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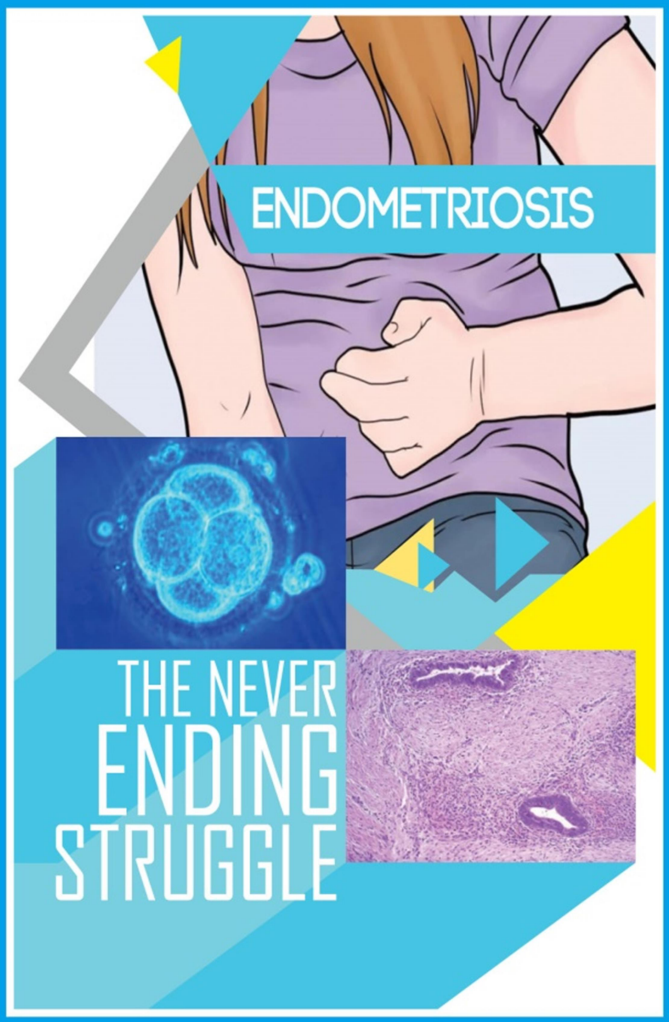

Editorial

Research Articles

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EDITORIAL

RESEARCH ARTICLE

EDITORIAL

The Role of Biomarkers in Diagnosing the Early Stage of Endometriosis

Wachyu Hadisaputra

Endometriosis is a disease that could lead to social problems in women.[1] Delay in the diagnosis of endometriosis is not uncommon, even in developed countries. Previous studies in England and USA found that diagnostic delay could reach 8 and 12 years, respectively.[2,3] These delays may occur at the individual level of women and medical level. At medical level, delay may be caused by the suppression of symptoms due to intermittent hormonal treatment, failure of physicians to notice menstruation complaints and inadequate gynecologic examinations.[2]

Endometriosis is a chronic inflammatory condition associated with defects in immune system. It is clinically characterized by the presence of endometrial tissue outside the uterine cavity.[4,5] Peritoneal fluid in endometriosis patient contained an increased level of active macrophages secreting various inflammatory products, including growth factors, cytokines and tumour markers.[6] Increase in vascular epithelial growth factor (VEGF) was known as a mediator of angiogenesis, which enables endometrial cells to proliferate. Development of endometrial tissue was also caused by various cytokines such as interleukin-6 (IL-6) and tumour necrosis factor-alpha (TNF-α). Furthermore, IL-6, Interleukin-8 (IL-8) and matrix metalloproteinase (MMP) were also associated with the implantation process of endometrial tissue. Interestingly, increased level of these cytokines was not only found in peritoneal fluid, but also in the serum of endometriosis patients.[7-9]

A lot of studies have described the relation between various biological markers and endometriosis diagnosis. However, IL-6, TNF-α, MMP-2, and VEGF were found to have a higher correlation with the incidence of endometriosis compared to other biological markers.[10] Thus, these markers can be a predictor for diagnosing the early stage of endometriosis if used along side with a combination of clinical symptoms, signs and other laboratory examination.

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Research Article

How Long is the Safest Inter-Delivery Interval in Women with Previous History of Cesarean Delivery?

Berapa Lama Jarak antar Kehamilan Teraman pada Perempuan dengan Riwayat Seksio Sesarea?

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Abstract

Objective: To investigate the association between inter-delivery interval and uterine rupture in women with previous CD.

Methods: The formulation question was how long is the safest inter-delivery interval to minimalize the risk of uterine rupture. The authors investigated in three databases including Pubmed, Cochrane, and Embase database. Inclusion criteria were abstract answering the clinical question, written in English language, and full-text paper availability.

Results: One systematic review, six cohort studies, and 1 case-control study were collected to compare the inter-pregnancy interval to the risk of uterine rupture. The author retrieved seven articles suitable to the inclusion criteria after excluding ten articles screened by the abstract and language. Then, the author added one article used in the systematic review. Hence, the critical appraisal based on Validity, Importance, and Applicability (VIA) was performed for eight articles.

Conclusion: The inter-delivery interval ≥18 months is the safest time to avoid uterine rupture. Prostaglandin analogue induction should be avoided and for patients with a history of past cesarean using a single-layer closure to be educated about the increased risk.

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Keywords: cesarean delivery, inter-delivery interval, uterine rupture, vaginal birth after cesarean delivery

Abstrak

***Tujuan**: Untuk mengetahui hubungan antara jarak antar kehamilan dengan ruptur uterus pada perempuan dengan riwayat SC sebelumnya.*

***Metode**: Formulasi pertanyaan ialah berapa lama jarak antar kehamilan teraman untuk meminimalisasi risiko ruptur uterus. Peneliti menginvestigasi dari tiga database meliputi Pubmed, Cochrane, dan Embase. Kriteria inklusi ialah abstrak yang menjawab pertanyaan klinis, ditulis dalam Bahasa Inggris, dan keberadaan artikel.*

***Hasil**: Satu ulasan sistematik, 6 studi kohort, dan 1 kasus control digunakan untuk membandingkan jarak antar kehamilan dengan risiko ruptur uterus. Peneliti mengambil 7 artikel yang sesuai dengan kriteria inklusi setelah mengeksklusi 10 artikel berdasarkan abstrak dan bahasa. Peneliti menambahkan satu artikel yang terdapat di dalam ulasan sistematik. Oleh karena itu, penilaian kritis berdasarkan validitas, kepentingan, dan penerapan pada 8 artikel.*

***Kesimpulan**: Jarak antar kehamilan ≥18 bulan merupakan waktu paling aman untuk mencegah ruptur uterus. Induksi dengan analog prostaglandin sebaiknya dihindari dan pada pasien dengan riwayat SC menggunakan satu lapis sebaiknya diedukasi untuk peningkatan risiko.*

[Maj Obstet Ginekol Indones 2018; 6-2: 71-77]

***Kata kunci**: jarak antar kehamilan, persalinan pervaginam setelah seksio sesarea, ruptur uterus, seksio sesarea*

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INTRODUCTION

In the world, the rate of cesarean delivery (CD) has increased sharply in the last few decades from 6% to 27.2% in the most developed regions. There was an increasing trend of CD between 1990 and 2014 which the global average CD rate raised about 12.4% (from 6.7% to 19.1%).[1] In the United States, this rate increased from 5% in 1970 to 31% in 2007. It was related to the increasing maternal age, decreasing of instrumental deliveries usage, decreasing of vaginal delivery after previous cesarean section (VBAC), and also increasing in medically indicated labour inductions.[2] American College of Obstetricians and Gynecologist (ACOG) reported that the rate of VBAC has declined from 28.3% in 1996 to 8.5% in 2006 due to the reports of increasing risk for uterine rupture and complications during VBAC.[3]

Uterine rupture is the most catastrophic complication for women attempting VBAC.[4,5] The Maternal-Fetal Medicine Units (MFMU) Network Study explained the incidence of symptomatic

uterine rupture was 0.69% of 18,000 women performing the trial of labour (TOL).[6] One risk factor influencing uterine rupture is inter-delivery and inter-pregnancy interval. Short inter-delivery and inter-pregnancy have been associated with poor maternal and neonatal outcomes, such as preterm birth, low birth weight, preterm premature rupture of membranes (PPROM), placenta accrete, and uterine rupture as the worst risk.[7] The pathophysiology of uterine rupture is in accordance with the healing of the lower uterine segment after CD. Short inter-delivery time causes lack of complete healing of the uterine scar which contributes to ineffective uterine contractility and poor lower segment thinning that increases the risk of uterine dehiscence or rupture.[8]

Therefore, the authors would like to know the association between inter-delivery interval and uterine rupture in women with previous CD. Appraisal was done with one systematic review[9] and seven studies[4,7,8,10-13] related to this topic to answer this evidence-based case report (EBCR). Although there were a lot of studies conducted on this topic; however, there is still no formal publication of EBCR.

The question formulation in this case report study was how long should the inter-delivery interval be to minimalize the risk of uterine rupture. To answer the question, the authors search the literature study starting from systematic review or meta-analysis as the highest hierarchy of study to expert opinion as of the lowest confidence of the study. Although this report is uncommon for scientific publication in Indonesia, the authors hope that this publication can help the obstetrics and gynecologists to improve their practice.

Case Resume

In this case, a 33-year-old female P1A0 came for the routine postpartum control. The patient preferred vaginal birth in that pregnancy; however, the obstetrician findings of oligohydramnios and post-term pregnancy suggested to CD. After CD, the obstetrician did the double-layer uterine closure. Now, the patient plans to get pregnant again because of her age; but she still intends to give exclusive breastfeeding. She asked the doctor how long she should postpone before the next pregnancy and whether she can deliver vaginally for the next pregnancy.

The patient asked herself what was the minimal inter-delivery interval that has minimal risk of uterine rupture. Through some searching, the patient heard that World Health Organization (WHO) recommends 24 months as the safe inter-delivery interval, but she was not satisfied with the information yet. Therefore, to gather the most appropriate inter-delivery interval in women with a history of CD, the authors conduct five steps of EBCR, consisting of formulation of the question, searching the evidence, appraisal of the study, applying the answer, and assessing the outcome.

Formulation of the question

How long is the minimal inter-delivery interval to minimize the risk of uterine rupture?

Searching the evidence

To answer the practical question above, three databases were investigated including PubMed, Cochrane database, and Embase database. In PubMed, the search included keywords using the MeSH, namely ("Birth Intervals"[Mesh] AND "Uterine Rupture"[Mesh]) and MeSH descriptor: [Birth Intervals] AND MeSH descriptor: [Uterine Rupture] in Cochrane database. Meanwhile, the authors used the keywords "uterine rupture" AND "inter-pregnancy interval" in Embase. All studies related to this topic were accepted due to the lack of systematic review or meta-analysis. Finally, 11 articles were found in PubMed, 1 article in Cochrane database, and ten articles in Embase. The articles were screened using the criteria consisting of abstracts answering the clinical question, written in English language, full-text paper availability, and omitting all duplication papers. Therefore, from this strategy of searching, the authors obtained one systematic review and six articles that continued to the next process of appraisal. The critical appraisal steps used in this article was written by Agustin CA et al.[9]; Emmanuel B, et al.[7]; Emmanuel B, et al.[4] Roy K, et al.[10]; Thomas DS, et al.[11]; David MS, et al.[12], Matthew AE, et al.[13] Due to lack of inconsistency in the appraisal of systematic review, all studies were reviewed and recruited by the systematic review. The authors found one study that was not included in the strategy of searching the evidence. Therefore, the authors included the study by Wilson HH et al. into our appraisal (described in figure 1).

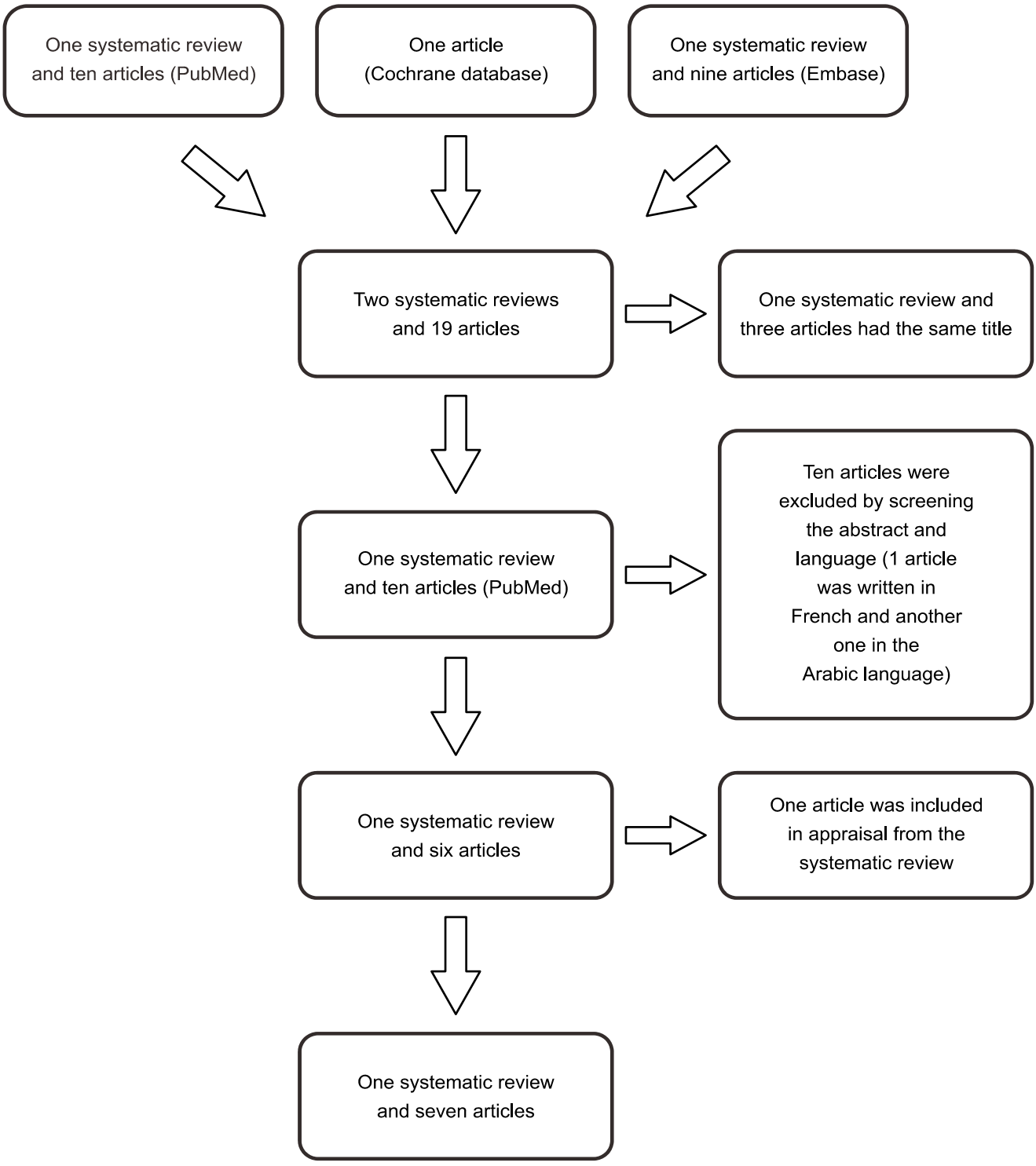

Figure 1. Flowchart of selecting articles using in EBCR

Appraisal of the studies

To appraise the scientific evidence of 8 articles, the guideline from Consolidated Standard of Reporting Trials (CONSORT) for retrospective studies and A Measurement Tool to Assess Systematic Reviews (AMSTAR) for systematic review was used. The tables 1, 2, and 3 below describe the appraisal form from the study based on VIA (validity, importance, and applicability) methods.

Table 1. Validity of the Studies Included in the Analysis**

Study	Type of study	Focused research question	Selection criteria	Primary outcome	Number of studies	Number of subjects	Validity appraisal	Reliability assessment	Similarity of the studies (homogeneity)
Conde-Agudelo A, et al	Systematic review	Yes	Yes	Uterine rupture	4	5164	Yes	No	Not mentioned
Bujold E, et al	Research article	Yes	Yes	Uterine rupture		1527			
Bujold E, et al	Research article	Yes	Yes	Uterine rupture		1768			
Kessous R, et al	Research article	Yes	Yes	Pregnancy complications and adverse outcomes		3176			
Shipp TD, et al	Research article	Yes	Yes	Uterine rupture		2409			
Stamilio DM, et al	Research article	Yes	Yes	Uterine rupture, composite major morbidity, and blood transfusion		13331			
Huang WH, et al	Research article	Yes	Yes	The rate of successful VBAC		1185			
Eposito MA, et al	Research article	Yes	Yes		43				

Table 2. Importance of the Studies Included in the Analysis

Study	Overall results (treatment preference)	RR	95% CI
Conde-Agudelo A, et al	Long intervals (birth intervals ≥19 or 25 months and interpregnancy interval ≥6 months)	Not mentioned	Not mentioned
Bujold E, et al	Interdelivery interval >24 months	2.65	1.08-5.46
Bujold E, et al	Interdelivery interval ≥18 months	2.8	1.2-6.6
Kessous R, et al	Not significant difference among ≤12, 13-18, 19-24, ≥25 months		
Shipp TD, et al	Interdelivery interval >18 months	3.0	1.2-7.2
Stamilio DM, et al	Interpregnancy interval ≥6 months	2.66	1.21-5.82
Huang WH, et al	Not significant difference between <19 and ≥19 months		
Eposito MA, et al	Interpregnancy interval ≥6 months	3.92	1.09-14.30

Table 3. Applicability of the Studies Included in the Analysis

Study	The source of data	Apply the result to patient care	Considering all clinically important outcomes	Other clinical outcome (s) or risk factor (s)
Conde-Agudelo A, et al	3 cohort and 1 case-control studies	Yes	Yes	
Bujold E, et al	Sainte-Justine Hospital, Montreal, Canada	Yes	Yes	Single-layer uterine closure at the previous CD (OR 4.33; 95% CI 1.70-10.98) increased the risk of uterine rupture
Bujold E, et al	Sainte-Justine Hospital, Montreal, Canada	Yes	Yes	Previous single-layer closure (OR 7.5; 95% CI 3.2-17.6) increased the risk of uterine rupture
Kessous R, et al	Soroka University Medical Center, Southern region of Israel	Yes	Yes	Long inter-delivery interval more than 24 months had higher rate of gestational diabetes mellitus and higher rates of CD; short interval group had lower birth weight and higher prevalence of low Apgar score at 1 and 5 minutes
Shipp TD, et al	Brigham and Women's Hospital, Massachusetts, USA	Yes	Yes	Induced with oxytocin (OR 4.9; 95% CI 1.7-14.6) increased the risk of uterine rupture
Stamilio DM, et al	Seventeen Hospitals in the Northeastern, USA	Yes	Yes	Interpregnancy interval <6 months had higher risk for composite morbidity (OR 1.95; 95% CI 1.04-3.65) and blood transfusion (OR 3.14; 95% CI 1.42-6.95)
Huang WH, et al	Irvine and Long Beach Memorial Medical Center, California, USA	Yes	Yes	
Eposito MA, et al	Women and Infants' Hospital, Rhode Island, USA	Yes	Yes	

Applying the answers

Inter-delivery interval has been associated with obstetric outcomes; one of them is the uterine rupture in women with previous history of CD. Report of WHO Technical Consultation on Birth Spacing in 2005 recommended the inter-pregnancy interval was at least 24 months to reduce the risk of adverse maternal, perinatal, and infant outcomes. This interval was consistent with the recommendation of breastfeeding for two years. Apart from that, WHO considered 2 years as the number which easily remembered in the program rather than "18 months" or "27 months".[14] Meanwhile, the Society of Obstetricians and Gynecologist of Canada (SOGC) in 2005 stated that inter-delivery interval more than 18 months had the lowest risk factor for uterine rupture.[15] The differences between the guidelines are the reason of interest related to searching of the evidence about the association between inter-delivery interval and the risk of uterine rupture. Apart from that, patients always ask the doctor for the minimal inter-delivery interval that is safe for the next pregnancy in women with history of CD. Therefore, this interval becomes our concern to answer the practical questions.

In this EBCR, one systematic review, six cohort studies, and 1 case-control study were collected to compare the inter-pregnancy interval to the risk of uterine rupture. The authors retrieved seven articles suitable to the inclusion criteria after excluding ten articles screened by the abstract and language. Then, one article was used in the systematic review, so this critical appraisal based on VIA was performed for eight articles.

Systematic review by Conde-Agudelo A, et al.[9] involved 3 cohort studies and 1 case-control study presented that there was an increasing risk of uterine rupture in women with short interval, whereas short birth interval in the study was defined as less than 19 or 25 months or inter-pregnancy interval was less than six months. Meanwhile, in one cohort study in the systematic review did not find an association between inter-delivery interval and uterine rupture. Due to lack of reliability assessment in the systematic review, all studies included in Agustin CA study were searched.

The authors found similar results among studies conducted by Bujold E, et al.[7], Bujold E, et al.[4], Shipp TD, et al.[11], Stamilio DM, et al.[12], and Eposito MA, et al.[13] They concluded that short inter-delivery interval was associated with the increased risk of uterine rupture; however, the definition of short interval was different among those studies. Bujold E, et al.[7] in their study showed that single-layer closure and inter-delivery interval ≤24 months significantly increased the risk of uterine rupture. Therefore, single-layer closure (OR 4.33; 95% CI 1.70-10.98) and inter-delivery interval ≤24 months (OR 2.65; 95% CI 1.08-5.46) were two independent factors related to uterine rupture. In this study, the use of prostaglandin during labor was very low (<1%). It is very essential because the use of it has been shown as significant factor associated with uterine rupture. In later study by Bujold E, et al.[4] explained the similar results to a previous study where inter-delivery interval less than 18 months (OR 2.8; 95% CI 1.2-6.6) and single-layer closure (OR 7.5; 95% CI 3.2-17.6) were factors contributed to uterine rupture. The difference between this and previous study was in the inter-delivery interval limitation. In the later study, they found that the 18 months of inter-delivery interval was enough to minimalize the risk of uterine rupture. This study described a similar result to a study by Shipp TD, et al.[11] They concluded that inter-delivery interval ≤18 months (OR 3.0; 95% CI 1.2-7.2) and induced with oxytocin (OR 4.9; 95% CI 1.7-14.6) were associated with the risk of uterine rupture. Meanwhile, Stamilio DM, et al.[12] and Eposito MA, et al.[13] used the inter-pregnancy interval term rather than inter-delivery interval. Stamilio DM, et al.[12] explained that short inter-pregnancy interval of fewer than 6 months increased the risk for uterine rupture in patients attempted the VBAC (OR 2.66; 95% CI 1.21-5.82), composite morbidity (OR 1.95; 95% CI 1.04-3.65), and blood transfusion (OR 3.14; 95% CI 1.42-6.95). This study also revealed that patients with short inter-pregnancy interval had lower haemoglobin level on average, was younger, and was less likely to develop gestational diabetes and chronic hypertension. This study also stated finding literature concluding radiographic and hysteroscopic evidence that cesarean scar development is incomplete for as long as 6 or 12 months post-operatively. While, in the case-control study by Eposito MA, et al.[13], the risk of uterine rupture increased in patients with inter-pregnancy interval <6 months (OR 3.92; 95% CI 1.09-14.3).

