by ultrasound testing, showing a bag with a

diameter of 20 mm or more.[3,4] But a fetus does not exist. The most common cause of this condition is chromosomal abnormalities. It caused less fertilization and decreases the number of chromosomes that can stop cell division of the zygote. Other factors can be poor quality of egg and sperm. It has been reported that the cause of one-third of spontaneous abortion before eight weeks of pregnancy is blighted ovum.[5,6] In general, it is believed that chromosomal abnormalities cause 50% of the first trimester abortion.[1]

The most common of these chromosomal abnormalities can be reciprocal displacement (62%), Robertsonian translocation (16%), inversion (16%), deletion (3%) and duplication (8%).[5,1] The purpose of the study was to investigate the GPx-1 (glutathione peroxides-1) gene polymorphism, in pregnant women with blighted ovum. GPx-1 gene produces glutathione peroxides-1, an important cytosolic peroxidase. This is the most important antioxidant enzyme derived from the cells of the body in cytosol.[6] The enzyme is a selenoprotein which reduces free radicals in cells.



The Original Reaction GPx-1

Glutathione peroxidase-1 has a tetramer structure which each monomer weighing 22 KDa and is a part of selenocysteine in the active site. Selenocysteine works directly to substrate peroxide electrons and it is oxidized during this process.[7,8] Five isoforms of glutathione peroxidase-1 are encoded by different genes. The most frequent type in the cytoplasm of all mammalian glutathione peroxidase-1 is GPx-1 and its preferred substrate is hydrogen peroxide (H_2O_2). Glutathione peroxidase-1 with the original reaction reduces free radicals and oxygen species in the cells. As a result, the cellular DNA is protected from damage. One of the properties of this protein cytosolic tail of a polialanine (ALA) in the N-terminal region, which consists of three, five, six, or seven alanine repeats in this region. Since during pregnancy women are under severe stress, keeping the body stable is very important, so the study of body fluids antioxidants and antioxidant activity of these gene is most important. GPx-1 somatic cells are also an important antioxidant, which express nearly in all our cells in the cytosol. In this study, the relationship between polymorphisms of this gene in women with blighted ovum is investigated. Intracellular antioxidant glutathione peroxidase-1, one of the most toxic substances that prevent the accumulation of intracellular catalase monomers, is even more efficient. GPx-1 gene expression pattern in different conditions, in men and women, in normal circumstances and stressful, in resting cells or growing together. GPx-1 genes are located in chromosome 3P21.3.[7] This gene includes two exons and gene Pro198Leu, which is one of the most important of these, is the polymorphism genes GPx-1 plays an important role in cancer, rheumatoid arthritis, diabetes and arthrosclerosis.[10,11]

METHODS

In this case-control study, 34 patients and 34 controls were involved. An informed consent letter was obtained from all participants approved by University of Guilan's institutional organization.

For collecting saliva sampling, the subjects rinsed their mouth with distilled water and collected about 5 ml of their unstimulated saliva samples in a sterile falcon tube. The samples were immediately centrifuged to remove cell derbies and kept at -20°C until examination. Then DNA was extracted from saliva being check for the correct extraction by horizontal electrophoresis method using 1% agarose gel with ethidium bromide. The DNA molecules from extraction were made transparent in order to observed the band. Genotype Pro198Leu by RFLP-PCR method was then determined, i.e. the PCR reaction product for this polymorphism in the gene for GPx-1. Therefore, GPx-1 gene polymorphism in codon 198 is reproduced there. The position for identification of the enzyme can be created when that piece Apa1 is duplicated containing the C allele (Pro). In this case the cutting enzyme reasonable for cutting the pieces is 77 and 237 bp. On the other hand, if the fragment amplified with allele T (Leu) is the enzyme, it disappears and 314 bp fragments remain intact. So, based on the 3 genotype, there are 3 possible outcomes. The homozygous genotype Leu/Leu reveals only one band of 314 bp. Heterozygous genotype Pro/Leu

creates three bands of 314, 77, 237 bp and thus homozygous genotype Pro/Pro creates two bands, 77 and 237 bp.

Extraction of DNA and PCR

Salivary DNA was extracted by use of an extraction GPPKit (From sinaclon company) to ensure proper extraction by spectroscopy and gel electrophoresis. The replication of DNA was the next experimental step. Primers were designed using the program oligo 7 primer analysis software: F($_5$/GTGTGCCC-CTACGCAGGTA$_3$/) and a primer R($_5$/CACACAGTT-CTGCTGACACC$_3$/). The PCR 314 bp by using agarose gel electrophoresis. The final step was to identify the Pro198Leu GPx-1 polymorphic region using Apa1 restriction enzyme. The PCR product was digested by Apa1 and two 237, 77 bp mutant into two pieces in a homozygous 314 bp fragment. The three carriers in the individual heterozygote pieces 237, 314 and 77 bp were observed and the individual patient's homozygote the two pieces on the electrophoresis gel and 77, 237 bp joint was observed. Taking into account all patient samples and control data analyzed and the following results were obtained.

Genotypic and allelic frequencies of the glutathione peroxidase-1 gene analyzed in the patient and the control group were compared. The results in both tests and controls were compared using Chi-Square test. Data were analyzed by Med Calc (version 12.1).

RESULTS

In this study, 34 female patients of blighted ovum and 34 healthy women were control group the aged interval was 20 to 35 years. Among the 34 women with blighted ovum, 14% have Pro/Pro, 44% of heterozygous genotypes Pro/Leu and 41% homozygous genotype Leu/Leu, respectively. The genotype distribution differs between patient and control test with a not significant Chi-square test (p-value = 0.63).

On this basis, there were not significant differences between the two groups (patients and control persons) from genotype polymorphism GPx-1. The study will continue allelic between the patient and control possible. The frequency of allelic Pro and Leu among patients 63%, 36% and in the control group 68%, 32% respectively. The difference is in the allelic of both distribution and significance level p=0.72 (Table 1). The Pro198 Leu gene, polymorphism in the population GPx-1 is not associated with disease blighted ovum.

DISCUSSION

The results of the research were studied; significant differences between the control and the patient population in the distribution and abundance of allelic genes did not show GPx-1 catheterizations separately. Heterozygote T/C genotype in the population was impressive. It is important to carriers of the mutant allele C. In this study, the research about the disorder in pregnant women before three months with blighted ovum is abortion. One of the most important antioxidants in the body is enzyme GPx-1. This gene in the body of the patient and all healthy individuals varies in expression with a different activity level. Since the cells are always in the face of ROS molecules, these molecules (such as hydrogen peroxide, superoxide) are highly reactive. During a natural process in the cytosol of all cells, antioxidant substances (including antioxidant enzymes) react with these toxins.[10,12,13]

Table 1. Genotype and Allele Distributions of GPx-1 (Pro 198Leu) Polymorphism.

	Controls n (Frequency %)	Patient n (Frequency %)	p-Value
Genotype			p = 0.63
CC	15 (44%)	14 (41%)	
CT	16 (47%)	15 (44%)	
TT	3 (8.82%)	5 (14 %)	
Allele			p = 0.72
C	46 (68%)	43 (63%)	
T	22 (32%)	25 (36%)	

The study and much research have been done in relation to the genes responsible for the production of antioxidants. This is the first study on the relationship between gene polymorphism of glutathione peroxides 593C/T + 1 and blighted ovum. Studies have shown that higher levels of hydrogen peroxide on the cell Leu allele than that of Pro allele. The aminoacid proline in comparison with Leu, is more effective in enzyme activity.[11]

In a healthy person, free radicals, and defense system antioxidants are in a state of balance.[14] This polymorphism is evident other diseases such as bladder cancer,[13] atherosclerosis, rheumatoid arthritis, and Alzheimer's disease.[9,10,15] The role for allelic variation within the gene for the glutathione peroxidase 1 (GPx-1) in the risk or etiology of breast cancer was investigated.[9]

The frequency of the genotype TT GPx-1 gene has been reported to be significantly higher in women with breast cancer. However, no association exists between GPx-1 Pro198Leu and breast cancer risk.[13]

These results indicated that the TT genotype of the GPx-1 gene had no significant association between this polymorphism and blighted ovum. It could be suggested that in a larger patients' population a possible relationship between GPx-1 gene polymorphism and blighted ovum be traced. However, further research is required to clearly comment on the role of gene polymorphism in blighted ovum.

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REFERENCES

1. Hu YJ, Diamond AM. Role of glutathione peroxidase 1 in breast cancer Loss of heterozygosity and allelic differences in the response to selenium. Cancer research. 2003; 63(12): 3347-51.
2. Bashir S, Harris G, Denman MA et al. Oxidative DNA damage and cellular sensitivity to oxidative stress in human auto immune diseases. Annals Rheumatic Diseases. 1993; 52(9): 659-66.
3. Arsova-Sarafinovska Z, Matevska N, Eken A et al. Glutathione peroxidase 1 (GPx-1) genetic polymorphism, erythrocyte GPX activity, and prostate cancer risk. International urology and nephrology. Int Urol Nephrol J, 2009; 41(1): 63-70.
4. Lubos E, Loscalzo J, Handy DE. Glutathione peroxidase-1 in health and disease: From molecular mechanisms to therapeutic opportunities. Antioxidants Redox Signaling J, 2011; 15(7): 1957-97
5. Cox DG, Hankinson SE, Kraft P, and Hunter DJ. No association between GPx-1 Pro198Leu and breast cancer risk. Cancer Epidemiol Biomarkers Prev, 2004; 13(11 Pt 1): 1821-2.
6. De Vivo I, Hankinson SE, Colditz GA, and Hunter DJ. A functional polymorphism in the progesterone receptor gene is associated with an increase in breast cancer risk. Cancer Res, 2003; 63(17): 5236-8.
7. Baghbani F, Mirzaee S, Hassanzadeh-Nazarabadi M. Association of heteromorphism of chromosome 9 and recurrent abortion (ultrasound diagnosed blighted ovum): A case report. Iran J Reprod Med, 2014; 12(5): 357-60.
8. Sahar Shekoohi, Majid Mojarrad, Reza Raoofian et al. Chromosomal Study of Couples with the History of Recurrent Spontaneous Abortions with Diagnosed Blighted Ovum, Mashhad University of Medical Sciences, Mashhad, Iran. Int J Mol Cell Med, 2013; 2(4): 164-8.
9. Rushton DI. Simplified classification of spontaneous abortions. J Med Genet. 1978; 15(1): 1-9.
10. Bernard KG, Cooperberg PL. Sonographic differentiation between blighted ovum and early viable pregnancy. AJR Am J Roentgenol. 1985; 144(3): 597-602.
11. Nybo Andersen AM, Wohlfahrt J, Christens P et al. Maternal age and fetal loss: population based register linkage study. BMJ, 2000; 320: 1708-12.
12. Berek JS. Berek and Novaks gynecology. 14 ed. Philadelphia: Lippincott Williams and Wilkins; 2007: 749-7.
13. Faghihzadeh S, Babaee Rochee G, Lmyian M et al. Factors associated with unwanted pregnancy. J Sex Marital Ther. 2003; 29(2): 157-64.
14. Cardoso BR, Ong TP, Jacob-Fiho W et al. Glutathione peroxidase1 Pro198Leu polymorphism in Brazilian Alzheimer's disease patients: relations to the enzyme activity and to selenium status. J Nutrigenet Nutrigenomics. 2012; 5(2): 72-80. Epub 2012 Jun 7.
15. de Hean J B, Bladier C, Griffiths P, et al. Mice with a homozygous null mutation for the most abundant gluta-thione peroxidase, GPx-1, show increased susceptibility to the oxidative stress-inducing agents Paraquat and hydrogen peroxide. J Biol Chem. 1998; 273(35): 22528-36.

Research Article

Hypoxia Inducible Factor-1α in Correlation with Preeclampsia

Hubungan Kadar Hypoxia Inducible Factor-1α dengan Preeklamsia

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Abstract

Objective: To know the correlation between Hypoxia Inducible Factor (HIF)-1α level with preeclampsia.

Method: The study was conducted at the Obstetrics and Gynecology Department of Prof. Dr. R. D. Kandou Hospital. This research was carried out from April to June 2014. This study is a cross-sectional analytic approach in preeclampsia and normotensive pregnancy. We perform cubital venous blood sampling for inspection by 5 ml of serum and stored it in a refrigerator (-20°C). The level of HIF-1α serum was done by quantitative ELISA method in Prodia Lab Jakarta.

Result: Seventy six pregnant women with 38 normotensive pregnancies and 38 preeclampsia, showed a significant association between the average levels of HIF-1α in the serum of pregnant women with preeclampsia compared to normotensive pregnancies (p = 0.000).

Conclusion: There is a positive relationship between the levels of HIF-1α in serum of preeclampsia, in which HIF-1α levels in preeclampsia was higher than normotensive pregnancies.

[Indones J Obstet Gynecol 2016; 1: 19-22]

Keywords: hypoxia inducible factor-1α, preeclampsia

Abstrak

***Tujuan**: Mengetahui kadar HIF-1α serum pada preeklamsia.*

***Metode**: Penelitian dilakukan di bagian Obstetri dan Ginekologi RSUP Prof. Dr. R. D. Kandou. Penelitan ini dikerjakan mulai bulan April sampai Juni 2014. Penelitian ini merupakan penelitian analitik menggunakan pendekatan potong lintang pada preeklamsia dan kehamilan normotensi. Dilakukan pengambilan darah vena cubiti sebanyak 5ml untuk pemeriksaan serum dan disimpan dalam lemari es (-20°C). Pemeriksaan kadar HIF-1α serum dilakukan dengan cara kuantitatif, menggunakan metode ELISA.*

***Hasil**: Tujuh puluh enam perempuan hamil dengan 38 orang dengan kehamilan normotensi dan 38 orang dengan preeklamsia. Didapatkan hasil hubungan yang bermakna antara kadar rata-rata HIF-1α serum dalam ibu hamil preeklamsia dibandingkan dengan kehamilan normotensi (p = 0,000).*

***Kesimpulan**: Terdapat hubungan positif antara kadar HIF-1α serum pada preeklamsia, di mana kadar HIF-1α pada preeklamsia lebih tinggi secara signifikan dibandingkan kehamilan normotensi.*

[Maj Obstet Ginekol Indones 2016; 1: 19-22]

***Kata kunci**: hypoxia inducible factor-1α, preeklamsia*

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INTRODUCTION

The incidence of preeclampsia varies greatly from country to country.[1] Preeclampsia is a multisystem disorder that complicated at 3% - 8% of pregnancies in Western countries and is a major source of morbidity and mortality in the world.[2] Meanwhile, the prevalence of hypertension in Indonesia were obtained through measurements at age > 18 years was 25.8%.[3] Overall, 10% -15% of maternal deaths are directly related to the preeclampsia. Some epidemiological study support the hypothesis of genetic and immunological as the cause of preeclampsia.[4]

Until now, there has not been an ideal way to be able to predict the occurrence of preeclampsia at an early stage. This is due to the etiology and definitive pathogenesis of preeclampsia remains unclear, so that preeclampsia is still referred to as the disease of theories.[5]

In the early preeclampsia, there is a failure of cytotrophoblast invasion into the maternal spiral arteries. This will cause a decrease in uteroplacental perfusion that followed by the failure of the unit of fetoplasenta to get enough oxygen from the intervillus space that ultimately lead to a state of hypoxia in the placenta resulting in the release of a factor which so-called Hypoxia Inducible Factors (HIF). It is necessary to spur more cytotrophoblast cells to migrate into cytotrophoblast extravillus that will become invasive cytotrophoblast who will invade vascular endothelial, deeper into the spiral arteries. The entire process from the placenta to ensure adequate oxygen supply to the developing fetus during pregnancy.[6]

HIF-1α levels in preeclampsia is still not widely studied. If the levels of HIF-1α is known related to preeclampsia, it can be done as early as possible so the anticipation can reduce the morbidity and mortality of the mother and fetus. Therefore, the researchers wanted to know the levels of HIF-1α in patients with preeclampsia compared with normal pregnancy thus morbidity and mortality of maternal and fetus can be prevented or reduced as expected.

