ISSN 0857-6084

THAI JOURNAL OF OBSTETRICS AND GYNAECOLOGY

THE OFFICIAL JOURNAL OF
THE ROYAL THAI COLLEGE OF OBSTETRICIANS AND GYNAECOLOGISTS

VOL. 26 NO. 1 JANUARY - MARCH 2018

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Thai Journal of Obstetrics and Gynaecology

ISSN : 0857-6084. The Official Journal of the Royal Thai College of Obstetricians and Gynaecologists

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Office: 8th Floor, The Royal Golden Jubilee Bldg. 2, Soi Soonvijai, New Petchburi Road, Bangkapi, Bangkok 10310, Thailand
Published by: PIMDEE Co., Ltd. Tel: 0-2874-4401, 086-144-8500

Website: www.tci-thaijo.org/index.php/tjog, E-mail: Vorapong.P@chula.ac.th

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Thai Journal of Obstetrics and Gynaecology

ISSN 0857-6084

The Official Journal of the Royal Thai College of Obstetricians and Gynaecologists

Vol. 26 No. 1

JANUARY - MARCH 2018

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EDITORIAL

At the beginning of New Year 2018, it's time for beginning good things. May this year bring happiness, new inspirations and new success to all members of Royal Thai College of Obstetricians and Gynaecologists (RTCOG).

Editor in Chief and managing staff already attended the Thai Journal Citation Index meeting: **"The 5th Editors' Workshop"** under the TCI-TRF-Scopus Collaboration Project in Bangkok, Thailand on March 8th, 2018. Editorial Board of TJOG looks forward to continuously raising the quality of the TJOG and prepare journal for submission to be index in Scopus index.

We would like to thank past RTCOG executive board, past editor in chief, editorial board and staff, reviewers, all members of RTCOG, and all researchers for their kind contribution and support to TJOG.

This first issue of TJOG 2018 contains many interesting articles. One special article is "**Pre-implantation Genetic Diagnosis of Thalassaemias"**.

Prof. Vorapong Phupong, M.D.
Editor in Chief

Thai Journal of Obstetrics and Gynaecology
January 2018, Vol. 26, pp. 2-9

SPECIAL ARTICLE

Pre-implantation Genetic Diagnosis of Thalassemias

Wirawit Piyamongkol, M.D., PhD*.

** Department of Obstetrics and Gynaecology, Faculty of Medicine, Chiang Mai University, Chiang Mai 50200, Thailand*

ABSTRACT

Beta-Thalassemia major, beta-thalassemia-Hb E disease and Hb Bart's disease are severe hereditary anemia which are prevalent in Thailand and neighborhood countries. Thalassemia syndromes and hemoglobinopathy cost significant health and economic burden and sometimes maternal morbidity and mortality. The present strategy to reduce new cases is population screening, prenatal genetic diagnosis (PND) and the option for termination of affected pregnancy (TOP) following thoroughly genetic counselling. The advances of reproductive technology and molecular genetics facilitate genetic testing of the embryos prior to transfer into the womb, therefore, embryo selection is possible. Pre-implantation genetic diagnosis (PGD) consists of sampling techniques from the embryos and molecular genetic analysis techniques. Polar body biopsy, cleavage stage embryo biopsy or blastocyst biopsy can be used for sampling DNA material from the embryos. Polymerase chain reaction (PCR) is employed for the analysis of thalassemia mutations. PGD is an alternative to the traditional PND, providing the couples at risk of having severe thalassemia babies an opportunity to get pregnant with a healthy one without the need for TOP. Since 2004, a total of 64 PGD cycles have been performed at the Department of Obstetrics and Gynaecology, Faculty of Medicine, Chiang Mai University, including 37 alpha-thalassemia, 5 beta-thalassemia and 22 beta-thalassemia-Hb E disease, giving rise to 24 healthy pregnancies (27 babies).

Keywords: Embryo selection, hemoglobinopathy, multiplex fluorescent polymerase chain reaction (PCR), pre-implantation genetic diagnosis (PGD), thalassemias.

Correspondence to: *Wirawit Piyamongkol, M,D, PhD., Department of Obstetrics and Gynaecology, Faculty of Medicine, Chiang Mai University, 110 Intawaroros Road, Sripoom, Mueang, Chiang Mai 50200, Thailand, E-mail address: wirawit.p@cmu.ac.th*

Thalassemias

Globin genes mutations causing thalassemia syndrome and hemoglobinopathy are the commonest single gene disorder and cost significant health and economic burden worldwide. Beta-Thalassemia, Hb E disease and alpha-thalassemia are prevalent in Thai and neighborhood population. Homozygous beta-thalassemia (beta-thalassemia major), compound heterozygous beta-thalassemia-Hb E disease and homozygous alpha-thalassemia-1 (Hb Bart's disease) are accounted as the most severe forms[1].

Beta-Thalassemia, an autosomal recessive disorder, is caused by over 200 mutations within the beta-globin gene, characterized by anemia, spleenomegaly, and bone marrow expansion with skeleton deformities. The patients with beta-thalassemia

major or beta-thalassemia-Hb E disease suffer from severe anemia and need regular blood transfusion since the first year of life. Despite adequate blood transfusion, most patients die from complications of iron loading with an average age of 20 years old, unless iron chelators are provided regularly[2]. The access to bone marrow transplantation treatment is still limited and risky option.

Alpha-Thalassemia is more common than beta-thalassemia. However, the patients are infrequently seen in the hospital, as the most severe form (homozygous affected or Hb Bart's hydrop fetalis) leads to still birth and heterozygotes have no symptom and do not need health care. Homozygous alpha-thalassemia leads to the absence of alpha-globin chain synthesis and causes Hb Bart's hydrop fetalis syndrome. Mothers who carry Hb Bart's babies are likely to develop life-threatening complications, i.e. eclampsia, dystocia and hemorrhage. Therefore, the aim of detecting homozygous alpha-thalassemia is to prevent maternal morbidity and mortality.

Prevention and Control Strategy

Since the effective treatment of thalassemias is still limited, most countries who face thalassemias acquire prevention and control programs. Pre-conception or prenatal genetic screening is employed to all couples in order to identify couples at risk of having severe thalassemia offspring. Thoroughly genetic counselling is given to the couples at risk and choices of invasive prenatal diagnosis are offered. Either chrionic villous sampling at 11-13 weeks, amniocentesis at 16-20 weeks or fetal blood sampling at 19-24 weeks can be done for fetal genetic diagnosis. For the diagnosis of Hb Bart's hydrop fetalis, ultrasonography measuring peak systolic velocity of the middle cerebral artery (PSV-MCA) and fetal hydropic signs surveillance can be additional non-invasive options[3]. Negative results reassure the parents that their babies will be healthy, however, in case of affected babies termination of pregnancy is offered for parental decision[1].

Pre-implantation Genetic Diagnosis (PGD)

The first PGD attempt was demonstrated by sexing rabbit blastocysts[4]. However, in that experiment there was no surviving embryo. In human, in vitro fertilization was first successful in 1969[5]. This knowledge has made it possible to study preimplantation human embryos. The preliminary PGD model was performed in mouse embryos using biochemical testing for hypoxanthine phosphoribosyl transferase (HPRT) enzyme deficiency, which is the cause of Lesch-Nyhan syndrome[6]. DNA based PGD model for beta-Globin gene testing in mouse embryos was also successfully carried out[7]. It was exhibited that the development of Day 3 8-cell stage preimplantation human embryo was not compromised following the biopsy[8].

The first successful PGD pregnancies were performed for sex identification using PCR in order to avoid X-linked disorders[9]. IVF procedures was used to generate several embryos and then single blastomeres were biopsied at the cleavage stage[10]. Most of PGD cycles were performed for single gene disorders, some were for chromosomal abnormalities and sexing in families at risk of X-linked diseases. The patients decided to performed PGD because of moral or religious objection to pregnancy termination, a third possessed fertility problems as well as carrying a genetic condition, while a quarter already had termination of pregnancy and do not want to go through another[11].

Sampling Techniques

Sampling of DNA materials for genetic analysis can be carried out using polar body biopsy, cleavage stage Day 3 embryo biopsy or Day 5 blastocyst biopsy. The obtained samples can then by analyzed for the particular disease and healthy embryos can be chosen for transfer.

- ***Polar Body Biopsy***

Polar body biopsy is a form of preconception diagnosis. The procedure is supposed to be non-invasive to the embryos and allows longer analysis time comparing with cleavage stage embryo biopsy. The first polar body which is extruded from primary oocyte is used for genetic analysis[12]. The genetic results of the first polar body are complementary to that of the

oocyte. For example, if the first polar body of a heterozygous subject shows genetic mutation, it means that the oocyte is normal and can be chosen for fertilization. However, meiotic recombination (crossing-over) can turn the predicted results inaccurate. The chance of recombination of telomeric genes can be up to 50% and that of centromeric genes can be less than 1%. Therefore, there is a need to analyzed both first and second polar bodies in order to have comprehensive predictive results[13]. Therefore, the techniques are labor-intensive. In addition, paternal mutation cannot be analyzed by this technique, therefore, sex identification for X linked diseases and the diagnosis of mutations carried by male partners, especially autosomal dominant disorders, cannot be done. In the analysis of autosomal recessive conditions in which both parents are carriers, the oocytes with a mutation will be discarded, even they may generate a heterozygous embryo when fertilized with a sperm with normal allele. Moreover, post-zygotic events, i.e. mosaicism, are not diagnosed by polar body biopsy.

- Cleavage Stage Day 3 Embryo Biopsy

Following fertilization, the zygote undergoes cleavage cell division every 24 hours. On day 3 when the embryo is at 8 cells stage, 1-2 blastomeres can be biopsied without affecting the development of the embryo. More than 90% of the embryos can survive the biopsy procedure[8]. Blastomeres at this stage are totipotent, have not been assigned to be any specific tissues or organs yet. Cleavage stage embryo biopsy on day 3 used to be the most popular technique for PGD[11].

The procedure is performed by zona drilling and blastomere aspiration. The embryo is held using a holding pipette on a micromanipulator. Acid Tyrodes solution is applied to drill a hole in the zona pellucida[8]. The zona can be cut mechanically in a V shape by partial zona dissection (PZD)[14]. Drilling the zona using a laser gives more control of the size of the hole[15]. The blastomere is then gently aspirated through the hole using a biopsy pipette.

Cleavage stage biopsy possesses some disadvantages. Using this technique, only 1-2 cells can be obtained and only 24 hours is available for genetic analysis as most IVF centers prefer to transfer the embryos on day 4. Therefore, the analysis techniques need to be fast, sensitive and accurate. Because of the chance of chromosomal mosaicism in preimplantation embryos[16] and allele specific amplification failure or allele drop out (ADO) of the PCR assays from single cells, the analysis of two blastomeres (separately) from each embryo is recommended to reduce the chance of misdiagnosis.

- Blastocyst Biopsy

Blastocysts can be obtained from extended culture of IVF embryos. Blastocyst biopsy can be done on day 5 to day 6 post-fertilization when the blastocyst consists of about 120 cells. A blastocyst contains two cell types; inner cell mass which will develop into the embryo and trophectoderm which will develop into the placental membranes. A hole is drilled in the zona pellucida and the embryo is put back in the culture medium. When the trophectoderm cells herniate from the embryo[17], 10-30 trophectoderm cells can be biopsied for genetic diagnosis. The disadvantage of this technique is that the analysis of extraembryonic cells may not represent the inner cell mass or the embryo. With the longer in vitro development, the number of the surviving embryos reduces. Moreover, adding up with the genetic analysis time the endometrium is not appropriate for implantation. Recently, due to the improvement of extended embryo culture medium and techniques, there are more surviving embryos to blastocysts stage. Incorporating with embryo freezing techniques, blastocyst biopsy has gained in popularity worldwide. Additional advantages of this technique include more number of biopsied cells and more time for the analysis.

Molecular Genetic Diagnosis

Fluorescent in situ hybridization (FISH) was used for sexing[18], and detecting numerical chromosomal abnormalities and chromosome translocations[19]. Recently comparative genome hybridization array (aCGH) and NextGen Sequencing (NGS) have gained popularity for comprehensive chromosome analysis.

Polymerase chain reaction (PCR) is employed for diagnosis of single gene disorders, including Duchenne muscular dystrophy[20], Fragile X syndrome[21], Tay Sachs disease[22], Marfan's syndrome[23], Myotonic Dystrophy[24], Charcot Marie Tooth type 1A[25], familial adenomatous polyposis coli (FAPC)[26], Huntington's chorea[27], severe inherited skin diseases[28], sickle cell anaemia[29], spinal muscular atrophy[30], beta-thalassaemia[31], congenital adrenal hyperplasia[32], Lesch Nyhan syndrome[33], medium chain acyl CoA dehydrogenase (MCAD) deficiency[34] and alpha-thalassemia[35]. Data demonstates no significant difference in pregnancy rates between those from PGD and routine IVF cycles[11].

- Single Cell Polymerase Chain Reaction (PCR)

PCR is a powerful molecular technique for rapidly multiplying a specific DNA fragment to a level that can be further analyzed by other methods[36]. Various modified techniques have been developed for various purposes, including forensic assay, evolutionary biology, genetic screening, mutation analysis, PND and PGD of single gene disorders. More sophisticated and modern advanced techniques have been applied. However, amplification failure, ADO and contamination are essential problems during PCR at the single cell level. Due to the wide variety of mutations within the same and among different genes, particular analysis methods are needed for particular mutations.

- Amplification Failure (AF)

Amplification failure of single cell PCR was realized when the first series of PGD for X-linked disorders was misdiagnosed[37]. Y chromosome specific sequences were amplified from the biopsied blastomeres in order to avoid transfer of male embryos. The absence of amplification indicates a female embryo. However, amplification failure leads to the same diagnosis result. Amplification failure is not an unusual phenomenon during single cell PCR and can be found about 10%[38].

Possible causes of amplification failure include the isolated cell may be lost during transfer to the PCR tube or the cell may be anucleate or in the process of degeneration. For this reason, current protocols are designed not to interpret a missing result as normal, but as an affected genotype. This design will not lead to serious misdiagnosis in cases of amplification failure, but may reduce the number of un-affected embryos for transfer. Amplification failure may also be caused by the suboptimal conditions of the lysis protocol, primers combinations, PCR protocol or poor cell quality. Therefore, single cell PCR protocols need be thoroughly optimized before clinical apply.

- Allele Drop Out (ADO)

One common problem of single cell PCR is ADO or allele specific amplification failure when one of the two alleles in a heterozygous sample randomly fails to amplify[39]. Consequently only one allele is shown after PCR, giving the interpretation as a homozygote. This problem is particular to PCR with low copy number of DNA templates and can give rise to misinterpretation. Misdiagnoses that might have caused from ADO have been reported. ADO is especially crucial in the analysis of dominant disorders or recessive disorders with two different mutations (compound heterozygous). However, in PGD of a recessive disease, ADO would not lead to the transfer of an affected embryo, but will reduce the number of heterozygous (unaffected carrier) embryos for transfer.

ADO occurs about 2-20% of single cell PCR[40]. ADO may causes from several theories, the foremost of which are: DNA degradation causing PCR-refractory breaks in both DNA strands; and inaccessibility of the DNA templates because of imperfect PCR conditions or incomplete cell lysis. Possible methods in order to improve amplification efficiency and minimize ADO include using highly sensitive fluorescent PCR (F-PCR) techniques[41], increasing PCR denaturation temperature[40], and the use of different cell lysis buffers[42]. However, none of these methods seems to consistently eliminate ADO. Most experienced PGD laboratories can generally reduce ADO rates to 5-10%. Strategy to avoid misdiagnosis caused from ADO involves the addition of a polymorphic linked marker analysis together with the mutation analysis reaction as

a multiplex PCR[43] or in separate reactions after whole genome amplification (WGA)[26] for back up linkage analysis results. In addition, it is suggested to interpret the results from two cells of each embryo to reduce the risk of misdiagnosis from ADO[38].

- Contamination

Contamination is another crucial problem encountering single cell PCR. ICSI is recommended for fertilization in order to eliminate the chance of paternal (sperm) DNA contamination. All maternal cumulus cells need to be removed before insemination in order to reduce the risk of maternal DNA contamination. The biopsied blastomeres need to be washed several times in clean medium in order to get rid of any remaining cumulus cells or DNA that may remain in the culture medium prior to transferring into the PCR tubes. PCR set up in a DNA-free environment separating from the analysis area can reduce the chance of getting 'carry over' PCR products from previous experiments. All media and reagents need to be tested before use. Nested PCR by amplifying the first amplified products using the second set of primers situated internally to those used in the first reaction was recommended to prevent carry over contamination[44]. Some PGD centers employ a restriction enzyme to digest extraneous DNA in PCR mixture prior to adding target DNA[45].

Despite all efforts, contamination may still occur and lead to misdiagnosis. Misdiagnosis from maternal DNA was documented[27]. In case of mother carrying the mutation gene of a dominant disorder, maternal DNA contamination will not lead to misdiagnosis, but a reduced number of embryos for transfer. In case of recessive disorders, contamination of maternal heterozygous DNA in a homozygote affected cell will lead to a heterozygous interpretation, and the transfer of affected embryos. To reduce the chance of misdiagnosis from contamination, DNA fingerprinting is used to trace down the presence of contamination by co-amplifying a highly polymorphic marker with the test gene as a multiplex PCR. The genotype in an embryo that deviates from the 4 possible combinations of parental alleles indicates the presence of contamination.

- Nested PCR

PCR templates with very low copy number, especially a single copy, need more PCR cycles in order to produce enough PCR products for further analysis on traditional gel electrophoresis. However, the very last PCR cycles will be less efficient due to the reduced substrates, i.e. primers, dNTPs, polymerase enzyme, etc. For this reason, a second amplification with a fresh identical PCR mixture was employed in order to produce enough PCR products to a detectable level[46]. Nested PCR involving two successive amplification reactions is an improved technique for single cell PCR. The second reaction tube consists of fresh PCR mixture with a different set of primers. The second set of primers is designed to situate within the first amplicon and generates a shorter DNA fragment. This technique is useful not only in increasing sensitivity, but also increasing specificity and reducing risk of contamination(47).