Unfortunately, studies by Kessous R, et al.[10] and Wilson HH, et al. both showed different results

from above. Both studies did not express association between inter-delivery interval and risk of uterine rupture. Kessous R, et al.[10] stated that the risk of uterine rupture did not differ between the inter-delivery interval of less than 18 months and more than 19 months (p=0.131). This study only presented that short interval group had higher rates of preterm deliveries, lower birth weight, and prevalence of low Apgar score at 1 and 5 minutes. Huang WH, et al. similarly concluded that the difference between the group with inter-delivery interval greater and less than 19 months was not related to the symptomatic uterine rupture (p=1.00).

In Indonesia, there is still no consensus regarding VBAC and the minimal inter-delivery interval to reduce the risk of uterine rupture. Meanwhile, ACOG explained that most women with one previous cesarean delivery with a low-transverse incision should be counselled for the VBAC and offered the TOLAC. Misoprostol as prostaglandin analogue should not be used for the cervical ripening or labour induced patients with history of CD or major uterine surgery.[3] The guideline by Royal College of Obstetricians and Gynecologists (RCOG) states that planned VBAC is appropriate for the majority of women with singleton pregnancy of cephalic presentation at 37^{+0} weeks or beyond with a single previous lower segment cesarean delivery. However, VBAC is contraindicated in women with previous uterine rupture or classical cesarean scar and in women who have other absolute contraindications to vaginal birth such as major placenta previa. The success rate of planned VBAC reaches 72-75%. Before offering the TOLAC, the clinician has to make the individual assessment of the risk of uterine rupture.[16] One of the main factors is inter-delivery interval.

After appraising the studies conducted in some countries (USA, Israel, and Canada), two studies mentioned the safe inter-delivery interval more than 18 months, two studies concluded the safe inter-pregnancy interval more than six months, and the other one said inter-delivery interval should be more than 24 months. The mother has enough time to complete exclusive breastfeeding for six months although the WHO suggests that the breastfeeding should be continued up to 2 years.The authors recommend taking inter-pregnancy interval a minimum 18 months based on the two cohort studies done by Bujold E, et al. and Shipp TD, et al. Another reason for using 18 months as the cut-off is the finding of Stamilio DM, et al. that hysteroscopic and radiographic evidence stating incomplete scar healing 6-12 months. Hence, inter-delivery of 18 months is enough for a minimum complete scar healing. Nevertheless, the other factors which impact to increase the risk of uterine rupture are single-layer closure and oxytocin induction. In this era of National Health Coverage (*Jaminan Kesehatan Nasional*/JKN) in Indonesia, patients should be offered the TOLAC and VBAC if the requirement of minimal inter-delivery interval is fulfilled. Vaginal birth is surely more cost-effective and efficient than CD. In conclusion, when doing the counselling, the clinician should advise the TOLAC and VBAC regarding minimal inter-delivery interval and history of double-layer uterine closure to minimize the morbidity of uterine rupture.

Assessing the outcomes

Our patient would like to get pregnant as soon as possible because of her age and desired vaginal birth. Based on guideline by ACOG and RCOG, the patient with history of low-transverse incision in previous cesarean delivery can do the TOLAC and VBAC. Even, the success rate of TOLAC and VBAC in that condition reached 72-75%. But, the inter-delivery interval has to be considered to reduce the risk of uterine rupture. After doing the appraisal, the authors suggest that equal or more than 18 months of inter-delivery interval is enough to have the minimal risk of uterine rupture. The hypothesis to explain the relationship between short interval and risk of uterine rupture is that the scar requires minimal time to heal from reaching the full strength. To support this statement, a study done by Dicle O, et al.[17] reported that the zonal anatomy of uterus needed minimally six months to get back completely. Like stated before, Stamilio DM et al also found a similar finding but with a larger range of duration whereas through hysteroscopic and radiographic evidence it was stated that incomplete scar healing ranging from 6 to 12 months. Hence, inter-delivery of 18 months is enough for a minimum complete scar healing and also for completing exclusive breastfeeding for 6 months.

If the authors look at the neonatal outcome, Kessous R, et al.[10] said that short inter-delivery interval was associated with preterm labor, lower

birth weight, and higher prevalence of low Apgar score at 1 and 5 minutes. Low Apgar score impacts the neonatal outcome which can end in morbidity and even mortality. The history of double-layer uterine closure would minimalize the risk of uterine rupture because study conducted in Canada revealed the risk of it was increased in the previous single-layer closure.[4,7]

In this EBCR, the authors reported a woman with history of CD asking for the minimal interval for the second pregnancy to do the vaginal birth in the next pregnancy. In the previous CD, the doctor did the double-layer uterine closure. From this critical appraisal focused on one systematic review and seven articles collected from PubMed, Cochrane database, and Embase with specific criteria, the authors could summarise that the inter-delivery interval more than 18 months has the minimal risk of uterine rupture regarding the history of double-layer closure. Apart from that, for the next pregnancy, it is not recommended to be induced by misoprostol as the prostaglandin analogue. In conclusion, for the patient above, the authors advise minimal 18 months for next delivery and offer the TOLAC for the cost-effective and efficient in the era of JKN with considering the minimal risk of uterine rupture.

CONCLUSION

Based on evidence, the inter-delivery interval ≥18 months is the safest time to avoid uterine rupture. Prostaglandin analogue induction should be avoided and for patients with a history of past cesarean using a single-layer closure to be educated about the increased risk.

Conflict of Interest

The authors hereby declare that there is no conflict and financial interest in this EBCR study.

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Research Article

Levels of 25-Hydroxyvitamin D in Normotensive Pregnancy and Severe Preeclampsia

Kadar 25-Hydroxyvitamin D pada Kehamilan Normotensif dan Preeklamsia Berat

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Abstract

Objective: To determine the ratio of 25-hydroxyvitamin D levels in normotensive pregnancy and severe preeclampsia.

Methods: This study was an analytic cross-sectional study with t-test. The subject of this study consists of 17 samples normotensive pregnancy and 17 samples severe preeclampsia. This study was conducted and evaluated from August 2016 until December 2016 at Department of Obstetrics and Gynecology Faculty of Medicine Universitas Sam Ratulangi Prof. Dr. R.D. Kandou Hospital Manado and satellite hospital in Manado. Samples were taken from serum as much as 5 ccs and were analyzed using CLIA at Prodia clinical laboratory. Data were analyzed with SPSS version 20.0.

Results: By using the t- test, there were significant differences in 25-hydroxyvitamin D levels between normotensive pregnancy group (24.771 ± 6.9567ng/ml) and severe preeclamptic group (17.712 ± 3.7513ng/ml), p = 0.001.

Conclusion: Levels of 25-hydroxyvitamin D in normotensive pregnancy significantly higher compared to severe preeclampsia so it can be concluded that the levels of 25-hydroxyvitamin D were associated with preeclampsia.

[Indones J Obstet Gynecol 2018; 6-2: 78-83]

Keywords: 25-hydroxyvitamin D, normotensive, severe preeclampsia

Abstrak

***Tujuan**: Mengetahui perbandingan kadar 25-hydroxyvitamin D pada kehamilan normotensif dan preeklamsia berat.*

***Metode**: Penelitian ini merupakan suatu penelitian potong lintang bersifat analitik dengan uji t terhadap subjek yang terdiri atas 17 sampel normotensi dan 17 sampel preeklamsia berat. Penelitian dilaksanakan dan dievaluasi sejak bulan Agustus 2016 sampai Desember 2016 di Departemen Obstetri dan Ginekologi Fakultas Kedokteran Universitas Sam Ratulangi / RSUP Prof. Dr. R.D. Kandou Manado dan rumah sakit jejaring di Manado. Sampel diambil dari serum sebanyak 5 cc dan dikirim ke Laboratorium klinik Prodia untuk diproses dengan metode CLIA. Data dianalisis dengan SPSS versi 20.0*

***Hasil**: Dengan menggunakan uji t, didapatkan perbedaan bermakna kadar 25-hydroxyvitamin D pada kelompok normotensif (24,771 ± 6,9567) dan kelompok preeklamsia (17,712 ± 3,7513), p= 0.001.*

***Kesimpulan**: Kadar 25-hydroxyvitamin D pada kehamilan dengan normotensif lebih tinggi secara bermakna dibandingkan dengan preeklamsia berat sehingga dapat disimpulkan bahwa kadar 25-hydroxyvitamin D berhubungan dengan kejadian preeklamsia.*

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***Kata kunci**: 25-hydroxyvitamin D, normotensif, preeklamsia*

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INTRODUCTION

Preeclampsia is still one of the major complications during pregnancy, delivery and post-partum period. Preeclampsia, one of the causes of death other than bleeding and infection.[1] The effect can lead to the disruption of maternal and fetal well-being. Despite considerable progress in the field of obstetrics and perinatology services for antenatal and neonatal care, but nonetheless, preeclampsia remains one of the most common causes of maternal and perinatal morbidity and mortality.[2] Every year nearly 40.000 women, especially in developing countries die from preeclampsia or eclampsia. Preeclampsia or eclampsia are the second leading cause of maternal death in Pakistan.

The prevalence of preeclampsia ranges between 7%-10% of all pregnancies. In the United States, the incidence was 23.6 cases/1000 pregnancies. Whereas in the UK the incidence of severe preeclampsia ranges from 5/1000 pregnant women and 5/10,000 pregnant women in eclampsia. And the incidence of preeclampsia tends to increase from year to years.[3] In Indonesia, this disorder is still the top three highest contributors to maternal mortality after bleeding and infection, with incidence rates varying between 2.1 to 8.5%. The pathogenesis of preeclampsia until now remains unclear. Preeclampsia is a disorder characterised by hypertension and proteinuria occurred > 20 weeks of gestation. Around the world, about 2-3%

of all pregnant women can develop preeclampsia. This condition is a major cause of maternal and perinatal morbidity and mortality.[3,4]

Vitamin D plays an important role in calcium metabolism, immune system, proliferation and cell differentiation, the process of infection and cancer.[5,6] Within the last ten years, research on vitamin D prove that vitamin D effects on conception, pregnancy and health neonatal.[7] Vitamin D (cholecalciferol) formed by the skin during exposure to sunlight (ultraviolet radiation), and also absorbed from food. Absorption of cholecalciferol converted in the liver to 25(OH) cholecalciferol or 25- hydroxyvitamin D (25(OH)D), and then by 1α-hydroxylation in the kidney to 1,25 hydroxylase dihydroxycholecalciferol or 1,25(OH)2D3, most forms active group vitamin D.

Vitamin D receptors are also present in the placenta. Levels of 1,25(OH)2D3 increases during pregnancy through increased 1α-hydroxylase. The discovery of the levels of 25(OH)D in maternal and umbilical cord serum proved that the development of the placenta and fetus are directly related to vitamin D intake and sun exposure.[8] Levels of 25(OH)D were lower in the first trimester associated with the incidence of preeclampsia.[9] Intake of vitamin D during early life is required so that the risk of preeclampsia can be reduced up to 50% pregnancy.[10]

Magnus (2001) observed that the incidence of preeclampsia-related with seasons. The incidence of preeclampsia is reduced in summer with high sun exposure.[11] Vitamin D deficiency (levels of 25(OH)D <17.5 nmol/l) and insufficiency (levels of 25(OH)D <50nmol/l) was found in tropical countries, for example, India and Bangladesh with high sun exposure.[12] Green (2008) investigated the levels of 25(OH)D in reproductive age women in Jakarta and Kuala Lumpur, and it showed that 60% reproductive-aged women have vitamin D insufficiency.

Deficiency of vitamin D (25(OH)D) causes endothelial dysfunction through the molecular mechanism. Endothelial dysfunction that occurs in preeclampsia begins with exposure of the endothelial cell membrane by mediators released as a result of placental ischemia and hypoxia, among the products of lipid peroxidation, resulting in damage of the cell membrane. Disruption of cell membranes earlier can disturb endothelial function, and even cause damage to the entire structure of endothelial cells. As a result of damage to endothelial cells, the endothelial function as a mechanical barrier endothelial lost so that leakage that causes extravasation of fluid into the extravascular intra, in addition to producing endothelial function and NO PGI2 also decreased, resulting in vasoconstriction and increased blood pressure.[13-15] In addition to the endothelial damage also causes many other disorders, such as, decreased production of prostacyclin, platelet aggregation in areas of damaged endothelium which will also produce thromboxane A2. The typical changes in the glomerular capillaries form of glomerular endotheliosis, increased capillary permeability, increased coagulation factors.

There also are found vitamin D (25(OH)D) effect on trophoblast invasion and angiogenesis when implantation process, so this is an important factor in the pathophysiology preeclampsia.[5] Many other studies that try to prove the pathogenesis of preeclampsia associated with defence vitamin D, for example regarding the response, oxidative stress and angiogenesis.[13-15]

OBJECTIVE

This study was performed to investigate the levels of 25-hydroxyvitamin D in normotensive pregnancy and severe preeclampsia.

METHOD

This research is an analytical cross-sectional study with t-test to compare the levels of 25-hydroxyvitamin D in normotensive pregnant women and severe preeclampsia. This research was conducted and evaluated from August 2016 to December 2016 in the Department of Obstetrics and Gynecology, Faculty of Medicine, Universitas Sam Ratulangi / Prof. Dr. R.D. Kandou Manado and hospital networks in Manado to research subjects who meet the inclusion and exclusion criteria. The inclusion criteria include pregnant women 20 weeks gestation to term with severe preeclampsia, superimposed preeclampsia and eclampsia and willing to participate in research. Exclusion criteria included pregnant women with chronic hypertension, heart disease, liver, kidney, diabetes mellitus, fetal growth retardation, and refuse to join the study. Subjects consisted of 17 samples and 17 samples of normotensive severe preeclampsia. After anamnesis, physical examination and have

informed consent, serum samples were taken from as many as 5 ccs and put into a sterile sample container, centrifuged and stored at -20°C and then processed in Prodia a clinical laboratory method chemiluminescent immunoassay (CLIA). This study has also been approved by the Integrated Health Research Unit (UPKT) Prof. Dr. R.D. Kandou Manado. Data were analyzed with SPSS version 20.0.

RESULT

In Table 1 shows the subject of research in the normotensive group most aged 21 to 35 years is 13 people (76.47%), Minahasans many as 14 people (82.36%), job IRT 11 (64.7%), multigravida as many as 11 people (64.71%) and the wedding one time as many as 15 people (88.24%). While in severe preeclampsia group there are at most ages 21-35 years as many as 9 people (52.94%), Minahasans 10 (58.83%), job IRT 16 people (94.12%), multigravida 10 people (58.82%) and the wedding one time as many as 15 people (88.24%).

Table 1. Research Subject Characteristics

Characteristics	Normotensive		Preeclampsia	
	n	%	n	%
Age				
< 20 years	-	0	2	11.76
21-35 years	13	76.47	9	52.94
> 36 years	4	23.53	6	35.30
Ethnic				
Minahasa	14	82.36	10	58.83
Sangihe	1	5.88	4	23.53
Gorontalo	1	5.88	1	5.88
Etc	1	5.88	2	11.76
Occupation				
Housewife	11	64.7	16	94.12
Private employee	1	5.88	1	5.88
Government employee	4	23.53	-	-
Student	1	5.88	-	-
Parity				
Primigravidity	6	35.29	7	41.18
Multigravidity	11	64.71	10	58.82
Marriage				
1	15	88.24	15	88.24
> 1	2	11.76	2	11.76

Table 2. Variable Distribution of Age and Blood Pressure (BP) In normotensive Pregnancy and Severe Preeclampsia

Variable	Normotensive Pregnancy (n = 17)	Severe Preeclampsia (n = 17)	p
Age	31.41 ± 5.455	30.94 ± 9.692	0.863
Systolic BP	122.35 ± 10.326	168.24 ± 14.246	0.000
Diastolic BP	78.82 ± 9.275	108.82 ± 6.002	0.000

Table 3. 25-Hydroxyvitamin DLevels in Normotensive Pregnancy and Severe Preeclampsia

Variable	Normotensive Pregnancy (n = 17)	Severe Preeclampsia (n = 17)	p
Kadar 25-hydroxyvitamin D (ng/ml)			
Mean ± SD	24.771 ± 6.9567	17.712 ± 3.7513	0.001
Median	24.700	17.500	
Highest value	14.8	7.9	
Lowest value	40.7	22.4	

From the above table, for the age variable t-test (mean difference between two independent variables), it was found there was no significant difference between the age groups of normotensive and severe preeclampsia group, so it was concluded there was no correlation between the incidence of severe preeclampsia with maternal age. Whereas for the variable systolic blood pressure and diastolic blood pressure test and the Mann-Whitney there are significant differences between the systolic and diastolic blood pressure in normotensive and severe preeclampsia group, so it was concluded there is a correlation between the incidence of severe preeclampsia with systolic and diastolic blood pressure.

In Table 3, 25-hydroxyvitamin D levels in pregnancy with normotensive obtained the highest levels of 40.7 ng/ml and the lowest levels in normotensive 14.8 ng/ml. As for severe preeclampsia 25-hydroxyvitamin D levels high of 22.4 ng/ml while 25- hydroxyvitamin D levels low of 7.9 ng/ml.

By using the t-test (mean difference between two independent variable), it was concluded that there are significant differences in vitamin D levels in each of the normotensive group (24.771 ± 6.9567) and severe preeclampsia group (17.712 ± 3.7513), (p = 0.001), meaning that there is a relationship between the incidence of severe preeclampsia with blood levels of vitamin D (Table 3 and Figure1).

Shapiro-Wilk normality test showed that the distribution of data levels of vitamin D in the normotensive group and Severe Preeclampsia were normally distributed (p = 0.57 and p = 0.13).

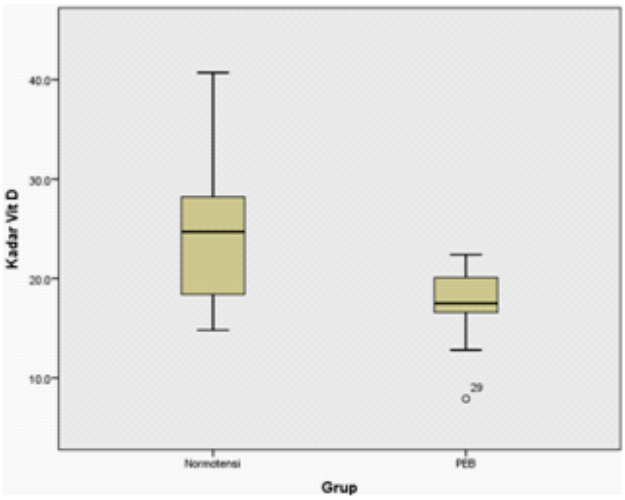

Figure 1. Graphs the levels of 25-hydroxyvitamin D in normotensive pregnancy and severe preeclampsia

DISCUSSION

The basic characteristics of the study sample include age, ethnicity, occupation, parity, marriage with sample number 34 and is divided into two groups: group with normotensive pregnancy and severe preeclampsia group. Table 1 can be seen from the distribution of study subjects based on the age of the mother is 16 to 46 years. The highest percentage in the age group 21-35 years both in the normotensive group as many as 13 people (76.47%) and severe preeclampsia group were nine people (52.94%). Followed age > 36 yrs namely the normotensive group of 4 people (23.53%) and severe preeclampsia six people (35.3%). In this study, the Minahasa tribe is the tribe most studied as many as 14 people in the normotensive group (82.36%) and ten men in the severe preeclampsia group (58.83%), because the study was conducted in the city of Manado, North Sulawesi where Minahasans a majority interest. In this study, it was found that the incidence of severe preeclampsia increases in the productive age and IRT. This contrasts with research conducted by SQ Wei et al in June 2012 which reported that there was no significant difference between preeclampsia and nonpreeclampsia terms of maternal age, education and ethnicity.[5]

In this group, the highest percentage obtained parity characteristics are good multigravida in normotensive group (64.7%) and severe preeclampsia (58.82%). This is due to the distribution of samples high in this group. Preeclampsia often occurs at a young age and primiparous, it is suspected because of the existence of a mechanism immunology.[13,16] In Table 3 shows the relationship of vitamin D (25(OH)D) in pregnancy normotensive, and severe preeclampsia in which it was concluded there are significant differences in the levels of vitamin D (25(OH)D) in the normotensive and severe preeclampsia, with an average value levels vitamin D (25(OH)D) in normotensive 24.771 ± 6.9567 ng/ml while in severe preeclampsia group was 17.712 ± 3.7513 ng/ml (p = 0.001). This suggests there is a correlation between levels of vitamin D (25(OH)D) and the incidence of severe preeclampsia.

Research in Surabaya Hanifa et al. (2015) that compared 18 women of severe preeclampsia and 18 normotensive women found a significant difference between levels of vitamin D (25(OH)D) in the normotensive with severe preeclampsia. Levels of vitamin (25(OH)D) in normotensive

(11.7 ± 4.8) compared with severe preeclampsia (15.9 ± 4.5). The hypothesis about the levels of vitamin D (25(OH)D) maternal determine the risk of preeclampsia.[18]

From several studies reported that a deficiency of vitamin D (25(OH)D) related to an inflammatory process due to endothelial dysfunction. Is a layer of endothelial cells lining the vascular wall facing the lumen and attached to the subendothelial tissue consisting of collagen and various glycosaminoglycans including fibronectin.[17] Formerly considered that endothelial function is as a structural barrier between the circulation of the surrounding tissue, but it is now known that the endothelial function of regulating vascular tone, prevents thrombosis, regulate the activity of the fibrinolytic system, preventing the adhesion of leukocytes and regulates vascular growth. Issued endothelial vasoactive substances include nitric oxide (NO) which is also called endothelial-derived relaxing factor (EDRF), endothelial-derived hyperpolarising factor (EDHF), prostacyclin (PGI2), bradykinin, acetylcholine, serotonin and histamine. Among other vasoconstrictor substance endothelin, platelet activating factor (PAF), angiotensin II, prostaglandin H2, thrombin and nicotine. Deficiency of vitamin D (25(OH)D) causes endothelial dysfunction through the molecular mechanism. Where the result of a deficiency of vitamin D (25(OH)D) causes the release of most of the proinflammatory factors including the increase in NFkB (nuclear factor-kB), interleukin-6, a decrease in the vitamin D receptor, 1α-hydroxylase, and calcium levels. Wherein the calcium associated with vitamin absorption D.[5,20,21] If endothelial impaired in by many things such as oxidative stress, shear stress hemodynamic, hypercholesterolemia and exposure of inflammatory cytokines, the regulatory function becomes abnormal and is called endothelial dysfunction.[18-21] Deficiency of vitamin D (25(OH)D) in pregnancy predisposes proinflammatory response and oxidative stress resulting in endothelial dysfunction that is the hallmark of preeclampsia. There also are found vitamin D (25 (OH)D) effect on trophoblast invasion and angiogenesis when implantation process, so this is an important factor in the pathophysiology preeclampsia.[5] Several studies have also reported a deficiency of vitamin D (25(OH)D) and the risk of preeclampsia. Marya et al. suggest that the intake of calcium and vitamin D can lower blood pressure, thereby reducing the incidence of preeclampsia. Other researchers such as Haugen et al. (2009) Haryana et al. (2012), Abdulbari (2013), Dharma et al. (2005) also suggests the same result.[14,15,19-21]

CONCLUSION

From this study, it can be concluded that the obtained 25-hydroxyvitamin D levels were lower in patients with severe preeclampsia. The results of the analysis of the relationship of 25-hydroxyvitamin D levels with the incidence of severe preeclampsia in getting that blood levels of 25-hydroxyvitamin D a pregnant woman is associated with severe preeclampsia.