Preeclampsia is a pregnancy-specific syndrome such as reduced organ perfusion due to vasospasm and endothelial activation, which is characterized by increased blood pressure and proteinuria. The onset of hypertension accompanied with proteinuria in gestational age over 20 weeks. Preeclampsia is common in second or third trimester of pregnancy with the highest incidence at 32 weeks of gestational.[6,7]

Previously, the diagnosis of preeclampsia is established based on the presence of the triad of edema, hypertension and proteinuria, but often the triad does not appear and manifest at more severe stages. Nowadays, diagnostic of preeclampsia is to find proteinuria and hypertension. Edema is no longer included because it often appears in normal pregnancy.[8]

Proteinuria occurred due to a defect in the kidney, abnormalities of the endothelium in particular, which is one of the pathogenesis of preeclampsia. As a result, proteinuria that occurred is a long process which has taken place further damage to the glomerulus of kidneys so proteinuria can not be the gold standard for early diagnosis of preeclampsia.[6]

Until now there is no theory about the preeclampsia that considered correct, that is why preeclampsia was called "the disease of theories". Various theories regarding the etiology of preeclampsia should be taken into account in the observation that hypertensive disorders in pregnancy often arise on women whom been exposed to the villi coriallis at the first time, women who were exposed to villikorialis at many times like in the gemelli or hydatidiform mole, women who had a previous vascular disorders and women who are genetically have a predisposition to hypertension in pregnancy.[6,7]

METHODS

The study was conducted at the Obstetrics and Gynecology Department of Prof. Dr. R. D. Kandou General Hospital. This research was carried out from April to June 2014. This study is a cross-sectional analytic approach in preeclampsia and normal pregnancy. Inclusion criteria for mothers who had agreed to participate in the study after receiving an explanation about the research and term that meet the criteria of severe preeclampsia and normal pregnancy. Exclusion criteria are pregnant women with chronic diseases such as diabetes mellitus, kidney disorders, cardiac disorders, chronic hypertension, cancer, multiple pregnancies and intrauterine fetal death.

Cubital venous blood sampling by 5 ml of serum was done and stored in a refrigerator (-20°C), then sent to Jakarta by using ice packs to maintain storage temperatures, the examination of HIF-1α proceed in Prodia Laboratory, Jakarta. The level of HIF-1α serum was done by quantitative ELISA method.

Table 1. Subject Characteristics based on Age, Parity, and Number of Marriages

Characteristics	Group				p
	Normotension		Preeclampsia		
	n	%	n	%	
Age (year)					
< 35	29	76.3	27	71.1	0.602
≥ 35	9	23.7	11	28.9	
Parity					
Primigravida	12	31.6	22	57.9	0.021
Multigravida	26	68.4	16	42.1	
Number of marriages					
1 time	37	97.3	32	84.2	0.047
> 1 time	1	2.7	6	15.8	

RESULTS

This study was conducted on 76 pregnant women who meet the criteria for inclusion in the Polyclinic Obstetrics and Gynecology and maternity room BLU RSUP Prof. Dr. R. D. Kandou, Manado. Thirty eight controls and 38 patients with preeclampsia.

Table 2. The Correlation of Hypoxia Inducible Factor 1 alpha between Preeclampsia, and Normotension Pregnancy

	Mean	SD	p
Normotension	0.092	0.046	0.000
Preeclampsia	0.175	0.212	

DISCUSSION

Basic characteristics of the study sample consisted of several variables including age, parity, number of marriages and the levels of Hypoxia Inducible Factor-1α, 76 pregnant women were divided into 2 groups : group with normotensive pregnancy and preeclampsia group.

Preeclampsia is a symptoms that often occur as a complication of pregnancy and caused by a lot of factors, one of which is the age of the mother during pregnancy is already advanced, it means a higher risk of preeclampsia in pregnant women over the age of 35 years. In a study involving 76 pregnant women, it turns out the largest number in the group under 35 years of age and pregnant with normotensive, while the second highest number in the group under 35 years of age but pregnant with preeclampsia, which found no significant differences between age with preeclampsia. This result contrasts with the results obtained by Duckitt (2005), that the age associated with the incidence of preeclampsia, especially extreme age (> 40 years) had a 2-fold risk of preeclampsia, both primipara and multipara.[8]

Other risk factors that may increase the risk of complications of preeclampsia is primigravida/ first pregnancy. The risk of preeclampsia is generally decreased in the second pregnancy compared to the first pregnancy. Explanation indicates a decreased risk is recurrent maternal exposure and adaptation to specific antigens of the same partner. The difference in risk might explain the gap between births. Have sex with different partner will increase the risk of preeclampsia.[9] In this study, we found a significant positive association between preeclampsia in primigravida than multi gravida. These results are similar to research conducted by Roberts et al (2009) and Nurdi AA (2013), that nulliparity have almost 3-fold risk for preeclampsia. In multipara shows there is a reduced risk that may be caused by maternal exposure to repetitive and adaptation to specific antigens of same couples.[8]

The number of marriages was also said to be one of the risk factors for preeclampsia which in this study, there is a significant correlation between married one time with more than 1 times. This is consistent with research of Skjaerven R (2002) where women who are having sex with different partner will increase the risk of this preeclampsia.[9] This is due to excessive maternal inflammatory response against foreign antigens from different sperm, resulting in a series of events including shallow trophoblast invasion, damage to the formation of spiral arteries, placental infarction and release of pro-inflammatory cytokines that enter the systemic circulation.[10]

Significant relationship between the average levels of HIF-1α in the serum of pregnant women with preeclampsia compared to normotensive pregnancy supports the hypothesis that there is an increment of HIF-1α levels in patients with preeclampsia compared with normotensive pregnancies. There is a lot of evidence to support the occurrence of hypoxia on preeclampsia. Lunell et al., explain that there is a decline of 50% in the utero utero placental circulation in patients with preeclampsia. Caniggia suggested that preeclampsia may occur due to failure increased responsiveness or sensitivity of trophoblast cells to oxygen.[11]

Hypoxia Inducible Factor-1α (HIF-1α) is a key regulator of the cellular response to low oxygen tension and centered on oxygen homeostasis. It is a heterodimer transcription factor composed of two subunits, namely α and β. When HIF-1β is constitutively active, HIF-1α is sensitive to oxygen, are rapidly inactivated and degraded into normoxia state. In a state of low oxygen, HIF is a key regulator of a number of target genes that can induce anaerobic process, reducing the consumption of oxygen or cause angiogenesis to stabilize the vascular environment. HIF can function at all stages of development and placental differentiation. Reshef Tal et al., conducted experiments on mice reported that the increase in HIF-1α can lead to growth retardation (IUGR) and clinical symp-

toms of preeclampsia. This is similar to research conducted by Rajakumar et al., reported that HIF-1α levels did not differ in term pregnancies compared preterm pregnancies without preeclampsia, where as HIF-1α levels increased two-fold in preeclamptic pregnancies compared with normal.[12,13] Stacy Zamudio et al also reported that in the hypoxia state, HIF-1α levels increased by 13 times.[14]

With so much evidence included in this study which found significant differences between the levels of HIF-1α in preeclampsia compared with normotensive so it can be inferred that HIF-1α levels can be used as a predictor of preeclampsia.

CONCLUSION

There is a positive relationship between the levels of HIF-1α in serum of preeclampsia, in which HIF-1α levels in preeclampsia was significantly higher than normotensive pregnancies

REFERENCES

1. Carty DM, Delles C, Dominiczak AF. Preeclampsia and future maternal health. J Hypertens. 2010; 28: 1349-55.
2. Duley L. The global impact of preeclampsia and eclampsia. Semin Perinatol. 2009; 33: 130-7.
3. Badan Penelitian dan Pengembangan Kesehatan Kementerian Kesehatan RI. Riset kesehatan dasar. Jakarta: Kementerian Kesehatan RI; 2013.
4. Maynard SE, Karumanchi SA, Thadhani R. Hypertension and kidney disease in pregnancy. In: Brenner BM, editor. Brenner and Rector's The Kidney. 8th ed. Philadelphia, PA: WB Saunders; 2007.
5. Angsar D. Hipertensi dalam kehamilan. Dalam: Ilmu Kebidanan ed. 4 Jakarta. Yayasan Bina Pustaka Sarwono Prawirohardjo. 2008: 530-67.
6. Cunningham FG, Leveno KJ, Bloom SL et al. Pregnancy hypertension. In: Twickler D, Wendel GD (Eds). Williams Obstetrics 23rd. McGraw Hill Book Company. 2010: 706-56.
7. Sibai B, Dekker G, Kupferminc M. Preeclampsia. Lancet. 2005; 365: 785-99.
8. Duckitt K and Harrington D. Risk factors for preeclampsia at antenatal booking: systematic review of controlled studies. BMJ. 2005; 330(7491): 565-7.
9. Skjaerven R, Wilcox AJ, Lie RT. The Interval Between Pregnancies and The Risk of Preeclampsia. N Engl J Med, 2002; 346 1 (3): 33-8.
10. Matthiesen L, Berg G, Ernerudh J et al. Immunology of Preeclmapsia. Chem Immunol Allergy. 2005; 89: 49-61; Formosa M. The paradox of pregnancy: an update on immunology of early pregnancy. Malta Med J. 2008;20(02): 10-4.
11. Caniggia l, Winter JL. Adriana and Luise Castellucci Award Lecture 2001 Hypoxia Inducible Factor-1. Oxygen Regulation of Trophoblast Differentiation in Normal and Preeclamptic Pregnancies-A Review. Placenta,2002; 16: 47-57.
12. Tal R. The role of hypoxia and hypoxia inducible factor 1 alpha in preeclampsia pathogenesis. Biol Reprod. 2012; 87(6): 134,1-8.
13. Tal R, Shaish A, Barshack I et al. Effects of hypoxia-inducible factor 1 alpha over expression in pregnant mice. Am J Pathology. 2010; 177(6): 2950-62.
14. Zamudia S, Wu Y, Letta F et al. Human placental hypoxia inducible factor 1 alpha expression correlates with clinical outcomes in chronic hypoxia in vivo. Am J Pathol, 2007; 170(6): 2171-9.

Research Article

Gynecology Cancer in Relationship with Obesity

Hubungan Kanker Ginekologi dengan Obesitas

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Abstract

Objective: To know the relationship between obesity and gynecology cancer.

Method: This study use case control study design for 250 gynecology patients (125 controls and 125 cases) in Prof. Dr. R. D. Kandou Manado Hospital from 1 July to 30 November 2015. The data was collected by measuring Body Mass Index (BMI) and filing out self-administered questioners.

Result: From the 250 subjects, the study group (125 subjects), 72 subjects have obesity (57.6%) and 97 subjects have multiple parities (77.6%) with 58 subjects diagnosed with cervical cancer (46.4%). In the control group (125 subjects), 71 subjects have normal weight (56.8%) and 67 subjects have multiple parities (53.6%) with 64 subjects diagnosed with ovarium cysts (51.2%). Using multivariate logistic regression, the overweight and obese subjects have 7 folds higher risk to develop gynecology cancer compared to those with normal or underweight subjects. Those with multiple parities and grande multipara subjects have 3 folds higher risk to develop gynecology cancer compared with those who are nullipara and primipara.

Conclusion: A significant correlation is found between obesity and gynecology cancer using multivariate logistic regression analysis (p=0.000, OR=6.9 (95% CI = 3.62-13.13).

[Indones J Obstet Gynecol 2016; 1: 23-30]

Keywords: gynecology cancer, multiple parities, obesity

Abstrak

***Tujuan**: Untuk mengetahui adanya hubungan obesitas dengan kanker ginekologi.*

***Metode**: Penelitian ini menggunakan desain penelitian case control pada 250 pasien ginekologi (125 pasien kontrol dan 125 pasien kasus) di RSUP Prof. Dr. R. D. Kandou Manado dari tanggal 1 Juli 2015-30 November 2015. Pengumpulan data dilakukan dengan mengukur Indeks Massa Tubuh (IMT) dan mengisi kuesioner secara mandiri.*

***Hasil**: Dari 250 pasien yang mengikuti penelitian, pada kelompok kasus (125 orang) didapatkan IMT obesitas sebanyak 72 orang (57,6%), serta multiparitas sebanyak 97 orang (77,6%) dengan diagnosis terbanyak kanker serviks 58 orang (46,4%). Sedangkan pada kelompok kontrol (125 orang) didapatkan IMT ideal sebanyak 71 orang (56,8%), serta multiparitas sebanyak 67 orang (53,6%), dengan diagnosis terbanyak kista ovarium 64 orang (51,2%). Berdasarkan analisis statistk regresi logistik multivariat, kelompok yang tergolong berat badan lebih dan obesitas mempunyai risiko hampir 7 kali menderita kanker ginekologik daripada yang kategori IMT-nya sangat kurus dan ideal. Pada pasien dengan riwayat multipara dan grande multipara mempunyai risiko 3 kali lipat lebih banyak menderita kanker ginekologi daripada pasien dengan riwayat nulipara dan primipara.*

***Kesimpulan**: Setelah dilakukan analisis statistik regresi logistik multivariat (p = 0,000; OR = 6,90 (95%CI = 3,62-13,13) didapatkan hubungan yang bermakna antara obesitas dengan kanker ginekologi.*

[Maj Obstet Ginekol Indones 2016; 1: 23-30]

***Kata kunci**: kanker ginekologi, multiparitas, obesitas*

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INTRODUCTION

The population structure is greatly changed due to the advancement in the science and technology of medical world and financial capacity. Along with the sophistication, the pathogenic pattern from infectious disease and malnutrition becoming more non-infectious and lifestyle associated ailments. This condition is concurred with the Transition Epidemiology Theory introduced by Omran in 1971.[1] Several countries in the world or areas in one country may be in different level of transition. Health condition and disease characteristics are associated with financial growth and social structure in the society. Industrialism is the cause of mortality and morbidity dominated by chronic diseases such as cardiovascular disease, cancer, diabetes and obesity.[1] Currently obesity is a global epidemic and is showing a more prominent impact. The prevalence of obesity in developing countries has increased 3 folds according to the World Health Organization.[2,3] Obesity has become one of ten health problems in the world and top 5 in developing countries. Obesity in adult prevalence in the world reached 400 millions in 2005 and in 2015 it is predicted that will increased to 700 millions.[3] In the developing countries, obesity is shown in a high frequency. Data from the WHO in 2008 the preva-

lence of obesity in Indonesian adult is 9.4% with 2.5% men and 6.9% women proportionately.[1] Previous survey in 2000 shown that only 4.7% Indonesian have obesity (approximately 9.8 million population).[2] In a short span of 8 years the prevalence increases two folds. We need to be aware of the increasing rate because life style changes to a more sedentary and relaxed fashion as a result of technology advancement.[2,3] Obesity is a manifestation of overweight meaning there are an abundant excess of fat in the body. Obesity differs from overweight in the weight difference. While overweight may possibly is caused by muscle or bone mass, or excess water (like in body builders). However, those two words (obesity and overweight) both signified a more than normal weight based on height.[1]

The criteria for obesity are based on Body Mass Index (BMI). BMI is a comparison between weight and height. The measurement is as follow: weight (kg)/(height (m) x height (m).[1,2] If not addressed properly, obesity may have serious impact on health. Several studies show that obesity is tightly linked with several risk factors for degenerative diseases such as type II diabetes mellitus, hypertension, dyslipidemia, heart problem and even cancer.[1]

Obesity is associated with an increased risk for several types of cancer. Malignancy caused by obesity is varied widely but 40% of the highest are endometrium cancer and esophageal adenocarcinoma. In the long run, obesity problem will continue to flourish and in 2030 will add 500,000 new cases in the United States. This analysis also found that if every adult cut off 1 point of their BMI, equivalent to 1 kg average adult weight, then it will prevent the increase by 100,000 less cases.[4,5]

Several mechanisms may be responsible in the association between obesity and the increased risk of cancer: fat tissue produces excess estrogen and a high level of estrogen is associated with increased risk of breast cancer, endometrium cancer and several other cancers. Obese individual usually has an increased of insulin and insulin-like growth factor-1 (IGF-1) in their blood stream (this condition is known for hyperinsulinemia or insulin resistance syndrome) which cause the development of several tumors. Fat cells produced hormones called adipokines which stimulates or inhibits cell growth. For example, Leptin which was found in abundance in obese individual can encourage cell proliferation and lesser level of adiponectin in obese individual may have anti-proliferative effect. Fatty cells also play a role in the regulation of other tumor cells growth. Obese individual often has subacute inflammation associated with an increased risk of malignancy.[6] Other mechanisms such as the alteration of immune response, radiation exposure and oxidative stress.[1,3,4] Cancer is still the second highest cause of death in developed countries after heart disease. In the United States, most often found cancer type in women is breast cancer followed by lung, colorectal and endometrium cancer. The highest mortality rate is caused by lung cancer followed by breast, colon, ovarium and pancreas. By evaluating the relationship between obesity and malignancy, Bergstrom evaluate the impact of obesity in cancer for European and concluded that 36,000 cases can be avoided annually by deplete the overweight and obese prevalence by 50%. Calle also found that more than 90,000 deaths annually can be avoided by Americans if their adult BMI maintained at < 25 kg/m^2. Additionally, the increase of BMI also related to the increase of mortality in breast, uterus, cervix and ovarium cancer. For example, women with the most weight cohort (BMI ≥ 40 kg/m^2) have 62% death rate for cancer compared to women with normal weight. The increase of death rate for obese women with cancer showed an increased malignancy incidence in obese women compared to lean women. The prognosis for obese women with cancer is uncomely. Up until now many data are unreliable but suggestively leading to a conclusion signifying the close relationship between obesity and malignancy such as endometrium, cervix, breast in post menopause women, ovarium, colon and gall bladder.[5]

METHODS

This study used cross sectional case control study design for 250 gynecology patients in Prof. Dr. R. D. Kandou Manado Hospital from 1 July to 30 November 2015. The data collected by using BMI measurement and self-administered questioner.