- Fluorescent PCR (F-PCR)

Conventional techniques of analyzing PCR products using electrophoresis include ethidium bromide or silver staining or radioactive labelled primers, which are either less sensitive or time consuming. The use of F-PCR with an improved sensitivity and specificity is useful for single cell PCR[48]. Oligonucleotide primers are attached with fluorescent molecules generating amplified products labelled with fluorescent dye. When these F-PCR products migrate under electrophoresis to the point where laser intersects, fluorescent molecules will be triggered and generate a signal with a specific wavelength which can be detected by a CCD (charged couple device) sensor and analyzed by computer software. F-PCR products from a single cell can be identified after only 35 cycles of amplification. This excludes the need for nested PCR and accelerates the analysis. Using F-PCR, size standards can be run in the same lane, consequently, size determination is as accurate as a single base pair difference.

- Multiplex PCR

Molecular analysis of single cell PCR for PGD can be done only once. Multiplex PCR permits more than one locus to be analyzed by combining unrelated sets of primers in a PCR reaction(35). Each combination of primer sets in multiplex PCR needs to be optimized for the relative primer concentrations, annealing temperatures and reaction buffers in order to minimize interaction between unrelated primers or PCR products. The PCR products of different loci can be differentiated by designing the primers to generate different amplified fragments sizes. The analysis of multiplex PCR products is easier on F-PCR. By labelling the primers with different dyes, the fragments from different sets of primers can be simply identified, even those with the same fragment size. This facilitates the analysis of multiple loci from a single cell, i.e. the analyses of more than one disease or different mutations and polymorphic markers. The additional polymorphic marker can be useful for a backup linkage analysis results and contamination detection[35].

- PGD of Thalassemias

PGD for thalassemias at the Department of Obstetrics and Gynaecology, Faculty of Medicine, Chiang Mai University started in 2004. Day 3 cleavage stage embryo biopsy was employed during the early years and then Day 5 blastocyst biopsy has been routinely performed during the last few years. Multiplex F-PCR protocols were developed for PGD of beta-thalassemia[31], and alpha-thalassemia[35]. Multiplex F-PCR protocol incorporating with mini-sequencing was also developed for more beta-thalassemia mutations and beta-thalassemia-Hb E disease. A total of 64 PGD cycles have been performed, including 37 alpha-thalassemia, 5 beta-thalassemia and 22 beta-thalassemia-Hb E disease, giving rise to 24 healthy pregnancies (27 babies). No contamination or misdiagnosis was detected. More PGD protocols for more mutations of thalassemias and other single gene disorders using advanced techniques are underdeveloped.

Conclusions

Severe thalassemias and hemoglobinopathy are prevalent and cause significant health and economic problems in Thailand and neighbor countries. Effective treatment is still limited. The present strategy to reduce new cases is population screening, prenatal diagnosis and termination of affected pregnancy. PGD is an alternative to the traditional PND, providing the couples at risk of having affected babies an opportunity to start a pregnancy with a healthy one without the need for TOP. PGD center at Chiang Mai University was established in 2004 and performed 64 PGD cycles, giving rise to 24 pregnancies (27 babies).

Potential conflicts of interest

The author declare no conflict of interest.

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Thai Journal of Obstetrics and Gynaecology
January 2018, Vol. 26, pp. 10-17

OBSTETRICS

Early versus Conventional Feeding and Onset of Lactation in Emergency Cesarean Parturient Mothers

Tunyatorn Srisataporn, M.D.*,
Rungruedee Jeerasap, M.D. *.

** Department of Obstetrics and Gynaecology, Khon Kaen Hospital, Khon Kaen 40000, Thailand*

ABSTRACT

Objectives: To study the onset of lactation between early and conventional feeding in emergency cesarean parturient mothers.

Materials and Methods: Emergency cesarean parturient mothers under spinal block with intrathecal morphine were randomized into two groups. The early feeding group received clear liquid diet 200 milliliter at 6 hours postoperation and then stepped diet as tolerate. The conventional group took a sip of water at 24 hours postoperation then stepped to liquid, soft and regular diet. All participants were informed about signs and symptoms of maternal perception of onset of lactation that consisted of breast fullness, breast tingling and milk leakage. Then they had to record timing if she had any one of signs and symptoms of onset of lactation.

Results: Two hundred and thirty-eight participants were participated in the study, 119 in each group. The onset of lactation was 30.1 ± 12.9 hours and 46.3 ± 10.8 hours in early feeding and conventional feeding group, respectively ($p < 0.01$). Only mild symptoms of bowel ileus were found in both groups.

Conclusion: Early feeding in emergency cesarean parturient mothers had a significant earlier onset of lactation when compare with conventional feeding without serious adverse gastrointestinal complications.

Keywords: Onset of lactation, emergency cesarean section, early feeding, conventional feeding.

Correspondence to: *Tunyatorn Srisataporn, M.D., Department of Obstetrics and Gynaecology, Khon Kaen Hospital, Khon Kaen 40000, Thailand, Email address: may.tunyatorn@gmail.com*

การรับประทานอาหารเร็วเปรียบเทียบกับการรับประทานอาหารตามขั้นตอนกับการเริ่มหลั่งของน้ำนมเต็มเต้าในมารดาหลังคลอดที่ผ่าตัดคลอดฉุกเฉิน

ธัญธร ศรีสถาพร, รุ่งฤดี จีระทรัพย์

บทคัดย่อ

วัตถุประสงค์: *เพื่อศึกษาถึงระยะเวลาการเริ่มหลั่งของน้ำนมเต็มเต้าในมารดาหลังคลอดที่ผ่าตัดคลอดฉุกเฉิน เปรียบเทียบระหว่างการรับประทานอาหารเร็ว และการรับประทานอาหารตามขั้นตอน*

วิธีการดำเนินวิจัย: *มารดาหลังคลอดที่ผ่าตัดคลอดฉุกเฉินโดยระงับความรู้สึกด้วยการฉีดยาชาและมอร์ฟีนเข้าช่องไขสันหลัง ถูกสุ่มแบ่งเป็น 2 กลุ่ม ได้แก่ กลุ่มที่รับประทานเร็ว รับประทานน้ำหวาน 200 มิลลิลิตร 6 ชั่วโมงหลังผ่าตัด แล้วรับประทานอาหารอื่นได้เท่าที่ต้องการ และกลุ่มที่รับประทานตามขั้นตอน จิบน้ำที่ 24 ชั่วโมงหลังผ่าตัด และรับประทานอาหารเหลว อ่อน และปกติ ตามลำดับ และผู้เข้าร่วมวิจัยทุกคนได้รับข้อมูลเรื่องอาการและอาการแสดงของการเริ่มหลั่งน้ำนมเต็มเต้า ประกอบด้วย เต้านมคัดตึง เสียวแปลบที่บริเวณเต้านม และมีน้ำนมไหล ซึ่งจะมีการบันทึกเวลาเมื่อมีอาการหรืออาการแสดงอย่างหนึ่งของการหลั่งน้ำนมเต็มเต้า*

ผลการศึกษา: *มีมารดาเข้าร่วมการศึกษา 238 คน กลุ่มละ 119 คน พบการหลั่งของน้ำนมเต็มเต้าที่ 30.1 ± 12.9 ชั่วโมง และ 46.3 ± 10.8 ชั่วโมง ในกลุ่มที่รับประทานอาหารเร็วและกลุ่มที่รับประทานอาหารตามขั้นตอน ตามลำดับ ($p < 0.01$) พบเพียงอาการท้องอืดเล็กน้อยทั้งสองกลุ่ม*

สรุป: *มารดาหลังผ่าตัดคลอดฉุกเฉินที่รับประทานอาหารเร็ว มีระยะเวลาการเริ่มหลั่งของน้ำนมเต็มเต้าเร็วกว่ากลุ่มที่รับประทานอาหารตามขั้นตอน อย่างมีนัยสำคัญทางสถิติ โดยไม่พบภาวะแทรกซ้อนของระบบทางเดินอาหารที่รุนแรง*

คำสำคัญ: *การเริ่มหลั่งของน้ำนมเต็มเต้า, การผ่าตัดคลอดฉุกเฉิน, การรับประทานอาหารเร็ว, การรับประทานอาหารตามขั้นตอน*

Introduction

Lactogenesis is the term meaning the initiation of lactation and divided into 2 stages. Lactogenesis I begins at last 12 weeks of pregnancy. Lactogenesis II or onset of lactation (OL), resulting in a copious increase of milk, begins at 2-3 days postpartum. The gold standard for documentation of OL is test weighing. Infants were weighed before and after breastfeeding three times a day continuously to determine milk transfer in each feed then plot graph to find out the maximal point of milk production. However, this method is expensive, invasive and impractical to use in general[1]. Maternal perception of OL is another way to detect OL that consists of breast fullness, breast tingling and milk leakage. This method is practical, noninvasive and inexpensive[1].

Several studies found mothers who had early OL had longer breastfeeding rate and duration[1, 2, 3]. Routes of delivery can affect OL. Previous study found cesarean section had longer time to OL compared with vaginal delivery[4]. Cesarean delivery affects OL due to post-operative pain and fatigue, anemia due to intra operative blood loss, interference from medications caused delay to access the baby and prolonged post-operative starvation when compare with vaginal delivery[5].

Lactating mother increases metabolism and consumes 25% of total energy to produce breast milk[6]. There were several studies had early feeding in post cesarean mothers under spinal block from thirty minutes to eight hours postoperation. They found early post-operative feeding was safe, no significant bowel ileus, earlier ambulation, shorter length of hospital stay and more maternal satisfaction compare to delayed feeding[7, 8]. One study showed early feeding in uncomplicated post cesarean mothers under spinal block also had earlier OL compare with conventional feeding[9].

However, there was no previous study of OL in emergency cesarean section compare between early feeding and conventional feeding. The aim of the current study was to compare OL between early and conventional feeding in emergency cesarean parturient mothers.

Materials and Methods

This randomized controlled trial was conducted at Khon Kaen Hospital. Postpartum emergency cesarean mothers under spinal block with intrathecal spinal morphine were enrolled into the study. Inclusion criteria were postpartum emergency cesarean mother whom performed operation between 06.00 a.m. – 04.00 p.m., singleton and term pregnancy, normal breasts and nipples, neonatal birth weight 2,500 gram or more, rooming-in mother and infant. Exclusion criteria were uncontrolled maternal medical complications such as severe hypertension, poor-controlled diabetes mellitus, heart diseases, serious intraoperative and postpartum complications such as early postpartum hemorrhage, contraindication for breastfeeding such as HIV infection, neonatal birth asphyxia (APGAR score at 1 minute ≤ 7), congenital fetal abnormalities such as tongue ties, clef lip, clef palate.

After operation, mothers with infants were transferred from recovery room to postpartum ward or private room and informed about the study when she was fully consciousness and prompted to receive study information usually within 3-4 hours postoperatively. All participants whom voluntary to join the study were written informed consent and randomized into two groups, early feeding and conventional feeding group, by computer generated list with allocation concealment by sequentially opaque envelopes.

The early feeding group received clear liquid diet 200 milliliters at 6 hours postoperation and then stepped diet as tolerate, the conventional group took a sip of water at 24 hours postoperation then stepped to liquid, soft and regular diet, respectively. They were informed about signs and symptoms of maternal perception of OL that consisted of breast fullness, breast tingling and milk leakage. Then they had to record timing if she had any one of signs and symptoms of OL. The clocks at postpartum wards and private rooms were set for standard time. The maternal perception of OL would be asked by staff nurses who did not involve in the previous procedure three times daily to remind mothers

and confirm signs and symptoms of OL.

The standard postoperative cesarean section care, breast feeding support such as initiated breastfeeding within 1 hour as soon as both mother and infant prompted, positioning and latch on, breastfeeding 10-12 times all day and night were performed similarly in both groups. Demographic characteristics, operative outcomes, gastrointestinal complications, neonatal outcomes, time to ambulation and length of stay were recorded.

The current study was the first study about maternal perception of OL between early feeding and conventional feeding in post emergency cesarean parturient mothers. Though, the sample size calculation was based on the pilot study in thirty participants by use of mean ± standard deviation of OL in early and conventional feeding group (34.5 ± 9.9 hrs and 37.7 ± 7.8 hrs, respectively), α = 0.05, power of 80% and 10% for dropout. Finally, the sample size was one hundred and nineteen per group. This study was approved by Khon Kaen Hospital Institute Review Board in Human Research.

Data analysis was performed with SPSS 17 software. Student t-test and Mann-Whitney U test were used to compare continuous variables depends on distribution between the groups. Chi square and Fisher's exact tests were used for categorical variables as appropriate. Cumulative rate of time to OL was analyzed by survival analysis. The Shapiro-Wilk test was used to test distributions for normality. Differences were considered statistically significant when the p value was < 0.05.

Results

This randomized controlled trial was conducted between January to August 2016. A total of 254 emergency cesarean parturient mothers were eligible, 16 declined to participate the study. Finally, 238 emergency cesarean parturient mothers underwent randomization into early feeding and conventional feeding group, 119 each. Participants flow was shown in Fig. 1.

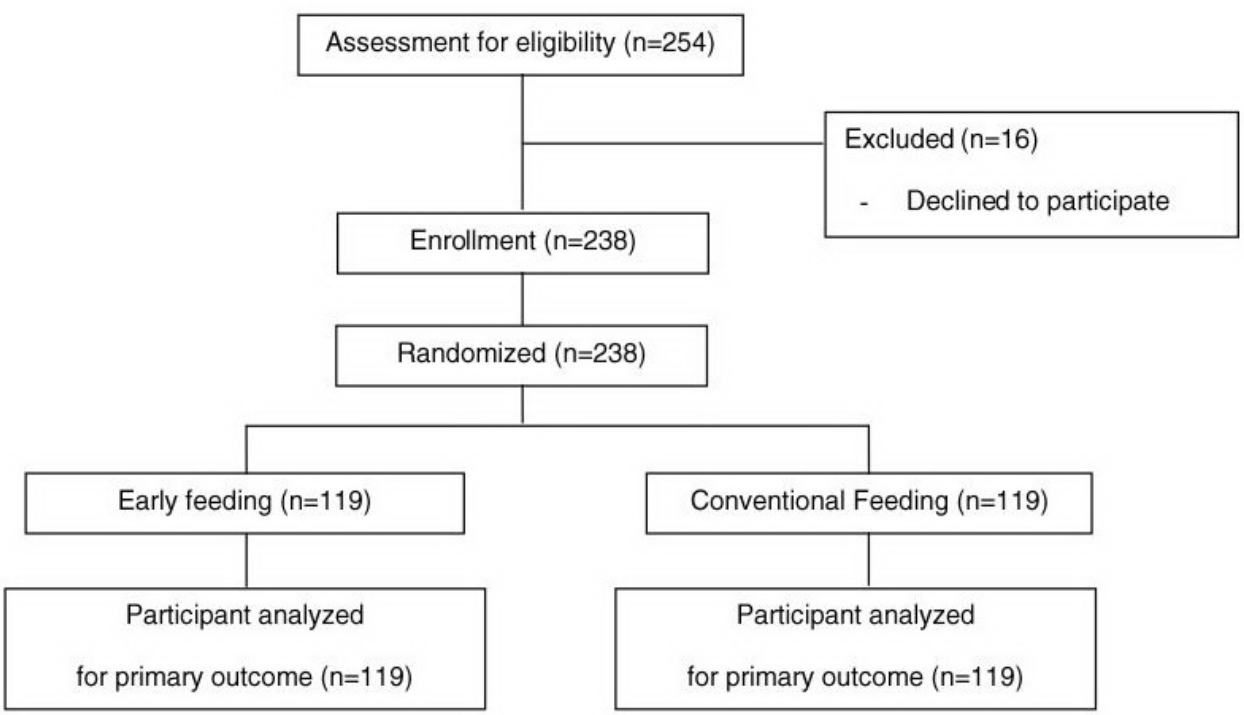

Fig. 1. Participants flow.

Baseline characteristics were presented in Table 1. Age, parity, breastfeeding experience, BMI were similar in both groups. The most common indication for cesarean section was cephalopelvic disproportion, 50 (42%) in early feeding and 59 (49.6%) in conventional feeding group. The other indications were previous cesarean section, failed induction of labor, abnormal presentation and placenta previa with labor. Mean of preoperative withhold diet time was similarly in both groups (11.8 hours and 11 hours in early feeding and conventional feeding group, respectively). There was no different in operation time, intraoperative blood loss, pre and postoperative hematocrit level between both groups. No serious intraoperative and postoperative complications were found. Neonatal birth weight and Apgar scores were not different between group. Few cases in both groups had mild degree of nipple sores that corrected when changed position and latched on of breastfeeding by lactating nurse.

Table 1. Demographic characteristics in early and conventional feeding.