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Research Article

The Prevalence and Risk Factors of Constipation in Pregnancy

Prevalensi dan Faktor-Faktor Risiko Konstipasi dalam Kehamilan

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Abstract

Objective: To estimate the prevalence of constipation in pregnancy and correlation between gestational age, dietary fiber intake, water comsumption, and physical activity.

Methods: This study used cross-sectional design with samples of 174 healthy pregnant women undergoing antenatal care at Obstetrics and Gynecology Outpatient Clinic RSCM during August - October 2016. Data were collected using questionnaire. Diagnosis of constipation was based on ROME III criteria, dietary fiber is measured using *Food Frequency Questionnaire* (FFQ), and physical activity was measured using *International Physical Activity Questionnaire* (IPAQ). Chi-square and Fisher's exact test were conducted to evaluate the association between variables.

Results: The prevalence of constipation in pregnant women observed in this study was 13.2% (95% CI 8.3-18.1). The most frequent complaints included straining, incomplete evacuation, and anorectal obstruction. Dietary fiber intake was low in 81.03% subject. with average dietary fiber intake of 18.97 gram/day. There was no significant association between constipation and gestational age (OR 4.36, 95%CI 0.51-37.48 for second trimester and OR 2.04, 95%CI 0.25-16.7 for third trimester), dietary fiber intake (OR 0.82, 95%CI 0.28-2.39), water consumption (OR 1.38, 95%CI 0.56-3.41), and physical activity (OR 1.167, 95%CI 0.28-4.87).

Conclusion: Prevalence of constipation in pregnant women is 13.2%. There is no significant correlation between gestational age, dietary fiber intake, water consumption, and physical activity.

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Keywords: constipation, pregnant woman, ROME III

Abstrak

***Tujuan**: Mengetahui prevalensi dan hubungan antara usia kehamilan, asupan serat, konsumsi air, dan tingkat aktivitas fisik dengan konstipasi pada ibu hamil.*

***Metode**: Penelitian ini merupakan penelitian potong lintang dengan jumlah sampel 174 perempuan hamil yang sehat yang berkunjung untuk melakukan pemeriksaan antenatal di poliklinik rawat jalan RSCM selama Agustus-Oktober 2016. Data dikumpulkan melalui pengisian kuesioner. Diagnosis konstipasi berdasarkan kriteria ROME III, pengukuran asupan serat dengan kuesioner Food Frequency Questionnaire (FFQ), pengukuran tingkat aktivitas fisik dengan kuesioner International Physical Activity Questionnaire (IPAQ). Uji chi square dan Fisher dilakukan untuk menilai hubungan antar variabel.*

***Hasil**: Prevalensi konstipasi pada perempuan hamil pada penelitian ini 13,2% (IK95% 8,3-18,1) Keluhan tersering yaitu mengedan keras, buang air besar (BAB) yang tidak lampias, dan sensasi tidak dapat mengeluarkan tinja saat BAB. Sebanyak 81,03% subjek asupan serat per harinya kurang dengan rata-rata asupan serat 18,97 gram/hari. Tidak terdapat hubungan yang bermakna antara usia kehamilan (OR 4,36, IK95% 0,51-37,48 untuk trimester 2 and OR 2,04, IK 95% 0,25-16,7 untuk trimester 3), asupan diet harian (OR 0,82, IK95% 0,28-2,39), asupan cairan (OR 1,38, IK95% 0,56-3,41), dan tingkat aktivitas fisik (OR 1,167, IK95% 0,28-4,87).*

***Kesimpulan**: Prevalensi konstipasi pada perempuan hamil sebanyak 13,2%. Tidak terdapat hubungan yang bermakna antara usia kehamilan, asupan serat, konsumsi air, dan tingkat aktivitas fisik.*

[Maj Obstet Ginekol Indones 2018; 6-2: 84-88]

***Kata kunci**: konstipasi, perempuan hamil, ROME III*

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INTRODUCTION

Constipation is the second most common gastro-intestinal problem after vomitus that often suffered by pregnant women. Constipation during pregnancy occurs about 11% to 38%.[1-3] The existence of constipation may contribute to increase in living cost and medical treatment, decrease productivity and quality of life as well as inducing permanent ailment such as pelvic muscle dysfunction. Constipation also can delay recovery time of digestion function after delivering baby and increase the prevalence of hemorrhoid. Hormonal changes, fetal and placental development, diet and physical activity alterations are factors that can caused constipation in pregnancy.[4-9] In Indonesia there are a lot of study about constipation, however, almost all of the subjects are children and elderly, while pregnant women are still very low. The purpose of this study to know the prevalence of constipation and its association with gestational age, fiber intake and water consumption, and the level of physical activity with constipation in pregnant women.

METHODS

This cross-sectional study was performed during the period of August to October 2016 in Dr. Cipto Mangunkusumo hospital at Obstetrics and Gynecology outpatient clinic with 174 subject samples collected by consecutive sampling. This study obtained ethical clearance from Research and Development Unit Faculty of Medicine Universitas Indonesia. Each subject signed an informed consent before participation. Inclusion criteria are pregnant woman age 20-35 years old who came to outpatient clinic in Department of Obstetrics and Gynecology, Dr. Cipto Mangunkusumo Hospital, and can read and write fluently. Exclusion criterias included history of constipation because of organic disease such as colon carcinoma, IBS, severe enteritis, rectocele and abdomen operation in last 30 days, history of bowel surgery (other than appendectomy), current treatment for thyroid disease, and has a smoking habit.

The study questionairre included validated ROME III questionairre for diagnose constipation, Bristol Stool chart for assessed stool consistency, FFQ for fiber dietary intake and IPAQ for physical activity level. Other data collected by questionnaire included demographics, medication use, gestational age, amount of water intake, medical history, and smoking habits.

The diagnosis of constipation is defined by Rome III criteria, which chronic disorder form of constipation is characterized by two or more of the symptoms: straining, lumpy or hard stools, sensation of incomplete evacuation, sensation of anorectal obstruction, manual maneuvers to facilitate defecation (digital evacuation) at least 25% of defecation, and fewer than 3 defecation per week. In addition, loose stool without using laxatives should be rare, and criteria for Irritable Bowel Syndrome (IBS) must not be met. Chronic definition is given if the onset of the symptoms are present in the last 6 months and the duration of the symptoms are present at least 3 months. Because the researcher wanted to see the constipation in pregnant women according to her gestational age, then we modify the duration in the criteria into two months. Daily fiber intake recommendation for pregnant women is 25 g/day whereas sufficient water intake recommendation is at least 8 glasses per day.

Categorical data are presented in percentage. Prevalence estimates with exact 95% confidence intervals (CI) were calculated. To determine the relationship between the variable using chi-square test and Fisher with level of significance as p value < .05.

RESULTS

In this study, we found 23 (13.2%) from 174 patients were diagnosed with constipation based on ROME III criteria.

Table 1. Prevalence of Constipation on Pregnant Woman

Characteristic	n	Percentage	CI 95%
Constipation	23	13.2	8.3-18.1
Non-Constipation	151	86.8	
Total	174	100.0	

Table 2 shows that three constipation symptoms based on ROME III criteria, most are straining, incomplete evacuation, and anorectal obstruction. Only 43.5% of subjects were diagnosed constipation that complain the frequency of defecation <3x/week. The most common stool consistency on subjects with constipation based on *Bristol stool chart* was normal stool (type 4) (60.9%). Meanwhile, only 13% of subjects had hard consistency and lumpy form that is in accordance with constipation stools. From 174 subjects, we found 81.03% of subjects were less fiber consumption, <25 gram per day.

Subjects of this study consumed 18.7 gram of fiber per day in average. From constipation group, 78.3% subjects had inadequate fiber intake. For fluid/water intake level, we found that most subjects from all groups had adequate fluid intake, by consuming 8 glass of water per day minimum, with an average of 8.76 glass per day. In subjects of this study, largest level of activity found was moderate activity, found in second and third trimester.

Table 3 shows factors associated with constipation. Unexpectedly, in relation to constipation, there were no significant relationships between constipation with gestational age (OR 4.36, 95% CI 0.51-37.48 for second trimester and OR 2.04, 95% CI 0.25-16.7 for third trimester), dietary fiber intake (OR 0.82, 95% CI 0.28-2.39), water consumption (OR 1.38, 95% CI 0.56-3.41), and physical activity (OR 1.167, 95% CI 0.28-4.87).

Table 2. Characteristic of Subjects

Parameter	Constipation n=23 (13.22%)	No Constipation n=151 (86.78%)
Constipation symptoms		
Straining	17 (73.9)	4 (2.6)
Lumpy or hard stool	13 (56.5)	1 (0.7)
Incomplete evacuation	17 (73.9)	8 (5.3)
Anorectal obstruction	14 (60.9)	0 (0)
Manual maneuvers	5 (21.7)	0 (0)
less than 3x/week	10 (43.5)	14 (9.3)
Stool Consistency~Bristol Stool chart		
Type 1	3 (13.0)	5 (3.3)
Type 2	1 (4.3)	5 (3.3)
Type 3	3 (13.0)	20 (13.2)
Type 4	14 (60.9)	88 (58.3)
Type 5	2 (8.7)	19 (12.6)
Type 6	0 (0)	11 (7.3)
Type 7	0 (0)	3 (2.0)
Fiber intake		
Adequate	5 (21.7)	28 (18.5)
Inadequate	18 (78.3)	123 (81.5)
Fluid intake		
Adequate	14 (60.9)	103 (68.21)
Inadequate	9 (39.1)	48 (31.79)
Level of Physical Activity		
Highly active	3 (13.04)	27 (17.88)
Sufficiently active	13 (56.52)	70 (46.36)
Insufficiently active	7 (30.43)	54 (35.76)

Table 3. Factors Associated with Constipation

Factors	Constipation n=23	No Constipation n=151	p Value	OR (CI95%)
Gestational Age				
Trimester 1	1 (5.88)	16 (94.1)		Reference
Trimester 2	9 (21.4)	33 (78.6)	0.254[b]	4.364 (0.508-37.481)
Trimester 3	13 (11.3)	102 (88.6)	0.694[b]	2.039 (0.249-16.671)
Fiber intake				
Adequate (≥25 g/day)	5 (15.15)	28 (84.8)	0.776[b]	0.820 (0.280-2.395)
Inadequate	18 (12.7)	123 (87.2)		
Fluid intake				
Adequate (>8 glass/day)	14 (11.9)	103 (88.0)	0.485[a]	1.379 (0.558-3.409)
Inadequate	9 (15.8)	48 (84.2)		
Level of physical activity				
Highly active	3 (10.0)	27 (90.0)		Reference
Sufficiently active	13 (15.6)	70 (84.3)	1.000[b]	1.167 (0.279-4.871)
Insufficiently active	7 (11.5)	54 (88.5)	0.553[b]	1.167 (0.441-6.330)

Categorical data displated in number (percentage) a Chi-square test, b Fisher test

DISCUSSION

Constipation prevalence in this study is 13.2% (CI 95% 8.3 - 18.1). Previous study in Asia, especially in Shanghai, China by Shi et al[9] found the constipation prevalence about 13.01%. The result is quite similar to this study. Small differences were found in a study by Bradley et al and Derbyshire et al. According to the study by Bradley et al, which was conducted in *University of Iowa Hospitals and Clinics*, US, the constipation prevalences among pregnant women is 24% (CI 95%, 16-33%), 26% (CI 95% 17-38%), 16% (CI 95% 8-26%), and 24% (CI 95% 13-36%) in first trimester, second trimester, and third trimester and 3 months *post-partum*. Beside that, according to Derbyshire E et al who conducted a study in pregnant woman in England, prevalence of constipation is 28.4%.[10,11] Both of the studies used Rome II Criteria as a diagnostic foundation of constipation. This discrepancy can be caused by the variances in measurement tools, which was used to diagnose the constipation. In this study, we use the ROME III Criteria. In addition, those differences may be caused by dietetic custom of women from different ethnic. According to the study by Smith et al[12], which was conducted a study to compare dietary pattern of Asian and Caucasian, the Caucasian people tend to consume higher protein and sugar, but contain lesser amount of fiber. Hence, this can contribute to higher constipation prevalence in pregnant women.

From the level of fiber consumption, there are 81.03% of the subjects that have less level of fiber consumption with an average consumption of fiber as much as 18.97 grams/day. This is in line with the study of Sri Wahyuni, cross sectional study at Gowa district in 2013 on 66 subjects, it found that there are 54.5% of pregnant women who rarely ate vegetables and fruit (≤1x / week). This study also found 100% of the subjects have low fiber intake (adequate fiber intake: 25 grams/day), even in those who eat vegetables often also still have a low fiber intake.[13] Study of Raissa et al (2012) in panti wreda elders, the fiber intake level were 100% low. Zulaika (2011) also showed low fiber intake in adults with normal and obese status. Fiber intake level in junior high school in Rahmania's (2012) study also showed deficit fiber intake level. Similar result were also written by Badrialaily (2004) that the average fiber intake in GMSK and Kehutanan students were not different (7.8 g/kap.day). All of this showed fiber intake level in Indonesian population are still low, whether it is in pregnant women, elders, teenagers, students with nutritional knowledge, and adults with normal and obese status.[14-17]

In relation to fiber intake in this study, there was no significant relationship between fiber consumption and constipation (p=0.776). This result is concomitant with Ambarta and Fitriani.[18,19] Other studies in pregnant women that compatible with our study were Derbyshire and Anderson.[11] Althought several studies showed high fiber intake had no relation with constipation, but there were some studies with large sample size showed that there was negative correlation between fiber intake and constipation. Increasing fiber intake in constipation was still as an early management besides of doing physical activity.

In relation to fluid intake, there was no significant correlation between fluid intake and constipation (p=0.485). The result corresponded to a study conducted by Raissa[14], but it was not similar as a study conducted by Fitriani[19]. Almost of subjects had enough water intake. Constipation was found more among subjects who had mild and moderate physical activities compared to intense physical activities. However, statistycal analysis showed that there was no significant difference between physical activities and constipation (p=0.553). This corresponded to a study conducted by Derbyshire[11], but it was not similar as a study conducted by Sanjoaquin[20]. Factor causing the insignificant difference between physical activities and constipation was dependent on subjects' memories and perception on estimating how much time they spent for doing physical activities as questioned in IPAQ questionnaire.

There were some limitations in this study, such as we could not control the recall bias which there was some difficulties for subjects to do recalling about the defecation pattern in the last 2 months. In addition, they had to recall their fiber intake pattern and the physical activities. In addition, this study could fulfill the minimal sample size but the proportion of subjects between two groups was still imbalanced. This issue could affect the power or the precision of this study. This study was done at one centre where had homogenous population therefore our result may not be generalizable to other groups of women.

CONCLUSIONS AND RECOMMENDATIONS

Constipation is the gastrointestinal tract problems that are quite common in pregnancy. Our study show that the prevalence of constipation using the Rome III criteria is 13.2%. There is no significant relationship between gestational age, daily fiber intake, water consumption, and level of physical activity on the incidence of constipation in pregnancy. Further prospective multicenter studies with a larger number of the samples are required to be conducted.

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Research Article

Oral versus Vaginal Misoprostol for Labour Induction : A Comparative Study

Misoprostol Oral versus Pervaginam untuk Induksi Persalinan : Suatu Studi Komperatif

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Abstract

Objective: To compare the efficacy and safety of hourly titrated oral misoprostol in solution (OMS) with vaginal misoprostol (PV) for labor induction.

Methods: Randomized Controlled Trial (RCT), double blind-add on the study was conducted from January-November 2016 in delivery ward of Moh. Hoesin general hospital. Women ≥30 weeks of gestation with an unfavorable cervix (Bishop score ≤6) and an indication for labor induction were randomly assigned to receive titrated oral or vaginal misoprostol. The OMS group received a basal unit of 20 ml misoprostol solution (1 μg/ml) every 1 hour for four doses and then were titrated against individual uterine response. In the absence of regular uterine contractions, the dose was increased to 40 ml hourly for four doses and then 60 ml for four doses. The vaginal group received 25 μg every 4 hours until attaining a more favorable cervix for three doses. All the subjects received amylum placebo. In labor within 12 hours was the primary outcome.

Results: A total of 30 women were enrolled in this study. One subject in the OMS group was dropped out due to eclamptic seizure. The average interval from induction until in labour in OMS group was 5.75±3.14 hour and 6.60±4.46 hour in PV group (p = 0.56). In labour stage was achieved within 12 hours in 14 women (100%) in OMS group and 14 women (93.3%) in PV group (p = 1.00). Vaginal delivery was achieved within 24 hours in 13 women (92.9%) in OMS group and 15 women (100%) in PV group. The incidence of uterine hyperstimulation/ tachysystolic was 7.1% in OMS group compared with 13.3% in PV group. Fetal distress was found only 1 case (7.1%) in OMS group. There was no difference in the maternal and neonatal outcome of labor in both the groups.

Conclusion: Oral titrated in solution, and vaginal route of administration of misoprostol for induction of labour are equally effective and safe.

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Keywords: hourly titrated oral misoprostol in solution, oral misoprostol, randomized controlled trial, vaginal misoprostol

Abstrak

***Tujuan**: Membandingkan efektivitas dan keamanan pemberian larutan misoprostol titrasi peroral/ titrated oral misoprostol in solution (OMS) dan misoprostol pervaginam (PV) untuk induksi persalinan.*

***Metode**: Randomized Controlled Trial (RCT), double blind-add on dilakukan Januari-November 2016 di kamar bersalin RSUP Dr. Moh. Hoesin Palembang. Terdapat 30 subjek wanita hamil dengan usia gestasi ≥30 minggu dan skor Bishop ≤6 yang memenuhi indikasi induksi persalinan; selanjutnya dirandomisasi menjadi 2 kelompok, yaitu OMS dan PV. Subjek pada kelompok OMS menerima misoprostol peroral 20 ml/jam (1 μg/ml) sebanyak 4 dosis. Bila kontraksi uterus yang regular belum timbul, dosis dinaikkan menjadi 40 ml/jam sebanyak 4 dosis. Bila kontraksi regular belum timbul, dosis dinaikkan menjadi 60 ml/jam sebanyak 4 dosis. Subjek pada kelompok PV menerima misoprostol pervaginam 25 μg/4 jam sebanyak 3 dosis. Setiap subjek juga menerima plasebo amilum. Parameter keberhasilan penelitian adalah keberhasilan mencapai inpartu ≤12 jam.*

***Hasil**: Dari 30 subjek, 1 orang pada kelompok OMS drop out dari penelitian karena eklampsia berulang. Rerata interval induksi-inpartu pada kelompok OMS 5,75±3,14 jam, sedangkan kelompok PV 6,60±4,46 jam (p = 0,56). Sebanyak 14 subjek (100%) pada kelompok OMS dan 14 subjek (93,3%) pada kelompok PV mencapai inpartu ≤12 jam (p = 1,00). Partus pervaginam ≤24 jam dicapai 13 subjek (92,9%) pada kelompok OMS dan 15 subjek (100%) pada kelompok PV (p = 0,48). Kasus hiperstimulasi/takisistolik uterus ditemukan pada 1 subjek (7,1%) pada kelompok OMS dan 2 subjek (13,3%) pada kelompok PV. Komplikasi gawat janin ditemukan pada 1 subjek (7,1%) pada kelompok OMS. Tidak ditemukan perbedaan bermakna luaran maternal dan neonatus pada kedua kelompok.*

***Kesimpulan**: Larutan misoprostol titrasi peroral memiliki efektivitas dan keamanan yang sama dengan misoprostol pervaginam untuk induksi persalinan.*

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***Kata kunci**: larutan misoprostol titrasi oral perjam, misoprostol peroral, misoprostol pervaginam, uji klinik acak berpembanding*

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INTRODUCTION

Induction of labor is defined as the initiation process of uterine contractions with the help of medical pharmacology or medical action before the onset of spontaneous parturition.[1] Approximately 20% of births in the United States and Britain begins with induction of labor, whereas the incidence of labor induction in Africa and Asia are 4.4% and 12.1% of all deliveries, respectively. This proportion will continue to increase significantly almost several years.[2-4]

Misoprostol is a synthetic analog of prostaglandin E1 that is inexpensive, stable at room temperature, easily stored, and simple in usage for cervical ripening and induction of labor. However, misoprostol can cause fetal distress due to uterine hyperstimulation or tachysystole uterus. To avoid hyperstimulation or tachysystole uterus and to shorten the induction interval up to labor, misoprostol should be given in small, effective doses, in a high frequency, and titrated according to uterine response. Oral titrated misoprostol solution administration or oral misoprostol in solution (OMS) fulfilled all aforementioned criteria. In addition, divided doses of misoprostol proved to be disadvatageous due to a difficult and imprecise tablet cutting, rendering the dosage to be inappropriate. OMS administration does not only allow the proper dosage, but misoprostol can remain active in the solution for 24 hours.[5]

Cheng, et al. compared the efficacy and safety of OMS and vaginal misoprostol for induction of labor in pregnant women 34-42 weeks with a Bishop score ≤ 6. OMS were given with an initial dose of 20 mcg/ hour and repeated for every hour until adequate uterine contractions were achieved. When the contractions are not adequate after four times of administration, the dose was increased to 40 mcg/hour and repeated every hour until adequate contraction, with a maximum of 4 doses. When the contractions were not adequate after 8 hours from the start of induction, then the dose was increased to 60 mcg/hour until adequate uterine contractions were achieved with a maximum of 4 doses. If the patient has become in labor, then misoprostol is stopped. When the contractions become inadequate before the active phase of labor, then the introduction of OMS can be repeated, starting at a dose of 10 mcg/hour and can be raised to 20 mcg/hour, or up to 40 mg/hour based on the response of the uterus to achieve adequate uterine contractions. Delivery within 24 hours was achieved in 94.1% of the 101 women who were randomly assigned oral titrated misoprostol solution, compared with 53.8% of the 106 women given misoprostol vaginally (p = 0.01). No women of the OMS group developed uterine hyperstimulation, while 11.3% in the vaginal misoprostol group did. Although more women experienced nausea in the OMS group (10.9%), the newborn Apgar score in this group is better (>7 at 1 minute first) than the vaginal group. Cheng, et al. concluded that low doses of titrated misoprostol are associated with a low incidence of uterine hyperstimulation and cesarean section compared with vaginal misoprostol in women with the immature cervix.[6] In another literature, Cheng concluded OMS is more effective and superior to vaginal misoprostol. By administering OMS, the rate of vaginal delivery becomes higher which decreases the rate of cesarean section.[7]

This study aims to compare the efficacy and safety of oral titrated misoprostol solution (OMS) with vaginal misoprostol (per vaginal misoprostol/ PV) in women undergoing labor induction.