Sampling criteria for the study groups are: patients coming to Prof. Dr. R. D. Kandou Hospital, diagnosed with gynecology cancer, obese and non-obese and willing to be respondents. Sampling criteria for control groups are: patients coming to Prof. Dr. R. D. Kandou Hospital, diagnosed with gynecology disease but not malignant, obese and non-obese and willing to be respondents.

RESULTS

This study is a case control study by measuring subjects' BMI and analyzing questioners filled out by the subjects who came to Prof. Dr. R. D. Kandou Manado Hospital. Study group is consist of 125 female subjects with gynecology cancer and control group is consists of 125 female subjects with non-gynecology cancer.

Table 1. Subjects Characteristic Distribution

		(n)	(%)
Women Subject Status	Cases	125	50
	Control	125	50
	Total	250	100
Age	≤ 20	8	3.2
	21-30	14	5.6
	31-40	47	18.8
	41-50	101	40.4
	> 50	80	32
	Total	250	100
Ethnic Group	Minahasa	169	67.6
	Mongondow	24	9.6
	Gorontalo	10	4
	Sangihe	37	14.8
	Others	10	4
	Total	250	100
BMI	Underweight	29	11.6
	Normoweight	101	40.4
	Overweight	42	16.8
	Obesity	78	31.2
	Total	250	100
Occupation	Housewives	201	80.4
	Civil Clerk	20	8
	Employee	19	7.6
	Farmer/Fishwives	3	1.2
	Others	7	2.8
	Total	250	100
Education	Primary School Graduate	32	12.8
	Junior High School Graduate	78	31.2
	High School Graduate	110	44
	Undergraduate	30	12
	Total	250	100
Marital Status	Married	236	94.4
	Single	14	5.6
	Total	250	100

Parities	0-1	86	34.4
	> 1	164	65.6
	Total	250	100
Diagnosis	Cervical Cancer	58	23.2
	Endometrium Cancer	16	6.4
	Ovarium Cancer	51	20.4
	Mioma	48	19.2
	Endometriosis Cysts	4	1.6
	Ovarium Cysts	64	25.6
	Solid Ovarium Tumor	9	3.6
	Total	250	100

In Table 1 we have the characteristics distribution of the 250 subjects willing to participate in this study. The age group of this study is 41-50 years old 40.4%, Minahasa ethnic race 67.6% with normal BMI 40.4%, high school graduate 44% and housewives 80.4%. From the overall respondents 94.4% are married with multiple parities 65.6%. The most common diagnosis from the subjects in our study is ovarium cysts 25.6%.

Table 2. Characteristic Distribution in Study and Control Groups

Variable	Cases		Control	
	n	**%**	**n**	**%**
Age				
≤ 20	0	0	8	6.4
21 - 30	5	4	9	7.2
31 - 40	23	18.4	24	19.2
41 - 50	50	40	51	40.8
> 50	47	37.6	33	26.4
Total	125	100	125	100
BMI				
Underweight	10	8	19	15.2
Normoweight	30	24	71	56.8
Overweight	13	10.4	29	23.2
Obesity	72	57.6	6	4.8
Total	125	100	125	100
Occupation				
Housewives	99	79.2	102	81.6
Civil Clerk	10	8	10	8
Employee	12	9.6	7	5.6
Farmer/Fishwives	3	2.4	0	0
Others	1	0.8	6	4.8
Total	125	100	125	100

Variable	Cases		Control	
	n	**%**	**n**	**%**
Education				
Primary School Graduate	19	15.2	13	10.4
Junior High School Graduate	29	23.2	49	39.2
High School Graduate	64	51.2	46	36.8
Undergraduate	13	10.4	17	13.6
Total	125	100	125	100
Parities				
0 - 1	28	22.4	58	46.4
> 1	97	77.6	67	53.6
Total	125	100	125	100
Diagnosis				
Cervical Cancer	58	46.4	0	0
Endometrium Cancer	16	12.8	0	0
Ovarium Cancer	51	40.8	0	0
Mioma	0	0	48	38.4
Endometriosis Cysts	0	0	4	3.2
Ovarium Cysts	0	0	64	51.2
Solid Ovarium Tumor	0	0	9	7.2
Total	125	100	125	100
Variable	**Obese**		**Non-Obese**	
	n	**%**	**n**	**%**
Diagnosis				
Cervical Cancer	35	28	23	18.4
Endometrium Cancer	14	11.2	2	1.6
Ovarium Cancer	34	27.2	17	13.6
Total N Diagnosis	125	100%		
Variable	**Obese**		**Non-Obese**	
	n	**%**	**n**	**%**
Diagnosis				
Mioma	21	16.8	27	21.6
Endometriosis Cysts	1	0.8	3	2.4
Ovarium Cysts	12	9.6	52	41.6
Solid Ovarium Tumor	0	0	9	7.2
Total N Diagnosis	125	100%		

Our study group shown in Table 2, 50 subjects (40%) are in the age group between 31-50 years old, 72 subjects with obesity BMI (57.6%), high school graduate 64 subjects (51.2%), housewives 99 subjects (79.2%) with multiple parities 97 subjects (77.6%) and diagnosed with cervical cancer 58 subjects (46.4%). In the control group 51 subjects (40.8%) are in the age group between 41-50 years old, 71 subjects with normal weight BMI (56.8%), junior high school graduate 49 subjects (39.2%), housewives 102 subjects (81.6%) with multiple parities 67 subjects (53.6%) and diagnosed with ovarium cysts 64 subjects (51.2%).

In the control group, from 48 subjects that are diagnosed with myoma utery, 21 subjects have

obesity (16.8%), from 4 subjects that are diagnosed with endometrium cysts, 1 subject has obesity (0.8%), from 64 subjects diagnosed with ovarium cysts, 12 have obesity (9.6%), from 9 subjects diagnosed with solid ovarium tumor, all of them have normal weight.

In the study group, from 58 subjects that are diagnosed with cervical cancer, 35 subjects have obesity (28%), from 16 subjects with endometrium cancer, 14 subjects have obesity (11.2%), from 51 subjects diagnosed with ovarium cancer, 34 subjects have obesity (27.2%).

From those characteristics table above, we follow through with a logistic regression analysis test to address the happening of gynecology cancer with obesity, education, marital status, history of diabetic parities, gynecology malignancy history, non-gynecology malignancy history, vegetable dietary, fruit dietary, exercise, alcohol consumption history, smoking history and the use of contraception as the risk factors.

From the multivariate logistic regression analysis for those risk factors in the relationship to gynecology cancer, obesity and parities have significant relationship in the development of gynecology cancer; p = 0.000, OR = 6.90 (95%CI = 3.62-13.13), and p = 0.004, OR = 2.95 (95%CI = 1.41-6.19), respectively.

Those who are overweight and obese have a risk of 7 folds higher than those with normal weight to have gynecology malignancy. Those who have multiple and grand multiple parities have 3 folds higher risk to develop gynecology cancer compared to the nullipara and primipara.

DISCUSSION

This study was conducted in Prof. Dr. R. D. Kandou Manado Hospital since July to November 2015. The subjects are 125 women with gynecology cancer and 125 women with non-gynecology cancer. All study subjects have fulfilled the inclusion and exclusion criteria and signed the consent form to participate in this study. This study is done with 2 steps; fill out the questioners and physical examination.

Cancer is still the second highest cause of death in developed countries after heart disease. Obesity is related to the increase risk for several types of cancer. The rate of cancer caused by obesity varied largely, but 40% is endometrium cancer and esophageal adenocarcinoma. The increase of BMI also related to the increase of mortality in breast, uterus, cervical and ovarium cancer. For example, women from the heaviest weight cohort (BMI > 40 kg/m^2) have a death rate for cancer 62% higher than women with normal weight. The increase of death rate for obese women with cancer showed that the increase of cancer incidence in obese women has worse prognosis than those with normal weight. There is an analysis that showed if an adult decrease 1 point of their BMI, equivalent to 1 kg of adult average weight, then the increase of incidence will be avoided.

The subjects in this study are patients of Prof. Dr. R. D. Kandou Manado Hospital. There are 125 women in the study group with 50 subjects in the age group of 41-50 years old (40%), 72 subjects with obesity BMI (57.6%), high school graduate 64 subjects (51.2%), housewives 99 subjects (79.2%) and diagnosed with cervical cancer 58 subjects (46.4%). In the control group we have 125 subjects with 51 subjects in the age group of 41-50 years old (40.8%), normal weight BMI 71 subjects (56.8%), junior high school graduate 49 subjects (39.2%), housewives 102 subjects (81.6%), and diagnosed with ovarium cysts 64 subjects (51.2%).

We measure the relationship significance of obesity in the development of gynecology cancer in the patients of Prof. Dr. R. D. Kandou Manado Hospital. Obesity is defined with BMI > 30 kg/m^2.

Obesity is related to the increase of malignancy risk in gynecology by several pathways. Mechanisms such as insulin resistance, adipokine, sex steroid, inflammation and genetic polymorphism may play a role in the development. A holistic understanding of obesity relationship to gynecology cancer may help avoid malignancy from happening and provide a better prognosis.

In this study we use multivariate logistic regression analysis to see the relationship between risk factors and gynecology cancer. We found that obesity (p = 0.000, OR = 6.90 (95%CI = 3.62-13.13)). Those who are overweight and obese have 7 times higher risk for gynecology malignancy than those with normal or underweighted.

This finding supports previous results which evaluate the role of obesity in cancer incidence. Bergstrom looked into the role of obesity in European cancer and found that by decreasing the

prevalence of overweight and obese patients by 50% there will be 36,000 less cancer cases annually. Calle also found that more 90,000 deaths can be avoided yearly in the United States if every adult can maintain their BMI to less than 25 kg/m^2. Additionally the increase of BMI also related to the increase of death rate in breast, uterus, cervical and ovarium cancer patients. For instance, women from the heaviest weight cohort (BMI > 40kg/m^2) have 62% higher death rate than women with normal weight. The increase in obese women with cancer showed an increase in cancer incidence in obese women compared to lean women and the outcome for obese women is significantly worse. Lahmann also found other risk factors for epithelial type ovarium cancer: BMI. A study found that women with > 30 kg/m^2 BMI (obesity) have 1.59 relative higher risk to develop ovarium cancer compared to those with normal BMI.[3,5]

In a study in the United States it was found that the increase of BMI and waist measurement is one of the risk factors related to endometrium cancer but it is still unknown whether those factors are determinant for the prognosis. Specifically, women obesity (BMI > 30kg/m^2) increase the risk of endometrium cancer by 76% and women with > 0.8530 waist measurement increase the risk of endometrium cancer by 33%. Freidenreich et al found that women with 30-40 kg/m^2 BMI have 1.78 more risk for endometrium cancer than women with normal BMI. Menopause women with obesity produce more estrogen than necessary. It is owed to the peripheral conversion in the adipose tissue from androgen secreted by adrenal gland and ovarium to estrone by aromatase. Long term exposure to uninhibited estrogen will cause changes in some spectrum of endometrium proliferation such as hyperplasia of endometrium/polyp to endometrium cancer.

The relationship between obesity and ovarium cancer is still unknown. Several studies suggest an existence of relation. But several other contradict the notion. Endogen hormone is believed to be one of the causes of ovarium cancer but the conclusion for the role of obesity in ovarium malignancy is still undecided.

In a study by Ovarian Cancer Association Consortium it is found that obesity may increase the risk of ovarium cancer with borderline histology subtype and low grade invasive type.[7]

There is still a lack of study in regard of the relationship between BMI and cervical cancer. In one study, an increased risk in women with excess body weight and obese to cervical cancer especially cervical adenocarcinoma and cervical squamous cell carcinoma is found but in a low significance.[8] It is seemed that this is due to the decrease of body immunity in those with obesity whereas one of the several causes of cervical cancer is Human Papilloma Virus infection.[9]

Based on the multivariate logistic regression statistical analysis of the risk factors related to gynecology cancer, we found that obesity and parities (p = 0.004, OR = 2.95 (95%CI = 1.41-6.19)) have significant result. Those with multiple pregnancies and grandemultipara have 3 folds higher risk to gynecology cancer than those with nullipara and primipara. This result support other previous results.

The initial result was delivered by Rigoni-Stern. In 1842, Rigoni-Stern published an analysis of death caused by cancer by the number of incidence in Verona from 1760 to 1839. They found that married women and widows may develop cervical cancer but the occurance is rare in unmarried women and nuns. They concluded that certain functional changes in the utery are related to pregnancy or delivery which causes the predisposition for cervical cancer. This carcinogenesis theory is supported by Broussais study who previously speculated that there might be a relationship between chronic irritation or trauma to neoplasm. Cervical continuous laceration, abration and infection related to indecent obstetric care or multiple parities may act as a causing factor. This finding is explained by Weinberg and Gastpar several years later that women from lower financial strata has higher risk for cervical cancer. Peluchi also study the relationship of multiple parities and the decrease of ovarium cancer risk where those with multiple parities have 0.6-0.8 more risk relatively to ovarium cancer than nullipara.[10-12]

CONCLUSION

We found significant result in the statistical analysis for the relationship of obesity and gynecology cancer. Those who are overweight and obese have 7 folds higher risk for gynecology cancer than those with normal and underweight. In obese grandemultipara the number of gynecology cancer incidence is higher than nullipara. Those multipara and grandemultipara have 3 folds higher risk for gynecology cancer than those who are nullipara and primipara.

REFERENCES

1. Affandi B, Gunardi ER, Santoso BI. Kesehatan Reproduksi dan Obesitas. Divisi Kesehatan Reproduksi Departemen Obgin FKUI. 2014.
2. Gustafsson F, Kragelund CB, Torp-Pederson C et al. Effect of Obesity and Overweight on Long Term Mortality in Congestive Heart Failure, Influence Of Left Ventricular Systolic Function, Eur Heart J. 2006; 26: 58-64.
3. WHO, Obesity: Preventing and Managing The Global Epidemic: Report of a WHO Consultation. Technical Report Series. Geneva, Switzerland. 2000: 894.
4. Hariadi dan Ali, Arsad Rahim. 2005. Hubungan Obesitas dengan Beberapa Faktor Risiko Penyakit Jantung Koroner di Laboratorium Klinik Prodia Makassar Tahun 2005. http://arali2008.files.wordpress.com/2008/09/obesitas-dan-jantung-koroner.pdf
5. Pamela, Dyah R. Overweight dan Obesitas sebagai Suatu Risiko Penyakit Degeneratif. 2011.http://www.suyotohospital.com/index.php?option=com_content&view=article&id=115:overweight-dan-obesitas-sebagai-suatu-risiko-penyakit degeneratif&catid=3: artikel&Itemid=2
6. Vvan Kruijisdjiik RC, van der Wall E, Visseren FL. Obesity and cancer the role of dysfuctional adipose tissue. Cancer epidemiology, biomarkers and prevention: a publication of the American Association for Cancer Research, cosponsored by t Am Soc Prev Oncol. 2009: 2569-78.
7. Calle EE, Kaaks R. Overweight, obesity and cancer: Epidemiological evidence and proposed mechanisms. Nat Reviews Cancer. 2004: 579-91.
8. Wadpadji S. Naskah Lengkap Pertemuan Ilmiah Tahunan Ilmu Penyakit Dalam Jakarta: Pusat Informasi dan Penerbitan Departemen Ilmu Penyakit Dalam FK UI, 2004: 79-89.
9. Tjandraningrum. Pengaruh Diet rendah Kalori Seimbang Indeks Glikemik Rendah dan Tinggi Terhadap Berat Badan, Lingkar Pinggang dan komposisi Tubuh Perempuan Obese. Tesis. Program Studi Ilmu Gizi Kekhususan Gizi Klinik Program Pendidikan, Pasca Sarjana FK UI. Jakarta: 2008.
10. Vucenik I, Stains JP. Obesity and cancer risk: evidence, mechanisms and recommendations. Annal of the New York Academy of Sciences 2012: 37-43.
11. Marti A, Marcos A, Martinez JA. Obesity and immune function relationships. Obesity reviews: an official J Int Associat Study Obes 2001: 131-40.
12. Copeland J Larry. Premalignant Lesions of the Cervix. In: Text book of Gynecology. Philadelphia: WB Saunders Company. 2000: 1225-56

Research Article

The Incidence of Anal Sphincter Ruptures and Risk Factors

Kejadian Ruptur Sfingter Ani dan Faktor-faktor Risikonya

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Abstract

Objective: To analyze the incidence of anal sphincter ruptures and to evaluate risk factors of obstetric anal sphincter ruptures in Dr. Cipto Mangunkusumo Hospital.