Characteristics	Early feeding (n = 119)	Conventional feeding (n = 119)	p value
Age (years), mean (SD)	28.1 (5.9)	28.2 (6.3)	0.96
Primiparous, n (%)	50 (42)	42 (35.2)	0.28
Breastfeeding experience, n (%)	69 (57.9)	77 (64.7)	0.28
BMI > 30 kg/m^2, n (%)	32 (26.8)	54 (45.3)	0.26
Indication for cesarean section, n (%)			0.51
Cephalopelvic disproportion	50 (42)	59 (49.6)	
Previous cesarean with labor	48 (40.3)	48 (40.3)	
Failed induction	3 (2.5)	1 (0.8)	
Abnormal presentation	15 (12.6)	10 (8.4)	
Placenta previa with labor	3 (2.5)	1(0.8)	
Pre-operative withhold diet (hours), mean (SD)	11.8 (4.1)	11 (4)	0.1
Hematocrit (%), mean (SD)			
Preoperative	36.2 (3.7)	36.3(3.5)	0.89
Post-operative	36 (3.3)	36.6(3.8)	0.21
Birth weight (grams), mean (SD)	3160.4 (414.3)	3251.6 (448.8)	0.13
APGAR at 1 min, mean (SD)	8.8 (0.5)	8.7 (0.5)	0.23

BMI: Body mass index, SD: Standard deviation

The primary outcome was maternal perception of OL. The current study found significance earlier OL in early feeding compare to conventional feeding group (30.1 ± 12.9 hours and 46.3 ± 10.8 hours, respectively, $p < 0.01$). The earliest and longest OL were at 6 hours and 62 hours in early feeding group while at 18 hours and 68 hours in conventional feeding group. The early feeding group had OL mostly within Day 1 and 2 while the conventional group had OL mostly within Day 2 and 3 postpartum. There was no delay OL (OL > 72 hrs) in both groups (Table 2). Cumulative incidence of OL in early feeding group was higher than conventional feeding group during the study period (Fig. 2).

Gastrointestinal outcomes were divided into

mild and severe bowel ileus. Mild symptoms of bowel ileus (anorexia and nausea) was 14 (11.8%) in early feeding group and only 5 (4.2%) in conventional feeding group with no statistical significance. Severe bowel ileus including vomiting, need to retain nasogastric tube or abdominal radiography were not found in the present study. All of them ambulated in first 24 hours. Early feeding group had earlier ambulation and shorter length of stay than conventional feeding group with statistical significance. There was no excessive infant weight loss (> 10% of birth weight) and neonatal jaundice were found in this study (Table 3).

Table 2. Onset of lactation between early and conventional feeding.

Onset of lactation	Early feeding (n= 119)	Conventional feeding (n= 119)
By mean (SD), hours	30.1 (12.9)	46.3 (10.8)
By min-max, hours	6-62	18-68
By day of postpartum		
Day 1	58 (48.7)	7 (5.9)
Day 2	45 (37.8)	58 (48.7)
Day 3	16 (13.4)	54 (45.3)
> Day 3*	0	0

* Delayed onset of lactation (OL > 72 hours)

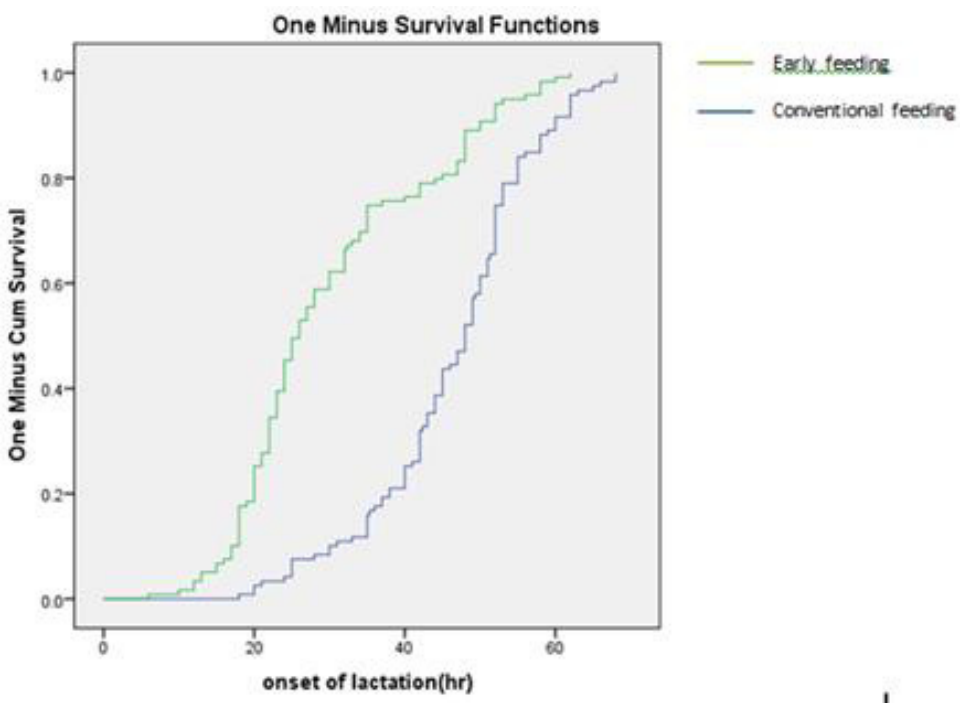

Fig. 2. Cumulative incidence of OL between early and conventional feeding.

Table 3. Gastrointestinal outcomes, onset of ambulation, length of stay between early and conventional feeding.

Variables	Early feeding (n = 119)	Conventional feeding (n = 119)	p value
Bowel ileus, n (%)	14 (11.8)	5 (4.2)	0.07
- Mild	14 (11.8)	5 (4.2)	
- Severe	0	0	
Onset of ambulation (hours), mean (SD)	12.1 (3.8)	14.9 (3.8)	< 0.01
Length of stay (days), mean (SD)	2.6 (0.8)	3.2 (0.6)	< 0.01

Discussion

Maternal perception for OL (breast fullness, breast tingling and milk leakage) is a very useful method for detection of OL because parturient mother could recognize of milk exceed enough to nurse her infant at first time. Moreover, previous study found that postpartum women were able to recall delayed OL even at seven months postpartum with high sensitivity and acceptable specificity (93.6% and 62.5% respectively)[10]. In addition, maternal perception of OL can apply in general, especially in low resource settings.

OL as the primary outcome in the present study, it showed OL in early feeding group had 16 hours earlier when compared to conventional feeding group with statistical significance (30.1 ± 12.9 hours and 46.3 ± 10.8 hours, respectively). OL in conventional feeding in emergency cesarean section in the current study was similar to the previous study[11]. Nearly half of mothers (48.4%) in early feeding group had OL within the first day of postpartum while conventional feeding group found only 5.8%, it was approximately 8 times earlier. OL in conventional feeding mostly (48.4%) occurred in the second day of postpartum. The cumulative incidence of OL in early feeding group was higher than conventional group at all study periods (Fig. 2). These finding confirmed that early feeding in emergency cesarean parturient mothers had a significant benefit in promoting earlier OL. Delayed OL (OL > 72 hrs) could affect to the neonate by excessive neonatal weight loss (> 10 percent of birth weight) which caused dehydration, electrolyte imbalance or even neonatal death[3]. Mothers who had delayed OL decreased breastfeeding rate and duration of breastfeeding[12]. Factors affected delayed OL included primigravida, obesity, diabetes, stress and cesarean section[8]. Delayed OL was found in both elective and emergency cesarean section[4, 11]. However, there was no delayed OL in early feeding group in one study with uncomplicated cesarean section while 11.1% of delayed OL had found in conventional feeding[9]. Surprisingly, delayed OL was not found in the current study even in conventional feeding group and no excessive neonatal weight loss and breastfeeding jaundice detected. It might be explained by proper breastfeeding support and adequate postoperative pain control could help mothers in early initiating of breastfeeding and nursing her infant. According to cesarean section rate has tendency to increase worldwide[13], we hope that early feeding in emergency cesarean parturient mothers could be one of the best choices to promote early breast milk production followed by increasing in rate and duration of breastfeeding.

Breastfeeding and taking care of baby all day and night, post cesarean parturient mothers seems to have more energy from early diet than those of other uncomplicated abdominal surgeries. Early feeding in post cesarean mother had more energy than conventional feeding in 7-10 day postpartum due to early feeding of regular diet[14]. Several studies started early feeding in post elective cesarean mothers in various times range from thirty minutes to eight hours without significant serious gastrointestinal

outcomes[7, 8, 15]. Moreover, one study showed early feeding had lesser bowel ileus symptoms than conventional feeding[8]. Postoperative early feeding is safe and well tolerate because cesarean section is uncomplicated operative technique, short operative time than other abdominal surgeries and less bowel interference. However, we decided to start early feeding at 6 hours postoperation because the study was conducted in emergency cesarean section that might have a chance of inadequate withhold diet and caused aspiration. The present study found only mild symptoms of bowel ileus higher in early feeding compare to conventional feeding group (11.7% and 4.2%, respectively) but there was no statistical significant. There was no serious gastrointestinal outcome in this study.

In this study, early feeding had earlier ambulation and shorter length of hospital stay similar to others[7, 15]. Spinal block with intrathecal opioid provides a long period of postoperative analgesia for 24 hours. Thus, the mothers in this study had earlier time to ambulate in both early and conventional group compare to spinal block without intrathecal opioid in other studies[7, 8].

The strength of this study was the first randomized controlled trial about OL and feeding types in emergency cesarean parturient mothers. The limitation of this study was OL and maternal confidence of breastfeeding before and after OL was not evaluated. OL in emergency cesarean parturient mothers under general anesthesia should be taken into account for the further study.

Conclusion

In conclusion, early feeding in emergency cesarean parturient mothers had a significant earlier onset of lactation when compare with conventional feeding without serious adverse gastrointestinal outcomes.

Potential conflicts of interest

The authors declare no conflict of interest.

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Thai Journal of Obstetrics and Gynaecology
January 2018, Vol. 26, pp. 18-26

OBSTETRICS

Incidence of Intrapartum Abnormal Fetal Heart Rate Pattern in Siriraj Hospital

Kanittha Boonchuan, M.D.*,
Kanokwaroon Watananirun, M.D.*,
Dittakarn Boriboonhirunsarn, M.D., M.P.H., Ph.D.*.

** Department of Obstetrics and Gynaecology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok 10700, Thailand*

ABSTRACT

Objectives: To determine the incidence of intrapartum abnormal fetal heart rate (FHR), possible associated factors and pregnancy outcomes.

Materials and Methods: A total of 900 low-risk pregnant women were enrolled in this retrospective cohort study. Obstetric, labor and delivery data were collected. Incidence of intrapartum abnormal FHR pattern was determined, according to the National Institute of Child Health and Human Development (NICHD) classification. Comparisons were made between those with and without abnormal FHR pattern to evaluate possible associated factors and pregnancy outcomes.

Results: Mean maternal age was 29.1 years, 55.7% were nulliparous, and mean gestational age at delivery was 38.1 weeks. Incidence of abnormal FHR pattern was 30.7% (30.3% and 0.4% in NICHD category II and III, respectively). Among these, 46.6% and 39.7% occurred in active and deceleration phase of labor, respectively. Univariate analysis showed that rate of abnormal FHR pattern was more common among nulliparous women (RR 1.22, 95% CI 1.003-1.5, p = 0.045). Cesarean delivery was required in 28.9% of cases with abnormal FHR pattern. Birth asphyxia was significantly more common among those with abnormal FHR pattern (7.2% vs. 3.7%, p = 0.016). Multivariate analysis demonstrated that only nulliparity was significantly associated with abnormal FHR pattern (adjusted OR 1.35, 95%CI 1.01-1.82, p = 0.045).

Conclusion: Incidence of intrapartum abnormal FHR pattern was 30.7% and nulliparity was the only independent associated factor. The condition significantly increased the risk of birth asphyxia.

Keywords: Abnormal fetal heart rate pattern, electronic fetal monitoring, incidence, nulliparity.

Correspondence to: *Kanokwaroon Watananirun, M.D., Department of Obstetrics and Gynaecology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok 10700, Thailand, Telephone: (662) 419-7000, Ext 4637, FAX: (662) 418-2662, Email address: kanokwaroon.wat@mahidol.ac.th*

อุบัติการณ์การเต้นผิดปกติของหัวใจทารกขณะเจ็บครรภ์คลอดในโรงพยาบาลศิริราช

กณิษฐา บุญชวน, กนกวรุณ วัฒนนิรันตร์, ดิฐกานต์ บริบูรณ์หิรัญสาร

บทคัดย่อ

วัตถุประสงค์: *เพื่อศึกษาอุบัติการณ์ ปัจจัยเสี่ยง และผลของการตั้งครรภ์ ของการเต้นผิดปกติของหัวใจทารกขณะเจ็บครรภ์คลอด*

วัสดุและวิธีการ: *ทำการศึกษาสตรีตั้งครรภ์ที่มีความเสี่ยงต่ำจำนวน 900 ราย ที่มาคลอดที่โรงพยาบาลศิริราช ทำการเก็บรวบรวมข้อมูลทางสูติศาสตร์ ข้อมูลการคลอด และผลของการตั้งครรภ์ จากนั้นทำการคำนวณอุบัติการณ์การเกิดการเต้นผิดปกติของหัวใจทารกขณะเจ็บครรภ์คลอด ตามการแบ่งกลุ่มของ NICHD ทำการเปรียบเทียบข้อมูลต่างๆ ระหว่างกลุ่มที่มีและไม่มีการเต้นผิดปกติของหัวใจทารกขณะเจ็บครรภ์คลอด เพื่อประเมินปัจจัยที่เกี่ยวข้อง และผลของการตั้งครรภ์*

ผลการศึกษา: *สตรีตั้งครรภ์มีอายุเฉลี่ย 29.1 ปี เป็นครรภ์แรกร้อยละ 55.7 อายุครรภ์เฉลี่ยเมื่อคลอด 38.1 สัปดาห์ อุบัติการณ์การเกิดการเต้นผิดปกติของหัวใจทารกขณะเจ็บครรภ์คลอดเท่ากับร้อยละ 30.7 ซึ่งเป็นกลุ่ม NICHD category II ร้อยละ 30.3 และ NICHD category III ร้อยละ 0.4 พบว่าการเต้นผิดปกติของหัวใจทารกเกิดในระยะ active phase ร้อยละ 46.6 และเกิดในระยะ deceleration phase ร้อยละ 39.7 และสตรีดังกล่าวคลอดโดยการผ่าท้องคลอดร้อยละ 28.9 พบอุบัติการณ์การเต้นผิดปกติของหัวใจทารกในสตรีที่คลอดครั้งแรกสูงกว่าครรภ์หลังอย่างมีนัยสำคัญทางสถิติ (RR 1.22, 95% CI 1.003-1.5, p = 0.045) และพบว่าอัตราการเกิดภาวะขาดออกซิเจนแรกคลอดในกลุ่มที่เกิดการเต้นผิดปกติของหัวใจทารกสูงขึ้นอย่างมีนัยสำคัญทางสถิติ (ร้อยละ 7.2 และร้อยละ 3.7, p = 0.016) จากการวิเคราะห์ multivariate analysis พบว่าการคลอดครั้งแรกเพิ่มความเสี่ยงต่อการเกิดการเต้นผิดปกติของหัวใจทารกอย่างมีนัยสำคัญทางสถิติ (adjusted OR 1.35, 95%CI 1.01-1.82, p = 0.045)*

สรุป: *อุบัติการณ์การเกิดการเต้นผิดปกติของหัวใจทารกขณะเจ็บครรภ์คลอดเท่ากับร้อยละ 30.7 และปัจจัยเสี่ยงที่สัมพันธ์กับภาวะนี้คือการคลอดครั้งแรก ภาวะนี้เพิ่มความเสี่ยงต่อการเกิดภาวะขาดออกซิเจนในทารกอย่างมีนัยสำคัญทางสถิติ*

คำสำคัญ: *การเต้นผิดปกติของหัวใจทารก, การตรวจติดตามการเต้นของหัวใจทารก, อุบัติการณ์, การคลอดครั้งแรก*

Introduction

Detection of abnormal fetal heart rate (FHR) pattern during intrapartum period indicated non-reassuring fetal condition. Without appropriate and immediate care, it may cause detrimental effect to the fetus, such as fetal hypoxia, acidosis and birth asphyxia. On the other hand, with suitable management, the complications can be prevented[1]. Thus, correct interpretation of fetal heart rate pattern is important. Fetal heart rate patterns are currently categorized into 3 categories according to The National Institute of Child Health and Human Development (NICHD) as demonstrated in Table 1[2] with different degree of severity and appropriate management has been recommended accordingly[2-7].

In terms of occurrence of different categories of FHR pattern, a previous study reported that majority of cases (77.9%) had normal FHR pattern (NICHD category I), and 22.1% had NICHD category II, while NICHD category III occurred in only 0.004%[8]. Another study reported the incidence of variable deceleration FHR pattern of 51.07%[9]. The differences might be due to differences in population characteristics or management guidelines in different settings. In addition, multiple factors also play important role in the occurrence of abnormal FHR pattern, including gravidity, amniotic fluid volume, meconium stain amniotic fluid, tocolytic drug administration[10-14].

When abnormal FHR pattern is detected, decision on appropriate route of delivery depends on the degree of abnormality and its improvement after appropriate management. For women with NICHD category II FHR pattern, if the abnormalities improve after appropriate management, vaginal delivery can be permitted. Otherwise, cesarean delivery should be performed. On the other hand, for women with NICHD category III FHR pattern, emergency cesarean delivery is usually mandatory[4, 5, 15, 16].

However, to date, there is still limited information on this important issue of abnormal FHR pattern in Siriraj Hospital and in Thailand. Therefore, this study was conducted to investigate the incidence of intrapartum abnormal FHR pattern in Siriraj Hospital. In addition, possible associated factors and pregnancy outcomes were also evaluated. The results of this study will provide more information and insights on this issue and can help obstetricians in giving better care of pregnant women for better pregnancy outcomes.

Table 1. Fetal heart rate classification system[2].