METHOD

This study was a Randomized Controlled Trial (RCT), double-blind, add-on study and had received ethics approval from the Ethics Committee of Dr. Moh. Hoesin Hospital Palembang. The study involved 30 pregnant women who meet the inclusion criteria during the period from January to November 2016. Inclusion criteria for the study were pregnant women ≥30 weeks, fulfilling an indication of induction of labor, a single live fetus pregnancy, Bishop score ≤6, cephalic presentation, normal fetal heart rate patterns, and willing to participate in the study by signing a letter of approval (*informed consent*). Exclusion criteria included pregnant women with contraindications of vaginal delivery, previous cesarean section, a history of surgery on the uterus, intrauterine fetal death, parity > 5, the presence of adequate uterine contractions, and abnormal fetal heart rate patterns or fetal distress. Drop out criteria were a history of allergy to misoprostol; patients experience side effects that heavy drug misoprostol (*adverse effects*), such as anaphylactic shock, imminent uterine rupture and uterine rupture; additional diagnoses that can stop the study procedures (eg, eclampsia, impending eclampsia, HELLP syndrome, and so on, which leads to the abdominal termination); or the patient does not comply with the study protocol. Withdrawal criteria were patients who decide to stop participating in the study on their own without any coercion.

The samples were divided into two groups derived from simple randomization using randomization tables. At the titrated oral misoprostol group (OMS), a solution of misoprostol was given orally according to the study protocols, and one placebo tablet which will be divided into

⅛ parts and administered vaginally in the posterior fornix according to the study protocols. In the group of vaginal misoprostol (PV), one placebo tablet was dissolved in 200 ml of water in a glass and administered according to the appropriate protocol, and one tablet of misoprostol 200 mcg which will be divided into ⅛ parts (25g) and administered in the posterior vaginal fornix. Regimens in the two groups of these samples were administered without the knowledge of researchers (blinded). Material samples have been coded "OMS" or "PV" and put in a sealed envelopes which have been given a random number based on the randomization tables by individuals not directly involved in the study. Before the envelope is closed and sealed, the envelope code is recorded on a special sheet and stored separately in a sealed envelope to be opened at the time of data analysis is complete. Researchers and patients did not know the contents of the drug given to the patient.

Misoprostol effectiveness was assessed by achieving successful in labour ≤12 hours after induction began. OMS and PV safety was assessed by observing drug side effects in both maternal and neonatal outcomes. Failed induction was defined as failure to achieve inabour after 12 hours from the start of administration of misoprostol. Uterine hyperstimulation was defined as the presence of excessive uterine contraction with fetal heart rate abnormalities. Changes in fetal heart rate were defined as persistent decelerations, tachycardia, or a decrease in *short-term* variability. Tachysy stole uterus was defined as the presence of uterine contractions > 5 times within 10 minutes, which lasted an average of > 30 minutes without changes in fetal heart rate.

In OMS protocol, one tablet misoprostol (200 μg) was dissolved in 200 ml water in a medical measurement bottle and mixed evenly. Misoprostol solution should be used within 24 hours after dissolved. OMS was given with an initial dose of 20 ml/hour and repeated every hour until adequate uterine contractions were achieved. When the contractions did not occur or not adequate after four times of administration, the dose was increased to 40 ml/hour and repeated every hour until adequate contractions were achieved, with a maximum of 4 doses. When the contractions were not adequate after 8 hours from the start of induction, then the dose was increased to 60 ml/hour until adequate uterine contractions were achieved with a maximum of 4 doses. Adequate uterine contractions were defined as the presence of three or more uterine contractions in a 10-minute period with a duration ≥ 30 seconds.[7]

When adequate uterine contractions have been achieved in 1 hour, misoprostol was subsequently terminated. If the patient has reached in labour, then misoprostol is stopped. When the contractions become inadequate after parturients entered labour, acceleration with oxytocin is possible to administer at least 2 hours after the last administration of misoprostol. Acceleration of labor is achieved with oxytocin 5 IU mixed in 500 ccs of Ringer Lactate. During the first 15 minutes, oxytocin is given ten drops/min, then increased five drops every 15 minutes until the adequate contraction is reached or a maximum of 40 drops/minute. If the Bishop score has reached ≥ 9, amniotomy may be done according to the doctor's discretion. If there is a failed induction, parturients are managed by standard procedure at the Obstetrics and Gynecology Department of Dr. Moh. Hoesin Hospital Palembang.

In PV protocol, the initial dose of 25 μg vaginal misoprostol is administered in the posterior vaginal fornix. This dose can be repeated every 4 hours to achieve adequate uterine contractions. Adequate uterine contractions were defined as the presence of three or more uterine contractions in a 10-minute period with a duration ≥ 30 seconds. When adequate uterine contractions have been achieved in 1 hour, then misoprostol is subsequently terminated. If the patient has reached in labour, then misoprostol is stopped. When the contractions become inadequate after parturients entered labour, acceleration with oxytocin is possible to administer at least 2 hours after the last administration of misoprostol. If the Bishop score has reached ≥ 9, amniotomy may be done according to the doctor's discretion. If there is a failed induction, parturients are managed by standard procedure at the Obstetrics and Gynecology Department of Dr. Moh. Hoesin Hospital, Palembang, Indonesia.

Fetal heart rate and uterine contractions are closely monitored using cardiotocography from the beginning of induction until delivery. Data were analyzed using SPSS 18. Dichotomous variables were compared between the two groups using Chi-square test, whereas continuous variables were compared using the Student t-test. A p

value less than 0.05 was considered as statistically significant.

RESULTS

A total of 30 subjects were divided into two groups; the OMS (15 pregnant women who received induction treatment with oral titrated misoprostol solution and vaginal placebo) and PV groups (15 pregnant women who received induction treatment with vaginal misoprostol and placebo oral titration solution). After follow-up, there is one subject on which the OMS group who dropped out because of recurrent eclamptic seizures.

Table 1 shows the demographic characteristics of the subjects and indications of induction of labor. No significant difference was found between the demographic characteristics of subjects in both groups. Highest indication of induction in both groups was preeclampsia. However, the numbers of subjects with pre-eclampsia were higher in PV group compared to the OMS group.

The average interval from induction (starting after the first dose of misoprostol) until in labour in the OMS group was 5.75 ± 3.14 approximately 60 minutes faster than the 6.60 ± 4.46 hours found in the PV group. The average induction to delivery interval occurs more quickly in the OMS group (10.11 ± 6.17 hours) than the PV group (11.33 ± 6.24 hours). However, statistical analysis revealed that the difference was not significant (p = 0.599). The mean dose of misoprostol in the OMS group was 4 times higher than the PV, but it is reasonable from the standpoint of drug pharmacodynamics.

All subjects (100%) in the OMS group reached in labour ≤12 hours, while the PV group had one subject (6.7%) with failed induction. The diagnosis of the subject who had a failed induction by vaginal misoprostol is a post-termpregnancy (gestational age 41-42 weeks). This particular subject was induced with IV oxytocin and managed to achieve spontaneous labor. The use of oxytocin in the OMS group is less than the PV group. All subjects in the PV group achieved spontaneous delivery, whereas in the OMS group there is 1 subject (7.1%) who needed abdominal termination due to prolonged latent phase and 1 subject (7.1%) terminated by forceps extraction due to maternal ventricular septal defect.

Table 1. Characteristics of the Subjects

Variables	OMS		PV		p value
	n	%	n	%	
Mean maternal age (years)	28.64 ± 6.54	30.67 ± 6.14	0.397*		
Mean gestational age (weeks)	37.79 ± 2.36	38.67 ± 27.9	0.369*		
Mean Bishop score	3.57 ± 1.02	3.40 ± 0.99	0.648*		
Bishop score					
≤ 4	7	50	8	53.3	1.000
5-6	7	50	7	46.7	**
Body mass index					
Nonobese (18-24.9)	3	21.4	4	26.7	1.000
Obese (≥25)	11	78.6	11	73.3	***
Parity					
Nullipara	9	64.3	8	53.3	0.825
Multipara	5	35.7	7	46.7	**
Induction indication					
Preeclampsia	5	35.7	6	40	
Postterm/postdate	0	0	4	26.7	
Oligohidramnion	4	28.5	2	13.3	0.145
PROM	2	14.2	2	13.3	****
Others	3	21.4	1	6.7	

** Unpaired T-test; p = 0.05*
*** Chi-square test*
**** Fischer exact test*
*****Pearson correlation test*

Table 2. Labour Outcome Characteristics

Variables	OMS		PV		p value
	n	%	n	%	
Induction-inlabour interval (hours)	5.75 ± 6.54	6.60 ± 4.46	0.560*		*
Induction-active phase interval (hours)	7.25 ± 2.36	8.07 ± 5.53	0.686*		
Induction-delivery interval (hours)	10.11 ± 1.02	11.33 ± 6.24	0.599*		
Total misoprostol dose (μg)	218.57 ± 147.27	50.00 ± 23.15			
Vaginal delivery ≤ 12 hours					
Yes	9	64.3	10	66.7	1.000
No	5	35.7	5	33.3	**
Vaginal delivery ≤ 24 hours					
Yes	13	92.9	15	100	0.483
No	1	7.1	0	0	***
Oxytocin acceleration					
Yes	5	35.7	6	40	1.000
No	9	64.3	9	60	**
Failed induction					
Yes	0	0	1	6.7	1.000
No	14	100	14	93.3	***
Delivery type					
Spontaneous	12	85.7	14	93.3	
Operative	1	7.1	0	0	0.316
vaginal					****
Abdominal	1	7.1	0	0	

** Unpaired T-test; p = 0.05*
*** Chi-square test*
**** Fischer exact test*
*****Pearson correlation test*

Table 3. Maternal and Neonatal Outcome Characteristics

Variables	OMS		PV		p value
	n	%	n	%	
Hyperstimulation					
Yes	1	7.1	1	6.7	1.000*
No	1	92.9	14	93.3	
	3				
Tachysystolic uterus					
Yes	0	0	1	6.7	1.000*
No	1	100	14	93.3	
	4				
Uterine rupture					
Yes	0	0	0	0	1.000*
No	1	100	15	10	
	4			0	
Postpartum hemorrhage					
Yes	0	0	0	0	1.000*
No	1	100	15	10	
	4			0	

Variables	OMS		PV		p value
	n	%	n	%	
Shivering					
Yes	4	28.6	6	40	0.700*
No	1	71.4	9	60	
	0				
Fever					
Yes	0	0	0	0	1.000*
No	1	100	15	10	
	4			0	
Nausea					
Yes	1	7.1	0	0	0.483*
No	1	92.9	15	10	
	3			0	
Vomiting					
Yes	1	7.1	0	0	0.483*
No	1	92.9	15	10	
	3			0	
Diarrhea					
Yes	0	0	0	0	1.000*
No	1	100	15	10	
	4			0	
Meconium staining					
Yes	6	42.9	5	33.3	0.885*
No	8	57.1	10	66.7	
Fetal distress					
Yes	1	7.1	0	0	0.483*
No	13	92.9	15	100	
APGAR score at 1 min					
<8	3	21.4	3	20	1.000*
≥8	11	78.6	12	80	
APGAR score at 5 min					
<8	2	14.3	2	13.3	1.000*
≥8	12	85.7	13	86.7	
NICU admission					
Yes	1	7.1	2	13.3	1.000*
No	13	92.9	13	86.7	
Perinatal death					
Yes	0	0	1	6.7	1.000*
No	14	100	14	93.3	

**Fischer exact test*

Table 3 shows maternal and neonatal outcomes on the use of drugs in both groups. There were no significant differences in maternal outcome variables between the two groups. In the OMS group of, there is one subject (7.1%) who developed uterine hyperstimulation following administration of oral misoprostol 480 ml within 11 hours. The induction on this particular subject is antepartum eclampsia. While in the PV group, there is one subject (6.7%) who developed uterine hyperstimulation following administration of vaginal misoprostol 75 mg in 12 hours and 1 subject (6.7%) with a tachysystole uterus. The induction indications of these subjects were fetal

congenital abnormality and preeclampsia respectively. Subjects from the PV group complained more chills (40%) than the OMS group (28.6%). Misoprostol was immediately discontinued in case of uterine hyperstimulation and tachysystole uterus. Furthermore, patients were given 10 mg of nifedipine and fetal heartbeats were monitored closely. Neonatal outcomes are relatively similar in both groups. Fetal distress was found in 1 subject (7.1%) with a gestational age of 31 weeks and an indication of antepartum eclampsia, which received OMS. Fetal distress occurs during the active phase due to hypoxia.

All subjects (100%) in the OMS group reached inlabour ≤ 12 hours, while 14 subjects (93.3%) of the PV group reached inlabour ≤ 12 hours. There were no efficacy differences between oral titrated solution misoprostol with vaginal misoprostol in achieving inlabour ≤ 12 hours (p = 1.000). Furthermore, this study resulted with a cut-off point of induction-inlabour interval at 4.75 hours. Based on the cut-off point, there were no differences between the oral misoprostol and vaginal misoprostol groups (p = 1.000, p > 0.05).

DISCUSSION

The study found no significant differences of mean age, mean gestational age, mean Bishop score, parity, and induction indications of labor between the two groups. The most common indication of labor induction is hypertension in pregnancy. A study by Rouzi, Madhavi, and Cheng also had similar results, but the most common indication in their study was post-term pregnancy. The third study was an RCT designs, but single blinded. The inclusion criteria between these studies were similar; Rouzi and Cheng used an inclusion criteria of 34-42 weeks' gestation; while Madhavi examined only 38-41 weeks gestation term. Furthermore, Madhavi used 60 samples, Rouzi used 160 samples, Rouzi and Cheng used a larger sample size of 207 samples.[5-8]

The study also found no significant difference between the mean induction-inlabour and induction-labor intervals between groups. The mean induction-inlabour interval in the OMS group were 5.75 ± 3.14 hours and 6.60 ± 4.46 hours in the PV group. Rouzi, et al. also failed to observe a significant difference in the induction-in labour interval. In his study, the mean induction-labor interval in the OMS group was 17.6 ± 8.5 hours and 20.2 ± 18 hours in the PV group.[5] Madhavi also found no significant differences in induction to delivery interval between OMS and and PV groups. The mean induction-labor ineterval in the OMS group was 13.83 hours and 13.82 hours in PV group (p = 0.994).[8]

Conversely, Cheng, et al. obtained significant difference of intervals until labor between OMS and PV groups. The mean induction - vaginal delivery interval was 8.2 hours in the OMS group and 17.6 hours in the PV group (p <0.01). The latent phase was 6.5 hours in the OMS group and 13.4 hours in the PV group; while the active phase was 1.6 hours in the OMS group and 3.4 hours in the PV group (p <0.01).[6,7] In contrast to these results, Ashalatha observed a significant difference in mean induction - labor interval in both groups. According to Ashalatha, vaginal misoprostol has a shorter induction - labor interval shorter compared with oral titrated misoprostol, with 17.8 hours in the PV group and 27.9 hours in the OMS group, with a mean difference of 10.1 hours.[9] The study by Cheng had inclusion criterias and study protocols that were almost similar to this study, but the large number of samples resulted in significant differences regarding the effectiveness between the two groups. Ashalatha, et al. used misoprostol dose titration per hour, but with a larger sample size of 245 samples. Madhavi used a protocol of OMS dose per 2 hours with a small sample size (n = 60), and the results were similar to the results of this study. Differences in failed induction criterias may also lead to a diversity of results. Factors such as infection, sweeping of the membrane, and amniotomy were also not controlled in this study.

The mean dose of misoprostol in the PV group is lower than the OMS group. This is due to the pharmacokinetics of misoprostol, which is different for each route of administration. Onset of action of oral misoprostol started at 8 minutes, a maximum half-life at 30 minutes, and a duration of 2 hours. When administered vaginally, the onset of action of misoprostol begins after 20 minutes of administration, the half-life of up to 70 minutes, and a duration of 4 hours. Therefore, vaginal misoprostol remains effective for a longer time and the total dose required for induction of labor is lower.[10]

This study did not observe any oxytocin acceleration incidence difference between the two groups. Acceleration oxytocin were indicated in 5 subjects (35.7%) in the OMS group and 6 (40%) in the PV group. Madhavi also obtain similar results. In the study by Madhavi, 26.7% of the subjects in the OMS group and 20% of the subjects in the PV group required oxytocin acceleration (p = 0.542).[8] Conversely, Cheng, et al. only found 10.9% of the subjects in the OMS group of CSOs who required oxytocin. This percentage is much less than 53.8% of the subjects in the PV group that required oxytocin acceleration (p = 0.01).[6] Another study by Ashalatha found that fewer oxytocin acceleration were indicated in the PV group (39%) compared to OMS (58.2%) group.[10]

In this study, all subjects in the PV intervention group is treated by spontaneous labor. Whereas in the OMS group, spontaneous labor occured in 12 subjects (85.7%), extraction forceps and cesarean section respectively in 1 subject (7.1%). Indication for forceps extraction is maternal cardio decompensation with a ventricular septal defect, so it is actually not related to the labor disruption at the second stage. Indication of cesarean section on the subject of this study is prolonged latent phase. There were no significant differences regarding the type of delivery between the OMS and PV groups according to Madhavi (p = 0.43).[8] Cheng, et al. also concluded that the incidence of uterine hyperstimulation and cesarean sections are lower when misoprostol is administered orally in titrated solution.[6]

The study found no differences in maternal outcomes between the two groups. Uterine hyperstimulation was found in one subjects with a diagnosis of $P_3 G_4 A_0$ 31 weeks pregnant with antepartum eclampsia, single live fetus and head presentation in the OMS group, which then reached the active phase. Uterine hyperstimulation was managed with fetal resuscitation, administration of nifedipine, and planned abdominal termination. But in the preparatory period of operation, the subject reached the second stage of labour. The fetus was born spontaneously with only 1400 grams birth weight and an Apgar score of 3/5/7. After undergoing intensive care in the NICU for 21 days, the baby's condition is healthy and stable. Nausea, vomiting, and shivering were quite observable symptoms which disappears after two hours of observation. Uterine hyperstimulation in the PV group was found in one subject a diagnosis of $G_1 P_0 A_0$, pregnant for 31 weeks, single live fetus with a head presentation + polyhydramnios + congenital abnormalities (there are findings of deformities of the heart, spinal deformity, single umbilical artery, echogenic bowel, hydrocele, ascites and claw hand) with a biophysical profile score of 8. When uterine hyperstimulation occured, the subject had reached 6 cm cervical dilatation and the fetus experienced tachycardia. Approximately 10 minutes after hyperstimulation of the uterus, the fetus is born spontaneously with 1 minute Apgar score of 1, and 5 minutes later the fetus died. The cause of perinatal death in this case is likely due to major congenital abnormalities. Madhavi only found 1 subject (3.3%) in the PV group who experienced uterine hyperstimulation and was treated by administration of terbutaline 250 mcg via subcutaneous injection. Misoprostol side effects such as nausea, vomiting, diarrhea, and fever were not found in the study by Madhavi.[8] Cheng et al. reported that oral and vaginal misoprostol are equally safe for the mother. Cheng reported misoprostol mild side effects such as nausea (10.9%), vomiting (8.9%), and diarrhea (5%) in the OMS group.[6]

In the OMS group, there were 6 cases of thick meconium staining. While in PV group, there were 5 cases of thick meconium staining. This incident may be triggered by the high-risk pregnancies, such as post-term pregnancy accompanied by oligohydramnios, premature rupture of membranes more than one day, and preeclampsia/ eclampsia. Madhavi reported 2 cases (6.7%) of thick meconium staining in the OMS group and 2 cases (6.7%) in the PV group. Both cases of thick meconium staining in the OMS group underwent cesarean sections, where the first case were caused by a non-reassuring CTG and the other cases by arrest of labor. In the PV group, two cases with thick meconium staining underwent cesarean section due to non-reassuring CTG. The four neonates in the study by Madhavi did not require NICU care and did not experienced respiratory failure syndrome.[8] The same was reported by Ashalatha, where the incidence of thick meconium staining is more common in oral compared to vaginal misoprostol.[9] In this study, we observed one neonate from the OMS group who needed NICU treatement, where the gestation age was 31 weeks, the fetus is born prematurely with low birth weight (1400 grams), and the newborn developed respiratory failure syndrome. While in

PV group, there are two neonates who required NICU care. One case was treated in the NICU because of a congenital heart defect, and the other due to respiratory failure syndrome.

There were no differences in the efficacy of oral titrated misoprostol solution and vaginal misoprostol in achieving inlabour ≤ 12 hours. Similar results were also obtained from the study by Madhavi. Madhavi concluded that administration of oral and vaginal misoprostol has the same effectiveness for labor induction.[8] Zvandasara et al. conducted a similar research at the University Hospital of Zimbabwe involving 69 pregnant women in the O|MS group and 65 pregnant women in the PV group. The study concluded that the effectiveness and safety of OMS is similar with PV for induction of labor, even in poor countries where intrapartum supervision is often inadequate. Subjects induced by OMS have a faster induction- initiation of uterine contraction interval (OR = 0.94; 95% CI = 0.42-2.12), but with a longer duration of labor (OR, 0.36; 95% CI 0,16- 0.79). Acceleration of oxytocin were more common in the OMS group.[11] In contrast, Cheng, et al. reported that oral titrated misoprostol may furtherly shorten the interval from induction to vaginal delivery compared to vaginal administration. In addition, the percentage of vaginal delivery ≤ 12 hours were more common in the OMS group compared to PV (p = 0.01, RR = 8.44 [4.52 to 15.76]). The percentage of vaginal delivery ≤ 24 hours are also more common in the OMS compared to PV; with a *p-value* = 0.01 and RR 13.61 (5.49 to 33.78). Failed induction is more common in PV group.[6]

This study is a double blind RCT-add on, thus ensuring the strength of the end results. Randomization in this study aims to create similar characteristics between groups. This study also conducted a double-blinding, where researchers and survey respondents did not know the status of the respondent whether they were included in the intervention or non-intervention group. The strength of this design can minimize confounding factors that may lead to bias in the results. Placebo used in this study is made of starch which is inert, does not have a pharmacological effect, and mimics the appearance, taste, and smell of misoprostol (Cytotex). The weakness of this study is the small sample size of just 30 people, thus resulting in the possibility of low precision. In addition, several confounding factors that can accelerate the onset of labor such as infection, sweeping of the membrane, and amniotomy were also not controlled in this study.