Method: We reviewed 2009 vaginal deliveries based on the analysis of obstetric data base and patient records of our department during 2012. Cases and control subjects were chosen randomly and patient's records were reviewed for the following variable: maternal age, parity, gestational age, labor induction, duration of 2nd stage labor, use of forceps, use of vacuum, use of episiotomy, birth weight, and presentation of the baby.

Result: There were 91 (4.53%) anal sphincter ruptures during period of study (91 of 2009 patients). An univariate analysis of these 91 case and 91 randomly selected control subjects show that primiparity (p = .000), gestational age (p = .016), duration of second-stage labor (p = .000), forceps delivery (p = .000), vacuum delivery (p = .001), episiotomy (p = .000), and birth weight (p = .000) increased the risk for anal sphincter ruptures. In multivariate re-gression models, only 5 of the 10 predictor variables were significantly related to the likelihood of having a severe perineal trauma greater than second degree. Primiparity (p = .023; OR 2.74, 95% [CI], 1.15-6.51), forceps delivery (p = .000; OR 18.18, 95% [CI] 3.84-86.07), vacuum delivery (p = .005; OR 6.83, 95% [CI] 1.77-26.42), episiotomy (p = .015; OR 2.86, 95% [CI] 1.23-6.65), and birth weight (p = .000; OR 0.99, 95% [CI] 0.997-0.999).

Conclusion: Damage of the anal sphincter resulting in a third- or fourth- degree perineal tear is a relatively rare but severe complication of vaginal delivery. We found that factors as sociated with anal sphincter ruptures were primiparity, forceps, vacuum, episiotomy and birth weight.

[Indones J Obstet Gynecol 2016; 1: 31-36]

Keywords: anal sphincter ruptures, third- or fourth- degree perineal tear, vaginal delivery

Abstrak

***Tujuan**: Untuk menganalisis kejadian ruptur sfingter ani dan mengevaluasi faktor risiko obstetrik ruptursfingter ani di Rumah Sakit Dr. Cipto Mangunkusumo.*

***Metode**: Kami meneliti secara retrospektif 2009 kelahiran pervaginam berdasarkan analisis data base obstetrik dan catatan pasien di departemen kami selama tahun 2012. Kasus dan kontrol yang dipilih secara acak dan catatan pasien ditinjau untuk melihat variabel-variabel berikut usia ibu, paritas, usia kehamilan, induksi persalinan, lama persalinan stage 2, penggunaan forseps, penggunaan vakum, penggunaan episiotomi, berat lahir dan presentasi bayi.*

***Hasil**: Ada 91 (4,53%) kejadian ruptur sfingter ani selama periode penelitian (91 dari 2009 pasien). Sebuah analisis univariat dari 91 kasus dan 91 kontrol yang dipilih secara acak menunjukkan bahwa primipara (p= 0,000), usia kehamilan (p = 0,016), lama persalinan stage kedua (p = 0,000), forseps (p = ,000), vakum (p = ,001), episiotomi (p = 0,000, dan berat bayi lahir (p = 0,000) meningkatkan risiko ruptur sfingter ani. Dalam model regresi multivariat, hanya 5 dari 10 variabel prediktor secara signifikan terkait dengan kemungkinan memiliki ruptur perineum yang berat lebih besar dari derajat kedua. Primipara (p = 0,023; OR 2,74, 95% [CI] 1,15-6,51), forseps (p= 0,000, OR 18,18, 95% [CI] 3,84-86,07), vakum (p = 0,005; OR 6,83, 95% [CI] 1,77-26,42), episiotomi (p = 0,015, OR 2,86, 95% [CI] 1,23-6,65), dan berat bayi lahir (p = 0,000; OR 0,99, 95% [CI] 0,997-0,999).*

***Kesimpulan**: Kerusakan sfingter ani mengakibatkan robekan perineum derajat ketiga atau keempat adalah komplikasi yang relatif jarang namun bila terjadi mempunyai komplikasi yang berat dalam persalinan pervaginam. Dalam studi ini, kami menemukan bahwa faktor yang terkait dengan ruptur sfingter ani adalah primipara, forseps, vakum, episiotomi, dan berat bayi lahir.*

[Maj Obstet Ginekol Indones 2016; 1: 31-36]

***Kata kunci**: persalinan pervaginam, robekan perineum derajat ketiga atau keempat, ruptur sfingter ani*

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INTRODUCTION

Obstetric anal sphincter ruptures may be seen at the time of birth ('overt') or may be detected only after additional ultrasound investigation, after birth ('occult'). As many as 85% of women who give birth vaginally will experience trauma to the perineum and 3-12% will be the anal sphincter muscle. Tear in the anal sphincter muscles will cause disruption to the muscles of the pelvic floor in the future. Damage of the anal sphincter resulting in a third- or fourth- degree perineal tear is a relatively rare but severe complication of vaginal delivery. The incidence of 'overt' anal sphincter injury has previously been reported in about 2.5% of vaginal deliveries with mediolateral episiotomy and about 11% with midline episiotomy.[1] However, 33% of women sustain occult anal sphincter

rupture during vaginal delivery.[2] The most plausible explanation for an occult rupture is either that an injury that has been missed or it has been wrongly classified as a second-degree tear. Forceps delivery, midline episiotomy, first vaginal delivery, larger baby, shoulder dystocia and a persistent occipito-posterior position have been identified as the main risk factors for the development of a third- fourth- degree tear.[3]

It might seem logical to assume that an increased grade of tear should be associated with increased severity of anal incontinence. However, while some studies have shown an association between symptoms of anal incontinence and increased degree of rupture[4,5], others have found there is no relationship.[6]

Anal incontinence after childbirth may be due to injury to the anal sphincter or its innervation, or both.[7] A rupture involving the anal sphincter during vaginal delivery has great bearing on a woman's future continence. Primary sphincter repair, performed by obstetricians immediately after delivery, has traditionally been regarded as providing a good outcome. However, recent studies in a total of 70 patients have reported subsequent anal incontinence in 29-48% of women three months to three years after primary sphincter repair.

In addition, patients sustaining third- or fourth-degree perineal tears are at a higher risk for the development of infection and rectovaginal fistulae.[8,9] As a result, the number of women requesting caesarean section is constantly growing in western European countries, thereby causing controversy between obstetricians on how to reduce maternal intrapartum and postpartum complications to provide optimal care of child bearing patient. It has therefore been the subject of several studies to identify potential risk factors associated with the development of perineal lacerations during vaginal delivery. Identified maternal and delivery variables reported in previous works include parity, maternal age, race, use of episiotomy, birth weight, assisted vaginal delivery, and induction of labor.[8-10]

The aims of the present work were to identify the incidence of anal sphincter rupture and to evaluate risk factors of obstetric anal sphincter tears. All women who had experienced second and third degree tear over a 12 month period in one obstetric unit of a teaching hospital were included in this study.

METHODS

This was an observational retrospective case-control based on register study. These analysis of obstetric variables using a 1:1 ratio of cases and control subjects. The information was taken from the Hospital Discharge Register equated to ICD-10 codes O70.2 (third- degree) and O70.3 (fourth- degree). The two data sources were linked together using the mothers' unique personal identification numbers. The degree was classified according to standard definitions: a third- degree rupture involves the external anal sphincter and a fourth- degree rupture affects both the anal sphincter and anorectal mucosa.[11] The degree of perineal trauma was assessed by obstetricians.

Women included in this study delivered their children (between January 2012 and December 2012) at the Department of Obstetrics and Gynecology Dr. Cipto Mangunkusumo Hospital, all deliveries were studied and analyzed with respect to risk factors for development of anal sphincter ruptures.

During the study period of 12 months, there were 2009 women undergoing vaginal delivery. Patients with multiple pregnancies (n = 71), induction of labor (n = 70), breech deliveries (n = 7), episiotomy (n = 94), forceps deliveries (n = 24), vacuum deliveries (n = 25). After strict application of in and exclusion criteria, data were divided into 2 groups: 1 group (cases) including all patients (n = 109) with laceration of the perineum greater than second degree. Perineal tears were classified into four degrees according to the international classification of diseases.[12] A first- degree tear involved the forche, the perineal skin, vaginal epithelium but not the underlying fascia and muscles. A second- degree tear also involved the fascia, muscles, perineal body but not the anal sphincter. A third- degree tear involved the anal sphincter, but does not extend through the rectal mucosa. A fourth- degree tear was defined as extending through the rectal mucosa. The second group (controls) was selected randomly on the basis of a blinded protocol from women undergoing vaginal delivery without anal sphincter ruptures.

All delivery records were studied and the following parameters were registered: age, parity, gestational age, induction of labor, duration of second stage labor, episiotomy, forceps delivery, vacuum delivery, presentation of the fetus, and birth weight.

Researches data obtained were recorded in a special form provided, then were tabulated and analyzed with the help of SPSS (Statistic Package for Social Science) computer software v.16 for Windows. Distribution of maternal and obstetrical predictor variables was compared with the use of T-Test and Chi Square Test. P value less than 05 was considered statistically significant. Multivariate logistic regression analysis was performed to evaluate to influence of potentially influencing the occurrence of third- or fourth- degree perineal tears considered in the logistic regression model.

RESULTS

In 2009 vaginal deliveries that were reviewed during study period, the incidence of anal sphincter ruptures was 4.53% (91 of 2009 patients). Mean maternal age in the sample group was 26.81 years and 27.71 years in controls (not significant, p > 05). Results of univariate analysis of maternal characteristics and delivery details of cases and control are listed in Tables 1 and 2. As shown, there were no significant differences in the age, induction, and presentation of the baby. Women with greater than third- degree tearing were more likely to be primiparity than the controls (p = .000). Furthermore, gestational age (p = .016), duration of second-stage labor (p = .000), forceps delivery (p = .000), vacuum delivery (p = .001), episiotomy (p = .000), birth weight (p = .000) were significantly associated with the occurrence of third- and fourth- degree perineal tears between the 2 groups (Table 2). Table 3 shows the results of a multivariate logistic regression model. Only 5 of the 10 predictor variables were significantly related to the likelihood of having a severe perineal trauma greater than second degree. Primiparity (p = .023; OR 2.74, 95% confidence interval [CI], 1.15-6.51), forceps delivery (p = .000; OR 18.18, 95% confidence interval [CI] 3.84-86.07), vacuum delivery (p = .005; OR 6.83, 95% confidence interval [CI] 1.77-26.42), episiotomy (p = .015; OR 2.86, 95% confidence interval [CI] 1.23-6.65), and birth weight (p = .000; OR 0.99, 95% confidence interval [CI] 0.997-0.999) were all significantly more common in women who sustained a third- degree tear than in those women who did not.

Table 1. Maternal Characteristics of the Study Population and Univariate Analysis of Cases and Controls by T-Test and Chi Square Test.

Characteristic	Cases	Controls	Statistical significance (p ≤ .05)
Mean age	26.81	27.71	0.320
Primiparity (yes/no)	21 (83.3%)	3 (16.7%)	0.000*
Mean gestational age	38.33	37.33	0.016*
< 35 wk	5 (31.3%)	11 (68.8%)	
35 - 36 wk	10 (40%)	15 (60%)	
37 - 38 wk	17 (37.8%)	28 (62.2%)	
39 - 40 wk	43 (59.7%)	29 (40.3%)	
Postdates > 40 wk	16 (66.7%)	8 (33.3%)	

Table 2. Delivery Details of the Study Population and Univariate Analysis of Cases and Controls by T-Test and Chi Square Test.

Characteristic	Cases	Controls	Statistical significance (p ≤ .05)
Labor induction (yes/no)	36 (51.4%)	34 (48.6%)	0.879
Mean duration of 2nd stage (min)	23.54	14.29	0.000*
Forceps (yes/no)	21 (87.5%)	3 (12.5%)	0.000*
Vacuum (yes/ no)	21 (84%)	4 (16%)	0.001*
Episiotomy (yes/ no)	69 (73.4%)	25 (26.6%)	0.000*
Mean birth weight (g)	3329.89	2769.34	0.000*
Below 3000 g	25 (32.5%)	52 (67.5%)	
3000 g to 4000 g	62 (61.4%)	39 (38.6%)	
Above 4000 g	4 (100%)	0 (0%)	
Presentation (buttock/head)	4 (57.1%)	3 (42.9%)	1.000

** = meaningful*

Table 3. Outcome of Multivariate Logistic Regression Analysis on Variables that Potentially Influence the Incidence of 3rd/4th Degree Lacerations.

Characteristic	Odds Ratio (95% CI)	Statistical significance ($p \leq .05$)
Primiparity (yes/no)	2.74 (1.15 - 6.51)	0.023*
Gestational Age	1.203 (0.968 - 1.496)	0.096
Duration of 2nd stage	0.989 (0.949 - 1.031)	0.604
Forceps (yes/no)	18.181 (3.841 - 86.068)	0.000*
Vacuum (yes/no)	6.834 (1.768 - 26.415)	0.005*
Episiotomy(yes/no)	2.862 (1.231 - 6.651)	0.015*
Birth Weight	0.998 (0.997 - 0.999)	0.000*

= meaningful

DISCUSSION

Anal sphincter ruptures are an uncommon complication of childbirth, although these tears are uncommon, we have shown that primary sphincter repair in these women is often unsatisfactory and associated with morbidity. The present study depicts risk factors that are associated with anal sphincter ruptures during spontaneous vaginal deliveries at Dr. Cipto Mangunkusumo Hospital. The goal of the present work was to identify for third and fourth degree perineal lacerations. Ninety one (4.53%) out of 2009 patients experienced ≥ third-degree perineal tears during vaginal delivery. Using cases and control subject univariate analysis revealed age, primiparity, gestational age, induction of labor, duration of second stage labor, forceps delivery, vacuum delivery, episiotomy, birth weight, and presentation of the baby as risk factors for disruption of the anal sphincter. The effect of unalterable maternal factors such as age, weight on the frequency of severe ruptures has been investigated, but the results are varying in different studies.[13,14]

Age differences between cases and controls statistically and clinically 1 years were found not meaningful. This is probably caused by the number of patients with relatively a few cases, so it did not give significant differences.

Primiparity is one of the most important risk factors, since primiparous have up to a 10-fold increased risk of anal sphincter ruptures. In keeping with other studies, we found that primiparous women were at greater risk of sustaining a third degree tear than women who had already had a vaginal delivery. This probably relates to relative in elasticity of the perineum. Differences in the elasticity and strength of connective tissue between nulliparous and parous women could be one explanation. There are few studies on those differences. A previous report by Petersen and Uldbjerg demonstrated that the content of hydroxyproline and the strength of the collagen in the uterine cervix of multiparas is reduced. If other risk factors are also present the attending obstetrician should anticipate the possibility of a major tear.[15-17] From our research, it was found that primiparity play an important role in the risk of anal sphincter ruptures.