Fetal heart rate category	Characteristics
Category I	Baseline rate of 110–160 beats per minute
	Moderate baseline fetal heart rate variability
	Late or variable decelerations are absent
	Early decelerations may be present or absent
	Accelerations may be present or absent
Category II	Not categorized as category I or category III
Category III	Absent baseline fetal heart rate variability with any of the following:
	Recurrent late decelerations
	Recurrent variable decelerations
	Bradycardia
	Sinusoidal pattern

Materials and Methods

After approval from the Siriraj Institutional Review Board (SIRB), a retrospective cohort study was conducted at Department of Obstetrics and Gynaecology,

Faculty of Medicine, Siriraj Hospital during January and May 2016. A total of 900 low-risk, singleton pregnant women during first stage of labor were consecutively enrolled. Women who were scheduled for cesarean delivery, had severe maternal complications such as pregnancy-induced hypertension, overt diabetes, heart disease of functional class III or IV, etc., documented fetal anomaly, fetal arrhythmia, and fetal death were excluded. At 95% significance level, a sample size of at least 801 was required based on the rate of abnormal fetal heart rate pattern of 25% from a pilot study, with 3% allowable error.

Management of labor and delivery were provided according to institutional guideline. Baseline characteristics and related clinical data were recorded, including age, parity, gestational age, pre-pregnancy weight, height, maternal and fetal complications, route of delivery, and neonatal outcomes. Electronic FHR monitoring was offered to all women during labor and delivery. All fetal heart rate tracings were assessed by attending obstetricians under staff supervision, to determine abnormalities of FHR patterns. Management of abnormal FHR pattern was provided as appropriate, according to NICHD recommendation, which was adopted as institutional guideline. All fetal heart rate tracings were reviewed retrospectively by experienced staff before final classification was recorded.

Descriptive statistics were used to describe various variables, using mean, standard deviation, number, and percentage, as appropriate. Incidence of abnormal FHR pattern was estimated. Student t test and Chi square test were used in comparison of variables between those with normal and abnormal FHR pattern as appropriate. Relative risk (RR) and 95% confidence interval (CI) were estimated to determine association between various characteristics and abnormal FHR pattern. Logistic regression analysis was performed to determine independent associated factors with abnormal FHR patter, adjusted for potential confounders. Adjusted odds ratio (OR) and 95% confidence interval (CI) were estimated. A p value of < 0.05 was considered statistical significance.

Results

A total 900 pregnant women who delivered during the study period were included in this study and medical records were reviewed and documented. Table 2 showed baseline characteristics of the participants. Mean maternal age was 29.1 years and 55.7% were nulliparous. Complications during pregnancy included gestational diabetes (8.2%), gestational hypertension (10%), and suspected IUGR (2.4%).

Table 2. Baseline characteristics of the patients (N=900).

Characteristics	Mean ± SD
Mean maternal age ± SD (years)	29.1 ± 6.2
Mean BMI ± SD (kg/m^2)	21.9 ± 4.1
	N (%)
Nulliparous	501 (55.7)
BMI category	
Normal	570 (63.3)
Underweight	168 (18.7)
Overweight	162 (18.0)
Complications during pregnancy	
Gestational diabetes	74 (8.2)
Gestational hypertension	90 (10)
Antenatal suspected IUGR	22 (2.4)

Table 3 showed labor and delivery characteristics of the participants. Mean gestational age at delivery was 38.1 weeks and only 2.2% delivered at < 34 weeks. Thin and thick meconium stained amniotic fluid was observed in 8.6% and 3.4%, respectively. Majority of cases delivered vaginally (61.8%) and mean birth weight was 3015.4 g.

Table 3. Labor and delivery characteristics of the patients (N=900).

Characteristics	N (%)
Mean gestational age ± SD (weeks)	38.1 ± 1.7
Mean birth weight ± SD (g)	3015.4 ± 465.1
Gestational age at delivery	
< 34 weeks	20 (2.2)
34 - 36^{+6} weeks	84 (9.3)
≥ 37 weeks	796 (88.5)
Meconium stained amniotic fluid	
Thin	77 (8.6)
Thick	31 (3.4)
Route of delivery	
Vaginal delivery	556 (61.8)
Cesarean section	344 (38.2)

Table 4 showed characteristics of fetal heart rate pattern during labor. Incidence of abnormal FHR pattern was 30.7% (30.3% and 0.4% in NICHD category II and III respectively). Among these, 46.6% and 39.7% occurred during active and deceleration phase of labor, respectively.

Table 4. Characteristics of fetal heart rate pattern during labor (N=900).

Characteristics	N (%)
NICHD category	
Category I	623 (69.2)
Category II	273 (30.3)
Category III	4 (0.4)
Timing of abnormal fetal heart rate pattern	N = 277
Latent phase	38 (13.7)
Active phase	129 (46.6)
Deceleration phase	110 (39.7)
Thick	31 (3.4)
Route of delivery	
Vaginal delivery	556 (61.8)
Cesarean section	344 (38.2)

Table 5 showed analysis of risk of abnormal FHR patterns according to various characteristics. Rate of abnormal FHR pattern was significantly more common among nulliparous than multiparous women (33.5% vs. 27.3%, respectively; RR 1.22, 95% CI 1.003-1.5, p=0.045). Other factors including maternal age, pre-pregnancy BMI, gestational age, complication during pregnancy, meconium stained amniotic fluid were not significantly associated with abnormal fetal heart rate pattern.

Table 5. Risk of abnormal fetal heart rate patterns according to various characteristics.

Characteristics	N	Normal FHR N = 623	Abnormal FHR N = 277	RR (95% CI)	p value
Age group					
< 20 years	60	40 (66.7%)	20 (33.3%)	1.08 (0.73, 1.54)	0.78
20-34 years	666	456 (68.5%)	210 (31.5%)	1.0	
≥ 35 years	174	127 (73%)	47 (27%)	0.85 (0.65, 1.12)	0.245
BMI category					
Normal	570	395 (69.3%)	175 (30.7%)	1.0	
Underweight	168	105 (62.5%)	63 (37.5%)	1.23 (0.98, 1.56)	0.082
Overweight	162	123 (75.9%)	39 (24.1%)	0.8 (0.59, 1.08)	0.131
Parity					
Nulliparous	501	333 (66.5%)	168 (33.5%)	1.22 (1.003, 1.5)	0.045
Multiparous	399	290 (72.7%)	109 (27.3%)	1.0	
Gestational diabetes	74	55 (74.3%)	19 (25.7%)	0.82 (0.55, 1.23)	0.321
Gestational hypertension	90	64 (71.1%)	26 (28.9%)	0.93 (0.66, 1.31)	0.682
Suspected IUGR	22	14 (63.6%)	8 (36.4%)	1.19 (0.68, 2.08)	0.566
GA at delivery (weeks)					
< 34	20	11 (55%)	9 (45%)	1.46 (0.89, 2.4)	0.175
$34\text{-}36^{+6}$	84	61 (72.6%)	23 (27.4%)	0.89 (0.62, 1.28)	0.52
≥ 37	796	551 (69.2%)	245 (30.8%)	1.0	
Meconium stained AF					
No	792	554 (69.9%)	238 (30.1%)	1.0	
Thin	77	51 (66.2%)	26 (33.8%)	1.12 (0.81, 1.56)	0.499
Thick	31	18 (58.1%)	13 (41.9%)	1.4 (0.91, 2.14)	0.159

Table 6 showed pregnancy outcomes between those with and without abnormal FHR pattern. Cesarean section rate was significantly less in women with abnormal FHR pattern compared to others (28.9% vs. 42.2%, p<0.001), and only 19.1% required emergency cesarean section from the condition. Among those with abnormal FHR pattern, 66.3% had cesarean section from non-reassuring FHR pattern. 1-minute Apgar score of < 7 was significantly more common among those with abnormal FHR pattern (7.2% vs. 3.7%, p=0.016). NICU admission of fetus was not significantly associated with abnormal fetal heart rate pattern. All of the 4

cases with FHR pattern in NICHD category III delivered by cesarean section and none of the neonates had birth asphyxia.

Logistic regression analysis was performed to determine independent risk factors associated with abnormal FHR pattern and the results were shown in Table 7. After adjusting for potential confounders, only nulliparity was significantly associated with abnormal FHR pattern (adjusted OR 1.35, 95%CI 1.01-1.82, p=0.045).

Table 6. Outcomes of pregnant women between those with and without abnormal fetal heart rate patterns.

Characteristics	Normal FHR N = 623	Abnormal FHR N = 277	p value*
Route of delivery			< 0.001
Vaginal	359 (57.6%)	197 (71.1%)	
Cesarean section	264 (42.4%)	80 (28.9%)	
Indication for cesarean section			< 0.001
Non-reassuring FHR	NA	53/80 (66.3%)	
Others	264/264 (100%)	27/80 (33.7%)	
1-minute Apgar scores <7	23 (3.7%)	20 (7.2%)	0.016
NICU admission	13 (2.1%)	6 (2.2%)	0.939

* Chi square test

Table 7. Logistic regression analysis of risk factors associated with abnormal FHR pattern.

Risk factors	Adjusted OR	95% CI	p value
Nulliparous	1.35	1.01-1.82	0.045
Age			
20-34 years	1.0		
< 20 years	0.95	0.53-1.70	0.866
≥ 35 years	1.08	0.52-2.26	0.839
Meconium stained AF			
No	1.0		
Thin	1.15	0.7-1.9	0.580
Thick	1.73	0.83-3.6	0.142
GA at delivery			
37-40 weeks	1.0		
< 34 weeks	1.9	0.77-4.69	0.166
34-36+6weeks	0.85	0.52-1.42	0.541

Discussion

Intrapartum fetal heart rate monitoring is a procedure that used for fetal surveillance during labor. Fetal heart rate pattern is classified into 3 categories according to NICHD[2]. Category I is a normal FHR pattern that reflected of normal fetal oxygenation status

and complication or birth asphyxia are uncommon. Whereas category II and category III are abnormal FHR patterns that reflect some degree of fetal acidosis and fetal hypoxia, which may cause birth asphyxia and complication[2]. When abnormal FHR pattern is detected, appropriate management should be provided in a timely fashion to minimize neonatal morbidity and mortality. Multiple factors have been related with abnormal fetal heart rate pattern including gravidity, amniotic fluid volume, meconium stain amniotic fluid.

The result of this study showed that incidence of intrapartum abnormal FHR pattern was 30.7%, with mostly was in NICHD category II (30.4%). Among these, 46.6% and 39.7% occurred in active phase and deceleration phase of labor, respectively. The results were similar to previous studies that a majority of FHR pattern was in category I and II. Jackson M, et al[8], reviewed FHR pattern in 48,444 term singleton pregnant women with intrapartum fetal heart rate monitoring and found that the incidence of abnormal FHR pattern category II was 22.1% and only 0.004% were in category III. Salim R, et al[9], examined FHR pattern of 513 term singleton pregnant women in latent phase of labor and reported the incidence of NICHD category II variable deceleration was 51.1%.

Previous studies have reported that meconium-stained amniotic fluid and oxytocin used were associated with abnormal FHR pattern[13, 17]. A more recent study reported that hydramnios, oligohydramnios, and the presence of meconium-stained amniotic fluid were independently associated with abnormal FHR pattern[12]. However, nulliparity was the only factor that significantly associated with abnormal FHR pattern in this study. The differences might partly due to differences in definitions of abnormal FHR classification of some previous studies. In relation to multiparous women, nulliparous women usually have longer duration of labor that might also increase the probability of detection of abnormal FHR pattern. However, explanation of this relationship needs to be further investigated in future researches.

In terms of delivery route, the results showed that only 28.9% of women with abnormal FHR pattern had cesarean delivery, and, among them, 66.3% were conducted for FHR abnormalities. When abnormal FHR pattern develops, initial management with intrauterine resuscitation is provided (i.e., encouraging women to adopt the left lateral position, treatment of maternal hypotension, administrating facial oxygen and lowering or discontinuing of labor stimulation) and cesarean delivery is performed if the abnormalities do not resolve. The results of this study reflected that majority of FHR abnormalities resolved after such management was provided. The results also demonstrated that abnormal FHR pattern contributed to only 15.4% (53 of 344) of all cesarean delivery, which was lower than a previous study, which reported that 27% of intrapartum cesarean delivery was conducted for abnormal FHR patterns[18]. The differences might be from differences in study population and management guideline.

The results of the study showed that birth asphyxia was significantly more common among women with abnormal FHR pattern. This would be the results of fetal hypoxia and acidosis. Similar results of increased in adverse neonatal outcomes among abnormal FHR pattern have been reported previously, including 1-minute Apgar score of < 7, fetal acidosis, and NICU admission[8, 9, 12].

There were some limitations of the current study to be mentioned. The sample size and power might be inadequate when comparisons were made between groups. This study included patients from a single, tertiary care hospital that the findings may not be generalizable to other populations. In addition, there might be some variations in clinical practice in management and decision of cases with abnormal FHR pattern.

The results of this study provided more information and insights into pregnant women with intrapartum abnormal FHR. The condition was relatively common, even among low-risk women. Appropriate management could resolve the abnormalities in majority of cases. However, birth asphyxia was still more common among these women. Although the condition might not be accurately predictable, careful surveillance and timely detection of abnormalities and appropriate management could help improving the pregnancy outcomes. Future

studies are needed to investigate the importance of FHR abnormalities, possible predictive factors, and related care process improvements.

Conclusion

In conclusion, incidence of intrapartum abnormal FHR pattern in Siriraj Hospital was 30.7%. Cesarean delivery was required in 28.9% of cases. Only nulliparity was significantly increased the risk of FHR abnormalities. The condition significantly increased the risk of birth asphyxia.

Potential conflicts of interest

The authors declare no conflict of interest.

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Thai Journal of Obstetrics and Gynaecology
January 2018, Vol. 26, pp. 27-33

OBSTETRICS

Membrane Stripping to Reduce Postdate Pregnancy: A Randomized Controlled Trial

Piriya Tuttinapanich, M.D.*,
Reuthairat Tungmunsakulchai, M.D.*,
Maleechat Sripipattanakul, M.D.*,
Thumwadee Tangsiriwatthana, M.D.*.

** Department of Obstetrics and Gynecology, Khon Kaen Hospital, Khon Kaen 40000, Thailand*

ABSTRACT

Objectives: To compare delivery before 40 weeks of gestation between the pregnant women who had membrane stripping and those with no intervention.

Materials and Methods: One hundred and seventy-eight pregnant women, gestational age of 38 weeks or more who attended antenatal care clinic at Khon Kaen Hospital from January to July, 2016 were randomized into two groups: membrane stripping group and no intervention group. The proportion of pregnant women who delivered before 40 weeks of gestation was analyzed.

Results: Baseline characteristics were similar between groups. The proportion of women who delivered before 40 weeks of gestation in membrane stripping group was significantly higher than no intervention group (69.3% VS 51.1%, p=0.01) (RR=0.6, 95% CI 0.4-0.9). There was no significant difference in cesarean section rate, maternal complications and neonatal outcomes between groups.

Conclusion: Membrane stripping can reduce postdate pregnancy.

Keywords: Postdate pregnancy, membrane stripping.

Correspondence to: *Piriya Tuttinapanich, M.D., Department of Obstetrics and Gynecology, Khon Kaen Hospital, Khon Kaen 40000 Thailand, E-mail: piriya.tt@gmail.com*

การเลาะถุงน้ำน้ำคร่ำ เพื่อลดการตั้งครรภ์ที่อายุครรภ์มากกว่า 40 สัปดาห์: การควบคุมแบบสุ่ม

พิริยา ทัตตินาพานิช, ฤทัยรัตน์ ตั้งมั่นสกุลชัย, มาลีชาติ ศรีพิพัฒนะกุล, ทุมวดี ตั้งศิริวัฒนา

บทคัดย่อ

วัตถุประสงค์: *เปรียบเทียบสัดส่วนการคลอดก่อนอายุครรภ์ 40 สัปดาห์ ในสตรีตั้งครรภ์ที่ได้รับการเลาะถุงน้ำคร่ำเทียบกับสตรีตั้งครรภ์ที่ไม่ได้รับการเลาะถุงน้ำคร่ำ*

วัสดุและวิธีการศึกษา: *สตรีตั้งครรภ์ที่มีอายุครรภ์มากกว่าหรือเท่ากับ 38 สัปดาห์ ทั้งหมด 178 คน ที่มาฝากครรภ์ที่ห้องฝากครรภ์ รพ.ขอนแก่น ในช่วงเดือนมกราคม ถึง กรกฎาคม 2559 ได้รับการสุ่ม เป็น 2 กลุ่ม คือ กลุ่มที่ได้รับการเลาะถุงน้ำคร่ำ และกลุ่มที่ไม่ได้รับการเลาะถุงน้ำคร่ำ เปรียบเทียบการคลอดก่อนอายุครรภ์ 40 สัปดาห์ ในสตรีตั้งครรภ์ทั้งสองกลุ่มทั้งสองกลุ่ม*

ผลการวิจัย: *ลักษณะทางประชากรศาสตร์ไม่แตกต่างกันระหว่างทั้ง 2 กลุ่ม กลุ่มที่เลาะถุงน้ำคร่ำ คลอดก่อนอายุครรภ์ 40 สัปดาห์ ร้อยละ 69.3 ส่วนกลุ่มที่ไม่ได้รับการเลาะถุงน้ำคร่ำ คลอดก่อนอายุครรภ์ 40 สัปดาห์ ร้อยละ 51.1 ซึ่งมีความแตกต่างกันอย่างมีนัยสำคัญทางสถิติ อัตราการผ่าตัดคลอด ภาวะแทรกซ้อนต่อมารดาและทารกทั้งสองกลุ่ม ไม่มีความแตกต่างกัน*

สรุป: *การเลาะถุงน้ำคร่ำ สามารถลดการตั้งครรภ์ที่อายุครรภ์มากกว่า 40 สัปดาห์*

คำสำคัญ: *การเลาะถุงน้ำคร่ำ, อายุครรภ์มากกว่า 40 สัปดาห์*

Introduction

The postdate pregnancy is gestational age (GA) more than 40 weeks to 41^{+6} weeks. The incidence of postdate pregnancy of Khon Kaen Hospital (KKH) is 17%. It is often associated with increased risk of perinatal morbidity and mortality[1]. Fetal and neonatal mortality rates increase sharply after 40 weeks of gestation. It is believed that utero-placental insufficiency, meconium aspiration and intrauterine infection are the underlying causes of the increased perinatal mortality rates in these cases[2]. In 2006, Nicholson, et al found that the nadir of neonatal morbidity, including birth injuries, was around 38 weeks of gestation and then increase in a continuous fashion thereafter[3].