CONCLUSION

Oral titrated misoprostol in solution and vaginal misoprostol are equally effective for achieveing in labour within 12 hours. There was no difference in maternal and neonatal outcome of labour in both the groups.

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Research Article

The Risk of Infection Human Papilloma Virus Infection in Acceptors of Depot Medroxyprogesterone Acetate Contraceptions

Risiko Infeksi Human Papilloma Virus pada Akseptor Kontrasepsi Depot Medroxyprogesterone Acetat (DMPA)

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Abstract

Objective: Cervical cancer is the second most prevalent cancer in women around the world and the most common cancer in women causing death. This study aims to analyze the connection between infection of human papilloma virus (HPV) 16/18 and cervical changes in the acceptors of Depot Medroxyprogesterone Acetate (DMPA) Contraceptions and nonacceptors of Depot Medroxyprogesterone Acetate (DMPA) Contraceptions.

Methods: The research was conducted at the Public Service Institution of Dr. Wahidin Sudirohusodo hospital, and private midwife clinics for seven months from December 2015 to June 2016. The research design is cross-sectional with. The samples were forty acceptors of Depot Medroxyprogesterone Acetate (DMPA) and forty non-acceptors of Depot Medroxyprogesterone Acetate (DMPA) contraception. Prevalence of HPV 16/18 and cervical cytology changes were examine using the polymerase chain reaction and liquid base cervical cytology.

Results: The results showed there was no significant relationship between long-term use of DMPA contraceptives with HPV 16 and 18. There was no significant relationship between long-term use of DMPA contraceptives with cervical cytology changes. There was no significant relationship between HPV 16 and 18 infections with the occurrence of cervical cytology changes in long-term use of DMPA contraceptives.

Conclusion: The long-term use of DMPA contraceptive does not increase the risk of HPV 16 and 18 infections. Also does not cause cervical cytology changes that lead to cervical malignancy.

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Keywords: cervical cytology changes, Depot Medroxyprogesterone Acetate (DMPA) contraception, HPV 16/18 infection

Abstrak

***Tujuan**: Kanker serviks merupakan kanker kedua terbanyak pada wanita di seluruh dunia saat ini dan merupakan kanker terbanyak pada wanita yang menyebabkan kematian. Penelitian ini bertujuan mengetahui hubungan antara infeksi human papilloma virus 16/18 dan perubahan sitologi serviks pada akseptor kontrasepsi DMPA dibandingkan dengan non akseptor kontrasepsi DMPA.*

***Metode**: Penelitian ini menggunakan rancangan potong lintang. Penelitian ini dilakukan di BLU RS Dr. Wahidin Sudirohusodo, rumah sakit jejaring serta Bidan Praktek Swasta. Penelitian ini dilakukan selama 7 bulan, yaitu mulai bulan Desember 2015- Juni 2016. Sampel sebanyak 40 akseptor kontrasepsi DMPA dan 40 non akseptor kontrasepsi DMPA. Infeksi HPV 16/18 dan perubahan sitologi serviks diperiksa melalui polymerase chain reaction dan Sitologi Serviks Berbasis Cairan.*

***Hasil**: Hasil penelitian menunjukkan tidak terdapat hubungan bermakna antara penggunaan kontrasepsi DMPA jangka panjang dengan infeksi HPV 16 dan 18. Tidak terdapat hubungan bermakna antara penggunaan kontrasepsi DMPA jangka panjang dengan terjadinya perubahan sitologi serviks. Tidak terdapat hubungan bermakna antara infeksi HPV 16 dan 18 dengan terjadinya perubahan sitologi serviks pada penggunaan kontrasepsi DMPA jangka panjang.*

***Kesimpulan**: Penggunaan kontrasepsi DMPA jangka panjang tidak meningkatkan risiko terjadinya infeksi HPV 16 dan 18. Juga tidak menyebabkan terjadinnya perubahan sitologi serviks yang mengarah kepada keganasan serviks.*

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***Kata kunci**: infeksi HPV 16/18, kontrasepsi Depot Medroxyprogesterone Acetate (DMPA) Contraceptions, perubahan sitologi serviks*

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INTRODUCTION

Cervical cancer is the second most prevalent cancer in women around the world today and the most common cancer in women that causes the death of primarily young women. Based on the World Health Organization (WHO) statistical data, there are about 500,000 new cases and 250,000 deaths each year.[1] In general, higher incidences are found in developing countries, and these countries contribute 83% of reported cases annually. Economically advantaged countries have significantly lower cervical cancer rates and add only 3.6% of new cancers. This incidence disparity highlights successes achieved by cervical

cancer screening programs in which Papanicolaou (Pap) smears are regularly obtained. In 2006, the American Cancer Society estimated 9,710 new cases and 3,700 deaths from this malignancy.[2] Data from the Ministry of Health in Indonesia, cervical cancer and breast cancer still the highest prevalence among malignant gynecologic tumors. In 2013, the prevalence of cervical cancer was 0.8%, and breast cancer is 0.5%.

HPV 16 and HPV 18 because this is a high-risk HPV found in almost all cervical carcinoma. In the United States and Europe, HPV 16 is the most prevalent type found in approximately 50% of cases, while types 18, 31 and 45 found in approximately 25 - 30% of cases.[3]

Progesterone contained in the Combined Oral Contraceptives (COCs) causes depletion of squamous epithelium thus it is more susceptible to HPV infection.[4] The relationship between the use of DMPA contraceptive with HPV infection is debatable. Research says that epithelial atrophy becomes more susceptible to damage, so it becomes more susceptible to HPV infection. This may explain that DMPA is positively associated with oncogenic HPV infection. We found that the use of DMPA results in depletion of the epithelium.[5]

Progesterone is previously considered as a major candidate hormone in the cervical neoplastic caused by the immunosuppressive effects and the likely associated with HPV infection. Human papilloma virus tends to infect the cells with progesterone receptors. Both HPV 16 and 18 contain progesterone and glucocorticoid response elements that increase the expression of oncogenic HPV E6 and E7, which are considered crucial in the transformation of cells with the gestagenic stimuli.[6]

By knowing HPV as the primary cause of cervical cancer, there is new development for the detection of HPV as screening for uterine cervical carcinoma. Therefore, HPV cannot be grown in culture, the HPV DNA testing with the methods of molecular biology is an accurate way to detect HPV infection and HPV typing by PCR (Polymerase Chain Reaction). In addition, early detection of cervical cytological changes in contraceptive DMPA acceptors is also important for the change in cervical cytology is a risk factor for HPV infection. For a screening of cervical cytological changes, the most commonly used today is conventional Pap smear. However, the conventional Pap smear has the limitations, i.e., the false negative rate of 14 - 33% and two-thirds are caused by the process of sampling or sample preparation. This leads to inaccurate and equivocal diagnosis. Currently, the major advanced screening technique is liquid-based cytology. The transition from conventional cytology to liquid-based cytology techniques is due to the increased sample quality, reproducibility, sensitivity, and specificity similar to molecular testing.[7]

METHOD

This research was conducted at several hospitals in Makassar, health centers and in Private Practice Midwife (BPS) Hj. Markarmah. Preparations that have been taken from the research subjects were sent to the Laboratory of Anatomical Pathology for liquid-based cervical cytology and the Laboratory of Microbiology for PCR. The research began in December 2015 - June 2016.

This was a cross-sectional study. The population in this research was contraceptive DMPA acceptors, and controls were those who did not receive DMPA acceptors. The sample was injected contraceptive DMPA acceptors, and the control group were women who did not use progestin injection hormonal contraceptive and met the inclusion criteria and have signed the informed consent to participate in the study.

All subjects who met the inclusion criteria were taken in accordance with the estimated sample size. Two examinations, i.e., PCR and liquid-based cytology with LC-Prep, were done on the subjects. Test results were recorded and then analyzed.

The data that have been collected and analyzed were then processed by computer using SPSS for windows. The statistical method used was Bivariate Analysis Chi-square test and Fisher's exact.

RESULTS

The basic characteristics of the samples showed that of the 80 research subjects involved, the largest age group was 31 - 45 years old (57.5%) with education level of more than nine years (71.25%). Employment status of the research subject was generally work (56.25%). The age at the first coitus was generally 20 - 25 years

(73.75%) with multiparity (63.75%). The length of contraceptive use was over three years (52.5%) with complaints of menstrual disorders (47.5%) and a complaint of menstrual disorders with vaginal discharge (50%). The research subjects using contraceptive DMPA were mostly aged 31 - 45 years (60%), with education level of more than nine years (55%). Employment status was not working (55%). All contraceptive DMPA acceptors were already married and the age at the first coitus was 20 - 25 years (77.5%) with multiparity (80%). The length of contraceptive use was mostly more than three years (52.5%) with the majority of complaints of menstrual disorders (97.5%). Based on the results of Chi-square test and Fisher's exact, the homogeneous characteristics of the research subjects obtained were age group, education, occupation, marital status, age at the first coitus, duration of contraceptive use and the type of complaint (p> 0.05). (Appendix, Table 1).

HPV 16 infection based on PCR showed that the research subjects who used contraceptive DMPA had positive for HPV 16 of 1 people (2.5%) and negative for HPV 16 of 39 people (97.5%). The research subjects who did not use contraceptive DMPA all had negative for HPV 16 of 40 people (100%) (Appendix, Table 2).

Table 1. Characteristics of the Subjects

Characteristics	DMPA Contraceptive					
	Yes		No		Total	
	n	%	n	%	n	%
Age (year)						
20 - 30	16	40	18	45	34	42.5
31 - 45	24	60	22	55	46	57.7
Education Level						
≤ 9 year	22	55	1	2.5	23	28.75
> 9 year	18	45	39	97.5	57	71.25
Employment						
Unemployment	18	45	27	67.5	45	56.25
Employment	22	55	13	32.5	35	43.75
Age first time coitus (year)						
20 - 25	31	77.5	28	70	59	73.75
> 25	9	22.5	12	30	21	26.25
Parity						
Nulli/Primipara	8	20	21	52.5	29	36.25
Multipara	32	80	19	47.5	51	63.75
Duration contraceptive use (year)						
≤ 3	19	47.5				
> 3	21	52.5				
Complain						
No complain	1	2.5				
Menstrual disorder	19	47.5				
Menstrual disorder and flor albus	20	50				

Table 2. HPV 16 Infections

Subject	HPV 16						p
	Positive		Negative		Total		
	n	%	n	%	n	%	
Contraceptive DMPA acceptors	1	2.5	39	97.5	40	100	
Non Contraceptive DMPA acceptors	0	0	40	100	40	100	1.000
Total	1	1.2	79	98.8	80	100	

HPV 18 infection based on PCR showed that the research subjects who used contraceptive DMPA had positive for HPV 18 of 1 people (2.5%) and negative for HPV 18 of 39 people (97.5%). The research subjects who did not use contraceptive DMPA all had negative for PCR 18 of 40 people (100%). Fisher's exact test results obtained by value p = 1.000 (p> 0.05). It means there is no relationship between hormonal contraceptive use in combination with the incidence of HPV 18 infection (Appendix, Table 3).

Cervical cytology based on liquid-based cervical cytology examination showed that the research subjects who used contraceptive DMPA found eight people who experienced cervical cytological changes (20%). While for non-contraceptive DMPA acceptors, there were six people who experienced cervical cytological changes. Most of the samples from both groups that did not experience changes in cervical cytology were 66 people (82.5%) (Appendix, Table 4).

DISCUSSION

This study shows that the use of contraceptive DMPA has not been associated with HPV 16/18 infection and cervical cytological changes. This study is conducted on contraceptive DMPA acceptors for this contraceptive type is one risk factor for cervical carcinoma with HPV infection.

In this study, we examined HPV 16 and HPV 18 because this is a high-risk HPV found in almost all cervical carcinoma. In the United States and Europe, HPV 16 is the most prevalent type found in approximately 50% of cases, while types 18, 31 and 45 found in approximately 25 - 30% of cases.[3]

Based on the results of Chi-square test and Fisher's exact, the homogeneous characteristics of the research subjects obtained were age group, education, occupation, marital status, age at the first coitus, duration of contraceptive use and the type of complaint (p> 0.05). Most of the research subjects had multiparity and used contraceptive DMPA.

To determine the effect of contraceptive DMPA to the HPV infection clearly, the risk factors that may increase HPV infections such as first sexual intercourse before age 20 years, parity more than four, having multiple sexual partners and smoking habits are excluded.

In this study, 80 subjects of the research found one case of HPV 16 infection based on PCR on contraceptive DMPA acceptor group with incidence rates of 1.2% and one case of HPV 18 infection with the incidence rate of 1.2%. There was no HPV infection in the control group. After Fisher's exact test to determine the relationship between contraceptive DMPA with HPV 16 and HPV 18 obtained results that were not statistically significant. This is consistent with research conducted by Morgan *et al*., which conducted follow up to 1,135 women (376 COC acceptors, 331 DMPA acceptors, and 428 non- contraceptive acceptors) for 18 months. They found new HPV infection in 269

Table 3. HPV 18 Infections

Subject	HPV 18						p
	Positive		Negative		Total		
	n	%	n	%	n	%	
Contraceptive DMPA acceptors	1	2.5	39	97.5	40	100	
Non Contraceptive DMPA acceptors	0	0	40	100	40	100	1.000
Total	1	1.2	79	100	80	100	

Table 4. Cervical Cytological Changes

Subject	cervical cytological changes						p
	Positive		Negative		Total		
	n	%	n	%	n	%	
Contraceptive DMPA acceptors	8	20	32	80	40	100	
Non Contraceptive DMPA acceptors	6	155	34	85	40	100	0.77
Total	14	17.5	66	82.5	80	100	

women and high-risk HPV infection in 157 women. However, after adjusting for age, number of sexual partners, new sexual partner, bacterial vaginosis infection and duration of the use of COCs and DMPA, the relation between the detection of new HPV infection with the use of COCs reduced even not statistically significant.[4]

The use of hormonal contraceptives such as depomedroxyprogesterone acetate (DMPA) has been associated with an increased risk of cervical cancer and is considered as a co-factor in cervical carcinogenesis. The increased risk of disease is observed in women who use hormonal contraceptives of long-term progesterone injection such as DMPA. Progesterone contained in the Combined Oral Contraceptives (COCs) causes depletion of squamous epithelium thus it is more susceptible to HPV infection.[4] Based on the research results, Tiffany G *et al.*, stated that use of DMPA within one year or more is related significantly to the detection of oncogenic HPV.[5]

Progestin contained in contraceptive DMPA can affect cervical cytology. The relationship between the use of DMPA contraceptive with HPV infection is debatable. Research says that epithelial atrophy becomes more susceptible to damage, so it becomes more susceptible to HPV infection. This may explain that DMPA is positively associated with oncogenic HPV infection. We found that the use of DMPA results in depletion of the epithelium.[5]

Progesterone is previously considered as a major candidate hormone in the cervical neoplastic caused by the immunosuppressive effects and the likely associated with HPV infection. Human papilloma virus tends to infect the cells with progesterone receptors. Both HPV 16 and 18 contain progesterone and glucocorticoid response elements that increase the expression of oncogenic HPV E6 and E7, which are considered crucial in the transformation of cells with the gestagenic stimuli.[6]

In this study, of the 80 research subjects found two cases of HPV infection, each infection with HPV 16 and HPV 18 in contraceptive DMPA acceptors. On the samples with positive HPV 16 infection found cervical cytological changes and on the samples with positive HPV 18 infection also found cervical cytological changes in the form of endocervical cells undergoing squamous metaplasia and found halo-perinuclear representation. Based on Fisher's exact test results, there was no association between HPV 16/18 infection with cervical cytological changes in the contraceptive DMPA acceptors.

Normal cervical cytology can be found in the latent phase of virus infection. In these circumstances, there are no lesions, but there is exposure to the virus without causing infection. In this phase, the virus cannot attach to the surface of cells or penetrate cells since there are no or less cell surface receptors specific for HPV. It can also occur in cases where the virus has entered the cell but has failed to do multiplication or no maturation of viral particles. In this phase, the HPV virus can only be detected with bio-molecular methods.

Genital HPV infection is very common, mostly asymptomatic, does not cause a change in the tissue and therefore is not detected on Pap smear. The prevalence of HPV in cervical cytology in women with normal Pap smears peaked at the age of 20 - 24 years.

Not all women infected with human papillomavirus (HPV) 16/18 have detectable levels of anti HPV-16/18 antibodies. Women who seroconvert develop low antibody levels and seroconversion occurs within months and varies among women. The slow and weak antibody response generated by HPV infections could be explained by its life-cycle in the host. HPV is shed within intact cells lining mucosal surfaces, which limits exposure of the host immune system to the virus. HPV infected cells that undergo lysis (i.e., koilocytes) are shed to the environment and infections do not produce viremia. Finally, infections produce a limited load of HPV antigenic proteins.[8]

Cervical cytological changes by HPV infection are also associated with higher levels of viral load in the host and the type of HPV. Previous studies conducted on cervical cell of women with cervical lesions (cancerous or pre-cancerous lesions) found that the average level of viral load is higher in women who are positive HPV.

This study has advantage and disadvantage. The advantage of this study is that confounding factors which can lead to a high incidence of HPV infection, primarily high-risk HPV such as age at the first coitus, parity, and number of sexual partners, are removed. This is done so that the effect of contraceptive DMPA against HPV infection,

primarily high-risk HPV, can be seen clearly. However, HPV DNA bio-molecular examination in this study is devoted to the high-risk HPV types 16/18 so that other high-risk HPV types cannot be detected.

CONCLUSION

The researcher concludes that out of 80 samples found HPV 16 infection of 1.2% and HPV 18 infection of 1.2%. The role of contraceptive DMPA against HPV 16/18 infection and cervical cytological changes cannot be proven through PCR and cervical cytology examination. In addition, the relationship between HPV 16/18 infection with cervical cytology changes in hormonal contraceptive DMPA acceptors has also not been proven. Despite of this study found no relationship between duration of use of contraceptive DMPA with HPV infection and cervical cytological changes, the two samples infected with HPV 16/18 are found in contraceptive DMPA acceptor groups that are also experiencing cervical cytological changes. The researcher suggests that further research on HPV types 16/18 and other HPV types of hormonal contraceptive DMPA acceptors is required. Further research with greater samples to determine the prevalence of HPV infection and cervical cytology changes in contraceptive DMPA users is also required.

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Research Article

The Role of Matrix Metalloproteinase-2 (MMP-2) in Serum and Peritoneal Fluid of Endometriotic Patients

Peran Matriks Metalloproteinase-2 (MMP-2) Serum dan Cairan Peritoneum pada Pasien Endometriosis

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Abstract

Objective: To determine the role of matrix metalloproteinase-2 (MMP-2) in serum and peritoneal fluid of endometriotic patients.

Methods: Research's design using cross-sectional method in Dr. Wahidin Sudirohusodo hospital and several other hospitals in Makassar within May 2015 until May 2016. Subjects were chosen using consecutive sampling technique. The examination using the ELISA method. The data were analysed using Fisher exact, t-independent, Mann-Whitney, and Spearman association.

Results: A total of 50 subjects were recruited in this study. Mostly the value of MMP-2 serum and peritoneal fluid in endometriosis group was higher compare to study control. There was significant different between the total of MMP-2 serum and peritoneal fluid. There was also a significant association between the value of MMP-2 serum and peritoneal fluid with endometriosis.

Conclusion: The value of MMP-2 serum and peritoneal fluid were higher in endometriotic patients compared to those without endometriosis. The higher the value of MMP-2 serum and peritoneal fluid, the higher the stage of endometriosis.

[Indones J Obstet Gynecol 2018; 6-2: 104-109]

Keywords: endometriosis, matrix metalloproteinase-2, MMP-2

Abstrak

***Tujuan**: Menentukan peran matriks metalloproteinase-2 (MMP-2) pada serum dan cairan peritoneum pasien endometriosis.*

***Metode**: Desain penelitan dengan metode potong lintang dilakukan di RS Dr. Wahidin Sudirohusodo dan jejaringnya di Makassar pada Mei 2015 sampai Mei 2016. Pemilihan subjek melalui teknik consecutive sampling. Pemeriksaan menggunakan metode ELISA. Analisis data menggunakan uji statistik Fisher exact, t independen, Mann-Whitney, dan uji korelasi Spearman.*

***Hasil**: Sejumlah 50 subjek direkrut pada studi ini. Rerata kadar MMP-2 serum dan cairan peritoneum kelompok endometriosis lebih tinggi dibandingkan kontrol (p<0,05). Terdapat perbedaan bermakna rerata antara kadar MMP-2 serum stadium III-IV dengan stadium I-II tetapi pada cairan peritoneum tidak bermakna. Kadar MMP-2 serum dan cairan peritoneum berkorelasi signifikan dengan kejadian endometriosis.*

***Kesimpulan**: Kadar MMP-2 serum dan cairan peritoneum lebih tinggi pada pasien endometriosis dibandingkan dengan pasien tanpa endometriosis. Semakin tinggi kadar MMP-2 serum dan cairan endometrium, semakin berat pula derajat endometriosis.*

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***Kata kunci**: endometriosis, matrix metalloproteinase-2, MMP-2*

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INTRODUCTION

Endometriosis is a benign gynecological disorder characterised by the presence of endometrial glands and stroma cells that grows outside the uterine cavity and is associated with pelvic pain and infertility. Ectopic endometrial tissue normally found in the pelvic cavity, but can also exist in all parts of the body. Signs and symptoms of endometriosis vary, tend to be progressive and recurrent and often creates difficulties for women and doctors.[1]

Until now, the aetiology and pathogenesis of endometriosis are not known with certainty. Endometriosis is found in about 3-10% of the female population of reproductive age. The incidence of endometriosis in women with dysmenorrheal is 60-80%, 30-50% of women with complaints of abdominal pain, and 30-40 women with infertility. This incidence is high enough to put endometriosis as one of the main reproductive problems nowaday. Additionally, the pathophysiology and the impact of clinical disorders caused by endometriosis and its management is still not as expected.[2] There is 63.6% incidence of endometriosis of infertility cases. At the Dr. Soetomo General Hospital obtained a trend of increased incidence of

endometriosis with 23% in 1980, 37% in 1990 and reached 50% in 2002.[3]

The cause of endometriosis has yet known with certainty. There are several theories put forward about the cause of endometriosis, one of the most common theories of the cause of endometriosis is regurgitation of menstrual blood, which allows the deployment of endometrial cells into the peritoneal cavity. Peritoneal endometriosis, which is the most common form of endometriosis occurs due to increase of metalloproteinases lesions. Red endometriosis is the most active type of endometriosis. In this type, there is increasing activity of angiogenesis which result in increase of blood flow in the endometriosis lesions. It also explains the high incidence of menstrual pain. Macrophages and other immuno competent cells surrounding the endometriosis lesions and issuing various types of cytokines catabolic intended to cause chronic inflammatory reactions and stimulate the formation of fibrotic tissue.[4]

The process of angiogenesis in excess endometrial tissue suspected as an important mechanism in the pathogenesis of endometriosis. The process of angiogenesis in addition to a role in the onset of endometriosis also plays a role in implantation, tissue remodeling and the process of metastasis.[2] Lately, research on endometriosis involving elements of peritoneal fluid and the process of angiogenesis open many new horizons of the pathogenesis of endometriosis, where the involvement of autocrine and paracrine factors thought to play a major role.[5]

In addition to VEGF, transforming growth factor-beta 1 (TGF-β1) and soluble endoglin; matrix metalloproteinase (MMP) is also widely studied as an angiogenesis factor in the occurrence of endometriosis. In a normal menstrual cycle, i.e. the inhibitors of MMPs and tissue inhibitors of matrix metalloproteinases (TIMPs), play a significant role in the remodelling of the endometrial tissue. MMP also plays an important role in physiological and pathological processes in humans which are embryogenesis, angiogenesis, wound healing and metastases.[6]

A previous study by Salata, suggests the approximate concentration of serum MMP-2, MMP-9, TIMP-1 and TIMP-2 did not reflect the severity of endometriosis, although MMP activity increased in the peritoneal fluid of women with endometriosis.[7]

Further research by Weigel *et al.*, reported there is a difference between MMP-2, MMP-9 and PCNA in stages of endometriosis and endometrial cancer.[8] Research by Jana *et al.* found MMP-2 plays an important role in pathological angiogenesis, and an imbalance ratio of MMP-2 and TIMP-2 are thought to modulate angiogenesis during the early development of endometriosis. These markers may assist in the evaluation of aggressiveness and invasive ability of endometriosis at different locations. The results obtained could explain the pathogenesis of endometriosis and the development of endometriosis therapy.[9]

Research on the role of MMP-2 as a marker of angiogenesis in endometriosis using serum and peritoneal fluid has never yet been studied in South Sulawesi. Therefore, the authors are interested in conducting research that aims to determine the relationship between levels of MMP-2 in serum and peritoneal fluid and the development of endometriosis.