Gestational age was associated with an increased risk for sphincter tears, which has been reported by Crawford et al. However, neither Sorensen et al nor Combs et al found such an association. We have no definite explanation for our finding. Gestational age was found to be an independent risk factor and an increased fetal weight is thus not the only explanation. Hormonal changes during pregnancy might alter connective tissue properties. The long standing effect of gravitational forces on the pelvic floor could also associate with changes in connective tissue.[18-20] Interestingly, gestational age was associated with a higher rate of sphincter damage in univariate analysis but did not prove to be an independent risk factor in the multivariate regression model.

Labor induction differences between cases and controls statistically and clinically 1 years were found not meaningful. This is probably caused by the number of patients with relatively a few cases, so it did not gives ignificant differences.

Duration of second stage was associated with a higher rate of sphincter damage in univariate analysis but did not prove to be an independent risk factor in the multivariate regression model.

Vaginal operative delivery, especially the use of forceps or vacuum, is a well-known cause of third- and fourth- degree perineal tears. The majority of research conducted in this field showed that forceps delivery significantly predicted anal sphincter injury.[8-10,21] However, in a retrospective study of 16,172 primigravid vaginal deliveries conducted by Gupta et al, instrumental delivery by the aid of forceps was not found to be an independent risk factor for sphincter damage with 36 (1.6%) of 2311 forceps deliveries resulting in third- degree lacerations. In contrast to several studies showing that anal sphincter injury is likely to complicate more than 60% of forceps deliveries, the incidence of 1.6% presented by Gupta and colleagues and another 13% in a prospective study investigating 93 females undergoing forceps delivery by de Parades et al is surprisingly low.[22,23] The present study clearly identified forceps and vacuum delivery as an independent risk factor for anal sphincter ruptures using cases and control. Instrumental delivery is known to increase risk for sphincter ruptures, and this risk is more pronounced with forceps compared with vacuum delivery. However, any intervention that substantially accelerates the last part of the second stage of labor could be harmful to the tissues of the pelvic floor.

Whether episiotomy is beneficial in the prevention of obstetric and anal sphincter ruptures (OASR) is an open question and under constant debate. Nowadays, limiting the use of episiotomy is recommended, since this appears to have a number of benefits such as less suturing and fewer complications.[24] We have previously reported that episiotomy is associated with a lower OASR rate in first births and a higher rate in second and subsequent births.[25] The role of episiotomy as a contributing factor for third- and fourth- degree lacerations is discussed controversially. By investigating a total of 50,210 vaginal deliveries, Angioli et al concluded that the episiotomy procedure per se, regardless of the type of episiotomy used, represents an independent risk factor for sphincter disruption.[26] Bek et al, Bodner et al, and Bodner-Adler and colleagues found an increased risk of anal sphincter tear when episiotomy was used.[21-28] By contrast, Poen et al, Shiono et al, and de Leeuw et al showed that episiotomy was protective against anal sphincter damage and fecal incontinence after vaginal delivery, and Hendriksen and coworkers and Buekens et al found no association between episiotomy and lesions of the anal sphincter.[2,22,29] In the present study, the use of episiotomy conferred an increased risk of severe perineal damage.

Many studies compare macrosomic infants to infants with lower birth weight and find a significant association between high birth weight and risk of anal sphincter ruptures.[15,16] Higher birth weight is associated with bigger head circumference, and some authors have reported a larger head to be a risk factor for sphincter damage.[30] In accordance with previous data, high birth weight was an independent risk factor for the occurrence of third- and fourth- degree perineal lacerations.[13] One simple reason may be the greater susceptibility and vulnerability to disruption of a perineum that is exposed to a greater tension with higher birth weight. From our statistical research conducted by us, it was found that a high birth weight had a role in the incidence of anal sphincter ruptures.

Presentation of the baby did not show significant thing in this study. This is probably caused by the number of patients with relatively a few cases, so it did not give significant differences.

CONCLUSION

In this study, we found that factors associated with anal sphincter ruptures were primiparity, forceps, vacuum, episiotomy, and birth weight. The most significant risk factors found for anal sphincter ruptures was forceps. Anal sphincter ruptures are an uncommon but serious complication of vaginal delivery. When multiple risk factors are present, special attention should be directed to preventing ruptures. Primary sphincter repair seems to be in adequate in at least half the women, often resulting in persistent symptoms. Because incontinence can be such a devastating social disability, the nature of sphincter repair deserves serious further attention. The goal of this study is to find out the causes of anal sphincter ruptures, so this incidence can be anticipated and be reduced.

REFERENCES

1. Sultan AH. Anal incontinence after childbirth. Curr Opin Obstet Gynecol, 1997; 9: 320-4.
2. De Leeuw JW, Vierhout ME, Struijk PC et al. Anal sphincter damage after vaginal delivery: functional outcome and risk factors for incontinence. Acta Obstet Gynecol Scand, 2001; 80: 830-4.
3. RCOG: London. The Management of third- and fourth- degree perineal tears. Royal College Obstet Gynecol Green-top Guideline, 2007: 29.

4. Nichols CM, Lamb EH, Ramakrishnan V. Differences in outcomes after third- versus fourth- degree perineal laceration repair: A prospective study. Am J Obstet Gynecol, 2005; 193: 530-6.
5. Norderval S, Oian P, Revhaug A et al. Anal incontinence after obstetric sphincter tears: outcome of anatomic primary repairs. Dis Colon Rectum, 2005; 48: 1055-61.
6. Starck M, Bohe M, Valentin L. The extent of endosonographic anal sphincter defects after primary repair of obstetric sphincter tears increases over time and is related to anal incontinence. Ultrasound Obstet Gynecol 2006; 27: 188-97.
7. Sultan AH, Thakar R. Third and fourth degree tears. In Perineal and Anal Sphincter Trauma, Sultan AH, Thakar R, Fenner DE (eds). Springer-Verlag: London, 2007: 33-51.
8. Richter HE, Brumfield CG, Cliver SP et al. Risk factors associated with anal sphincter tear: a comparison of primiparous patients, vaginal births after cesarean deliveries, and patients with previous vaginal delivery. Am J Obstet Gynecol, 2002; 187: 1194-8.
9. Christianson LM, Bovbjerg VE, Mc Davitt EC et al. Risk factors for perineal injury during delivery. Am J Obstet Gynecol, 2003; 189: 255-60.
10. Golgberg J, Hyslop T, Tolosa JE et al. Racial differences in severe perineal lacerations after vaginal delivery. Am J Obstet Gynecol, 2003; 188: 1063-7.
11. Power D, Fitzpatrick M, O'Herlihy C. Obstetric anal sphincter injury: how to avoid, how to repair. A literature review. J Fam Pract, 2006; 55(3): 193-200.
12. World Health Organization. International classification of diseases, 9th revision, clinical modification (ICD-9-CM). Geneva, Switzerland, 1996.
13. Hudelist G, Gelle'n J, Singer C et al. Factor predicting severe perineal trauma during childbirth: Role of forceps delivery routinely combined with mediolateral episiotomy. Obstet Gynecol, 2005; 192: 875-81.
14. Aukee P, Sundstrom H, Kairaluoma MV. The role of mediolateral episiotomy during labour: analysis of risk factors for obstetric anal sphincter tears. Acta Obstet Gynecol Scand 2006; 85(7): 856-60.
15. De Leeuw JW, Struijk PC, Vierhout ME, Wallenburg HC. Risk factors for third degree perineal ruptures during delivery. BJOG 2001; 108: 383-7.
16. Handa VL, Danielsen BH, Gilbert WM. Obstetric anal sphincter lacerations. Obstet Gynecol 2001; 98: 225-30.
17. Petersen LK, Uldbjerg N. Cervical hydroxyprolin concentration in relation to age. In: Lippert PC, Woessner JF, eds. The extracellular matrix of the uterus, cervix and the fetal membranes. New York: Perinatol Press, 1991: 138 -9.
18. Sorensen M, Tetzschner T, Rasmussen OO et al. Sphincter rupture in childbirth. Br J Surg 1993; 80: 392-4.
19. Crawford LA, Quint EH, Pearl ML, De Lancey JO. Incontinence following rupture of the anal sphincter during delivery. Obstet Gynecol, 1993; 82: 527-31.
20. Combs CA, Robertson PA, Laros RK Jr. Risk factors for third-degree and fourth- degree perineal lacerations in forceps and vacuum deliveries. Am J Obstet Gynecol, 1990; 163: 100-4.
21. Bodner-Adler B, Bodner K, Kaider A et al. Risk factors for third- degree perineal tears in vaginal delivery, with an analysis of episiotomy types. J Reprod Med, 2001; 46: 752-6
22. Gupta N, Kiran TU, Mulik V et al. The incidence, risk factors and obstetric outcome in primigravid women sustaining anal sphincter tears. Acta Obstet Gynecol Scand, 2003; 82: 736-43.
23. De Parades V, Etienney I, Thabut D et al. Anal sphincter injury after forceps delivery: myth or reality? A prospective ultrasound study of 93 females. Dis Colon Rectum, 2004; 47: 24-34.
24. Carroli G, Mignini L. Episiotomy for vaginal birth. Cochrane Database Syst Rev 2009; 3: CD000081.
25. Raisanen SH, Vehvilainen-Julkunen K, Gissler M et al. Lateral episiotomy protects primiparous but not multiparous women from obstetric anal sphincter rupture. Acta Obstet Gynecol Scand, 2009; 88(12): 1365-72.
26. Angioli R, Gomez-Marin O, Cantuaria G et al. Severe perineal lacerations during vaginal delivery: the University of Miami experience. Am J Obstet Gynecol, 2000; 182: 1083-5.
27. Bodner K, Bodner-Adler B, Wagenbichler P et al. Perineal lacerations during spontaneous vaginal delivery. Wien Klin Wochenschr, 2001; 113: 743-6.
28. Bek KM, Laurberg S. Risks of anal incontinence from subsequent vaginal delivery after a complete obstetric anal sphincter tear. BJOG, 1992; 99: 950-4.
29. Shiono P, Klebanoff MA, Carey JC. Midline episiotomies: more harm than good? Obstet Gynecol, 1990; 75: 765-70.
30. Andrews V, Sultan AH, Thakar R et al. Risk factors for obstetric anal sphincter injury: a prospective study. Birth, 2006; 33: 117-22.

exposed by 200 μM of curcumin. Group V : with exposed by 400 μM of curcumin, Group VI : with exposed by 800 μM of curcumin. Each group replicate five times, and each will be the nexamined by two kind of examination, proliferation and apoptosis index.

Proliferation Index

Proliferation index was measured by calorimetric examination which is called the method of MTT proliferation index. This method measuring absorbance of formazan, which it's produce by proliferative cells. The higher the absorbance value means more formazan produced, and indicates a growing number of proliferating cells.

Apoptosis Index

Apoptosis index examination by the method of labeling DNA fragmentation TUNEL system. Cell undergoing apoptosis was characterized by brown staining in the nucleus.

Ethics

Ethical clearance was obtained from Health Research Ethics Committee of Dr. Saiful Anwar General Hospital.

Statistical Analysis

Data are analyzed using one-way ANOVA test and followed by Least Significant Difference (LSD) to differences between groups. Post hoc test was used if the ANOVA was significant. P < 0.05 was considered statistically significant.

RESULTS

Culture of Complete Hydatidiform Mole

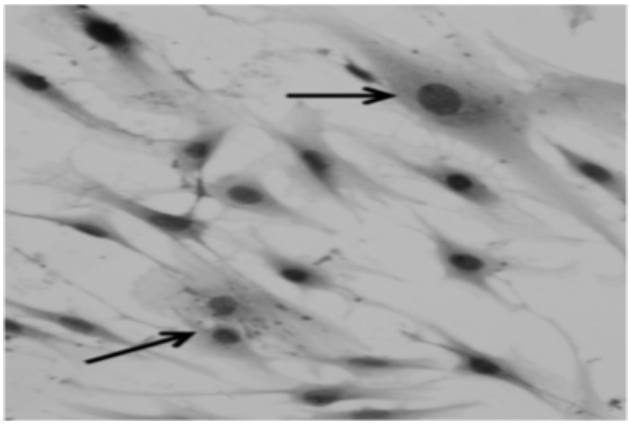

Figure 1. Trophoblast Cell Culture of Complete Hydatidiform Mole.

Proliferation Index Analysis

Proliferation index in CHM trophoblastic cells can be seen in Table 1 and Fig. 1. Table 1 shows mean data of proliferation index according to varrying doses of curcumin.

Table 1. Mean Data of Proliferation Index According to Varrying Doses of Curcumin

Treatment	Proliferation index Means ± SD	p-value
Control	0.96 ± 0.20^{a}	0.001
Curcumin 50 μM	1.10 ± 0.10^{a}	
Curcumin 100 μM	0.64 ± 0.37^{a}	
Curcumin 200 μM	0.36 ± 0.03^{c}	
Curcumin 400 μM	0.34 ± 0.02^{c}	
Curcumin 800 μM	0.32 ± 0.01^{c}	

Description : at the mean ± SD, if it contains different letters mean no significant difference (p-value <0.05) and if it contains the same letters mean no significant difference (p-value>0.05).

The table shows that treatment of varying doses of curcumin have different influence on mean proliferation index. ANOVA test showed significant differences (p<0,001) in proliferation index in CHM trophoblastic cells with various doses of curcumin treatment and control.

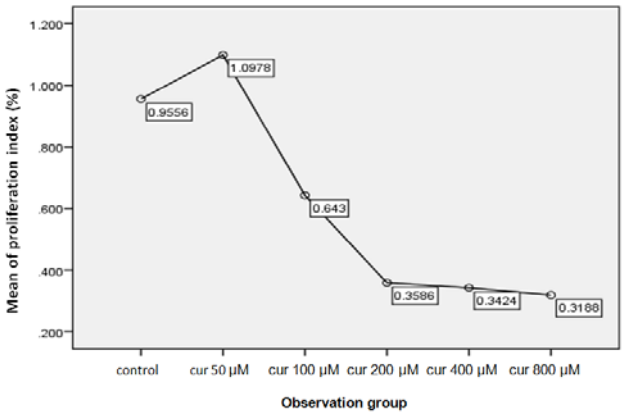

Figure 2. Trend Change in Mean Proliferation Index

Apoptosis Index Analysis

Description : cells undergoing apoptosis are characterized by brown-staining in the nucleus (marked with circles) and cells which not under-

going apoptosis showed no brown staining in the cell nucleus (marked with a circle cut off).

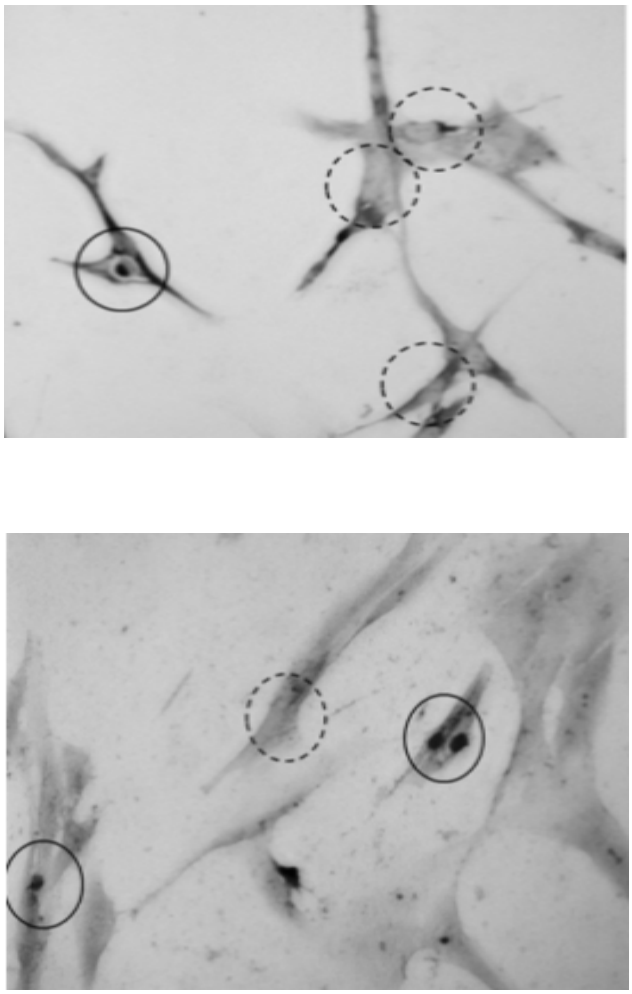

Figure 3. Immunohistochemical TUNEL Labeling System Introphoblast Cells CHM with 400x Magnification.