Stripping or sweeping the amniotic membrane is commonly practiced to induce labor. Digital separation of fetal membranes from the lower uterine segment is safe and easy to perform using circular movement of the examining fingers between the lower segment and the fetal membranes. It increases local release of prostaglandin $F_{2\alpha}$, activity of phospholipase A_2, frequency of uterine contractions and also cause mechanical dilatation of the cervix. Moreover, it can promote the spontaneous onset of labor, reducing the duration of pregnancy and reducing induction of labor using oxytocin, prostaglandins or amniotomy. Although membrane stripping has been associated with an increase risk of premature rupture of membranes, discomfort from the procedure, other systematic review of 1,525 women reported no evidence of serious finding [4-6].

Kashanian, et al., found that time to delivery was not different between membrane stripping group and no intervention group (7.7±6.9 and 7.1±5.6 days, p=0.61)[7]. In contrast, Boulvain and Ugwu, et al., reported that membrane stripping can reduce the incidence of postterm pregnancy[6,8].

There was no study about the safety and efficacy of membrane stripping in prevention of postdate pregnancy. Despite many studies demonstrated the effectiveness of membrane stripping in postterm pregnancy but their findings are still inconclusive. Therefore, the present study was conducted to evaluate whether membrane stripping could reduce postdate pregnancy.

Materials and Methods

This randomized controlled trial was conducted at Khon Kaen Hospital, Thailand from January to July, 2016. This study was approved by Khon Kaen Hospital Institute Review Board in Human Research. All participants were informed about the study and signed the consent form before enrollment.

We included pregnant women age 18 years old or more with gestational age 38 weeks or more, singleton pregnancy, cephalic presentation, no labor pain, and planned vaginal delivery. Pregnant women with HIV infection, placenta previa, rupture of membranes, previous uterine scar such as previous cesarean section, myomectomy, diabetes mellitus (overt or gestational type A_2), and pregnancy induced hypertension were excluded.

The pregnant women who had uncertain date, eg. had irregular period, wrong date, recent use of hormonal contraception, the gestational age was corrected using ultrasound[9].

Eligible participants were randomized by computer generated with block of four and randomly assigned into two groups; membrane stripping and no intervention groups. The random numbers were put in the sequentially sealed, opaque envelopes. Pregnant women who were randomized to membrane stripping group, membrane stripping was performed by digital separation of fetal membranes from the lower uterine segment using two circular movements once a week until delivery by residents or staffs of Obstetrics and Gynecology. Routine ANC was similarly provided in both groups. Induction of labor using either vaginal misoprostol or intravenous oxytocin was provided after 40 weeks. Primary outcome was delivery before 40 weeks of gestation. Secondary outcome was cesarean section rate. Women who delivered in other hospital were interviewed by phone.

The sample size was calculated from a pilot study. We used formula for test of difference in two independence proportions with alpha of 0.05, power of

80% and 10% dropouts. The sample size in each group was 89 cases.

$$n/group = [\frac{z_{\alpha/2}\sqrt{2pq} + z_{\beta}\sqrt{p_1q_1 + p_2q_2}}{p_1 - p_2}]^2$$

Analyses were based on the intention to treat. Categorical variables were analyzed by Chi-square test or Fisher's extract test. Continuous variables were analyzed by Student t-test or Mann-Whitney U-test depends on data distribution. The primary outcome was presented as relative risk with 95% confidence interval. Other outcomes were presents as percentage, mean with standard deviation, and median with interquartile range. P value less than 0.05 was represented statistical significance. Statistical analysis was performed using SPSS 17.0 software.

Results

One hundred and seventy-eight participants were randomly assigned into two groups, 89 cases in both groups. One participant in membrane stripping group dropped out due to birth before admission and one in no intervention group dropped out due to delivery at district hospital and the data was not available. Therefore, the totally 88 participants left in each group were analyzed (Fig. 1).

Baseline characteristics including age, parity and gestational age were similar in both groups (Table 1). The proportion of delivery before 40 weeks in membrane stripping group was significant higher than no intervention group (69.3% versus 51.1%, p=0.01, RR=0.6, 95% CI 0.4-0.9) (Table 2). Time to delivery in membrane stripping group was significantly shorter than no intervention group 5(2-8) versus 7.5(4.5-12) days, p<0.001, route of delivery was similar in both groups (vaginal delivery 84.1% versus 77.3% and cesarean section 15.9% versus 22.7%, p=0.25 in membrane stripping group and no intervention group, respectively) (Table 2).

There were four women (4.6%) who had premature rupture of membranes only in membrane stripping group. There were no serious maternal complications and neonatal outcomes were similar in both groups (Table 3).

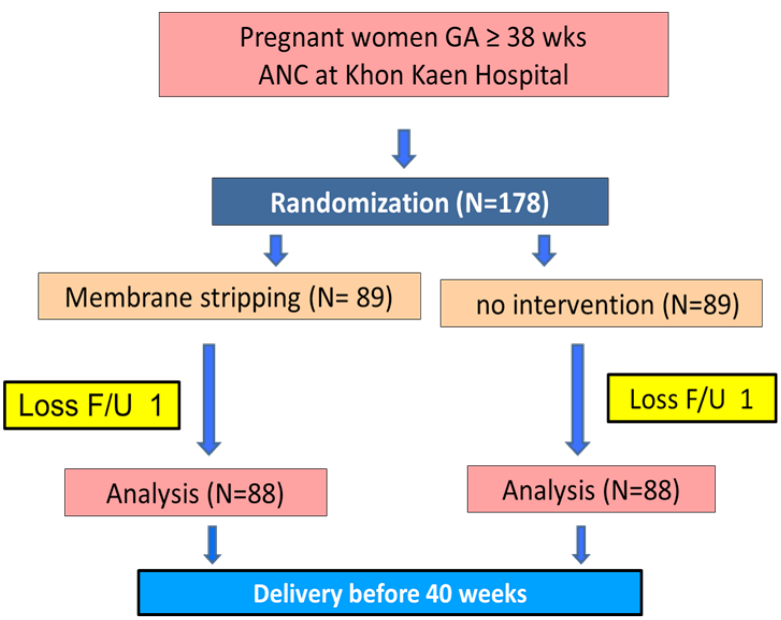

Fig. 1. Study flow chart.

Table 1. Baseline characteristics.

Characteristics	Membrane stripping (n= 88)	No intervention (n= 88)	p value
Age (yrs), median (IQR)	25 (21-30)	24.5 (21-29.5)	0.63
Parity			0.36
Nulliparous, no. (%)	53 (60.2)	47 (53.4)	0.975
Multiparous, no. (%)	35 (39.8)	41 (46.6)	0.944
Gestational age (weeks), median (IQR)	38^{+4} (38^{+2} - 39^{+1})	38^{+3} (38^{+1} - 39^{+0})	0.08

Table 2. Primary outcome and secondary outcomes.

Duration of delivery	Membrane stripping (n= 88)	No intervention (n= 88)	RR	95%CI	p value
Primary outcome					
Delivery before GA 40 weeks, no. (%)	61 (69.3)	45 (51.1)	0.6	0.4-0.9	0.01
Nulliparous	36 (59)	23 (51.1)			
Multiparous	25 (41)	22 (48.9)			
Secondary outcomes					
Time to delivery (days), median (IQR)	5 (2-8)	7.5 (4.5-12)	-	-	< 0.001
Route of delivery					0.25
Vaginal delivery, no. (%)	74 (84.1)	68 (77.3)	-	-	
Cesarean section, no. (%)	14 (15.9)	20 (22.7)	-	-	

Table 3. Maternal complications and neonatal outcomes.

Outcomes	Membrane stripping (n= 88)	No intervention (n= 88)	p value
Maternal complications			
Premature rupture of membrane (PROM), no. (%)	4 (4.6)	0	0.12
Vaginal bleeding, no. (%)	0	0	-
Chorioamnionitis, no. (%)	1 (1.1)	0	0.99
Neonatal outcomes			
Birth weight (g), mean (SD)	3,114.1 (373.2)	3,184.1 (389.2)	0.25
Meconium stain amniotic fluid	22 (25)	11 (12.5)	0.03
Birth asphyxia (APGAR ≤ 7)			
At 1 min, no. (%)	12 (13.6)	5 (5.7)	0.07
At 5 min, no. (%)	3 (3.4)	0 (0)	0.25
Admission to NICU, no. (%)	4 (4.6)	3 (3.4)	0.99

Discussion

Our findings supported the effectiveness of membrane stripping for induction of labor. Although, most of the previous studies performed membrane stripping in pregnant women gestational age more than 40 weeks and meta-analysis of 14 randomized controlled trials[6] found that membrane stripping in pregnant women with gestational age 38 weeks had no benefit, but the present study demonstrated that membrane stripping in pregnant women GA 38 weeks or more could successfully induce labor and had delivery before 40 weeks more than control group. The present study included pregnant women who were 38 weeks or more, then followed up until 40 weeks and inducedlabor after 40 weeks while other studies followed up until 42 weeks. The reason of conducting study in women with gestational age of 38 weeks or more was due to increase perinatal risk index, neonatal morbility and mortality rate due to meconium aspiration syndrome, uteroplacental insufficiency induced intrauterine growth restriction, fetal distress and fetal macrosomia, and increase cesarean section rate[1-3]. The present study showed that membrane stripping reduced postdate pregnancy as high as 40% or every five women who had membrane stripping could reduce postdate pregnancy in one woman (NNT=5). Approximately 60% of primigravida women in membrane stripping group delivered before 40 weeks. The other potential benefit of membrane stripping founded in the present study was significantly shorter time to delivery when compared to no intervention. The participants who had membrane stripping experienced spontaneous labor within 5 days after the procedure. Cesarean section rate in membrane stripping group was not increase which was comparable to other studies[6-8,10]. Premature rupture of membrane (PROM) occured in membrane stripping but the difference was not statistically significant[7, 8] which was consistent with our finding. Even though four participants in the membrane stripping group had premature rupture of membranes, however, none of them developed chorioamnionitis. Other complications of membrane stripping such as vaginal bleeding, which was reported in the previous study[6, 8, 11], was not found in the present study. Neonatal outcomes (birth asphyxia at 1 minute, 5 minutes, rate of admission NICU) were not different in both groups, which was similar to previous studies[6, 8, 11]. The present study showed more birth asphyxia at 1 minute in membrane stripping group compare to no intervention group, this might be due to the higher incidence of meconium stain in membrane stripping group (25% VS 12.5%).

The strength of the present study was randomized controlled trial and low dropout rate (1.1%) and limitation in the present study was patient satisfaction, especially in terms of maternal discomfort or pain during membrane stripping was not evaluated.

Membrane stripping is a useful procedure in low resource setting and help reducing postdate pregnancy. Other aspects should be assessed such as patient satisfaction, physicians satisfaction, and cost effectiveness of the procedure.

Conclusion

This study supported that membrane stripping can reduce postdate pregnancy without any maternal and neonatal complications.

Acknowledgements

The authors would like to thank nurses and staffs of Department of Obstetrics and Gynecology, Khon Kaen Hospital for their helps and supports.

Potential conflicts of interest

The authors declare no conflict of interest.

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Thai Journal of Obstetrics and Gynaecology
January 2018, Vol. 26, pp. 34-41

OBSTETRICS

The Comparison of Blood Loss After Vaginal Delivery Between Placental Cord Drainage and Cord Clamping Before Placental Delivery in Buddhachinaraj Phitsanulok Hospital

Sarawut Mithala, M.D.*,
Kanchapan Sukonpan, M.D.*,
Chokdee Chunlaphakhee, M.D.*.

** Department of Obstetrics and Gynecology, Buddhachinaraj Phitsanulok Hospital, Phitsanulok 65000, Thailand*

ABSTRACT

Objectives: To compare blood loss after vaginal delivery between placental cord drainage and cord clamping before placental delivery.

Materials and Methods: A randomized controlled trial was done on 180 pregnant women who were admitted for vaginal delivery in Buddhachinaraj Phitsanulok Hospital during 1st July 2015 to 30th June 2016. The patients were divided equally into two groups: a study group and a control group. In the study group, the placental cord was drained before placental delivery whereas in the control group, the placental cord was clamped. Postpartum blood loss and other complications were recorded.

Results: Median duration of the third stage of labor and postpartum blood loss were significantly different between two groups. The median (interquartile range) duration of the third stage in the study group (3 (2,4) minute) was shorter than the control group (4 (3,6) minute, $p < 0.05$). The median blood loss in study group (300 (250, 330) mL) was also lower than the control group (320 (300, 350) mL, $p < 0.05$). There was not significantly different in postpartum hemorrhage between the two groups.

Conclusion: Placental cord drainage can reduce postpartum blood loss and the duration of the third stage of labor. This technique is safe, simple and noninvasive to practice for reducing amount of postpartum blood loss and no serious complications occurred.

Keywords: Placental cord drainage, postpartum hemorrhage, third stage of labor.

Correspondence to: *Sarawut Mithala, M.D., Department of Obstetrics and Gynecology, Buddhachinaraj Phitsanulok Hospital, Phitsanulok 65000, Thailand, E-mail: sara_mith@hotmail.com*

การเปรียบเทียบปริมาณการเสียเลือดหลังคลอดในหญิงตั้งครรภ์ที่คลอดทางช่องคลอดระหว่างกลุ่มที่ปล่อยเลือดจากสายสะดือและกลุ่มที่ไม่ได้ปล่อยเลือดจากสายสะดือก่อนคลอดรกในโรงพยาบาลพุทธชินราช พิษณุโลก

ศราวุธ มิทะลา, กัญจน์พรรณ สุคนธ์พันธุ์, โชคดี จุลภาคี

บทคัดย่อ

วัตถุประสงค์: *เปรียบเทียบผลการเสียเลือดหลังคลอดในหญิงตั้งครรภ์ที่คลอดทางช่องคลอดระหว่างกลุ่มที่ปล่อยเลือดออกจากสายสะดือและกลุ่มที่ไม่ได้ปล่อยเลือดออกจากสายสะดือหลังคลอด*

วัสดุและวิธีการ: *งานวิจัยนี้เป็นแบบ Randomized controlled trial มีผู้เข้าร่วมการศึกษา 180 คนที่รับไว้รักษาในโรงพยาบาลพุทธชินราช พิษณุโลก และคลอดเองทางช่องคลอดในช่วง 1 กรกฎาคม 2558 ถึง 30 มิถุนายน 2559 โดยแบ่งเป็นกลุ่มศึกษาและกลุ่มควบคุมกลุ่มละ 90 คน กลุ่มศึกษาคือกลุ่มที่ปล่อยเลือดจากสายสะดือก่อนทำคลอดรก ส่วนกลุ่มควบคุมคือกลุ่มที่ไม่ได้ปล่อยเลือดจากสายสะดือก่อนทำคลอดรก การศึกษานี้ทำคลอดรกด้วยวิธี controlled cord traction ทั้งสองกลุ่ม และมีการบันทึกข้อมูลพื้นฐาน การดำเนินการคลอด และเปรียบเทียบปริมาณเลือดหลังคลอดรวมถึงภาวะแทรกซ้อนที่เกิดขึ้นขณะศึกษา*

ผลการศึกษา: *พบว่ามีความแตกต่างกันอย่างมีนัยสำคัญทางสถิติระหว่างปริมาณการเสียเลือดหลังคลอดและระยะเวลาการคลอดระยะที่สามของทั้งสองกลุ่ม ค่ากลางของปริมาณการเสียเลือดของกลุ่มศึกษา (300 (250, 330) มิลลิลิตร) มีปริมาณน้อยกว่ากลุ่มควบคุม (320 (300 ,350) มิลลิลิตร) อย่างมีนัยสำคัญทางสถิติ (pvalue < 0.001) ส่วนค่ากลางของระยะเวลาของระยะที่สามของการคลอดของกลุ่มศึกษา (3 (2, 4) นาที) น้อยกว่ากลุ่มควบคุม (4 (3, 6) นาที) อย่างมีนัยสำคัญทางสถิติเช่นกัน (p value <0.001) แต่ไม่มีความแตกต่างกันของจำนวนการตกเลือดหลังคลอด*

สรุป: *การปล่อยเลือดออกจากสายสะดือก่อนคลอดรกสามารถลดปริมาณการเสียเลือดหลังคลอดและระยะที่สามของการคลอดได้ เป็นวิธีที่สะดวกและปลอดภัยเพื่อลดปริมาณการเสียหลังคลอดและไม่มีภาวะแทรกซ้อนที่รุนแรงเกิดขึ้น*

คำสำคัญ: *ปล่อยเลือดจากสายสะดือ, ตกเลือดหลังคลอด, การคลอดระยะที่สาม*

Introduction

Postpartum hemorrhage (PPH) is one of the most common causes of maternal death worldwide[1, 2]. There are many causes of early postpartum hemorrhage, with the most common cause being uterine atony, which is caused by uterine overdistension, chorioamnionitis, tocolytic drugs, or prolonged labor. Nowadays, the World Health Organization (WHO)[3] recommendation for the prevention of PPH is the active management of the third stage of labor, consisting of three steps: intramuscular administration of 10 units of oxytocin immediately after anterior shoulder is delivered; placenta was delivered by controlled cord traction technique; followed by uterine massage. Furthermore, placental cord drainage in the third stage of labor involves unclamping the previously clamped and allowing the blood from the maternal side of placenta to drain freely, is one of the techniques for reducing postpartum blood loss and some complications, such as prolonged third stage, retained placenta.