METHOD

The study was conducted at Dr. Wahidin Sudirohusodo Hospital, Makassar and other networking Hospitals Department of Obstetrics and Gynecology, Faculty of Medicine, Universitas Hasanuddin in Makassar began in May 2015 until May 2016.

The method used is observational with a cross-sectional design. The study population was all patients who underwent laparoscopic in Dr. Wahidin Sudirohusodo Hospital, Makassar and other networking Hospitals Department of Obstetrics and Gynecology, Faculty of Medicine, Universitas Hasanuddin in Makassar. Samples are all patients with endometriosis that underwent laparoscopic with various operating indications that met the inclusion criteria and had signed a letter of approval. The sample in this study amounted to 29 women with endometriosis and 21 controls.

Doing anamnesis towards mothers willing to participate in this study and have filled the approval, noted on the questionnaire sheet. Recording the identity of the patient including their name, age, parity and the first day of the last menstrual period. Before laparoscopy performed blood sample for examination of serum MMP-2. At the time of laparoscopy peritoneal fluid is taken for examination of peritoneal fluid MMP-2. Laboratory

tests for the levels of MMP-2 serum and peritoneal fluid is carried out in the Laboratory of Microbiology Teaching Hospital Universitas Hasanuddin, Makassar.

To determine differences in the levels of MMP-2 well in the control group as well as in endometriosis used Fisher's exact test, Mann Whitney, independent T, and Spearman association. The data were processed using SPSS 18. Then the data are presented in tabular form with a p value less than 0.05 was considered significantly available.

RESULTS

Research has been conducted with cross-sectional design to determine the relationship between levels of MMP-2 in serum and peritoneal fluid with the incidence of endometriosis. The study was conducted in public service agency of Dr. Wahidin Sudirohusodo, Hospital Makassar and networking Hospital Department of Obstetrics and Gynecology, Faculty of Medicine, Universitas Hasanuddin in Makassar began in May 2015 until May 2016.

Measurement of MMP-2 levels in serum and peritoneal fluid were conducted on 29 women with endometriosis and 21 controls (without endometriosis). Fisher test results on the characteristics of this sample showed that there were significant differences between infertility and dysmenorrhea for both groups, but other characteristics did not differ significantly (p> 0.05) (Table 1).

The results of measurements of serum level of MMP-2 in both study groups showed a mean serum levels of MMP-2 of 6.03 ± 3.26 ng/ml in the endometriosis group and 1.49 ± 0.68 ng/ml in controls. Serum levels of the MMP-2 endometriosis group were higher than the control, and there is a significant difference (p = 0.000) between the two groups. The mean levels of MMP-2 were also higher peritoneal fluid in endometriosis than in controls (1.98 ± 1.61 ng/ml vs 0.61 ± 0.12 ng/ml), and significantly different for the two groups (p = 0.036) (Table 2).

Table 1. Respondent Characteristic

Respondent Characteristic	Endometriosis (n=29) n(%)	Control (n=21) n(%)	p
Age (year)			
20-35	24 (82.8)	14 (66.7)	0.189
36-45	5 (17.2)	7 (33.3)	
Marital status			
Married	26 (89.7)	17 (81.0)	0.434
Not married	3 (10.3)	4 (19.0)	
Body Mass Index (kg/m^2)			
Normal	25 (86.2)	20 (95.2)	0.383
Overweight	4 (13.8)	1 (4.8)	
Infertility			
Primer	16 (55.2)	8 (38.1)	0.009
Secondary	6 (20.7)	0	
Not infertile	7 (24.1)	13 (61.9)	
Dysmenorrhea			
Yes	27 (93.1)	3 (14.3)	0.000
No	2 (6.9)	18 (85.7)	
Contraception			
Yes	3 (10.3)	7 (33.3)	0.073
No	26 (89.7)	14 (66.7)	

Table 2. MMP-2 Level in the Study Sample

Sample	MMP-2 serum level (Mean ± DS ng/ml)	p*	MMP-2 peritoneal fluid level (Mean ± DS ng/ml)	p**
Endometriosis (n=29)	6.03 ± 3.26	0.000	1.98 ± 1.61	0.036
Control (n=21)	1.49 ± 0.68		0.61 ± 0.12	

**Independent T-test*
***Mann-Whitney test*

Table 3. MMP-2 Level in Endometriosis Stage

Endometrosis Stage	n	MMP-2 serum level (Mean ± DS ng/ml)	p*	MMP-2 peritoneal fluid level (Mean ± DS ng/ml)	p**
I-II	10	3.35 ± 1.41	0.000	1.17 ± 1.01	0.119
III-IV	19	7.45 ± 3.06		2.41 ± 1.72	

**Independent T-test*
***Mann-Whitney test*

The levels of MMP-2 on the stage of endometriosis showed the average serum levels of MMP-2 in stage I-II, 3.35 ± 1.41 ng/ml and 7.45 ± 3.06 ng/ml in stage III-IV. The mean serum levels of MMP-2 in stage III-IV is higher than stage I-II, and there is a significant difference between the two stages (p = 0.000). In peritoneal fluid, the mean levels of MMP-2 did not differ significantly between the two stages endometriosis' (p> 0.05) (Table 3).

Association test results showed there is a strong relationship and meaning between the levels of MMP-2 serum and endometriosis (r = 0.874; p = 0.000), as well as the levels of MMP-2 peritoneal fluid and endometriosis (r = 0.383; p = 0.006) (Table 4).

Table 4. The Association between MMP-2 dan Endometriosis Test Result

MMP-2 Level	Endometriosis	
	r	p
Serum	0.874	0.000
Peritoneal fluid	0.383	0.006

DISCUSSION

This study shows that infertility and dysmenorrheal have an effect on endometriosis than age, marital status, BMI and contraception. Endometriosis affects 10% of women of reproductive age. Clinical symptoms are dysmenorrhea, dyspareunia and pelvic pain, making women's health worsens. Patients with endometriosis often accompanied by symptoms of dysmenorrhea and infertility complaint. The complaint is the primary reason the patient to see a doctor.

Research proves infertility due to endometriosis also increased. Hadisaputra Research found that infertility is closely related to endometriosis. Approximately 20-40% of infertile women have endometriosis. The overall prevalence of patients with endometriosis in infertile women is higher compared with fertile women. In moderate to severe endometriosis, a higher ratio of infertile patients than fertile patients.[3,10]

The average age of diagnosis of endometriosis is between 25 to 35 years.[1] As shown in this study, the most established was in the age range of 20-35 years. Another study by Abdullah and Manuaba found that the mean age for each case is 32 years old and 34 years old. Although the onset can occur during adolescence, detection is often done too late. Peak prevalence of endometriosis is found, at age 40, but the risk of a woman suffering from endometriosis is 30-34 years (RR = 2.1), 35-39 years (RR = 4.4) and 40-44 years (RR = 6.1) at the age range 25-29 years.[2,5] Increasing age in women affects fecundity and lowers fertility through distortion of adnexal anatomy and overproduction of prostaglandins, metalloproteinases, cytokines and chemokines cause chronic inflammation that disrupts ovarian function, tubal or endometrial so that an interruption in the process of folliculogenesis, fertilisation or implantation.[1,10]

Although some studies suggest that obesity is associated with the incidence of endometriosis but in this study only obtained five patients with a BMI> 25. In the study by Manuaba in Makassar found that normal body mass index was also obtained in most cases of endometriosis.[5]

Research by Vercellini *et al.* showed that there is decreased in the incidence risk of endometriosis in women who are new to the contraceptive pills but increased in women who had used the contraceptive pills.[11] Research by Chapron *et al.* shows there is no relationship between the new user to use contraceptive pills with endometriosis. This study also indicates that the history of the use of contraceptive pills for primary dysmenorrhea related diagnosis endometriosis surgery at a later date, especially on deep infiltrating endometriosis. However, this does not mean that the use of contraceptive pills increases the risk of development of endometriosis. Contraceptive pill usage history can serve as a marker for women with endometriosis and deep infiltrating endometriosis while IUD use of the user's current (0-12 months) than former users (49-72 months), will increase the risk of endometriosis in former users.[12] Other research by Rambulangi *et al.*, shows the levels of MMP-2 serum and peritoneal fluid of women endometriosis is higher than the control. In this study, also obtained the levels of MMP-2 in serum and peritoneal higher in endometriosis than in controls.[13]

Elevated levels of MMP-2 allegedly due to the stimulation by inflammatory factors and activation MT1 MMP. This is supported by several studies showing an increase in MMP-2 along with some marker that is associated with inflammation and angiogenesis such as IL-6, TGF-β, TNF-α, MIF and VEGF.[14] This study also shows the levels of MMP-2 serum and peritoneal fluid of endometriosis is higher in women with stage III-IV than stage I-II, although the peritoneal fluid did not differ significantly. Association test results showed there is a strong association between high levels of MMP-2 serum and peritoneal fluid with endometriosis that may indicate the involvement of MMP-2 in the pathogenesis of endometriosis. The results are consistent with research Malvezzi *et al.*, also found elevated levels of MMP-2 serum of infertile women with stage III-IV higher than stage I-II.[15]

Pathogenesis of endometriosis regarding attachment scheme-aggression-angiogenesis (AAA) has been accepted. In this process, the role of MMP uncontested. MMP can degrade collagen and extracellular matrix components. The formation of the ectopic endometrial tissue is mediated by factors that facilitate adhesion to the peritoneal cavity, cell growth, increased aromatase activity, angiogenic, neurogenic/lymphogenic factors, and reinforced by the activity of MMP. MMP-2 plays an important role in the adhesion and proliferation of loose menstrual tissue as the pathogenesis of endometriosis. Increased circulation of MMP-2 in patients with severe pelvic endometriosis through the mechanism of major tissue remodeling happened due to disease progression, as a result of increased activity of MMP-2 systemic and local, as well as the high activity of ectopic aromatase tissues can increase the production of MMP-2, which can facilitate the invasion and progression of the disease, so that MMP-2 correlated with increasing degrees of endometriosis.[15]

CONCLUSIONS

The researchers concluded that there is a significant increase in the levels of both-2 serum and peritoneal fluid in patients with endometriosis compared to women who did not suffer from endometriosis. Also found the levels of MMP-2 were higher in severe endometriosis compared with mild endometriosis, but the peritoneal fluid has not found significant differences. The levels of MMP-2 serum or peritoneal fluid is significantly correlated with the incidence of endometriosis.

RECOMMENDATIONS

Researchers suggested that to do further study on MMP-2 and other endometriosis markers in determining the diagnosis and therapy development of endometriosis.

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Research Article

Methylation Profile of HOXA 11 Gene in Eutopic Endometrium on Endometriosis Patient with Infertility

Profil Metilasi Gen Hoxa 11 pada Endometrium Eutopik Pasien Endometriosis dengan Infertilitas

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Abstract

Objective: To investigate the HOXA11 gene profile on endometriosis patients with infertility in Indonesia.

Methods: This cross sectional study was conducted in Dr. Cipto Mangunkusumo Hospital from July 2015- June 2016. The subjects were endometriosis patients with infertility who have been confirmed histopathological. The control group was taken from non-endometriosis and fertile patients. Eutopic endometrium samples were taken and examined for the methylation of HOXA 11 gene.

Results: Both groups consist of six patients. The difference of methylation of HOXA 11 gene between those two groups is statistically significant (p=0.03). There was hyper methylation in endometriosis group.

Conclusion: There is a hyper methylation of HOXA 11 gene in eutopic endometrium of endometriosis patients with infertility. Thus, possibly can explain the poor endometrial receptivity in endometriosis patient and give a broad research area in epigenetic therapy of endometriosis.

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Keywords: endometriosis, epigenetic, HOXA 11, infertility, methylation

Abstrak

***Tujuan**: Teori epigenetik yang berkembang adalah terjadi hipermetilasi pada gen HOXA 11 sehingga terjadi penurunan ekspresi gen tersebut.*

***Metode**: Penelitian potong lintang ini dilakukan di RS Dr. Cipto Mangunkusumo pada Juli 2015 - Juni 2016. Subjek penelitian adalah pasien endometriosis yang terbukti secara histopatologi dengan infertilitas dan kelompok kontrol merupakan pasien non-endometriosis yang fertil. Status metilasi gen HOXA 11 dari sampel endometrium eutopik pada kedua kelompok ini diperiksa dan dibandingkan.*

***Hasil**: Enam pasien endometriosis dan enam pasien kontrol diambil sebagai subjek. Perbedaan tingkat metilasi gen HOXA 11 pada kedua kelompok ini berbeda secara signifikan dengan nilai p = 0,03 dengan perbedaan rerata peningkatan kadar metilasi pada kelompok pasien endometriosis sebesar 33%.*

***Kesimpulan**: Gen HOXA 11 yang berperan dalam reseptivitas endometrium mengalami hipermetilasi pada pasien dengan endometriosis dan infertilitas.*

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***Kata kunci**: endometriosis, epigenetik, hipermetilasi, HOXA11, infertilitas*

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INTRODUCTION

Endometriosis is a chronic recurrent disease which impacts to pain and infertility. A tissue like endometrium consisting of stroma and glands arising outside endometrium become the pathophysiology of this disease.[1] Endometriosis and infertility has a clinical association.[2] Previous theories have explained the association between endometriosis and infertility.[3] Latest study showed that there were 25-50% of infertile women suffering from endometriosis and about 30-50% endometriosis women having infertility.[4]

Numerous studies attempted to search the causal-effect relationship between endometriosis and infertility; however, the cause was still controversial. A current concept to estimate this relation is that endometriosis is a part of epigenetic disorder. Several studies conducted to determine the role of epigenetic factor on endometriosis patients with infertility because it caused poor effect to the endometrium receptivity.[1,5,6] Epigenetic means a branch of science focusing on the change of genetic expression on phenotype without any alteration on DNA sequence. This change contributes to variation of pathological symptoms.

Epigenetic regulation consists of DNA methylation or his tone modification.[7] Every modification of epigenetic is reversible and dynamic. There are several factors influencing DNA methylation such as environment, stress, and lifestyle. Dietary habit can also affect to epigenetic modification e.g. folate deficiency on neural tube defect because folate has a role in DNA methylation reaction.[8]

Several studies stated that there was hyper methylation on gene promotor, for example HOXA10 and HOXA11 gene, causing low expression of the gene. HOX gene is a progesterone target having dysregulation on endometriosis; never the less, this gene is essential in endometrium res-ponse to progesterone during decidualization and implantation. The progesterone resistance explained poor support of implantation and failure of treatment on endometriosis.[9]

The study of epigenetic on endometriosis has important role to diagnosis, management, and prognosis in future. Unfortunately, there is still no available data in Indonesia about the profile of methylation on HOXA11 gene. Therefore, this study aims to find out the HOXA11 gene profile on endometriosis patients with infertility in Indonesia.

METHODS

This was a cross sectional study to determine the difference on profile of methylation on HOXA11 gene on endometriosis patients with infertility and control group. This study was carried out at Dr. Cipto Mangunkusumo hospital from July 2015 to 2016 involving all endometriosis patients with infertility undergoing treatment.

The inclusion for cases group was 20-35-year-old women diagnosed endometriosis and confirmed by surgery and histopathology, having married and diagnosed infertility, having regular menstruation cycle between 21 and 35 days, and agreed to participate in this study. Meanwhile, for control cases, we recruited 20-35-year-old married women, having history of pregnancy and delivery, not diagnosed as infertility, having regular menstruation cycle between 21 and 35 days, not having dysmenorrhea, pelvic pain, dysuria or pain on defecation, pain during sexual intercourse, having normal gynecology result on ultrasound, Ca-125 result less than 19 U/ml, and agreed to participate in this study.

We excluded all women having endometrial cancer, ovary cancer, gastric cancer, leukemic, having endometritis, history of ectopic pregnancy, ongoing pregnancy, contraceptive user since last 6 months, having tubal occlusion or abnormal sperm analysis result on her couple. The data would be dropped out whether resigning from this study, the broken tissue to be difficult in analysing, and histopathological result on sample not endometriosis.

The subjects were taken by consecutive sampling with 6 subjects each for the minimal number of samples. Data were analysed through SPSS Statistics version 22 on IBM software. The analysis on methylation on HOXA11 gene in eutopic endometrium between endometriosis and control group was performed through independent t-test. This study has been approved by Ethical Committee on Dr. Cipto Mangunkusumo Hospital/ Faculty of Medicine Universitas Indonesia under number 757/UN2.F1/ETIK/2015.

RESULTS

There were 12 subjects consisting 6 subjects each on endometriosis and control group as inclusion and exclusion criteria. The characteristic data on each subject was shown at Table 1.

Table 1. The Subjects Characteristic Participating in this Study

Subjects	Age (years old)	Diagnosis	Fertility Status	Surgery	AFS Scoring
E1	35	Bilateral endometriosis cyst	P1, Secondary infertility for 8 years	Cystectomy laparoscopy, chromotubation, and adhesiolysis	IV
E2	27	Left ovary endometriosis cyst	Primary infertility for 3 years	Cystectomy laparoscopy and chromotubation	III
E3	35	Left ovary endometriosis cyst	Primary infertility for 3 years	Cystectomy laparoscopy and chromotubation	III

Subjects	Age (years old)	Diagnosis	Fertility Status	Surgery	AFS Scoring
E4	32	Bilateral endometriosis cyst	Primary infertility for 10 years	Bilateral cystectomy laparoscopy, chromotubation, and adhesiolysis	IV
E5	31	Left ovary endometriosis cyst	Primary infertility for 4 years	Left cystectomy laparoscopy and chromotubation	III
E6	33	Bilateral endometriosis cyst	Primary infertility for 4 years	Bilateral cystectomy laparoscopy, chromotubation, and adhesiolysis	IV

For methylation profile, the author performed DNA amplification process on promotor region of HOXA11 gene through Methylation-specific polymerasechain reaction (MSP) technique to detect the sequence having methylation. The specific primer was used on promotor region of HOXA11 gene by using software Methprimer. We also applied positive control from Epitech Methylated Human. The MSP result was going on electrophoresis in 2.8% agarose gel with 90 volts for 42 minutes.

The electrophoresis result was changed into methylation area using software "Image J". Every methylation level was the comparison between methylation region and positive control area (20944.78); then, we counted the mean of intensity on endometriosis group (64.02%) and control group (31.99%). The result pointed out that there was an increase of methylation in endometriosis group as 32.03% (Table 2).

Normality test using Shapiro Wilk showed both groups had significant value of 0.664 and 0.443. Therefore, these group had normal distribution. Independent t-test was run to determine the difference on methylation level between endometriosis and control group. Statistical analysis revealed there was significant difference between these group (p=0.036).

DISCUSSION

The strength of study was the first research in Indonesia approaching methylation profile on HOXA11 gene on endometriosis patients with infertility. There was significant difference on methylation percentage of HOXA11 gene which was found higher on endometriosis group. Epigenetic theory on DNA methylation stated that silencing gene could be found in increasing of methylation.[10,11] Nonetheless, this study did not find out the mRNA expression on HOXA gene due to budget limitation.

Table 2. Analysis Result on Semi Quantitative Data on Methylation Level of HOXA11 Gene

Sample Code	Status	MSP Result		Image J Semi Quantitative		Methylation Percentage (%)
		Methyl	Un-methyl	Methyl	Un-methyl	
E1	case	v	v	15305.5	13.739.87	73.08
E2	case	v	v	14257.7	12049.12	68.07
E3	case	v	v	12754.49	11752.82	60.90
E4	case	v	v	13591.21	14184.31	64.89
E7	case	v	x	10677.68	0	50.98
E8	case	v	v	13868.53	12.846.92	66.21
K1	control	x	v	0	5260.27	0.00
K2	control	v	v	11187.6	14464.78	53.41
K3	control	v	v	13982.06	19.451.57	66.76
K4	control	v	v	8886.09	13510.06	42.43
K5	control	v	v	6148.57	26450.88	29.36
K8	control	x	v	0	6843.34	0.00

X: band (-)
V: band (+)
Positive control: 20944.78

Recently, epigenetic study in endometriosis aims to understand infertility on endometriosis targeting to promising therapy invention. HOXA11 gene has their own role in implantation disruption on infertility cases caused by endometriosis.[12] Some studies revelaed that gene silencing of HOXA11 was led due to hyper methylation.[10,12]

Progesterone resistance on endometrium is a common condition found in endometriosis cases. Apart from that, endometriosis is known as estrogen-dependent disease to grow and reserve the tissue. Ovary and several other tissues such as adrenal and adipose produced estrogen. Previous study showed that there were inflammation reaction increasing aromatase activity in endometriosis; thus, it produced more estrogen on local tissue. Meanwhile, expression of HOXA11 gene was set by endogenous estrogen and progesterone.[13,14]

The HOXA11 gene is not a single epigenetic aberration responsible for infertility incidence on endometriosis patients. Several studies had shown the DNA methylation involvement on some genes to develop endometriosis, including HOXA10, E-cadherin, ER-α, SF-1, and PGR.[10] Natadisastra, et al. stated that methylation of HOXA10 gene in Indonesia was similar with other studies abroad. There was an increase of methylation on endometriosis patients compared to control group ($p=0.03$).[15,16]

Epigenetic study certainly offers hope to endometriosis patients with infertility. In addition to that, researchers have broad chances to investigate epigenetic both for therapy and prognosis. It hopes that further studies about gene target therapy on enzyme affecting epigenetic change. Therefore, it can manipulate the expression of HOXA11 gene to repair the methylation aberration as promising therapy to repair the endometrium receptivity on endometriosis patients with infertility.