Table 2. Mean Data of Apoptosis Index According to Varying Doses of Curcumin

Treatment	Apoptosis index Means ± SD	p-value
Control	14.4 ± 4.56[a]	0.001
Curcumin 50 μM	18.8 ± 5.02[a]	
Curcumin 100 μM	22.4 ± 7.13[a]	
Curcumin 200 μM	44.4 ± 7.40[b]	
Curcumin 400 μM	56.8 ± 13.16[b]	
Curcumin 800 μM	49.6 ± 16.15[b]	

Description: at the mean ± SD, if it contains different letters mean no significant difference (p-value <0.05) and if it contains the same letters mean no significant difference (p-value>0.05).

This table shows that treatment of varying doses of curcumin have different influence on mean apoptosis index. ANOVA test showed significant differences (p<0,001) in apoptosis index in CHM trophoblastic cells with various doses of curcumin treatment and control.

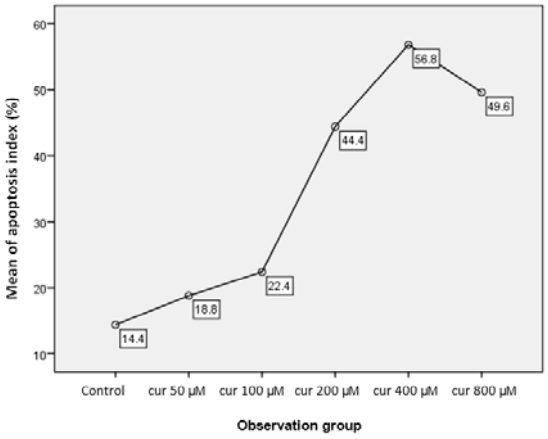

Figure 4. Trend change in mean apoptosis index.

DISCUSSION

Curcumin is a compound derivative of turmeric which proved capable of inhibiting tumor transformation, initiation and promotion, proliferation, invasion, angiogenesis and metastasis. Many studies have shown that curcumin modulates a variety of molecular targets, including growth factors and their receptors, transcription factors, cytokines, enzymes and genes that regulate apoptosis. Curcumin inhibits proliferation of cancer cells by holding them at different stages of the cell cycle and inducing apoptosis.[10-12]

Curcumin proved to inhibit cell proliferation, namely through inhibition of NFkB, in several types of cancer by lowering protein antiapoptosis (Bcl-2 and Bcl-XL), cell cycle regulators (cyclin-cyclin D-1 and D-2), growth factors (interleukin, TNF-α, VEGF) and increase apoptosis, by activating caspase.[10-12]

In this study, the one-way ANOVA test proved there were not significant differences in decreasing proliferation index between control group and curcumin dose 50 μM. And there were highly significant differences (p=0.001) indecreasing of proliferation index by curcumin administration of a dose of 100 μM, 200 μM, 400 μM and 800 μM compared with the control and curcumin dose of 50 μM (Table 1). Figure 1 shown that proliferation index

decreased with increasing doses of curcumin. However, there was no significant difference in reduction in proliferation index between the treatment dose administration of curcumin at a dose of 200 μM, 400 μM and 800 μM. So it means that statistically the third dose has the same capabilities in terms of reducing the proliferation index in cell culture of complete hydatidiform mole.

In this study, the one-way ANOVA test obtained a very significant difference in the mean apoptosis index of cells into six groups of sample observations, as shown by the p-value=0.001. With LSD in Table 2 shows that there is no significant difference in the mean apoptosis index of cells between the control group and the group treated with curcumin dose administration of 50 μM and also with a dose of 100 μM. This suggests that there is no effect of giving curcumin 50 μM and 100 μM against cell apoptosis index in cell culture complete hydatidiform mole. Although, it appears to be an increase in the mean value but the increasement was not statistically significant.

From this research shows that there are significant differences in the apoptosis index between the control group and the group given doses of curcumin 200 μM, 400 μM, ora dose of 800 μM. Figure 3 shown that apoptosis index increase with increasing doses of curcumin. However, there was no significant difference in increasing of apoptosis index between the group given doses of curcumin 200 μM, 400 μM, or 800 μM. In other words, the three doses of curcumin have the same ability to increasing the apoptosis cells index in cell culture of complete hydatidiform mole. Where the average value of the cell apoptosis index was highest at a dose of 400 μM. In this study a dose of 400 μM can be considered as the most optimal dose of curcumin in increasing cell apoptosis index.

CONCLUSION

Based on the results and discussion in this study it can be concluded that the administration of curcumin can lowering proliferation index and increasing apoptosis index in trophoblastic cell culture of complete hydatidiform mole. Where dose of curcumin that proved significant in reducing the proliferation index and increasing the apoptosis index in this study is a dose of 200 μM.

REFERENCES

1. Bess KA and Wood TL. Understanding gestational trophoblastic disease. AWHONN Lifelines, 2006; 10(4): 321-6.
2. Andrijono. Penyakit Trofoblas Gestasional. Edisi pertama. Divisi Onkologi Departemen Obstetri Ginekologi Fakultas Kedokteran Universitas Indonesia. 2007: 23-41.
3. Sastradinata I. Problematik Penyakit Trofoblas di Indonesia. Departemen Obstetri Ginekologi Fakultas Kedokteran Universitas Sriwijaya, 2009.
4. Schorge JO and Schaffer JI. Gynecologic Oncology: Gestational Trophoblastic Disease in Williams Gynecology. The McGraw-Hill Companies 2008; 37: 755-70.
5. Tangapazham RL, Sharma A and Maheswari RK. 2006. Multiple Molecular Targets in Cancer Chemo prevention by Curcumin. The AAPS J, 2014: 443-9.
6. Lin C and Lin JL. Curcumin: a Potential Cancer Chemo preventive Agent through Suppressing NFkB Signaling J. Cancer Mol, 2008; 4(1): 11-6.
7. Berkowitz RS and Goldstein DP. Current management of gestational trophoblastic disease. Am Cancer Soc, 2005; 112(3): 654-62.
8. Newlands ES. The management of gestational trophoblastic disease. Royal College Obstet Gynecol, 2010; 38: 1-11.
9. Anand P, Kunumakkara AB, Newman RA, Aggarwal BA. Bioavailability of curcumin: Problems dan Promises. Am Chem Soc, 2007; 4(6): 807-18.
10. Sharma RA, Gescher AJ and Steward WP. Curcumin: The story so far. Eur J Cancer 2005; 41: 1955-68.
11. Shishodia S, Sethi G and Aggarwal BB. Curcumin: Getting back to the roots. NY Academy of Science, 2005; 1056: 206-17.
12. Duvoix A, Blasius R, Delhalle S et al. 2005. Chemopreventive and therapeutic effect of curcumin. Luxemberg. Cancer letters. www.elsevier.com/locate/canlet.: 181-90.
13. Morikawa T, Matsuda H, Ninomiya K, and Yoshikawa M. 2007. Role of Curcumin in Cancer Therapy. J Current Problem Cancer, 2005; 31: 243-305.

Research Article

International Ovarian Tumor Analysis (IOTA) Scoring System to Predict Ovarian Malignancy Pre-operatively

Sistem Skoring Internasional Ovarian Tumor Analisis untuk Memprediksi Keganasan Ovarium Prabedah

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Abstract

Objective: To compare diagnostic performance of International Ovarian Tumor Analysis (IOTA) scoring method with Risk of Malignancy Index-4 (RMI-4) and Sassone Morphology Index to predict ovarian malignancy preoperatively.

Method: Retrospective study with 119 subject who underwent surgical removal of ovarian tumor and performed histopathological examination at Dr. Cipto Mangunkusumo Hospital on January to December 2013. Demographic status, ultrasound scans, CA-125 level and histopathological result were collected to calculate the score of each method. Sensitivity, specificity, positive predictive value, negative predictive value and accuracy were calculated by comparing each score with histopathology result. Comparison of diagnostic performance was analyzed by ROC curve.

Result: There were 51.26% subjects with benign tumor and 48.74% subjects with malignant tumor. Result was analyzed with sensitivity test (IOTA simple-rules, IOTA subgroup, RMI-4 and Sassone): 98%, 88%, 86% and 79%; specificity: 74%, 67%, 61% and 89%; positive predictive value: 78%, 72%, 68% and 87%; negative predictive value: 98%, 85%, 82% and 81%; and accuracy: 86%, 77%, 73% and 84%. AUC value for IOTA simple-rules, IOTA subgroup, RMI-4 and Sassone were: 0.86, 0.78, 0.73 and 0.84. Comparison of these results were significant with p = 0.000.

Conclusion: IOTA simple-rules had better sensitivity, negative predictive value and accuracy than IOTA subgroup, RMI-4 and Sassone morphology index to predict ovarian malignancy preoperatively.

[Indones J Obstet Gynecol 2016; 1: 42-46]

Keywords: iota, ovarian neoplasm, risk of malignancy, scoring

Abstrak

***Tujuan**: Membandingkan kemampuan diagnostik metode skoring International Ovarian Tumor Analysis (IOTA) dengan Risk of Malignancy Index-4 (RMI-4) dan Sassone Morphology Index dalam memprediksi keganasan ovarium prabedah.*

***Metode**: Uji diagnostik secara retrospektif pada 119 pasien yang menjalani pembedahan atas indikasi neoplasma ovarium dan dilakukan pemeriksaan histopatologi di RSUPN Dr. Cipto Mangunkusumo dari Januari hingga Desember 2013. Data demografi, ultrasonografi dan kadar CA-125 dikumpulkan untuk dikelola menurut metode skoring IOTA simple-rules, IOTA subgroup, RMI-4 serta Sassone dan dibandingkan dengan histopatologi. Nilai diagnostik dari keempat metode skoring dihitung dengan luaran: sensitivitas, spesifisitas, nilai prediksi positif, nilai prediksi negatif dan akurasi. Perbandingan ketiganya dihitung menggunakan kurva ROC.*

***Hasil**: Didapati 51,26% subjek dengan tumor jinak dan 48,74% subjek dengan tumor ganas. Dari perhitungan, didapat sensitivitas IOTA simple-rules, IOTA subgroup, RMI-4 dan Sassone adalah: 98%, 88%, 86% dan 79%. Spesifisitas: 74%, 67%, 61%, dan 89%. Nilai prediksi positif: 78%, 72%, 68%, dan 87%. Nilai prediksi negatif: 98%, 85%, 82%, dan 81%. Akurasi: 86%, 77%, 73% dan 84%. Nilai AUC IOTA simple-rules, IOTA subgroup, RMI-4 dan Sassone adalah: 0,86; 0,78; 0,73 dan 0,84. Perbandingan keempat nilai AUC ini memberikan hasil bermakna p = 0,000.*

***Kesimpulan**: IOTA simple-rules memiliki sensitivitas, nilai prediksi negatif dan akurasi lebih baik dibandingkan IOTA subgroup, RMI-4 dan Sassone Morphology Index dalam memprediksi keganasan ovarium prabedah.*

[Maj Obstet Ginekol Indones 2016; 1: 42-46]

***Kata kunci**: iota, kanker ovarium, keganasan ovarium, skoring, tumor ovarium*

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INTRODUCTION

Ovarian cancer is a primary malignancy of the ovary.[1] Approximately 192.000 new cases are discovered per year worldwide.[2] In United State, the prevalence of ovarian cancer is 23.100 cases per year, while in United Kingdom is 6.000 cases.[3,4] In Indonesia, according to the National Cancer Registry Indonesian Society of Gynecological Oncology (INASGO)[5], from 2000 - 2013, approximately 2930 cases were discovered. Ovarian cancer is the third most common malignancy in women after cervical and breast cancer.[6]

Survival rate of ovarian malignancy is very poor, a study was done in United Kingdom demonstrated that 5-years survival rate of early ovarian cancer was 73%, while in the advanced stage was 16%.[7] According to this study, it is important to detect in early stage, since delay in diagnosis correlates with

delay in treatment and more over poorer in prognosis. To minimize delay in diagnosis, it is important to evaluate the ovarian tumor whether it is a benign or malignant, because it will facilitate the referral to the tertiary level.

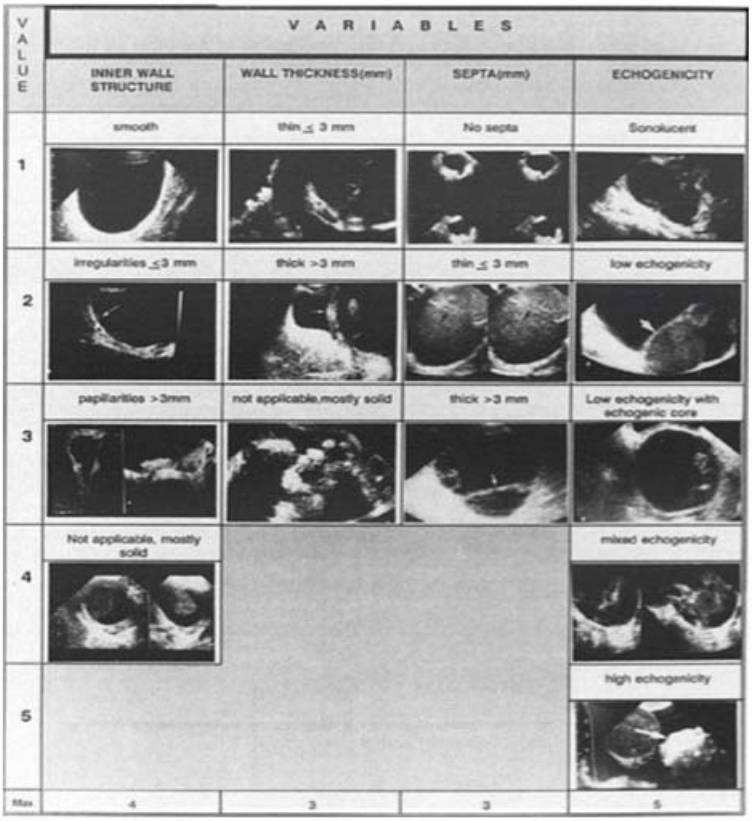
Ultrasonography has developed many scoring systems to predict ovarian malignancy, which is: Sassone Morphology Index (1991), Risk of Malignancy Index (Jacob, 1991), and International Ovarian Tumor Analysis (IOTA, 2000-2013).[8] Each of the scoring system has good sensitivity and specificity in predicting malignancy in ovarian tumor.

Unfortunately, there is no data that compare the diagnostic performance of each scoring systems and its applicability in Indonesian population. Therefore, this study aims to demonstrate the diagnostic performance of IOTA, Sassone Morphology Index and RMI-4.

METHODS

This was a retrospective study with subject population was patients who underwent surgical removal of ovarian tumor and histopathological examination in National General Hospital Dr. Cipto Mangunkusumo on January to December 2013. Medical records, along with the ultrasound scans, were reviewed by gynecological oncology consultant. Incomplete medical records or ultrasound scans and borderline histopathology tumor were excluded from this study.

Each scoring method was calculated on every subjects, based on IOTA simple-rules, IOTA subgroup, Sassone Morphology Index and RMI-4 to evaluate the tumor for malignancy possibility. The reference standard of this study was histopathology examination using World Health Organization classification. The operational definition of IOTA simple-rules, subgroup, RMI-4 and Sassone Morphology Index can be found on Table 1, 2, 3 and Figure 1. The outcome of the study was sensitivity, specificity, positive predictive value, negative predictive value and accuracy that were performed by ROC curve. Statistical significancy was determined by p value < 0.05. SPSS v.21 was used to do the statistical calculation.