According to the previous studies[4, 5, 6], showed that placental blood drainage is a noninvasive, simple and simple technique for shortening the length of the third stage labor and reducing the amount of postpartum blood loss. The information about the use of this method is still sparse in Thailand. Also, the previous studies in Thailand had small sample size[7] and unclear technique of the measurement of blood loss[8, 9] - so these shortcomings are a motivation for this study. The main objective is to compare blood loss after vaginal delivery between placental cord drainage and cord clamping before placental delivery in Buddhachinaraj Phitsanulok Hospital, Phitsanulok province. It is in the central region of Thailand, where the incidence of postpartum hemorrhage is about 4.2 percent per year.

Materials and Methods

This study was designed as a prospective randomized controlled trial. The sample size was calculated from Chalaew Sattamai's study[9] and approved by the ethics committee of Buddhachinaraj Phitsanulok Hospital. The pregnant women who were admitted to the labor room in Buddhachinaraj Phitsanulok Hospital from 1st July 2015 to 30th June 2016 and met the criteria were recruited. The inclusion criteria included Thai pregnant women who had 34-42 weeks of gestational age, vertex presentation, viable fetus, no detected fetal anomalies, estimated fetal weight < 4,000 gm, history of childbirth less than 4 times, no history of previous cesarean section, no history of antepartum hemorrhage and postpartum hemorrhage, no obstetric indications for cesarean section, no medical complication such as liver disease, coagulopathy and anticoagulant use. The exclusion criteria included third degree of episiotomy tear, chorioamnionitis, preeclampsia with severe features and delivery with cesarean section during the study. The protocol and informed consent were explained to all participants. For underage patients, the informed consent were explained to their guardians and signed by themselves.

The patients were randomized by block randomization to the study and control groups according to a code kept in a sealed envelope; it was opened when the doctors performed the vaginal delivery. The general maternal baseline characteristics, history taking, physical examination, and progression of labor of all pregnant women were recorded. In the study group, after cutting of umbilical cord, blood was freely released from the maternal side into a sterile container, until no blood was seen in the end of the cord. Then, controlled cord traction was performed to deliver the placenta[6]. Postpartum blood loss was assessed by a plastic collecting bag with scale and the released blood was drained to a sterile container and measured by a syringe. The duration of third stage of labor, degree of episiotomy, placental weight, neonatal weight, uterotonic drugs used, vital signs and blood transfusion were recorded. In the control group, the umbilical cord was clamped immediately after fetal delivery. Placental delivery was performed with controlled cord traction technique. All information was

recorded in the same method as the study group.

Postpartum hemorrhage is defined as a blood loss of 500 milliliters (mL) or more after completion of the third stage of labor[1]. The duration of the first, second, and third stages, and the duration of membranes ruptured until delivery, postpartum blood loss, retained placenta, placental cord rupture during placental delivery, manual removal of placenta, and blood transfusion were recorded in both groups.

Statistical analysis

Descriptive statistics were used to analyze demographic data, labor and delivery characteristics. Fisher's exact or Chi-square was used to compare the categorical variables, and Mann-Whitney test was used to compare continuous variables (median) between the two groups. A p value of less than 0.05 was considered a statistically significant difference.

Results

One hundred and eighty pregnant women were recruited and were randomly divided into two groups, so that there were 90 participants in each group. For the baseline maternal characteristics, there were not a significant differences between the two groups in maternal age, gravidity, parity, gestational age, body mass index (BMI), maternal weight gain and maternal underlying diseases (Table 1, 2).

There was no statistically significant difference in mode of delivery, duration of the first stage and second stage of labor, duration of rupture of membranes, birthweight, placental weight and uterotonic agents use (Table 3). There was a statistically significant difference in postpartum blood loss and duration of the third stage of labor. Median (interquartile range) of postpartum blood loss in the study group (300 (250, 330) mL) was lower than the control group (320 (300, 350) mL, $p < 0.001$). There were 5 and 6 cases of postpartum hemorrhage in the study and control groups, respectively. There were three cases in the control groups who had postpartum hemorrhage as 1,000 mL or more after completion of the third stage of labor (1,000, 1,200, 1,200 mL, respectively). They received a blood transfusion because of hypovolemic shock due to acute blood loss and hematocrit was 27, 25 and 24 percent respectively. PPH was detected in the study group but did not require blood transfusion because they had PPH less than 1,000 mL and no signs of hypovolemic shock or decreasing of hematocrit less than 30 percent after transfer to postpartum ward (Table 4). There was no statistically significant difference in PPH was observed between the two groups. The median (interquartile range) duration of the third stage in the study group (3 (2,4) minutes) was also shorter than the control group (4 (3, 6) minutes, $p < 0.001$). The mean volume of released blood was 39.9 mL. In the control group, one case had cord rupture during placental delivery. There was no manual placental removal in all participants.

Table 1. Maternal baseline characteristics.

Characteristics	Study group (n=90)	Control group (n=90)	p value
Age (year)	25 (21, 29)	24 (19, 32)	0.79
Gravid	2 (1, 2)	2 (1, 2)	0.51
Parity	0 (0, 1)	0 (0, 1)	0.60
Gestational age (weeks)	38 (37, 39)	39 (38, 40)	0.29
BMI (kg/m^2)	21.5 (19.3, 23.8)	21.2 (18.7, 23.5)	0.47
Maternal weight gain (kg)	14 (11, 17)	13 (10, 16)	0.43

* Mann-Whitney test, Data were represented as median (interquartile range)

Table 2. Maternal underlying diseases.

Underlying disease	Study group (n = 15)	Control group (n = 24)	p value
Chronic hypertension	3	1	0.62
Gestational hypertension	1	4	0.36
Preeclampsia without severe feature	1	2	1.00
Overt DM	1	0	1.00
GDMA1	2	1	1.00
GDMA2	0	1	1.00
Anemia	3	10	0.81
Hepatitis B viral infection	2	0	0.49
HIV infection	0	1	1.00
Latent syphilis	0	2	0.49
Thyroid goiter	0	1	1.00
Asthma	1	0	1.00
Hypercholesterolemia	1	0	1.00
G6PD deficiency	0	1	1.00

* Fisher's exact

Table 3. Labor and delivery characteristics.

	Study group (n=90)	Control group (n=90)	p value
Mode of delivery[1]			0.28
Normal delivery	85	80	
Vacuum extraction	5	10	
Progression of labor[2]			
First stage of labor (min)	437 (315, 660)	455 (270, 615)	0.19
Second stage of labor (min)	10 (5, 17)	11 (7, 17)	0.91
Duration of membrane ruptured (min)	145 (65, 269)	149 (60, 260)	0.90
Third stage of labor (min)	3 (2, 4)	4 (3, 6)	< 0.001
Uterotonic drugs used[1]			
Oxytocin (Syntocinon)	90	90	1.00
Ergometrine (Methergine)	20	32	0.07
Misoprostol (Cytotec)	6	12	0.21
Postpartum blood loss[2]	300 (250, 330)	320 (300, 350)	< 0.001

1 = Fisher's exact 2 = Mann-Whitney test

Table 4. Postpartum outcomes.

	Study group (n = 90)	Control group (n = 90)	p value
Episiotomy tear[1]			1.00
No tear	1	0	
First degree	1	2	
Second degree	88	88	
Birth weight (gm)[2]	3115 (2970, 3350)	3185 (2900, 3330)	0.87
Placental weight (gm)[2]	620 (600, 650)	620 (600, 650)	0.65
Postpartum hemorrhage[1]	5	6	0.5
Blood transfusion[1]	0	3	0.12
Hypovolemic shock[1]	0	3	0.12
Placental cord ruptured during placental delivery[1]	0	1	1.00

1= Fisher's exact 2 = Mann-Whitney test

There was no statistically significant difference in mode of delivery, duration of the first stage and second stage of labor, duration of rupture of membranes, birthweight, placental weight and uterotonic agents use (Table 3). There was a statistically significant difference in postpartum blood loss and duration of the third stage of labor. Median (interquartile range) of postpartum blood loss in the study group (300 (250, 330) mL) was lower than the control group (320 (300, 350) mL, $p < 0.001$). There were 5 and 6 cases of postpartum hemorrhage in the study and control groups, respectively. There were three cases in the control groups who had postpartum hemorrhage as 1,000 mL or more after completion of the third stage of labor (1,000, 1,200, 1,200 mL, respectively). They received a blood transfusion because of hypovolemic shock due to acute blood loss and hematocrit was 27, 25 and 24 percent respectively. PPH was detected in the study group but did not require blood transfusion because they had PPH less than 1,000 mL and no signs of hypovolemic shock or decreasing of hematocrit less than 30 percent after transfer to postpartum ward (Table 4). There was no statistically significant difference in PPH was observed between the two groups. The median (interquartile range) duration of the third stage in the study group (3 (2,4) minutes) was also shorter than the control group (4 (3, 6) minutes, $p < 0.001$). The mean volume of released blood was 39.9 mL. In the control group, one case had cord rupture during placental delivery. There was no manual placental removal in all participants.

Discussion

Our study showed postpartum blood loss and duration of the third stage of labor were significantly different in the study and control groups, while the general characteristics of participants were not different. On the other hand, the incidence of postpartum hemorrhage was no statistically significant difference between two groups. The outcomes of this study were similar to the previous studies[7, 9, 10] which could reduce postpartum blood loss and short of duration of the third stage of labor. The mechanism of placental cord drainage is reduction of the uterine bulkiness that can make a good uterine contraction so that can reduce blood loss after delivery. Therefore, releasing blood from the umbilical cord in the maternal side is one of the techniques that can reduce duration of the third stage of labor and postpartum blood loss. Although the incidence of PPH did not decrease significantly, but serious complications such as retained pieces of placenta, placental cord rupture, hypovolemic shock

and blood transfusion were not found in the placental cord drainage group.

In Thailand, the study of Surasak[11] showed that the postpartum hemorrhage was the most important factor in maternal health and postpartum complications such as health recovery, breast feeding and neonatal development. The incidence of PPH in Thailand was 11.89 percent compared with the information from World Health Organization (WHO), which is 24 percent worldwide. Therefore, placental cord drainage combined with the active management of the third stage of labor could reduce postpartum blood loss and improve postpartum complications. Regarding the safety of placental cord drainage, data from this study were congruent with Jeborry's study[12] that was simple, safe and noninvasive to practice, and there were no serious complications such as uterine inversion, retained placenta which was managed with manual placental removal, or increased the incidences of PPH. On the others hand, the outcome of this study was in contrast to Leduc D et al.'s study[13] which did not recommend placental cord drainage as a routine practice in third stage of labor because there was no significant difference in PPH. However, every complication was dependent on the experience of the doctors.

Although, there was no statistically significant difference in postpartum hemorrhage between placental cord drainage and cord clamping groups but the complications in the control group occurred more than the study group such as placental cord ruptured during placental delivery and hypovolemic shock who required blood transfusion. From these complications, the clinical benefit of placental cord drainage could reduce these serious complications.

However, this study did not show a significant difference of postpartum hemorrhage among both groups. Thus, further studies should be considered of more sample size to show the PPH and some serious complications obviously.

Conclusion

Placental cord drainage could reduce postpartum blood loss and the duration of the third stage of labor. This technique was safe and simple and noninvasive to practice for reducing postpartum blood loss, and no serious complications occurred.

Acknowledgement

The authors would like to thank Mr. Robert Moore, a professional English reviewer, for his kind help editing English grammar and Mr. Chatuphorn Suamee, a senior researcher, for an invaluable statistical consultation.

Potential conflicts of interest

The authors declare no conflict of interest.

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Thai Journal of Obstetrics and Gynaecology
January 2018, Vol. 26, pp. 42-51

GYNECOLOGY

Effect of Voiding Position on Uroflowmetry in Women with Anterior Vaginal Wall Prolapse Stage II and III

Anchalee Khuntong, M.D.*,
Rujira Wattanayingcharoenchai, M.D.*,
Wit Viseshsindh, M.D.**.

** Department of Obstetrics and Gynaecology, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok 10400, Thailand*

*** Department of Surgery, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok 10400, Thailand*

ABSTRACT

Objectives: To determine the effect of voiding position on uroflowmetry parameters in women with anterior vaginal wall prolapse stage II and III

Materials and Methods: A total of 51 women with anterior compartment prolapse stage II and III attending female pelvic medicine and reconstructive surgery clinic, Ramathibodi Hospital during June 2015 to April, 2016 were enrolled in the randomized controlled crossover study. After informed consent was obtained, participants were randomly allocated sequences of two voiding positions: sitting and modified squatting. The uroflowmetry was performed in both voiding positions for each participant. The post-void residual urine (PVR) volumes were measured using transabdominal ultrasound. Uroflowmetry parameters and PVR values were compared between the two different voiding positions.

Results: The mean age of the participants was 64.8 ± 9.1 years. The POP-Q staging was stage II in 30 (58.8%) and stage III in 21 (41.2%) women. There was no statistically significant difference in voided volume of women in sitting and modified squatting position which were 335.2 ± 160.1 and 362.7 ± 161.0 ml, respectively ($p > 0.05$). Mean maximum flow rate and mean average flow rate for the sitting (22.3 ± 11.2 and 10.7 ± 5.5 ml/s) and modified squatting position (23.8 ± 10.9 and 11.91 ± 6.4 ml/s) in the women were not significantly different ($p > 0.05$). PVR value in sitting voiding position was significant lower than in modified squatting position (52.6 ± 55.1 vs 75.0 ± 78.6) ($p < 0.05$).

Conclusion: Voiding positions either sitting or modified squatting does not affect urinary flow rate in women with anterior wall prolapse. Voiding in modified squatting position may results in higher post-void residual urine.

Keywords: Pelvic organ prolapse, voiding position, uroflowmetry.

Correspondence to: *Anchalee Khuntong, M.D., Department of Obstetrics and Gynaecology, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok 10400, Thailand .*
E-Mail: Anchaleekhuntong@gmail.com

การศึกษาผลของท่าการปัสสาวะต่ออัตราการไหลของปัสสาวะในสตรีที่มีการหย่อนของผนังช่องคลอดด้านหน้าระยะที่สองและสาม

อัญชลี ขุนทอง, รุจิรา วัฒนายิ่งเจริญชัย, วิทย์ วิเศษ

บทคัดย่อ

***วัตถุประสงค์**: เพื่อศึกษาผลของท่าการปัสสาวะต่ออัตราการไหลของปัสสาวะในสตรีที่มีการหย่อนของผนังช่องคลอดด้านหน้าระยะที่สองและสาม*

***วิธีการศึกษา**: งานวิจัยนี้เป็นการศึกษาแบบสุ่มควบคุมแบบไขว้กลุ่ม (Randomized controlled crossover study) ในสตรีที่มีภาวะหย่อนของผนังช่องคลอดด้านหน้าระยะที่สองและสามที่มารับการรักษาที่คลินิกนรีเวชระบบสืบพันธุ์และทางเดินปัสสาวะ โรงพยาบาลรามาธิบดี ในช่วงมิถุนายน 2558 – เมษายน 2559 จำนวน 51 ราย ผู้เข้าร่วมวิจัยจะได้รับการสุ่มลำดับท่าการปัสสาวะ ทุกรายจะได้รับการตรวจอัตราการไหลของปัสสาวะและวัดปริมาณปัสสาวะเหลือค้างหลังการปัสสาวะทั้งท่าปัสสาวะแบบนั่งปกติและปัสสาวะแบบนั่งยองประยุกต์*

***ผลการศึกษา**: อายุเฉลี่ยของผู้เข้าร่วมวิจัย 64.8 ± 9.1 ปี มีการหย่อนของผนังช่องคลอดด้านหน้าระยะที่สอง 30 ราย (ร้อยละ 58.8) และระยะที่สาม 21 ราย (ร้อยละ 41.2) ไม่พบความแตกต่างอย่างมีนัยสำคัญทางสถิติของปริมาณปัสสาวะที่ถ่ายออกมาจากการปัสสาวะในท่านั่งปกติ และท่านั่งยองประยุกต์ (335.2 ± 160.1 และ 362.7 ± 161.0 มิลลิลิตร) ($p > 0.05$) ค่าเฉลี่ยของอัตราการไหลของปัสสาวะสูงสุดและอัตราการไหลของปัสสาวะเฉลี่ยในท่านั่งปกติ (22.3 ± 11.2 และ 10.7 ± 5.5 มิลลิลิตรต่อวินาที) และท่านั่งยองประยุกต์ (23.8 ± 10.9 และ 11.91 ± 6.4 มิลลิลิตรต่อวินาที) ไม่แตกต่างกันอย่างมีนัยสำคัญทางสถิติ ($p > 0.05$) พบปริมาณปัสสาวะเหลือค้างหลังการปัสสาวะในท่านั่งปกติน้อยกว่าท่านั่งยองประยุกต์อย่างมีนัยสำคัญทางสถิติ (52.6 ± 55.1 และ 75.0 ± 78.6) ($p < 0.05$)*

***สรุป**: การปัสสาวะในท่านั่งปกติและท่านั่งยองประยุกต์ ไม่ส่งผลต่ออัตราการไหลของปัสสาวะในสตรีที่มีการหย่อนของผนังช่องคลอดด้านหน้าระยะที่สองและสาม แต่อย่างไรก็ตาม การปัสสาวะในท่านั่งยองประยุกต์จะส่งผลให้มีปัสสาวะคงค้างหลังการถ่ายปัสสาวะมากขึ้นกว่าการปัสสาวะในท่านั่งปกติ*

***คำสำคัญ**: อุ้งเชิงกรานหย่อน, ท่าการปัสสาวะ, ยูโรไดนามิค*

Introduction

Pelvic organ prolapse (POP) is one of the most troublesome problems in advanced age women with the reported prevalence varied from 24-50 %[1-5]. The reported prevalence in Thai menopausal and elderly women were 43% and 70%, respectively[6, 7]. According to the International Continent Society, POP is defined by a bulge or protrusion of pelvic organ and their associated vaginal segments into or through the vagina. It can be divided into anterior, apical, and posterior compartment prolapse[8,9]. Among the prolapse, anterior vaginal prolapse is the most common form of pelvic organ prolapse[2, 6, 10]. Due to the proximity of anterior compartment to the bladder and urethra, women with significant anterior compartment prolapse may have a functional outlet obstruction due to a "kink" in the normal urethral mechanism. The affected women may complain of hesitancy, slow or intermittent urine stream, frequent urination, incomplete bladder sensation, or urinary retention[10, 11]. This was confirmed by several studies which found abnormal voiding function test in prolapsed women, especially in anterior compartment and in advanced prolapse, using urodynamic study and uroflowmetry[12-17].