CONCLUSION

There was an epigenetic role including DNA methylation on HOXA11 gene in endometriosis patients with infertility. The methylation degree of HOXA11 gene on endometriosis group with infertility shows higher significantly than control group.

CONFLICT OF INTEREST

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

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Research Article

Effect of Postpartum Pelvic Floor Muscles Training in Pelvic Floor Muscles Strength on Postpartum Women with Stress Urinary Incontinence

Pengaruh Latihan Otot Dasar Panggul terhadap Kekuatan Otot Panggul pada Perempuan Postpartum dengan Stres Inkontinensia Urin

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Abstract

Objective: To evaluate the effect of pelvic floor training (Kegel exercise) on pelvic floor muscle strength in postpartum women with SUI.

Methods: Thirty-five birth vaginally postpartum women with SUI were experimentally enrolled. After four weeks of postpartum observation, the diagnosis of SUI confirmed, and all of these women were asked to complete the International Consultation on Incontinence Questionnaire-Sort Form (ICIQ-SF) questionnaire. The strength of the pelvic floor muscle measured with perineometer every once in 3 weeks for 12 weeks of Kegel exercise. SUI severity assessed with ICIQ-SF after completing the Kegel exercise. A paired t-test was used to compare measurement results between ICIQ-SF questionnaire and perineometer and multiple linear regression models was used for multivariate analysis. A p value of less than 0.05 was taken to be statistically significant.

Results: Findings show a significant difference between clinical variables (parity, neonates birth weight, perineal tear grade, BMI) and the improvement of pelvic floor muscles before and after performed the Kegel exercise (all p<0.05). The pelvic floor muscles strength significantly improved (p=0.000) after Kegel exercise both in ICIQ-SF questionnaire and perineometer measurement.

Conclusion: Pelvic muscles floor training or Kegel exercise improve pelvic muscles floor strength in postpartum women with SUI.

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Keywords: pelvic muscles floor training, postpartum, stress urinary incontinence

Abstrak

***Tujuan**: Untuk mengevaluasi efek latihan dasar panggul (latihan kegel) pada kekuatan otot dasar panggul pada wanita pasca-melahirkan dengan SUI.*

***Metode**: Tiga puluh lima perempuan postpartum dengan SUI dilibatkan dalam penelitian ini. Setelah 4 minggu observasi postpartum, diagnosis SUI dikonfirmasi dan semua partisipan diminta untuk melengkapi kuesioner the International Consultation on Incontinence Questionnaire-Sort Form (ICIQ-SF). Kekuatan otot lantai panggul diukur dengan perineometer setiap sekali dalam 3 minggu selama 12 minggu setelah latihan Kegel. Tingkat keparahan SUI dinilai dengan ICIQ-SF setelah latihan Kegel. Uji t berpasangan untuk membandingkan hasil pengukuran antara kuesioner ICIQ-SF dan perineometer dan model regresi linier berganda digunakan untuk analisis multivariat dengan tingkat kebermaknaan p<0,05.*

***Hasil**: Hasil menunjukkan perbedaan signifikan antara variabel klinis (paritas, berat bayi lahir, derajat ruptur perineum, IMT) dan peningkatan kekuatan otot dasar panggul sebelum dan sesudah latihan Kegel (semua p<0,05). Kekuatan otot dasar panggul meningkat secara signifikan (p=0,000) setelah latihan Kegel baik pengukuran dengan kuesioner ICIQ-SF maupun perineometer.*

***Kesimpulan**: Latihan otot dasar panggul atau latihan Kegel meningkatkan kekuatan otot dasar panggul pada perempuan postpartum dengan SUI.*

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***Kata kunci**: latihan otot dasar panggul, postpartum, stres inkontinensia urin*

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INTRODUCTION

Stress urinary incontinence (SUI) is defined as the involuntary loss of urine on effort or physical exertion, or on sneezing or coughing[1] results from either hypermobility of the vesicourethral segment due to weakness of the pelvic floor support or from intrinsic sphincter deficiency (ISD).[2] ISD is due to lack of coaptation of the urethral wall and is diagnosed when a well-supported or non-mobile urethra leaks urine in response to a slight increase in intra-abdominal pressure in the absence of detrusor contraction.[2] SUI often can occur during pregnancy and immediately after delivery (temporary or permanent basis) and this may be in addition to the risk of subsequently developing SUI in a woman's lifetime[3] and contribute to the psychosocial impact in a woman's life in physical activity, travel, social relationships, and emotional health.[4]

The prevalence of persistent SUI varies from 18% to 50%.[5-8] A systematic review of 33 population-based studies on the prevalence of postpartum urinary incontinence found that the pooled prevalence of any postpartum incontinence was 33% in all women during the first 3 months postpartum.[9] Age, obesity, diabetes, pelvic floor surgery, pregnancy, and delivery are the risk factors for the development of stress urinary incontinence in women[10] which a significant percentage of women have persistent symptoms in the postpartum period. Prenatal incontinence increases the risk of postpartum incontinence,[11] which in turn increases the risk of long-term persistent incontinence.[12] The antenatal development of stress incontinence leads to an 18-times higher risk of developing stress incontinence after delivery, and the most prevalent group is delivered vaginally.[13]

The function of the pelvic floor muscles is to lend structural support to the pelvic structures, the urethra, vagina, and rectum.[14] The strengthening of pelvic floor muscles is one of the first recommendations for the treatment of mild and moderate SUI.[15] Conservative management of stress urinary incontinence often involves pelvic floor training. Pelvic muscle floor training (also known as Kegel exercise) involve the voluntary contraction of the levator ani (pelvic floor) muscles to increase their tone, strength, and endurance.[16] Pelvic floor muscle training is one of the modalities for this treatment.[10] This training has no significant side effects and enables improvement in SUI symptoms, and if the outcome is unsatisfactory, the patient can be referred for further evaluation and possible surgical intervention.[17] Training of pelvic floor muscles during SUI has reached success rates of 56% to 75%.[18]

The assessment of muscular strength and endurance in postpartum women provides information about the severity of muscle weakness and is the basis for the planning of exercise programs for strengthening of pelvic floor muscles. The primary objective of this study was to evaluate whether the effect of pelvic floor exercises on pelvic floor muscle strength could be improved in stress urinary incontinence postpartum women and the secondary objective was to identify factors involved in stress urinary incontinence with post-partum women.

METHODS

Birth vaginally postpartum women with stress urinary incontinence were experimentally enrolled and underwent pelvic floor muscle training (Kegel exercise) in the present study at RSKDIA Siti Fatimah Makassar between December 2015 and June 2016. Postpartum women who reported with chronic diseases (hypertension, diabetes mellitus, chronic lung disease), perineal tear grade III-IV, cesarean delivery, postpartum of preterm birth, and complicated pregnancy or delivery were excluded from the study. All of the women who enrolled were fully informed about the study and gave their consent before enrollment. The study was approved by the Health Research Ethics Committee of Faculty of Medicine, Universitas Hasanuddin.

After 4 weeks of postpartum observation, the diagnosis of SUI confirmed and all of these women were asked to complete the International Consultation on Incontinence Questionnaire-Sort Form (ICIQ-SF) questionnaire. The strength of the pelvic floor muscle measured with perineometer (PFX09122, Laborie, Canada) every once in 3 weeks for 12 weeks of Kegel exercise. The severity of SUI assessed with ICIQ-SF after completing the Kegel exercise. A paired t-test was used to compare measurement results between ICIQ-SF questionnaire and perineometer. Multiple linear regression model was used for multivariate analysis. A p value less than 0.05 was considered to be statistically significant.

RESULTS

Thirty-five postpartum women with stress urinary incontinence were included in this study. Majority of these women were aged 20-30 years (48.6%), multipara (71.4%), neonates birth weight 3000-4000 gram (77.1%), perineal tear grade 2 (74.2%) and normal body mass index (BMI) (77.1%). The baseline characteristics of the women are summarized in Table 1.

Table 1. Baseline Characteristics

Characteristics (n=35)	n	%
Age (years)		
< 20	11	31.4
20 - 30	17	48.6
> 30	7	20

Parity		
Primipara	10	28.6
Multipara	25	71.4
Neonates birth weight (gram)		
< 3000 gram	8	22.9
3000 - 4000 gram	27	77.1
Perineal tear grade		
0	1	2.9
1	8	22.9
2	26	74.2
BMI (kg/m²)		
< 18	2	5.7
18 - 24.9	27	77.1
≥ 25.0	6	17.2

In Table 2, the present study also shows a significant difference between clinical variables (parity, neonates birth weight, perineal tear grade, BMI) and the improvement of pelvic floor muscles before and after the women performed the Kegel exercise (all $p<0.05$). Based on measurement methods, the pelvic floor muscles significantly improved ($p=0.000$) after Kegel exercise both in ICIQ-SF questionnaire and perineometer measurement (Table 3). A multiple logistic regression models to identify the factors involved in the pelvic floor muscles strength for Kegel exercise show none of the factors (parity, neonates birth weight, perineal tear grade, BMI) significantly affect the pelvic floor strength (Table 4).

Table 3. Comparison between ICIQ-SF and Perineometer Measurement

Measurement	before	after	p value
ICIQ-SF score	12.97 ± 2.75	9.03 ± 3.25	0.000
Perineometer	4.23 ± 1.19	6.34 ± 1.71	0.000

DISCUSSION

The present study found that pelvic floor muscles training increased pelvic floor muscles strength based on the results of perineometer and ICIQ-SF questionnaire. Perineometer measurements of pelvic floor muscle contractions show very good inter- and intrarater reliability compared to the

Table 2. Comparison of Pelvic Muscle Strength with Perineometer Measurement before and after Kegel Exercise

Variables (n=35)	before	after	p value
Parity			
Primipara	4.80 ± 1.03	6.50 ± 1.72	0.008
Multipara	4.00 ± 1.19	6.28 ± 1.74	0.000
Neonates birth weight (gram)			
< 3000	4.63 ± 1.19	7.13 ± 1.89	0.002
3000 - 4000	4.11 ± 1.19	6.11 ± 1.63	0.000
Perineal tear grade			
0	3.00 ± 0.00	5.00 ± 0.00	-
1	4.00 ± 1.69	6.50 ± 1.85	0.003
2	4.35 ± 1.02	6.35 ± 1.72	0.000
BMI (kg/m²)			
< 18.5	5.00 ± 1.41	6.00 ± 2.83	0.5
18.5 - 24.9	4.22 ± 1.22	6.33 ± 1.73	0.000
≥ 25	4.00 ± 1.10	6.50 ± 1.64	0.010

Table 4. Multivariate analysis Results to Identify Variables Associated with Pelvic Floor Muscle Strength

	Unstandardized Coefficients		Standardized Coefficients	t	Sig.
	B	Std Error	Beta		
Age	-0.061	0.046	-0.31	-1.333	0.193
Parity	-0.299	0.234	-0.303	-1.276	0.212
Birth weight	-0.001	0.001	-0.173	-1.034	0.31
Perineal tear grade	-0.124	0.387	-0.054	-0.321	0.751
BMI	0.015	0.066	0.035	0.222	0.826

Brink total and pressure scores.[19] Previous studies has reported the successful of pelvic floor muscles training for postpartum pelvic floor muscles strength and improvement in SUI. Pelvic floor muscles training is applied in 12- to 14-weeks and 6-months programs. A significant increase in pelvic floor muscles strength has been observed after 12-week programs.[20] Pelvic floor muscles strength was significantly higher in women with PFME at gestational age 36 weeks and 3 months after delivery than control.[21] Another study by Sampselle et al. suggested that pelvic floor muscles training can reduce the incidence of SUI at gestational ages of 35 weeks and at 6 weeks and 6 months postpartum by significantly increasing pelvic floor muscles strength compared with women who did not perform the training.[22]

Hormonal and metabolic changes associated with pregnancy and spontaneous healing of traumatic lesions due to vaginal childbirth might improve the postnatal of SUI. This is due to pelvic floor muscles play a urethral support role, and it may be aided by pelvic floor training.[23] Improvement in pelvic floor muscles strength in women during the postpartum period showed that pregnancy, with its hormonal and mechanic effects, is a very important risk factor for UI during pregnancy.[24]

Pregnancy is one of the main risk factors for the development of SUI in young women.[25] Studies in pregnant women with SUI have found significantly decreased pelvic floor muscles strength in incontinent pregnant women compared with continent pregnant women.[26] Young-aged postpartum women with SUI mainly caused by stretching, pressure and ischemic to the endopelvic fascia, levator ani muscles and nerves in the pelvic floor.[27] Keane et al. showed that the etiology of SUI in these pregnant women appears due to both quantitative and qualitative reduction in collagen.[28]

SUI risk also increased with parity or vaginal birth mode.[29] Multigravidity causes a decrease in PFM strength at a rate of 22-35% beginning at a gestational age of 20 weeks and lasting until 6 weeks postpartum.[30] Vaginal birth caused injury in levator ani and perineal nerves damage.[31] Most cases of incontinence after vaginal birth occur as the results of injury to the muscles of the pelvic floor and its inervasion lead to neuropathy in 42% of postpartum women particularly in multiparous women and the second stage of labor extension. Two months after vaginal delivery, the recovery of the pudendal nerve function approximately 60% of women, but others not. Nerve damage due vaginal delivery caused by a combination of strain and compression of the fetal head into the birth canal.[32] The enlargement of the uterus and increase in fetal weight with gestational age, especially at the third-trimester influence the incontinence mechanism. Both factors have direct pressure on the bladder that might lead to changing the bladder-neck position[33] and reducing bladder capacity, contributing to bladder pressure that exceeds urethral pressure[34] and results in urine leakage. Excess body weight, as well as birth weight, also increases abdominal pressure, which in turn increases bladder pressure and urethral mobility, leading to stress UI and also exacerbating detrusor instability and overactive bladder. Like pregnancy, obesity may cause chronic strain, stretching and weakening the muscles, nerves and other pelvic floor structure.[35] Degree of perineal tear either spontaneously or with episiotomy hypothesized have an influence on the pelvic floor dysfunction. Study found there was no difference in the urinary incontinence rate between episiotomy and spontaneous tears.[36]

Pelvic floor muscles training is the most commonly recommended conservative therapy for pregnant women with SUI. For women who are continent during pregnancy, this training may prevent urinary incontinence up to 6 months after delivery[37] and recommend in first-line conservative management programmes for women with stress, urge, or mixed, urinary incontinence.[38] Pelvic floor muscles training is more an effective treatment for SUI during pregnancy because it is a safe treatment without complications, inexpensive, simple to perform, does not require instruments, and can be done anywhere and anytime.[17]

CONCLUSION

Our study suggests pelvic muscles floor training or Kegel excersice improve pelvic muscles floor strength in postpartum women with SUI. Parity, neonates birth weight, perineal tear grade, and BMI contributed as risk factors in the present study.

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Research Article

The Prevalence of Occult Omental Metastases in Patients with Epithelial Ovarian Cancer

Prevalensi Occult Metastasis di Omentum pada Pasien Penderita Kanker Ovarium Epithelial

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Abstract

Objective: Studies regarding omentectomy on epithelial ovarian cancer are scarce with conflicting results; this study is aimed to investigate the prevalence of occult metastases in patients with epithelial ovarian cancer of the omentum.

Methods: A cross-sectional study design was used by evaluating the medical records of surgically staged ovarian cancer patients in Dr. Cipto Mangunkusumo Hospital, Jakarta, Indonesia during the period of January 2009 to December 2015.

Results: A total of 51 subjects were involved in this study. One (2%) of 51 subjects was found to have occult metastases in the omentum. The prevalence of metastases of early stage epithelial ovarian cancer in 2009-2015 is 33.3% (17 out of 51 subjects), whereas the omental involvement is found only in 2% subjects (1 out of 51).

Conclusion: The prevalence of occult metastases of early stage epithelial ovarian cancer in Dr. Cipto Mangunkusumo Hospital, Jakarta, Indonesia, from 2009-2015 in 2% (1/51 subjects).

[Indones J Obstet Gynecol 2018; 6-2: 119-122]

Keywords: cancer, epithelial, metastases, omentum, ovarian cancer, ovary, prevalence

Abstrak

***Tujuan**: Mengenai omentektomi dalam pembedahan kanker ovarium epitelial masih sangat terbatas dengan hasil yang bervariasi sehingga dilakukan penelitian ini di Rumah Sakit Dr. Cipto Mangunkusumo (RSCM).*

***Metode**: Penelitian ini menggunakan desain potong silang dengan mengambil rekam medis pasien kanker ovarium yang dilakukan pembedahan di RSCM pada bulan Januari 2009 - Desember 2015.*

***Hasil**: Sebanyak 1 subjek dari 51 subjek penelitian (2%) ditemukan occult metastasis pada omentum. Kejadian metastasis pada kanker ovarium epithelial stadium dini pada tahun 2009 - 2015 adalah sebesar 33,3% (17 dari 51 subjek), di mana keterlibatan omentum ditemukan pada 2% subjek (1 dari 51).*

***Kesimpulan**: Kejadian occult metastasis kanker ovarium epithelial stadium klinis dini yang dilakukan pembedahan di RSCM tahun 2009 - 2015 adalah sebesar 2% (1 dari 51 subjek).*

[Maj Obstet Ginekol Indones 2018; 6-2: 119-122]

***Kata kunci**: epithelial, kanker, metastasis, omentum, ovarium, prevalensi*

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INTRODUCTION

Ovarian cancer accounts for approximately 3% of all cancer occurring in women. The prevalence is relatively stable since 1992.[1] The main spread of ovarian cancer is through the trans-coelomicpath therefore it often involves abdominal viscera, peritoneum and omentum.[2,3] The omentum of early stage ovarian cancer patient often times don't appear to have macroscopic lesions, yet FIGO stated that omentectomy need to be performed for adequate surgery for all stages.[4] The role of omentectomy as a therapeutical modality for early stage ovarian cancer cannot be concluded.[4] The function of omentum is mainly as a defense against infection, omentum can also be used for various surgical procedure, be it in general surgery or gynecologic surgeries.[5-8] Complication of omentectomy may increase the risk of developing organ injury such as intestines, spleen and bleeding.[9,10] Vinokurov found that the prevalence of omental metastases in all stages of ovarian cancer is 73.9%.[11] Terauchi stated that the prevalence of omental metastases was 47% (9/19 subjects), indicating that omentum is one of the major spread locations of ovarian cancer, and omentectomy should be performed if possible.[12] Those studies didn't specifically explain the omentum condition or the stage of ovarian

cancer when the surgery performed. Other sources stated that the prevalence of occult metastases of epithelial ovarian cancer in omentum varied from 0 to 13%.[4,13-15] Studies regarding omentectomy in epithelial ovarian cancer surgery are scant, and the results were contradictory. We aim to investigate the prevalence of epithelial ovarian cancer in Indonesia.

METHODS

This study used cross sectional study design. Data were collected from medical records of patients diagnosed with ovarian cancer who went to Dr. Cipto Mangunkusumo Hospital during the period of January 2009 to December 2015. Data were taken from the Cancer Registry of Oncology Gynecology of Obstetric and Gynecological Department of Dr. Cipto Mangunkusumo Hospital, Faculty Medicine - Universitas Indonesia. Inclusion criteria were women who were diagnosed with ovarian cancer and had undergone surgical staging. Those who underwent surgery without omentectomy, those who had clinically advanced ovarian cancer, and patients with pathological results non-epithelial or incomplete data.

RESULTS

Subject's characteristics

A total of 401 subjects were recruited in this study. Of these, 120 were of epithelial ovarian cancer with primary surgery, and 105 were those who underwent omentectomy. Of these, 51 cases were found with early stage.

Table 1. Characteristics of Subjects

Characteristics	n	%
Clinical Stage		
Stage 1	41	80.4
Stage 2	10	19.6
Histopathology Stage		
Stage 1	35	68.6
Stage 2	7	13.7
Stage 3	8	15.7
Stage 4	1	2
Subtype		
Clearcell	19	37.2
Endometrioid	10	19.6
Mucinous	9	17.7
Serous	13	25.5
Omentum Pathology		
Negative	50	98
Positive	1	2
Macroscopic appearance of omentum		
Normal	51	100
Grade		
N/A	8	15.7
Low grade	14	27.4
Moderate	13	25.5
High grade	16	31.4

Table 2. Prevalence of Metastases of Epithelial Ovarian Cancer in RSCM from 2009-2015

		n	%
Metastases	No (-)	34	66.7
	Yes (+)	17	33.3
Total		51	100.0

Out of 51 subjects of early stage epithelial ovarian cancer, we found 17 from 51 patients with positive metastases (33.3%) on uterus, omentum, appendix, lymph nodes, or cytology. Therefore prevalence of early stage epithelial ovarian cancer in RSCM from 2009-2015 is 33.3% (17/51). From 51 clinically early stage epithelial ovarian cancer in RSCM from 2009-2015, we found 16 cases that experienced upstaging from histopathological examination and 35 cases remained as early stage (31.3% and 68.7%)

Table 3. Prevalence of Omental Occult Metastases of Epithelial Ovarian Cancer

Macroscopic	Histopathology	n	%
No macroscopic	Negative (-)	50	98
anomaly	Positive (+)	1	2
Total		51	100.0

Prevalence of occult metastases of early stage epithelial ovarian cancer in omentum in RSCM from 2009-2015 is 2% (1/51), the subject is clinically stage 2.