Benign if score < 9; Malignant if ≥ 9

Figure 1. Sassone morphology index[12]

Table 1. IOTA Simple-rules[9]

B-rules	M-rules
Unilocular	Irregular solid
Solid part with diameter < 7 mm	Ascites
Multilocular with regular border size < 100 mm	Multilocular with irregular border > 100 mm
Acoustic shadow	At least 4 papillary projections
No blood flow	Strong blood flow

Table 2. IOTA subgroup[10]

Unilocular	Multilocular	Weight	Solid component, no papillation	Weight	Papillation	Weight
	Ascites	2	Ascites	7	Ascites	3
	Number of locules	1	Irregular wall and:		Age ≥ 50 years	1
	Max lesion D ≥ 100 mm	1	Completely solid tumor	5	Number of papillations ≥ 4	2
	Age ≥ 50 years	1	Multilocular solid with max lesion D ≥ 100 mm	3	Papillary flow	2
			Other	1		
			Blood flow color score:		Blood flow color score:	
			No flow	-4	Very strong flow	2
			Minimal flow	-1		
			Moderately strong flow	0		
			Very strong flow	2		
			Max solid D:		Max solid D:	
			< 10 mm	-3	< 10 mm	-3
			10-19.9 mm	-1	10-19.9 mm	-1
			20-29.9 mm	0	20-29.9 mm	0
			30-39.9 mm	1	30-39.9 mm	1
			40-49.9 mm	2	40-49.9 mm	2
			≥ 50 mm	3	≥ 50 mm	3
			Bilateral	2		
			Acoustic shadow	-3	Acoustic shadow	-3
					Personal history of ovarian cancer	3
	Total < 3 → benign		Total < 4 → benign		Total < 2 → benign	
Benign	Total ≥ 3 → malignant		Total ≥ 3 → malignant		Total ≥ 3 → malignant	

Table 3. RMI-4[11]

	RMI-4
	U x M x S x CA125
U	Parameters: solid, multilocular, bilateral, ascites, metastasis Ultrasound score: 1 or 4. Put 1 if ≤ 1 parameters, 4 if > 1 parameters
M	Menopausal status: 1 or 4. Put 1 if premenopause, 4 if post menopause
S	Size of tumor mass: 1 or 2. Put 1 if size < 7 cm, 2 if size ≥ 7 cm
CA125	CA125 value

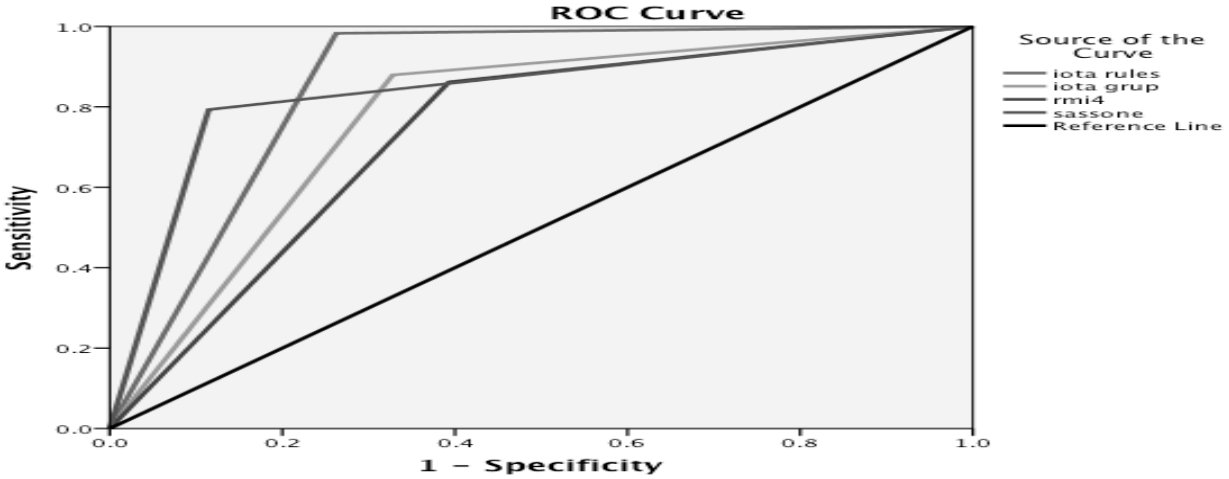


Figure 2. ROC curve of each scoring method

RESULTS

From this study, we confirmed that 61 (51.26%) subjects with benign tumor and 58 (48.74%) subjects with malignant tumor, with approximately 69% of the population were aged above 40 years and 4.2% were aged below 19 years. Mean size of tumor was 152 mm (50 - 480 mm) for benign and 139 mm (53 - 450 mm) for malignant tumor and the mean value of CA-125 was 129 U/ml (5 - 816 U/ml) for benign and 658 U/ml (7 - 7490 U/ml) for malignant tumor. The majority of the population with benign and malignant tumor was found in premenopausal status (68.9% and 58.6%, p = 0.246).

The scoring methods were applied to the subjects and resulted with sensitivity (IOTA simple-rules, IOTA subgroup, RMI-4 and Sassone): 98%, 88%, 86% and 79%; specificity: 74%, 67%, 61% and 89%; positive predictive value: 78%, 72%, 68% and 87%; negative predictive value: 98%, 85%, 82% and 81%; and accuracy: 86%, 77%, 73% and 84%. The AUC value for IOTA simple-rules, IOTA subgroup, RMI-4 and Sassone were: 0.86, 0.78, 0.73 and 0.84 respectively. Comparison of these results were significant with p = 0.000.

DISCUSSION

Compared with its predecessor study, the sensitivity of IOTA simple rules in this study was quite consistent. Timmerman et al[9] demonstrated that simple rules method had sensitivity of 95% and specificity of 91%. Validation of this system by Timmerman et al[9] and Kaijser et al[13] showed a sensitivity of 90%. The specificity result of this study was different from the initial study. There was a 20% difference. It can be explained from the morphological characteristics of benign tumors that truly benign and malignant tumors that suspected benign. Approximately 43.8% of benign tumors suspected malignancy had ascites, 31.3% had multilocular appearance with irregular border, 18.8% had strong blood flow and 18.8% had papil more than 4. These factors could contribute to increasing the false-positive interpretation. Based on further analysis, the presence of ascites correlated significantly in improving the false-positive rate (r = 0412; p = 0.005).

The sensitivity of IOTA subgroup in this study also had consistent result with previous research. Ameye et al[10] demonstrated that this subgroup method had sensitivity of 88% and specificity of 90%. There was a 23% difference in the specificity resulted from this study with the original research.

Morphological characteristic of the tumor was also considered as a factor that increased the number of false-positives result in this group. In multilocular dominant appearance group of tumor, 37% histopathological benign multilocular tumor was suspected malignant by this scoring method. This was due to several factors such as: ascites, number of locules, tumor size, and the age of the patient. Approximately, 62.5% histopathological-benign tumor which were suspected malignant had locules more than 5.25% had ascites, 100% had tumor size more than 100 mm, and 75% found in subjects over 50 years. In tumors with solid appearance, 44% of histopathological benign tumors

were suspected and classified as a malignancy. Approximately, 57% of this benign solid tumors had ascites, irregular border (42.9%), and appearance of blood flow (28.6%). In tumors with papillary projection, 60% of histopathological benign tumor were suspected malignant. All of histopathological benign tumors with papillary projection which suspected malignant had papil more than 4. The other parameters which contribute to increasing number of false-positives were: ascites (33.3%) and the appearance of blood flow (33.3%). Based on further analysis, the presence of ascites had a significant strong positive correlation in improving the false-positive rate (r = 0667; p = 0.027).

The sensitivity results for RMI-4 in this population was also consistent with previous research. Yamamoto et al[11] gained 86.8% sensitivity and 91% specificity. Wide differences between this study and Yamamoto's was also due to the characteristic of the tumor, size of the tumor and menopausal status. Approximately, 91.7% of histopathological benign tumors were diagnosed as malignant by RMI-4 had a tumor size above 100 mm, 41.7% of the population were in the post-menopausal state. Ultrasound scoring equal to 4 was also contribute to 68.8% of this group. Based on further analysis, ultrasound scoring equal to 4 had a weak positive correlation in increasing the number of false-positive but it was not significant (r = 0:25; p = 0.126).

Sassone's previous studies obtained a sensitivity of 100% and specificity of 83%.[12] Geomini et al[14] validated this scoring method and gained 84% sensitivity and 83% specificity. This study found a 21% false-negative. This was also due to some morphological characteristics of benign ovarian tumors in histopathological malignant tumors. This study found: 41.7% of tumors had regular wall, 50% with wall thickness less than 3 mm, and 41.7% had sonolucent or low echogenicity. Based on further analysis, it was found that the wall thickness of less than 3 mm had a weak positive correlation in increasing the numbers of false-negative (r = 0:25; p = 0.313).

The ROC curve showed that the AUC value of IOTA simple-rules and Sassone morphology index had over 80% (86% and 83%), demonstrating the diagnostic test had a strong interpretation. While IOTA subgroups and RMI-4 showed the AUC above 70% (78% and 73%) which showed moderate interpretation.

CONCLUSION

IOTA simple rules scoring system had better sensitivity, negative predictive value and accuracy than RMI-4 and Sassone Morphology Index in predicting ovarian malignancy. Careful interpretation needs to be done in a presence of ascites as this was correlated with false-positive.

REFERENCES

1. Berek JS, Natarajan S. Ovarian and Fallopian Tube Cancer. In: Berek JS, editor. Berek & Novak's Gynecology. 14th ed. Philadelphia: Lippincott Williams & Wilkins; 2007: 1457-9.
2. Arab M, Khayamzadeh M, Tehranian A et al. Incidence rate of ovarian cancer in Iran in comparison with developed countries. Ind J Cancer. 2010; 47(3): 322-7.
3. Piver MS, Frank TS. Epidemiology of ovarian cancer. Diagnosis and Management of Ovarian Disorders: Elsevier Science; 2003: 209-17.
4. Guppy AE, Nathan PD, Rustin GJ. Epithelial ovarian cancer: a review of current management. Clin Oncol (R Coll Radiol). 2005; 17(6): 399-411.
5. Indonesian Society of Gynecological Oncology. Data Nasional. 2014 [cited 2014June7]; Available from: http://www.inasgo.com/webrscm/Laporan/Data_Nasional.aspx.
6. Evans J, Ziebland S, McPherson A. Minimizing delays in ovarian cancer diagnosis: an expansion of Andersen's model of 'total patient delay'. Fam Pract. 2007; 24(1): 48-55.
7. Aslam N, Tailor A, Lawton F et al. Prospective evaluation of three different models for the pre-operative diagnosis of ovarian cancer. BJOG, 2000; 107(11): 1347-53.
8. Twickler DM, Moschos E. Ultrasound and assessment of ovarian cancer risk. AJR Am J Roentgenol. 2010; 194(2): 322-9.
9. Timmerman D, Testa AC, Bourne T et al. Simple ultrasound-based rules for the diagnosis of ovarian cancer. Ultrasound Obstet Gynecol. 2008; 31: 681-90.
10. Ameye L, Valentin L, Testa AC et al. A scoring system to differentiate malignant from benign masses in specific ultrasound-based subgroups of adnexal tumors. Ultrasound Obstet Gynecol. 2009; 33(1): 92-101.
11. Yamamoto Y, Yamada R, Oguri H, Maeda N, Fukaya T. Comparison of four malignancy risk indices in the preoperative evaluation of patients with pelvic masses. Eur J Obstet Gynecol Reprod Biol. 2009; 144(2): 163-7.
12. Sassone AM, Timor-Tritsch IE, Artner A et al. Transvaginal sonographic characterization of ovarian disease: evaluation of a new scoring system to predict ovarian malignancy. Obstet Gynecol. 1991; 78(1): 70-6.
13. Kaijser J, Bourne T, Valentin L et al. Improving strategies for diagnosing ovarian cancer: a summary of the International Ovarian Tumo Analysis (IOTA) studies. Ultrasound Obstet Gynecol. 2013; 41: 9-20.
14. Geomini P, Kruitwagen R, Bremer GL, Cnossen J. The accuracy of risk scores in predicting ovarian malignancy: a systematic review. Obstet Gynecol. 2009; 113: 384-94.

Research Article

Theurapeutic Response of Neoadjuvant Chemotherapy between Platinum and Ifosfamide Combination and Platinum, Vincristine and Bleomycin Combination in Cervical Carcinoma Stage IB2

Perbandingan Respons Terapi antara Kemoterapi Neoajuvan Kombinasi Platinum dan Ifosfamide dengan Kombinasi Platinum, Vincristine dan Bleomycin pada Karsinoma Serviks IB2

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Abstract

Objective: To evaluate the theurapeutic response and acute toxicity of neoadjuvant chemotherapy between the combination of Platinum and Ifosfamide, and the combination of Platinum, Vincristine and Bleomycin in Cervical Carcinoma Stage IB2 and then continued with radical hysterectomy and pelvic lymphadenectomy.

Method: Thirteen samples received neoadjuvant chemotherapy of Platinum and Ifosfamide and 17 samples received neoadjuvant chemotherapy of Platinum, Vincristine and Bleomycin, after receiving the neoadjuvant chemotherapy, clinically complete response samples underwent radical hysterectomy and pelvic lymphadenectomy (PI VS PVB = 3 VS 1). Histopathology examination was performed to evaluate the presence of malignant viable cells at the cervix, pelvic lymph node metastasis and parametrium metastasis. Acute toxicity evaluation was performed based on gastrointestinal, genitourinarius and hematology sign and symptom.

Result: Theurapeutic response of PI is 1.12 higher than PVB (p>0.05). Subanalysis of group response of PI is 1.962 higher than PVB. PI and PVB have the same risk to have pelvic lymph node metastasis, but not parametrial metastasis. There were no differences in terms of the risk of gastrointestinal, genitourinarius and hematologic toxicity between PI and PVB.

Conclusion: There was no statistical difference in clinical and pathological response, and also in acute toxicity between the two combination (p>0.05).

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Keywords: acute toxicity, cervical carcinoma stage IB2, neoadjuvant chemotherapy, response

Abstrak

***Tujuan**: Untuk mengevaluasi respons terapi dan toksisitas akut pada kasus karsinoma serviks stadium IB2 yang mendapatkan kemoterapi ajuvan kombinasi platinum dan ifosfamide dan kombinasi platinum, vincristine dan bleomycin, yang dilanjutkan dengan histerektomi radikal dan diseksi kelenjar getah bening.*

***Metode**: Sebanyak 13 sampel mendapatkan kemoterapi neoajuvan kombinasi platinum dan ifosfamide dan sebanyak 17 sampel mendapatkan kemoterapi neoajuvan kombinasi platinum, vincristine dan bleomycin. Pasca pemberian kemoterapi neoajuvan, sampel dengan respons komplit, dilanjutkan dengan tindakan histerektomi radikal dan diseksi masing-masing kelenjar getah bening pelvis (PI VS PVB = 3 VS 1). Penilaian histopatologi dilakukan untuk penilaian adanya malignant viable cell di masa tumor, metastasis kelenjar getah bening pelvis dan parametrium. Penilaian toksisitas akut dilakukan pada gastrointestinal, genitourinarius dan hematologi.*

***Hasil**: Respons terapi kombinasi PI adalah 1,12 kali lebih besar dibandingkan dengan kombinasi PVB (p>0,05). Subanalisis respons kelompok terapi kombinasi PI adalah sebesar 1,962 kali lebih dibandingkan dengan kombinasi PVB (p>0,05). Kombinasi PI mempunyai risiko yang sama dengan kombinasi PVB dalam hal ditemukannya metastasis kelenjar getah bening pelvis, tetapi tidak untuk metastasis pada parametrium. Pada pemberian kemoterapi kombinasi PI, tidak ditemukan perbedaan risiko terjadinya toksisitas gastrointestinal, genitourinarius dan hematologi selama terapi dibandingkan dengan kombinasi PVB.*

***Kesimpulan**: Tidak didapatkan perbedaan yang bermakna pada respons terapi secara klinis dan histopatologi, juga pada toksisitas akut antara kedua kombinasi (p>0,05).*

[Maj Obstet Ginekol Indones 2016; 1: 47-51]

***Kata kunci**: karsinoma serviks stadium IB2, kemoterapi neoajuvan, respons, toksisitas akut*

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INTRODUCTION

Cervical cancer is the third most diagnosed cancer and the fourth cause of female mortality worldwide. From 13 pathology center in Indonesia, cervical cancer is the first rank among all cancers (23,4% of 10 most common cancers in men and women). Data from university hospitals in 2007 stated that cervical cancer is the most gynecology

cancer, followed by ovarian, endometrial, vulva and vaginal cancer.[1,2]

A study on cervical cancer at Dr. Cipto Mangunkusumo Hospital Jakarta stated that 5 years survival rate of cervical cancer stage I, II, III and IV is 50%, 40%, 20% and 0%. Marisa et. al[3] in 2013 stated that 1-survival rate of cervical cancer stage IB-IIA that underwent radical hysterectomy and pelvic lymphadenectomy at RSCM is 96%, while 2-years is 90%, 3-years is 83%, 4-years is 73% and 5-years is 71%.