Most women suffering from this condition try to relieve their obstructive voiding symptoms by several methods including abdominal straining, suprapubic pressure, pushing the prolapse back inside to empty their bladder completely. Changing voiding position such as leaning forward or backward on the toilet seat, lift up theirs legs from the floor or urinate in a semi-standing position is also the adaptable method used by prolapsed women with voiding problems.

Uroflowmetry is a standard test, commonly used in screening for men and women with obstructive voiding problems[18, 19]. It is a simple, time-efficient, non-invasive test, performed by asking the patients urinate normally in a urinal or toilet fitted with a machine that has a measuring device. The machine will calculate the amount of urine voiding, the flow rate in seconds, and the length of time it takes to empty the bladder completely. Post-void residual urine (PVR) measurement is another test, usually performed with uroflowmetry, for evaluating voiding dysfunction. The test is performed by measuring the amount of urine left in the bladder after the end of micturition. PVR volume can be measured by either direct urethral catheterization or bladder ultrasound. Due to the comparable accuracy of bladder ultrasound in PVR measurement, compared to direct catheterization, it is now acceptable for use as an alternative to catheterization[20-22].

Although the study of the effect of voiding position on micturition has been studied for many years, but the results are varied and inconclusive[24-27]. The inconsistent findings of these studies may be due to different voiding position used in the studies and variations in study population. Most studies investigated in normal healthy subjects or in men with lower urinary tract symptoms (LUTS). At present, there is no published study that examined the effect of voiding position on voiding in women with pelvic organ prolapse.

In Thai culture, the two most common voiding positions in women are sitting and squatting. However, the ability to attain a squatting position or squatability is decreasing in elderly women. Therefore, the objective of this study is to compare uroflowmetry and PVR measurement between 2 voiding positions; sitting and modified squatting position in women with anterior vaginal wall prolapse stage II and III.

Materials and Methods

This was a randomized cross-over study, conducted in women attending female pelvic medicine and reconstructive surgery clinic, Department of Obstetrics and Gynaecology, Ramathibodi Hospital, Bangkok, Thailand, during June 2015 to April, 2016. This study was approved by the Ethics Committee on human rights related to research involving human subjects, based on Declaration of Helsinki, Faculty of Medicine,

Ramathibodi Hospital, Mahidol University.

The inclusion criteria were women with pelvic organ prolapse stage II and III, according to POP-Q staging and the leading edge of the most descended compartment was anterior, age between 40-80 years old, able to control micturition. The exclusion criteria included history of urinary tract infection within 1 week, had adrenergic, cholinergic, antidiuretics drugs or any drug which may have an effect on voiding, unwilling to participate the study. All eligible participants were informed about the research study and signed written informed consent.

On enrollment, all participants were informed about the uroflowmetry, PVR measurement, and details of each voiding position using picture card (Fig. 1). The demographic data including age, body mass index (BMI), parity, menopausal status, previous hysterectomy status and POP staging were collected. Then, they were randomly allocated sequences of producing two separate acts of micturition in the same day, using computer-generated numbers. Group one had uroflowmetry performed first in a sitting position and then in a modified-squatting position. Group two had uroflowmetry performed first in a modified-squatting position and then in a sitting position. The randomized treatment assignments were sealed in opaque envelopes and opened individually for each participant who agreed to be in the study.

Aquarius TT TM version 8 (Laborie, Mississauga, Canada) uroflowmeter was used in this study. All participants were asked to arrive with a comfortably full bladder for uroflowmetry and were instructed to void normally without any straining in both sitting and modified squatting positions. For the comfort of the participants, uroflowmetric study was performed in a private section, separated by room partition. Participants who had voided volume less than 150 ml were ask to come for a new measurement in the subsequent day. PVR volume was evaluate by CubeScanTM BioCon-700 bladder scanner (Mcube Technology, Seoul, Korea) immediately after complete each episode of micturition. The flow diagram of the study is shown in Fig. 2.

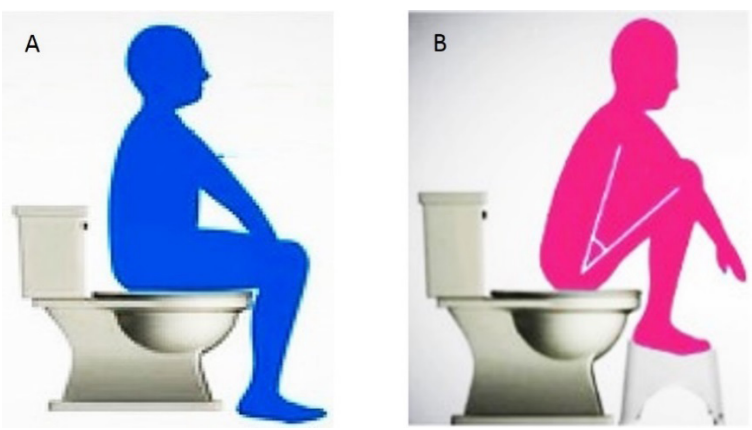

Fig. 1. Voiding position on the commode.

(A) Sitting position defined as sit back straight, feet rest on the floor, and both hands were placed on thigh.
(B) Modified-squatting position defined as sit leaning forward with forearm rest on the thigh, foot rest on the stool (up to 30 cm high), knee above anterior superior iliac spine level, and hug knees to the chest during urinate.

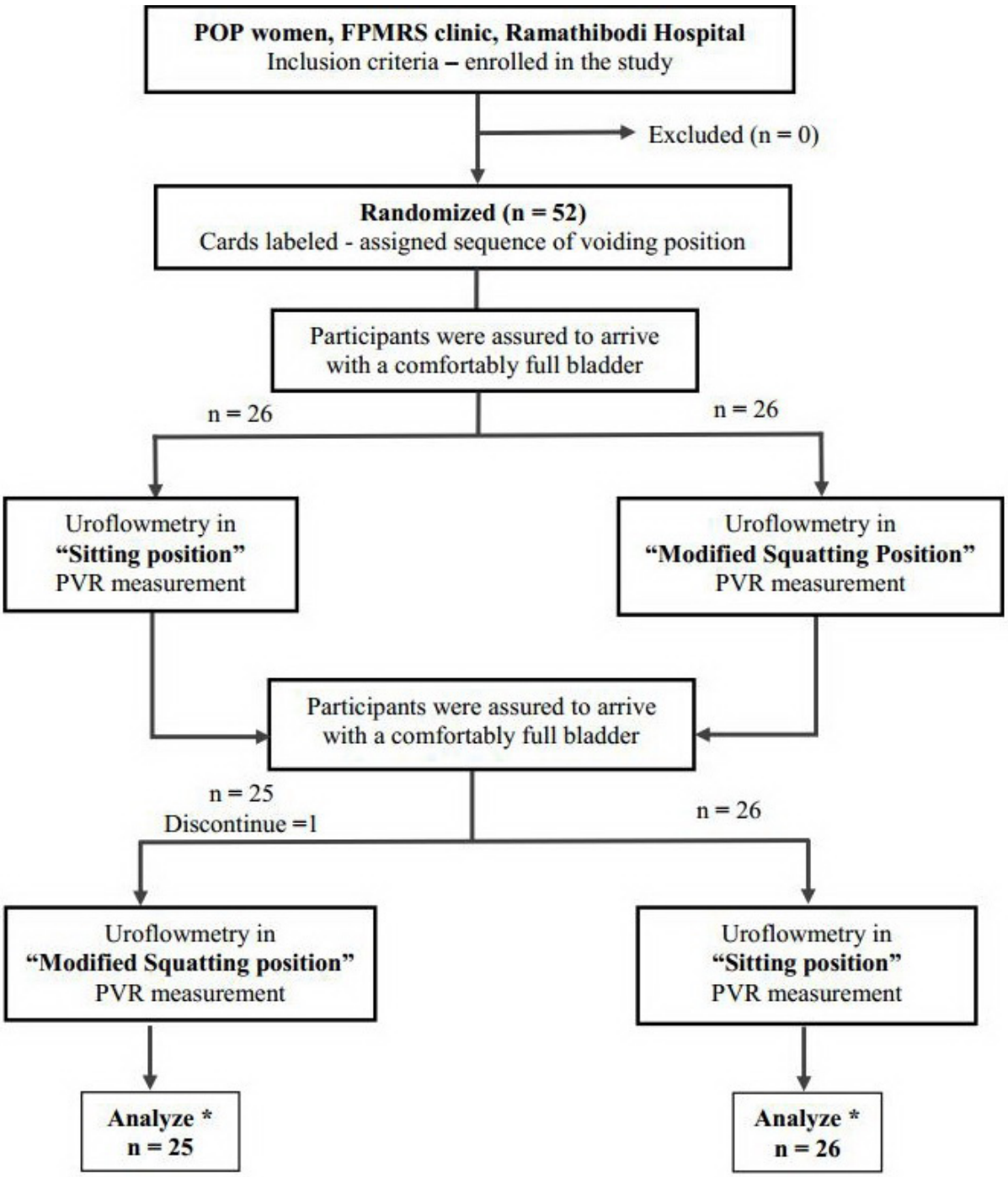

Fig. 2. Flow diagram of the study.

Sample size calculation

The sample size was calculated according to the following formula:

$$N = \left[\frac{(Z\alpha_2 + Z\beta)\,\delta}{\Delta} \right]^2$$

From the pilot study included 10 women with anterior vaginal wall prolapse stage II and III, mean maximal flow rate (Q_{max}) in sitting position was 24.09 ±7.21 mL/s, mean Q_{max} in modified-squatting position was 28.59 ± 9.10 mL/s, the different (Δ) Q_{max} was 4.5 mL/s. $Z\alpha$ was set as 1.96 with a type I error of 5%, $Z\beta$ was set as 1.28 with a power of 90%. The calculated number was then added with 20% of the calculated number of subjects who might be excluded due to data loss. Therefore, 52 subjects would need to be enrolled in the study.

Statistical analysis

Statistical analyses were performed using STATA version 14.0 software (Stata Corporation, College Station, TX). Continuous data were reported as the mean and standard deviation. Categorical data were shown as the number and percentage. Linear mixed-effect model was used to analyze repeated measures data. All reported probability values are two-tailed; $p < 0.05$ was considered to be statistically significant.

The uroflowmetric parameters and corresponding PVR values of two voiding postures are shown in Table 2. No participant in the study urinates less than 150 ml. There was no statistically significant difference in voided volume and voiding time between two voiding positions ($p > 0.05$). Mean Q_{max} during urination in sitting position and modified squatting was not different (22.28 ± 11.21 ml/s and 23.80 ± 10.88 ml/s, respectively). Women urinate in modified squatting position tended to have better average flow (Q_{aveg}) rate than that in sitting position, but the difference did not reach statistical significance. Mean PVR volume in modified squatting group was significant higher than in sitting group ($p < 0.01$).

Results

Of 52 women, one participant was excluded due to unwilling to perform the second uroflowmetry. Therefore, a total of 51 data was left for analysis. The mean age of study population was 64.9 ± 9.1 years. There were 6 participants with previous hysterectomy due to benign condition. The demographic characteristics and POP staging of subjects are presented in Table 1.

Table 1. Demographic Characteristics (N = 51).

Characteristics	n = 115
Age (years), mean ± SD	64.9 ± 9.1
BMI (kg/m^2), mean ± SD	26.1 ± 3.3
Parity, n (%)	
1	6 (11.8)
2	17 (33.3)
≥ 3	28 (54.9)
Menopause, n (%)	44 (86.3)
Previous hysterectomy, n (%)	6 (11.8)
POP-Q (anterior compartment), n (%)	
Stage II	30 (58.8)
Stage III	21 (41.2)
Most distal portion of prolapse, n (%)	
Above hymen	26 (51.0)
Below hymen	25 (49.0)

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Using the maximum flow rate less than 15 ml/s and post-void residual urine volume over 100 ml as cut-off point to classify abnormal uroflowmetry[28], there was no significant difference in abnormal flow rate ($p = 0.477$) and abnormal PVR ($p = 0.087$) between sitting and modified squatting position. (Fig. 3.)

Table 2. Uroflowmetric parameters and PVR in sitting and modified squatting position (N = 51).

Uroflowmetric parameters	Sitting position (mean ± SD)	Modified Squatting position (mean ± SD)	Difference (95%CI)	p value*
Q_{max} (ml/s)	22.28 ± 11.21	23.80 ± 10.88	1.52 (-0.46 - 3.49)	0.132
Q_{aveg} (ml/s)	10.65 ± 5.48	11.91 ± 6.40	1.26 (-0.01 - 2.53)	0.052
Voided volume (ml)	335.19 ± 160.12	362.65 ± 161.00	27.46 (-13.73 - 68.66)	0.192
Voiding time (sec)	41.75 ± 24.21	44.79 ± 27.76	3.04 (2.98 - 9.07)	0.322
PVR (ml)	52.63 ± 55.08	74.98 ± 78.60	22.35 (6.03 - 38.86)	0.007

Q_{max}, maximum flow rate; Q_{aveg}, average flow rate; *Linear mixed-effect model

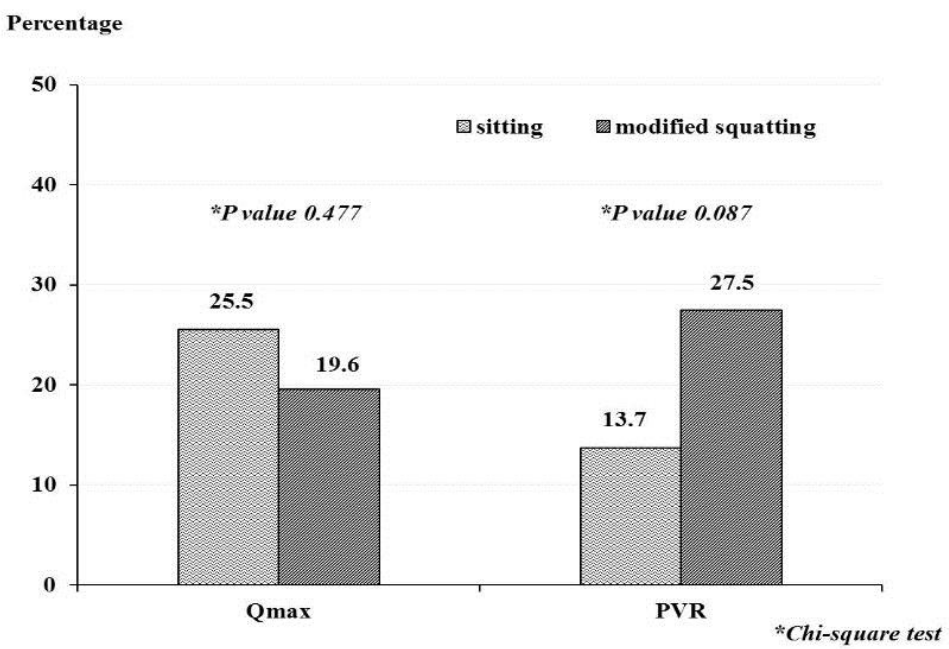

Fig. 3. Abnormal uroflowmetry: maximal flow rate and PVR volume.

Discussion

Pelvic organ prolapse is the troublesome problems in women especially in elderly. Women with pelvic organ prolapse may present with various symptoms including bulging mass, lower urinary tract symptoms, or bowel symptoms depend on compartment and degree of prolapse. Obstructive voiding symptoms, such as hesitancy, slow stream, feeling of incomplete emptying and frequent urination are the common bothered problems that affect quality of life in women with anterior compartment prolapse. The associations between anterior vaginal wall prolapse and voiding difficulty have been demonstrated in many studies[14-16]. Many prolapsed women tried to improve their voiding by several methods; changing their voiding position is the commonly used method in real life.

There are many studies examined the effect of different voiding positions on urinary flow rates and the PVR volume in normal women. Moore and colleague[23] found decreasing average and increasing flow rate in crouching over position, compared with sitting position. The result could be explained by the contraction of adductor muscle in crouching position interfere with pelvic floor muscle relaxation during urination. Most studies that compared voiding parameters between squatting, near squatting with sitting position, found better flow rate in both squatting and near squatting voiding position[24-27]. Rad and colleague's study found the increase in anorectal angle in squatting position which causes relaxation of puborectalis muscles. This may lead to easier bladder and bowel evacuation[29]. This findings were also supported by Rane and colleague's study[27] which found an increase in intra-abdominal pressure with no increase in detrusor pressure and increase levator hiatus dimension in squatting position. Pelvic floor muscle relaxation in squatting or near squatting position, together with passively increase intra-abdominal pressure seems to facilitate bladder emptying.

In the present study, we compared flow rate and PVR volume between two voiding positions; sitting and modified squatting position. The result was not similar to the previous report. Urine flow rate did not improve by a change in voiding position from sitting to modified squatting. Moreover, voiding in modified squatting position resulted in higher PVR volume. This could be explained by the physioanatomy changes in prolapsed women. Most women with pelvic organ prolapse usually had pelvic muscle weakness and relaxation, therefore increase in levator hiatus dimension in squatting position did not result in more relaxed pelvic floor. In anterior vaginal wall prolapse, a weakness supporting tissue may allow the bladder and urethrae drop down from their normal position. Thus, passively increased intra-abdominal pressure from modified squatting position could not help complete bladder emptying.