DISCUSSION

Prevalence of early stage epithelial ovarian cancer metastases in RSCM from 2009 - 2015

Out of 51 early stage epithelial ovarian cancer, we found 17 from 51 patients with positive metastases

(33.3%) in uterus, omentum, appendix, lymph node or cytology. The prevalence of early stage epithelial ovarian cancer in RSCM from 2009-2015 is 33.3% (17/51), literature recommend to perform routine surgical staging and we found 30% of upstaging from early stage ovarian cancer (stage 1 and stage 2), with omental involvement around 0-11% cases.[5,16,17]

Prevalence of upstaging of epithelial ovarian cancer clinically early stage in RSCM from 2009-2015

From 51 patients with clinically early stage ovarian cancer in RSCM from 2009-2015, we found 16 cases upstaged from the pathological examination and 35 cases remain early stage (31.3% and 68.7%.) This result is similar to Garcia Soto et al who studied epithelial ovarian cancer clinically early stage who underwent surgical staging with 86 subjects, where 25 out of 86 subjects (29%) were upstaged.[18]

Prevalence of occult metastases of epithelial ovarian cancer clinically early stage to the omentum

In this study we found occult metastases prevalence of epithelial ovarian cancer around 2% (1/51). From literature we found that the range of prevalence of occult metastases in the omentum is around 0-13%. This result is similar to other studies.[16,18-21]

Distribution of epithelial ovarian cancer metastases in omentum based on its subtype

We found that the subject who had metastases in the omentum were patients with serous subtype, we didn't found metastases on other subtypes. Using Fisher's exact test we found no correlation between these two variables (p=0.6). This result is similar to what Garcia Soto et al found in 2012 that the histology of ovarian cancer is an independent variable to metastases.[16,18]

Distribution of epithelial ovarian cancer based on its grade

We found that the subject with omental metastases were patients with poorly differentiated tumors. No metastases to the omentum from patients with good or moderately differentiated tumors. Using Fisher's exact test we found no correlation between the grade of tumor to the metastases. This result is contradictory with Ayhan's results that grade ($p<0.03$) is correlated significantly with the stage of the disease.[19]

CONCLUSION

The prevalence of occult omental metastases in early stage epithelial ovarian cancer in Dr. Cipto Mangunkusumo Hospital during the year of 2009 to 2015 was 2%.

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Research Article

Endoglin Expression (CD105) in Ephithelial Ovarian Cancer

Ekspresi Endoglin (CD105) pada Kanker Ovarium Tipe Epitelial

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Abstract

Objective: To address the endoglin expression (CD105) in the primary tumour and metastases tumour (omentum) and their relation with clinicopathological factors: stadium, differentiation level, and histological epithelial ovarian cancer.

Methods: The research was performed at Public Service Hall of Dr. Wahidin Sudirohusodo Hospital and educational networking hospital of Obstetrics and Gynecology Department of Faculty of Medicine Universitas Hasanuddin. The research design is cross-sectional with 55 samples consisting of 55 samples of primary tumours and 55 metastatic tumours. Imunohistochemistry examination was performed on all samples.

Results: The results show a significant relation between endoglin (CD105) at omentum metastases tumour and stadium and cell differentiation level of epithelial ovarian cancer. There is no significant relation between endoglin (CD105) expression at primary tumour of ovarian cancer and stadium and differentiation and type of histopathological cell. In addition, there is no significant relation between endoglin expression (CD105) in omentum metastases tumour and type of histopathological cell of ovarian cancer. There is a significant correlation (strong category) between endoglin expression at omentum metastases tumour and endoglin expression at primary tumour of epithelial ovarian cancer.

Conclusion: Endoglin expression in ovarian cancer metastatic tumour to omentum is correlated with clinical stage and differentiation level of ovarian cancer. And endoglin is one of the proangiogenic and prometastatic factors.

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Keywords: CD105, endoglin expression, epithelial ovarian cancer, immunohistochemistry

Abstrak

***Tujuan**: Mengetahui ekspresi endoglin (CD105) pada tumor primer dan tumor metastases (omentum) serta hubungannya dengan faktor klinikopatologik: stadium, derajat diferensiasi, dan jenis histopatologi kanker ovarium tipe epitel.*

***Metode**: Penelitian ini menggunakan rancangan potong lintang. Penelitian dilaksanakan di BLU RS Dr. Wahidin Sudirohusodo dan RS jejaring pendidikan Departemen Obstetri dan Ginekologi Fakultas Kedokteran Universitas Hasanuddin Makassar. Sampel penelitian sebanyak 55 orang dengan rincian masing-masing 55 sampel untuk kelompok tumor primer dan 55 kelompok tumor metastases omentum. Pemeriksaan imunohistokimia dilakukan terhadap semua sampel.*

***Hasil**: Hasil penelitian menunjukkan terdapat hubungan bermakna antara ekspresi endoglin (CD105) pada tumor metastases omentum dengan stadium dan derajat diferensiasi sel kanker ovarium epitelial. Tidak didapatkan hubungan bermakna antara ekspresi endoglin (CD105) pada tumor primer kanker ovarium dengan stadium, derajat diferensiasi dan jenis histopatologi sel. Juga tidak didapatkan hubungan bermakna antara ekspresi endogin (CD105) pada tumor metastases omentum dengan tipe histopatologi sel kanker ovarium. Terdapat korelasi yang signifikan (kategori kuat) antara ekspresi endoglin pada tumor metastases omentum dan ekspresi endoglin pada tumor primer kanker ovarium tipe epitelial.*

***Kesimpulan**: Ekspresi endoglin pada tumor metastases omentum kanker ovarium berhubungan dengan stadium klinis dan derajat diferensiasi sel pada kasus kanker ovarium. Selain itu, endoglin juga merupakan faktor proangiogenik dan prometastases.*

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***Kata kunci**: CD105, ekspresi endoglin, imunohistokimia, kanker ovarium epitelial*

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INTRODUCTION

Ovarian cancer is the fifth leading cause of death worldwide and is the most deadly cancer among all gynecology cancer. Annually, there are 204.000 women diagnosed with ovarian cancer, and 125.000 women died of the disease worldwide.[1]

The risk of ovarian cancer occurrence in women is 1 : 70, and is related to the age of the patients, which is approximately 61 years old. Eighty-five to ninety percent of diagnosed ovarian cancer is known as the epithelial type of ovarian cancer. Hereditary factor is the major risk factor for the disease, especially the presence of ovarian cancer history in the family. Approximately 5-10% of ovarian cancer cases were suspected inherited, while the remaining 90-95% are related to continuous ovulation cycle.[1]

Ovarian cancer is a very challenging clinical situation because initially, the disease does not provoke any symptom. Thus, more than two-third

of the cases are diagnosed in its late stage, where cancer has been spread outside the ovary or even widely metastasized.[2] Although nowadays, the aetiology of the disease is better understood, the presence of aggressive cytoreductive surgery and more combined modern chemotherapy, there is no significant statistical mortality rate decrease in the last 30 years.

In the last few years, the understanding of molecular biology, especially regarding angiogenesis in ovarian cancer, is improving, leading to the finding of some new therapeutic target and molecular prognostic factor. Hence, this triggers the development of some more radical therapy of ovarian cancer. Some factors that involve in angiogenesis are keys to better understand the mechanism and management of ovarian cancer. One of the factors is Endoglin (CD105).

Endoglin (CD105) is the glycoprotein membrane which is included in zona pellucida protein group. Endoglin is expressed strongly in proliferated vascular endothelial cells and has been identified as an accessory receptor for transforming growth factor-β (TGF-β). The endoglin gene in human is located on chromosome 9q34ter.[3]

The main role of endoglin is regulating the TGF-β-dependent vascular remodelling and angiogenesis. The signal produced by the TGF-β might induce various process, including proliferation, migration, and adhesion. TGF-β is expressed in endothelial cells of the embryo, infected tissue, healing tissue, inflamed arthritis synovial, and solid tumour. Currently, endoglin is suggested as the marker of neovascularization in solid tumours.[4]

In the studies of fertility, endocrinology, and reproduction, especially endometriosis, the role of endoglin has been improved, compared with its role in the cases of ovarian malignancies. The serum endoglin level was identified as a predictor factor of endometriosis, with the threshold of ≥ 11 ng/ml for endometriosis cases and <11 ng/ml for non-endometriosis cases.[5]

The role of endoglin in the spreading process of cancer has been discussed in several previous studies. In the study of gastric cancer, found a correlation of increased VEGF with endoglin expression in tumours metastasized to the lymph node, and suggested it as an important prognostic indicator. Similar result was found by, indicated a correlation of VEGF and endoglin expression in the cases of prostate cancer, and suggested that the increase of both VEGF and endoglin expression is associated with the low survival rate. In breast cancer, endoglin assessment has a higher specificity to assess the neovascularization compared with other pan-endothelial markers, such as CD31, CD34, and Von Willebrand Factor (vWF).[6,7]

The suppression of endoglin regulation may trigger apoptosis, induce significant DNA destruction by modulating some DNA restoration gene, increase the sensitivity of platinum, both in vivo and in vitro, and become a specific therapeutic target in epithelial ovarian cancer.[8]

Endoglin expression is frequently found in ovarian cancer effusion, both in the tumour cells and the reactive mesothelial cells. The extent of endoglin expression is correlated to the chemotherapy state, and endoglin expression is found higher in solid metastatic tumour than that in effusion.[9]

The role of endoglin in ovarian cancer has not been reported in a considerable number compared with that in other malignancies, such as gastric cancer, prostate cancer, and breast cancer. Therefore, the investigators sensed the need to conduct the study, which aims to understand the expression of endoglin (CD105) in primary and metastatic tumours (omentum) as well as its correlation with tumour clinicopathologic factors: staging, differentiation level, and histopathology of epithelial ovarian cancer.

METHOD

Study Location and Period

The study was conducted in several educational networking hospitals of Obstetrics and Gynecology Department of the faculty of medicine of Universitas Hasanuddin: RS Dr. Wahidin Sudirohusodo, RS Pendidikan UNHAS, RS Pelamonia, RSU Labuang Baji, RSI Faisal, RS Bhayangkara, RS Ibnu Sina, and RSUD Syekh Yusuf Gowa; as well as Pathology Laboratory of Pathology Anatomy Department of the faculty of medicine of Universitas Hasanuddin and Pathology Laboratory of RS Dr. Wahidin Sudirohusodo Makassar. The study was conducted after been approved by the local authorities and the patients in regard to the sampling process. The study was conducted from December 2015 until the period of the sample completion.

Study Design and Variables

The study used cross-sectional study design. The variables of the study consisted of: independent variable (endoglin expression), dependent variable (staging, differentiation level, histopathology of epithelial ovarian cancer), intervening variable (apoptosis inhibition, vasopermeability increase, proliferation, migration, and invasion), and confounding variable (hereditary factor, hormonal factor, radiation, and carcinogenic agents).

Population and Sample

The population of this study included all patients with epithelial ovarian cancer who were treated and underwent the surgery in RS Dr. Wahidin Sudirohusodo Makassar and several educational networking hospitals of Obstetrics and Gynecology Department of the faculty of medicine of Universitas Hasanuddin Makassar. Sample is the part of population. The sample of this study included all patients with epithelial ovarian cancer who were treated and underwent the surgery in RS Dr. Wahidin Sudirohusodo Makassar and several educational networking hospitals of Obstetrics and Gynecology Department of the faculty of medicine of Universitas Hasanuddin Makassar.

Data Collection Method

Patients who consented to participate in the study should sign the informed consent form then complete the questionnaire, consisted of history taking, physical examination, and another workup. The data were subsequently analyzed.

Data Analysis Technique

The data were categorised based on the purpose and type of the data, with appropriate statistic method, then processed with SPSS programme for Windows version 16.

RESULT

The study was an observational study with a cross-sectional design. This study aims to address endoglin (CD105) expression in primary tumour and metastatic tumour (omentum) of epithelial ovarian cancer and its correlation with some clinicopathology factors, such as clinical stage, differentiation level, and histopathology type. This study was conducted in several educational hospitals of obstetrics and gynecology department of faculty of medicine, Universitas Hasanuddin, Makassar, from December 2015 until the period of the sample completion.

There were 55 patients enrolled in the study (20-76 years old) where most cases, as much as 27 (49.1%), were younger than 45 years old. As many as 42 patients (76.4%) were unemployed. The highest parity was 1-3 parities, which was found in 23 patients (41.9%). Of all patients, 18 patients (32.7%) suffered from early-stage ovarian cancer, and 37 patients (67.3%) suffered from late-stage ovarian cancer. Serous type of ovarian cancer was found in 29 patients (52.7%), and mucinous type was found in 26 patients (47.3%). Poorly differentiated cell was found in 39 patients (70.9%), and well differentiated cell was found in 16 patients (29.1%) (Table 1).

Table 1. Characteristic of Sample

Characteristic	Total	
	n = 55	%
Age (year)		
<45	27	49.1
45-50	20	36.4
>50	8	14.5
Parity		
0	19	34.5
1-3	23	41.9
>3	13	23.6
Educational level		
Uneducated	6	10.9
≤ 9 years of education	32	58.2
> 9 years of education	17	30.9
Employment		
Unemployment	42	76.4
Employment	13	23.6
Clinical stage		
Early stage (I and II)	18	32.7
Late stage (III and IV)	37	67.3
Endoglin Expression (Primary)		
Negative	10	18.2
Positive	45	81.8
Endoglin Expression (Omentum)		
Negative	18	32.7
Positive	37	67.3

The distribution of endoglin expression based on the clinical stage in epithelial ovarian cancer tissue (primary tumor) showed that both negative endoglin expression and positive endoglin expression (the weak and the strong one) were found higher in late stage ovarian cancer, as much as 60.0% and 68.9%, respectively. The distribution of endoglin expression based on cell differentiation of epithelial ovarian cancer (primary tumour) showed that both negative and positive endoglin expression in the primary tumour of ovarian cancer was found higher in poorly differentiated cell, as much as 70% and 71.1%, respectively. The distribution of endoglin expression based on histopathology type of epithelial ovarian cancer (primary tumour) showed that both negative and positive endoglin expression in the primary tumour of ovarian cancer was found higher in serous type, as much as 60.0% and 51.1%, respectively. (Table 2).

The distribution of endoglin expression based on the clinical stage of epithelial ovarian cancer (omentum metastatic tumour) showed that negative endoglin expression was found higher in early-stage ovarian cancer (61.1%), while positive endoglin expression was found higher in late stage ovarian cancer (81.1%). The distribution of endoglin expression based on cell differentiation of epithelial ovarian cancer (omentum metastatic tumour) showed that negative endoglin expression was found higher in the well differentiated cell (61.1%), while positive endoglin expression was found higher in the poorly differentiated cell (86.5%). The distribution of endoglin expression based on histopathology type of epithelial ovarian cancer (omentum metastatic tumour) showed that negative endoglin expression was found higher in serous type (61.1%), while positive endoglin expression was found higher in mucinous type (51.4%). (Table 3).

Table 2. Distribution of Endoglin Expression based on Clinical Stage, Cell Differentiation, and Histopathology Type in Epithelial Ovarian Cancer Tissue (Primary Tumour)

Endoglin Expression	Clinical Stage							Cell Differentiation							Histopathology Type						
	Early		Late		Total		p	Well		Poor		Total		p	Mucinous		Serous		Total		p
	n	%	n	%	n	%		n	%	n	%	n	%		n	%	n	%	n	%	
Negative	4	40.0	6	60.0	10	100.0		3	30.0	7	70.0	10	100.0		4	40.0	6	60.0	10	100.0	
Positive	14	31.1	31	68.9	45	100.0	0.588	13	28.9	32	71.1	45	100.0	0.944	22	48.9	23	51.1	45	100.0	0.611
Total	18	32.7	37	67.3	55	100.0		16	29.1	39	70.9	55	100.0		26	47.3	29	52.7	55	100.0	

Table 3. Distribution of Endoglin Expression based on Clinical Stage, Cell Differentiation, and Histopathology Type in Epithelial Ovarian Cancer Tissue (Omentum Metastatic Tumour)

Endoglin Expression	Clinical Stage							Cell Differentiation							Histopathology Type						
	Early		Late		Total		p	Well		Poor		Total		p	Mucinous		Serous		Total		p
	n	%	n	%	n	%		n	%	n	%	n	%		n	%	n	%	n	%	
Negative	11	61.1	7	38.9	18	100.0		11	61.1	7	38.9	18	100.0		7	38.9	11	61.1	18	100.0	
Positive	7	18.9	30	81.1	37	100.0	0.002	5	13.5	32	86.5	37	100.0	0.000	19	51.4	18	48.6	37	100.0	0.385
Total	18	32.1	37	67.9	45	100.0		16	29.1	39	70.9	55	100.0		26	47.3	29	52.7	55	100.0	

There is a significant correlation of endoglin expression in primary tumour and endoglin expression in omentum metastatic tumour of ovarian cancer ($p<0.05$). The coefficient value of 0.771 indicated a strong correlation of endoglin expression in omentum metastatic tumour and endoglin expression in primary tumour of ovarian cancer (Table 4).

Table 4. Correlation of Endoglin Expression in Primary Tumor and Endoglin Expression in Omentum Metastatic Tumor of Epithelial Ovarian Cancer

Primary Endoglin	
Endoglin Omentum	rs = 0.771
	p = 0.000

DISCUSSION

The study showed a significant correlation (strong category) of endoglin expression in omentum metastatic tumour and endoglin expression in the primary tumour of epithelial ovarian cancer.

Statistically, this result showed no significant correlation between endoglin expression and clinical stage of epithelial ovarian cancer. Similar result was reported by Zhai (2011) and Satya *et al.* (2016), as well as Annika *et al.* (2011), concluded that regardless the cell type, endoglin expression was not correlated to tumour stage, FIGO classification, or volume of the remaining disease after surgery. Annika *et al.* (2011) observed endoglin expression in serous ovarian cancer by assessing the efusion fluid and the primary tumour. They found a stronger endoglin expression in patients with recurrent disease, both in the efusion fluid and in the primary tumour.[9-11]

Although there was no statistical correlation of endoglin expression and clinical stage, the data showed a linear tendency indicating that positive endoglin expression was found dominant in the late stage. Of 37 cases of late-stage ovarian cancer, 31 expressed positive endoglin.

Endoglin expression in primary tumour of ovarian cancer is not statistically significant at various level of cells differentiation. The result is in accordance with the result of Zhai (2011), Nikiteas *et al.* (2007), and Satya *et al.* (2016), which found that there was no correlation of endoglin expression and the differentiation level. Previous study showed that endoglin involved more in tumour progressivity, proven by its presence as cell differentiation marker in ovarian cancer. Previous studies did not specifically state cell differentiation type. Endoglin (CD105) is a part of Transforming Growth Factor (TGF)-β complex receptor, a pleiotropic cytokine which involved in cellular proliferation, differentiation, and migration.[6,10,11]

In this study, we did not find any significant statistical difference of endoglin expression and the histopathology of epithelial ovarian cancer. Similar result was found by Nikiteas *et al.* (2007), which showed no correlation between endoglin expression and the histopathology type of cancer. Data showed that positive endoglin expression was higher in serous type (52.7%) than in mucinous type (48.9%).[6]

From the statistical test, this study indicated a significant correlation of endoglin expression in omentum metastatic tumour and the clinical stage of epithelial ovarian cancer. Endoglin is suggested as a progressivity marker of various solid tumours and their metastases by many researchers. This is different with the study result of Annika *et al.* (2011), which concluded that, regardless of the cell type, endoglin expression is not correlated to the staging of the tumour, FIGO classification, or the volume of the remaining disease after surgery.

According to Annika *et al.* (2011), endoglin expression in solid metastases is higher than that in efusion. Similarly, a non-significant tendency indicated that there is a higher expression in a primary tumour than in efusion. There is no significant difference in endoglin staining of the solid metastatic tumour and primary tumour. The latter is consistent with our finding which indicated that there is no significant difference in endoglin staining of the solid metastatic tumour and primary tumour. However, we can not confirm the previous statement since we did not assess endoglin expression in the efusion fluid of ovarian cancer.[9]

In this study, we did not find a significant statistical correlation of endoglin expression and histopathology type of epithelial ovarian cancer. The data showed that positive endoglin expression was the slightly higher mucinous type (51.4%) compared to serous type (48.6%). Theoretically, serous type of epithelial ovarian cancer was found more frequently. The study of McCluggage (2011) found that serous adenocarcinoma is the most

frequent type of epithelial ovarian cancer, and most serous type epithelial ovarian cancer was found in late stage (III & IV). This finding is consistent with the result of our study, where we found 29 (51.8%) cases of serous type epithelial ovarian cancer, and most cases are late-stage ovarian cancer (67.8%).[12]

This study showed no significant correlation between endoglin expression increase and the clinical stage, differentiation level, and histopathology type of epithelial ovarian cancer primary tumour. However, there was a significant correlation of endoglin expression increase of omentum metastatic tumour and the clinical stage as well as differentiation level of epithelial ovarian cancer, but there was no significant correlation of endoglin expression increase of omentum metastatic tumour and histopathology type of epithelial ovarian cancer.

One of some possibilities that may appear is the presence of angiogenic factor molecular heterogeneity of ovarian cancer. That might be caused by the mutation of ALK-1 and ALK-5 proteins which then suppress/influence endoglin expression, both in protein level and mRNA level.[3]

ALK-1 or activin receptor-like kinase 1 is an enzyme that is encoded by ACVRL1 gene which is a receptor in TGF-β signaling pathway. While ALK-5 is a specific receptor enzyme for TGF-β1. Mutation and deletion of ALK-1 and ALK-5 are frequently found in various cancers of human.

There was a significant correlation between endoglin expression in primary tumours and endoglin expression in omentum metastatic tumour of ovarian cancer. The correlation of endoglin expression in omentum metastatic tumour and endoglin expression in primary tumours was considered as a strong correlation.

The increase of endoglin (CD105) expression might be detected in microvascular endothelial cells and in vascular endothelial cells of tissues with active ongoing angiogenesis, such as tissues that are in their regeneration process as well as tumour and its metastases or inflammation. The result of endoglin (CD105) staining showed more evident stain in the area with active angiogenesis, including tumour borders, metastases tumour, while less evident stain was seen in the central area of the tumour, and no stain was seen in normal tissues around a tumour.

This result is consistent with the results of previous studies which stated that measuring endoglin expression by assessing microvascular density is a proper prognostic indicator; the study found that the density of endoglin-positive vascular is associated with metastases of various solid tumours.[3]

High level of endoglin expression was associated with ovarian cancer growth and metastatic tumour. In tumour pathogenesis, endoglin plays some big roles, such as inducing tumour proliferation, invasion, and metastases. Metastases includes some gradual steps of malignant cells that spread from the primary tumour to other distant organs. The recruitment of endothelial cells and neovascularization play an important role in tumour progression and metastases. Important events of metastases including the ability of tumour to survive in circulation and to invade other tissues, initiate and maintain the growth, and form a proangiogenic micrometastases in the tissue, then eventually form a vascularization for the macrometastases tumour.

CONCLUSION AND RECOMMENDATION

The investigators concluded that endoglin expression in ovarian cancer primary tumour is not correlated to clinical stage, differentiation level, and histopathology of epithelial ovarian cancer. Endoglin expression in ovarian cancer metastatic tumour to omentum is correlated with clinical stage and differentiation level of ovarian cancer. Endoglin expression in ovarian cancer metastatic tumour to omentum is not correlated to histopathological of epithelial ovarian cancer. There is a strong correlation between endoglin expression in omentum metastatic tumour and endoglin expression in primary tumor of ovarian cancer. And endoglin is one of the pro angiogenic and pro metastases factors. Further studies are needed, considering the role of endoglin in therapeutic target of ovarian cancer has not been reported considerably. However, endoglin expression is a proper prognostic marker and a promising therapeutic target of antiangiogenesis process and anti-tumor; thus, further studies are needed. Deeper analyses are needed to explain the molecular mechanism of other angiogenic factors in ovarian cancer progressivity.

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