In stage IB2, the tumor size is bigger than 4 cm which makes it the most important predictor for local recurrence and smaller survival rate. The big tumor mass increases the risk of stromal invasion that leads to higher risk of lymph node metastasis. The neoadjuvant chemotherapy hopefully will decreased the size of the tumor so that the shrinkage of the tumor mass will decrease the risk of lymph node metastasis.[4]

Management for cervical cancer stage IB2 is still debatable among oncologists, as some prefer to have surgery while others have radiation/chemoradiation as the main management. Neoadjuvant chemotherapy before definitive management has been an option, Kim H.S. et al[5] stated that even neoadjuvant chemotherapy has decreased the rate of adjuvant radiation after surgery by decreasing the tumor mass and the risk of lymph node metastasis, but it does not improve the survival rate compared to primary radical hysterectomy. Study on neoadjuvant chemotherapy of Platinum, Vincristine and Bleomycine on cervical cancer stage IB2 stated that it gives better response prior the surgery, that are 12% for clinical complete response and 81% for 2-years-disease-free-survival rate.[6-10] González-Martín et. al.[11] also stated in a meta analysis that neoadjuvant chemotherapy followed by surgery is better than radiation only in terms of overall survival. Hwang YY et. al.[12] reported his study on 10-years-observation of 80 patients with stage IB-IIB cervical cancer with tumor size of 4 cm after neodjuvant chemotherapy with Cisplatin, Bleomycine and Vincristine continued by radical hysterectomy, that there were 75 patients with tumor shrinkage, with 5 and 10 years-survival rate of 82% and 79,4%. Hutapea et. al.[13] in 2011 reported 10 negative clinical response and 7 positive clinical response (n=17) based on RECIST with neoadjuvant chemotherapy (Cisplatin, Vincristine and Bleomycine). The positive clinical response consists of 2 clinical complete response and 5 clinical partial response. All clinical complete response revealed pathological complete response as well. Neoadjuvant chemotherapy of Cisplatin and Ifosfamide was also reported by Zanetta et. al.[14] on cervical cancer stage IB2, with significant response and overall response rate of 84% (CI 95% = 68%-94%) and with tolerable toxicity.

At RSCM, neoadjuvant chemotherapy has been used as part of cervical-cancer-stage-IB2 management. The combination of the chemotherapy regimens are Platinum-Vincristine-Bleomycine (which is used earlier) and Platinum-Ifosfamide. This study will evaluate the response of both combination at RSCM, based on the clinical response, pathological response and toxicity.

METHODS

The study is an ambispective cohort which took place from April 2013 - August 2014 at The Divison of Oncology Gynecology, Department of Obstetrics and Gynecology, Medical Faculty University of Indonesia, Dr. Cipto Mangunkusumo Hospital Jakarta. The study population is cervical cancer stage IB2 who received neoadjuvant chemotherapy, either combination of platinum, vincristine and bleomycine, or combination of platinum and ifosfamide, the followed by radical hysterectomy and pelvic lymphadenectomy. Total samples needed were 62 samples for each group.

Since ambispective cohort was used as study design, retrospective and prospective samples were used. Inclusion criterias include complete medical records, cervical cancer stage IB2 that has been confirmed histopathologically and has completed 3-cycle of neoadjuvant chemotherapy, either with combination of PVB or PI, followed by radical hysterectomy and pelvic lymphadenectomy, and patients who agreed to participate in the study. Exclusion criteria include incomplete medical records, patients with pelvic and/or paraaortic lymphadenectomy based on imaging, patients with lung or bone or liver metastasis based on imaging, patients who did not complete 3-cycle neoadjuvant chemotherapy, patients with other cancers and patients who refused to participate in the study.

All samples that fulfilled inclusion and exclusion criteria, were given 3-cycle neoadjuvant chemotherapy. Assessment of clinical response was performed 3 weeks after the last neoadjuvant chemo-

therapy at the latest. Samples with clinical complete response then underwent radical hysterectomy and pelvic lymphadenectomy. Histopathological examination of surgical specimen was performed to evaluate the malignant viable cells at the tumor mass, lymph node metastasis and parametrial metastasis. Patients with malignant viable cells at the tumor mass, lymph node metastasis and parametrial metastasis, then received adjuvant radiation/chemoradiation.

RESULTS

There were 13 samples that received combination of platinum and ifosfamide as neoadjuvant chemotherapy dan 17 samples that received combination of platinum, vincristine and bleomycine as neoadjuvant chemotherapy.

The age, education and profession distribution is found at Table 1. The most age distribution is 38-48 years, 8 samples (61.5%) from combination of PI and 10 samples (58.9%) from combination of PVB. There were 4 samples (30.8%) of PI combination and 7 samples (41.1%) of PVB combination at the age distribution of 49-59 years.

The education distribution showed that the most distribution is high school for combination of PI (38.4%) and elementary for combination of PVB (53%).

The chemotherapy response is divided into positive and negative response, in which positive response consists of clinical complete response and partial response, while negative response consists of stable disease and progressive disease. The response distribution can be seen on Table 2.

There were 3 (23.1%) clinical complete response of PI combination and 2 (11.8%) clinical complete response of PVB combination. Subanalysis was performed to evaluate the response of clinical complete response and nonclinical complete response, which consists of clinical partial response, stable disease and progressive disease (PI vs PVB = 3 : 10 vs 2 : 15).

The clinical complete response samples were then scheduled for radical hysterectomy and pelvic lymphadenectomy. Surgery was performed to all PI samples and only 1 PVB sample due to respectability reason (PI vs PVB = 3 vs 1).

Table 3. Distribution of Histopathological Result

Chemotherapy	MVC	LNM	PM
PI combination (n=3)	3 (100%)	3 (100%)	0 (0%)
PVB combination (n=1)	1 (100%)	1 (100%)	1 (100%)

MVB: Malignant Viable Cells; LNM: Lymph node metastasis; PM: Parametrialmetastasis

Table 1. Demographic Data and Cancer Stage

Demographic Data	Combination of PI (n=13)	%	Combination of PVB (n=17)	%
Age (years)				
38 - 48	8	61.5	10	58.9
49 - 59	4	30.8	7	41.1
≥ 60	1	7.7	0	0
Education				
Undergraduate	2	15.4	1	5.9
Elementary	4	30.8	9	53.0
Junior High	1	7.7	3	17.6
Senior High	5	38.4	4	23.5
Bachelor	1	7.7	0	0

Table 2. Distribution of Response based on Tumor Size after Neoadjuvant Chemotherapy (RECIST)

Response	Complete	Partial	Stable disease	Progressive
PI combination (n=13)	3 (23,1%)	3 (23,1%)	2 (15,4%)	5 (38,4%)
PVB combination (n=17)	2 (11,8%)	5 (29,4%)	1(5,9%)	9 (52,9%)

Based on histopathological examination, malignant viable cells and positive lymph nodes metastasion were found in all complete response samples. Parametrial metastasis occurred only in combination of PVB. See Table 3.

Acute toxicity was divided into 3 categories, which are gastrointestinal, genitourinary and hematology. All samples in both combination reported abdominal discomfort and nausea without the need of medical management. Genitourinary toxicity was not found at PI samples but found at 2 (11,7%) PVB samples. Hematology toxicity, that is Hb level 9.5-10.9 gr% or leucocyte < 3.900/m^3, was reported at 4 (30.8%) PI samples and 5 (29.4%) PVB samples. See Table 4.

Table 4. Distribution of Acute Toxicity

Toxicity	Gastrointestinal	Genitourinary	Hematology
Combination of PI (n=13)	13 (100%)	0 (0%)	4 (30.8%)
Combination of PVB (n=17)	17 (100%)	2 (11.8%)	5 (29.4%)

DISCUSSION

Total samples collected were 13 samples for PI combination and 7 samples for PVB combination. As the samples were not able to reach the minimal amount, it was realized that the power of the study might be less and be considered as a preliminary report.

The mean and median of age distribution for PI combination are 47.3 and 46 years old, while for PVB combination are 47.5 and 47 years old. The age distribution showed similarity between 2 groups. Data from INASGO 2009-2013 stated that the most age distribution is 36-45 years old, while in the study is 38-48 years old, so that it also showed similarity in the age distribution.

The main purpose of giving neoadjuvant chemotherapy is to decrease the tumor size to improve the respectability and decrease the risk factors for recurrency. Li et al.[15] stated that neoadjuvant chemotherapy would decrease the risk of LVSI and stromal infiltration which finally would decrease the risks of lymph node metastasis.

Recist was used to evaluate the clinical response, which was performed 3 weeks after the last course of neoadjuvant chemotherapy. The response was divided into positive and negative response. The positive response was then divided into complete response and partial response, while the negative response was divided into stable and progressive disease. The RR of positive and negative response is 1.2 (p>0.05). It showed that PI combination has a better chance to give positive response compared to PVB combination, although the p value showed insignificancy of the chance. Subanalysis RR was also calculated to evaluate the relation between the complete response and the group of partial response, stable and progressive disease. The RR is 1.96 (p>0.05), which showed that PI combination has also a better chance compared the PVB combination. The result of p value, which is > 0.05, showed that the chance although is 1.96 is statistically insignificant. The RRs could be considered as preliminary RRs in evaluating the clinical response of PI and PVB combination. Both insignificant values for both RRs might be due to the number of samples collected, so that further study with more samples is needed. The insignificant result might also due to bio-molecular reasons which in this study was not analyzed. Colombo et al[16], in a critical review stated that there was not enough data to show the superiority of each combination as neoadjuvant chemotherapy for cervical cancer before the definitive procedure, so a study to evaluate the chemotherapy combination is needed.

In histopathological response, the main purpose is to evaluate the presence of malignant viable cell, lymph node and parametrial metastasis. The histopathological response should correspond to the clinical response. The categories used are[17] : Pathological complete response, in which there is no more residual or viable tumor at the surgery specimen, tumor mass and/or lymph node (T0N0 M0). Near-complete or Microscopic Response, in which one or more focuses or malignant viable cells measuring less than 1 mm at the surgery specimen, tumor mass and/or lymph node. Pathological partial response, in which the residual mass measuring more than 1 mm at the surgery specimen, tumor mass and/or lymph node.

As it was mentioned that all clinical complete response of PI combination (n=3) underwent surgery while only 1 of PVB combination. The RR of malignant viable cell at the tumor mass and lymph node metastasis are 1 (p value can not be calculated because there was no comparison), which showed that there was no difference in the chance of pathological complete response between PI and

PVB combination in tumor mass and pelvic lymph node. The RR of parametrial metastasis is 4 (p>0.05), which not only showed that there was a positive association between the neoadjuvant chemotherapy and the parametrial metastasis, but also showed that PI combination has a better chance to eliminate the risk of parametrial metastasis compared to PVB combination.

These data showed that clinical complete response does not correspond to pathological complete response in the presence of malignant viable cell and lymph node metastasis. Pathological complete response should more be understood and taken into consideration as it plays important role in recurrency and survival.

RR for toxicity distribution of gastrointestinal is 1 (p value can not be calculated because there was no comparison) and of hematology is 1.04 (p>0.62), while of genitourinarius is 0 (p>0.05). It showed that there was no difference in acute gastrointestinal and hematology toxicity between PI and PVB combination, while there was no risk of genitourinarius toxicity in PI combination compared to PVB combination. The insignificancy of all correlation might be due insufficient amount of samples in both groups.

All samples with malignant viable cell and lymph node metastasis received adjuvant radiation/ chemoradiation.

CONCLUSIONS

Clinical and histopathological response and also the acute toxicity of PI and PVB combination as neoadjuvant chemotherapy does not differ significantly. The clinical response does not correspond to histopathological response, where it should be taken for consideration to evaluate the risk of recurrency and survival. Further study should be done not only to evaluate both clinical and histopathological response of neoadjuvant chemotherapy of PI and PVB combination, but also to evaluate which combination among several combination used by oncologist give a better result.

REFERENCES

1. Ferlay J, Shin H, Bray F et al. GLOBOCAN, Cancer Incidence and Mortality Worldwide: IARC Cancer Base 2008; 10
2. Nuranna L, Azis F, Cornain S et al. Cervical cancer prevention program in Jakarta, Indonesia: See and Treat model in developing country. J Gynecol Oncol, 2012; 23(3): 147-52.
3. Anindya M, Purbadi S, Prihartono J. Kesintasan pasien kanker serviks stadium awal pasca histerektomi radikal beserta faktor-faktor yang mempengaruhinya di RSUPN Cipto Mangunkusumo tahun 2005-2010. Jakarta: Universitas Indonesia 2013.
4. Yun-Hyun C, Dae-Yeon K, Jong-Hyeok K, et al. Comparative study of neoadjuvant chemotherapy before radical hysterectomy and radical surgery alone in stage IB2-IIA bulky cervical cancer. J Gynecol Oncol. 2009; 20(1): 22-7.
5. Kim HS, Sardi JE, Katsumata N, et al. Efficacy of neoadjuvant chemotherapy in patients with FIGO stage IB1 to IIA cervical cancer: An international collaborative meta-analysis. J Cancer Surg. 2013; 39: 115-24.
6. Sardi J, Sananes C, Giaroli A, et al. Results of a prospective randomized trial with neoadjuvant chemotherapy in stage IB, bulky, squamous carcinoma of the cervix. Gynecol Oncol 1993; 49: 156-65.
7. Stewart LA, Tiernay JF. Neoadjuvant chemotherapy and surgery versus standard radiotherapy for locally advanced cervical cancer: a meta-analysis using individual patient data from randomized controlled trials. Proc ASCO. 2002; 21: 207a.
8. Eddy GL, Manetta A, Alvarez RD, et al. Neoadjuvant chemotherapy with vincristine and cisplatin followed by radical hysterectomy and pelvic lymphadenectomy for FIGO Stage 1B bulky cervical cancer: a Gynecologic Oncology Group pilot study. Gynecol Oncol 1995; 57: 412-6.
9. Benedetti-Panici P, Greggi S, Colombo A. Neoadjuvant chemotherapy and radical surgery versus exclusive radiotherapy in locally advanced squamous cell cervical cancer: results from the Italian Multicenter Randomized Study. J Clin Oncol 2001; 20: 179-88.
10. Chang TC, Lai CH, Hong JH, et al. Randomized trial of neoadjuvant cisplatin, vincristine, bleomycin and radical hysterectomy versus radiation therapy for bulky Stage IB and IIA cervical cancer. J Clin Oncol 2000; 18: 1740-7.
11. González-Martín A, González-Cortijo L, Carballo N, et al. The current role of neoadjuvant chemotherapy in the management of cervical carcinoma. Gynecologic Oncology. 2008; 110: S36-S40.
12. Hwang YY, Moon H, Cho SH. Ten-year survival of patients with locally advanced, stage Ib-IIb cervical cancer after neoadjuvant chemotherapy and radical hysterectomy. Gynecol Oncol. 2001; 82: 88-93.
13. Hutapea J, Kampono N, Sutrisna B, et al. Ekspresi VEGF-C Sebagai Prediktor Respon Kemoterapi Neoajuvan Dan Metastasis Ke Kelenjar Getah Bening Pada Kanker Leher Rahim Lesi Besar Jakarta: Universitas Indonesia; 2011.
14. Zanetta G, Lissoni A, Pellegrino A, et al. Neoadjuvant chemotherapy with cisplatin, ifosfamide and paclitaxel for locally advanced squamous-cell cervical cancer. Annals Oncol. 1998; 9: 977-80.
15. Rui L, Shen-tao L, Jing-ge S, et al. Prognostic value of responsiveness of neoadjuvant chemotherapy before surgery for patients with stage IB2/IIA2 cervical cancer. Gynecol Oncol 2013; 128: 524-9.
16. Colombo N, Peiretti M. Critical review of neoadjuvant chemotherapy followed by surgery for locally advanced cervical cancer. Int J Gynecol Cancer. 2010; 20: S47-8.
17. Myrna C, Chanona-Vilchis J, Cetina L, et al. Prognostic significance of pathological response after neoadjuvant chemotherapy or chemoradiation for locally advanced cervical carcinoma. International Seminars in Surg Oncol 2006; 3:3:3.