Although, higher PVR volume was found in women voiding in modified squatting position, but the urine volume left in the bladder might not have any clinical importance. This was confirmed by similar proportion of abnormal PVR volume in both voiding positions found in this study.

Several previous studies reported abnormal voiding function in prolapse women, especially in anterior compartment and/or advanced prolapse[12-17]. In this study, we emphasized on the effect of two voiding positions on uroflowmetry in women with predominately anterior compartment prolapse. Some participants may have other compartments of prolapse, but with less severity, comparing to anterior compartment. To study the association between severity of prolapse and voiding parameters in different voiding positions, more number of sample size are enrolled to perform subgroup analysis either for different stages or compartments of prolapse in further study.

Uroflowmetry and PVR measurement were the voiding tests used in this study. Both tests are acceptable tests, widely used in evaluating women with obstructive voiding problems. As micturition is a dynamic event, it may be influenced by several factors such as age, sex and anatomical properties of lower urinary tract and adjacent tissues, voiding habit and environment. To avoid the influence on voiding test, a crossover randomized trial was used. This type of study could reduce inter-variable confounding between two acts of voiding. All participants were instructed to void normally,

without the need to strain during uroflowmetric testing.

This is the first study determined the effect of changing voiding position on voiding functions in prolapsed women. This study focused on the uroflowmetric parameters and PVR measurement. Problems related slow flow and high PVR including hesitancy, frequency, incomplete bladder sensation, overflow incontinence and recurrent UTI did not include in this study. The clinical effect of different PVR, patient's LUTS symptoms and preferred voiding posture are needed in further study.

Conclusion

Voiding position either sitting or modified squatting did not affect urinary flow rate in women with anterior wall prolapse stage II and III. Although, voiding in modified squatting position might results in higher post-void residual urine, but without evidence of clinical effect. Therefore, voiding in either sitting or modified squatting position is still the choice of each prolapsed women, depend on their comfort and preference.

Acknowledgments

The authors would like to thank all participants for their active co-operation in this study, all staff members in urogynecologic clinic and urodynamic unit, and Dr. Sasiwimon Rattanasiri for statistical consultation.

Potential conflicts of interest

The authors declare no conflict of interest.

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Thai Journal of Obstetrics and Gynaecology
January 2018, Vol. 26, pp. 52-58

GYNECOLOGY

Gel Pack Reduced Postoperative Pain in Benign Gynecologic Surgery: A randomized controlled trial

Wanlayanee Nuangpho, M.D.*,
Sukanya Srinil, M.D.*,
Thumwadee Tangsiriwatthana, M.D.*,
Maleechat Sripipattanakul, M.D.*.

** Department of Obstetrics and Gynecology, Khon Kaen Hospital, Khon Kaen 40000, Thailand*

ABSTRACT

Objectives: To examine the effectiveness of gel pack for reducing postoperative pain in patient who undergoes exploratory laparotomy for benign gynecologic surgery.

Materials and Methods: Twenty eight participants who underwent benign gynecological surgery under general anesthesia at Khon Kaen Hospital in March 2016 were randomized by computer generated into two groups: gel pack group (N=14) and control group (N=14). Gel pack was applied at 2 hours after operation for 20 minutes and pain score was measured using visual analog scale (VAS) at 2 (baseline), 6 and 24 hours, respectively. The VAS was divided into two grades by pain-intensity; mild (VAS < 4), and moderate to severe (VAS ≥ 4). The comparison of pain-intensity was analyzed by Fisher exact test ($p < 0.05$).

Results: Gel pack was statistically significant reduced postoperative pain from moderate-severe pain to mild pain intensity at 6 hours compared with control group (11 to 8 cases versus 14 to 14 case, $p = 0.01$, 95%CI 0.03-0.89). There was no statistically significant difference in opioid consumption, hospital stay and wound infection between two groups.

Conclusion: Gel pack can reduce postoperative pain at 6 hour in benign gynecological operation without complication.

Keywords: Gel pack, exploratory laparotomy, benign gynecologic surgery, postoperative pain.

Correspondence to: *Wanlayanee Nuangpho, M.D., Department of Obstetrics and Gynecology, Khon Kaen Hospital, Khon Kaen, 40000, Thailand, Phone: (043) 336789, E-mail: wnuangpho@gmail.com*

การศึกษาทดลองแบบสุ่มเรื่องการใช้ถุงเจลเย็นเพื่อลดระดับความเจ็บปวดหลังผ่าตัดในผู้ป่วยนรีเวชที่ไม่ใช่มะเร็ง

วัลยาณี เนื่องโพธิ์, สุกัญญา ศรีนิล, ทุมวดี ตั้งศิริวัฒนา, มาลีชาติ ศรีพิพัฒนกุล

บทคัดย่อ

วัตถุประสงค์: *เพื่อศึกษาประสิทธิภาพของการใช้ถุงเจลเย็นในการลดระดับความเจ็บปวดหลังผ่าตัด ในผู้ป่วยที่ได้รับการผ่าตัดทางนรีเวชที่ไม่ใช่มะเร็ง*

วิธีการศึกษา: *ผู้ป่วยนรีเวชที่ไม่ใช่มะเร็ง จำนวน 28 ราย ที่นัดมาผ่าตัดทางหน้าท้อง ในเดือนมีนาคม พ.ศ. 2559 ที่โรงพยาบาลขอนแก่น ได้รับการสุ่มแบ่งเป็น 2 กลุ่มๆ ละ 14 คน โดยกลุ่มทดลองจะได้รับถุงเจลเย็นวางบนแผลผ่าตัดที่ 2 ชั่วโมงหลังการผ่าตัด เป็นเวลานาน 20 นาที และผู้ป่วยทุกคนจะได้รับการดูแลอื่นๆ หลังผ่าตัดตามปกติ รวมถึงการประเมินความเจ็บปวดหลังการผ่าตัด โดยใช้ visual analog scale (VAS) โดยจะประเมินความเจ็บปวดที่ 2, 6 และ 24 ชั่วโมงหลังการผ่าตัด ตามลำดับ ซึ่งคะแนนความเจ็บปวดจะแบ่งเป็นระดับความเจ็บปวด 2 ระดับ คือ ระดับเจ็บปวดเล็กน้อย (mild; VAS < 4) และ ระดับเจ็บปวดปานกลางถึงรุนแรง (moderate to severe; VAS ≥ 4) เปรียบเทียบสัดส่วนของความแตกต่างของระดับความเจ็บปวดระหว่างสองกลุ่มทดลอง โดยใช้ Fisher's exact test (กำหนดค่า $p < 0.05$)*

ผลการศึกษา: *พบว่าระดับความเจ็บปวดที่ 6 ชั่วโมงหลังการผ่าตัด ในผู้ป่วยกลุ่มที่ได้รับถุงเจลเย็นที่มีความเจ็บปวดอยู่ในระดับเจ็บปวดปานกลางถึงรุนแรง มีจำนวนลดลงอย่างมีนัยสำคัญทางสถิติ จาก 11 ราย เป็น 8 ราย ส่วนกลุ่มที่ได้รับการดูแลตามปกติ มีจำนวนผู้ป่วยที่มีความเจ็บปวดอยู่ในระดับเจ็บปวดปานกลางถึงรุนแรงไม่เปลี่ยนแปลง มีจำนวน 14 ราย ตั้งแต่เริ่มการวิจัย และที่ 6 ชั่วโมงหลังการผ่าตัด ($p = 0.01$, 95%CI 0.03-0.89) นอกจากนี้ปริมาณการใช้ยาแก้ปวดมอร์ฟีน ระยะเวลาพักรักษาในโรงพยาบาล ตลอดจนการติดเชื้อบริเวณแผลผ่าตัดพบว่า ไม่แตกต่างกันในผู้ป่วยทั้งสองกลุ่ม*

สรุป: *ถุงเจลเย็นสามารถลดระดับความเจ็บปวดที่ 6 ชั่วโมงหลังการผ่าตัด ในผู้ป่วยนรีเวชที่ไม่ใช่มะเร็งได้ อย่างมีนัยสำคัญทางสถิติ และการใช้ถุงเจลเย็นไม่ก่อให้เกิดภาวะแทรกซ้อนเพิ่มเมื่อเปรียบเทียบกับผู้ป่วยที่ได้รับการดูแลตามปกติ*

คำสำคัญ: *เจลเย็น, ถุงเจลเย็น, ผ่าตัดนรีเวชทางหน้าท้อง, ลดปวดหลังผ่าตัด, นรีเวชทั่วไป*

Introduction

Postoperative pain in trans-abdominal surgery patients are mostly found within 24 hours after surgery, this is mainly due to soft tissue injury that leads to local inflammation and stimulation of surrounding nociceptors. Pharmacologic therapies are used to relieve this pain, in exchange of their side effects[1,2]. Alternatively, there are many non-pharmacological treatments which could ease the pain, with much lesser side effects such as acupuncture, massage, repositioning, breathing exercises, physical modalities and we were interested in heat and cold application within 24 hour of tissue injury[3]. Cryotherapy induced lowering the temperature of skin, which helps to decrease inflammatory process by reducing blood flow, slowing down nerve conduction velocity, inhibiting edema formation and reducing muscle spasm. Thus, lowering cellular metabolism and also lessen secondary injury by decreasing cellular metabolism[4]. Optimum temperature of cryotherapy is 10-15°C. It can be applied to the skin at surgery site for 20-30 minutes per session as immediately or within 12-72 hours after surgery[5]. This modality is used in many fields of surgery such as general surgery, ophthalmology, orthopedics, plastic, otolaryngeal surgery and some obstetrics and gynecologic surgery[6-10]. There are many types of cryotherapy, of these, ice pack is the most common used. However, cold gel pack (CGP) is also an interesting alternative as it could provide the optimum temperature of cryotherapy.

In an experiment, skin was applied for 20 minutes with ice pack, gel pack, frozen peas, and mixture of water and alcohol, and skin temperature was recorded; the result were 10.2 ± 3.5°C, 13.9 ± 4.1°C, 14.4 ± 3.0°C, and 10.0 ± 4.5°C, respectively[11]. Therefore, cold gel pack gives optimum temperature, its flexibility provides proper skin contact, convenient since its reusable, accessible, and low cost.

Nowadays, there is no study about CGP in patient who underwent benign gynecologic operation. The aim of our study was to examine the effectiveness of CGP for reducing postoperative pain in patient who underwent exploratory laparotomy for benign gynecologic surgery.

Materials and Methods

Twenty eights participants were enrolled to the study in March 2016. Inclusion criteria were a Thai woman who underwent benign gynecological surgery, under general anesthesia, and received postoperative morphine (body weight (BW) > 50 kg; Morphine 3 mg, BW < 50 kg; morphine 2 mg) every 4 hour as needed in the first 24 hours as pain controller. Participants who used narcotic drugs within 24 hours prior to operation, used of local anesthesia at surgical wound, had underlying diseases which take medicine that effected pain perception, ice hypersensitivity, hypothermia (body temperature below 35°C), history of Raynaud phenomenon and active skin lesion at surgical site were excluded.

The participants were randomly assigned into two groups (14 each) by computer generated using block of four. The random numbers were put into sequentially sealed opaque envelops. Randomization was performed after wound closing. The envelope which contained random number was picked up by scrub nurses. The skin suture methods included subcuticular and interrupted stitches (by nylon 4/0 or staples). Intervention group, cold gel pack (3M NexcareR size10cm x 25cm), frozen at (-10)-0°C for 1-2 hours and wrapped by 2-mm thick towel, was placed onto the surgical wound and covered by waterproof dressing (tegadermR without pad) at 2 hours after the procedure for 20 minutes. After removed CGP, the tegadermR without pad was checked that it was not detached and CGP was cleaned before reused. Control group received standard routine postoperative care. Individual informed consent was obtained before operation. Before using CGP, it was tested its sustain efficacy by left it at room temperature (30-35°C) and wrapped by 2-mm thick towel. We found that it could preserve the optimal temperature at 10-15°C for 3 hours. Postoperative pain scores were measured by visual analog scale (VAS) at 2 (before placed CGP), 6 and 24 hours after operation. Participants were asked to mark the vertical line on the 10-cm line and pain score was measured in centimeter by ruler. The participants were informed that "0" represented no pain, and "10" was the

worst pain. Outcome assessors were nurses at gynecologic ward who did not involve in the preceded processes. Both groups equally received standard postoperative care. Vital signs were recorded before placing CGP then recorded every 4 hours. The primary outcome was postoperative pain at 6 hours. Pain score was classified into two categories according to pain intensity, mild pain (VAS < 4) and moderate to severe pain (VAS 4-6 and 7-10, respectively)[12]. The secondary outcomes included opioid consumption, length of hospital stay, wound complication. Wound complication was observed after placed CGP and via telephone interview at 1 month after the procedure.

Sample size was calculated by using data from pilot study. We used formula for test of difference in two independence proportions with alpha of 0.05, power of 90% and 10% dropouts. The sample size was 14 participants per group.

Fisher's exact and Pearson Chi square test was used for categorical variables and for continuous variables student t-test or Mann-Whitney-U test were used depended on data distribution. The primary outcome was presented as percentage, relative risk with 95% confidence interval. Other outcomes were presented as mean and standard deviation and median with interquartile range. P value < 0.05 is considered statistically significant. Statistical analysis was performed by SPSS version 17 software.

Results

Twenty eight eligible participants were enrolled into the study. Fourteen received cold gel pack at two hours after operation and the other fourteen receive routine post-operative care (Fig. 1.).

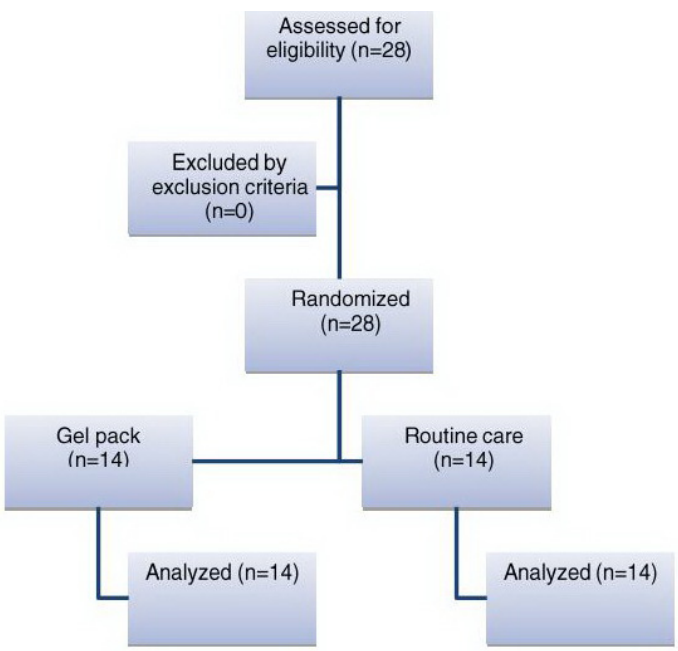

Fig. 1. Flow chart of participants' progress through the study.

Baseline characteristics including age, body mass index (BMI), operation, surgeon, type of incision, incision length, operative times, underlying diseases (hypertension, migraine and thalassemia) and base line postoperative pain at 2 hours were similar in both groups as presented in Table 1. The operations were classified into four groups which were total abdominal hysterectomy (TAH), TAH with adnexal surgery, adnexal surgery alone and other (adnexal surgery with repaired small bowel). There

was significant different in postoperative pain intensity at 6 hours between groups (RR 0.57, p = 0.01, 95%CI 0.03-0.89). At 6 hours after operation, in CGP group, the number of participants who rated their pain as moderate and severe pain decreased from 11 to 8 participants, while all of the participants in routine group, pain persisted in moderate to severe intensity as at baseline (Table 2).

Table 1. Baseline characteristics.

	Control (n=14)	CGP (n=14)	p value
Age, year, mean (SD)	43 (5.14)	42.78 (9.04)	0.76
BMI, kg/m^2, mean (SD)	23.42 (3.01)	24.07 (3.07)	0.62
Operation, n			0.62
TAH	3	2	
TAH with adnexal surgery	9	9	
Adnexal surgery	1	3	
Adnexal surgery with repaired small bowel	1	0	
Surgeon, n			0.43
Staff	10	8	
Resident	4	6	
Incision, n			0.25
Low midline	6	9	
Pfannenstiel	8	5	
Incision length, cm, mean (SD)	12.57 (2.20)	12.07 (1.59)	0.33
Operative times, minutes, mean (SD)	77.14 (23.43)	75.21 (26.66)	0.64
Underlying disease, n	2	5	0.38
Hypertension	2	3	
Migraine	0	1	
Thalassemia	0	1	
Postoperative pain at 2 hours, n			0.22
Mild	0	3	
Moderate to severe	14	11	

CGP; cold gel pack, BMI; body mass index, cm; centimeter

Table 2. Comparison of postoperative pain between two groups measured by VAS at 6 hours.

Postoperative time	Pain scale				
	Control (n=14)	CGP (n=14)	p value	RR	95%CI
6 hours, n			0.01	0.57	0.03-0.89
Mild	0	6			
Moderate to severe	14	8			

CGP; cold gel pack

Every participant in CGP group could tolerate to the intervention, no one asked to remove CGP before 20 minutes. When we recorded VAS, we asked every participant about the comfortable of placing CGP. There was no reported of shivering, sub-temperature or frost bite. There was no significant difference in opioid consumption, wound infection rate, length of hospital stay and postoperative pain at 24 hours as presented in Table 3.

Table 3. Comparison of secondary outcome between two groups.

Postoperative time	Control (n=14)	CGP (n=14)	p value	RR	95%CI
Morphine accumulated dose, mg (median (IQR))	15 (12-15)	12 (9-15)	0.09		
Postoperative hospital stay, day	3	3	1.00		
Wound complication	0	0	NA		
Postoperative pain at 24 hours, n			1.00	1	0.06-14.45
Mild	13	13			
Moderate to severe	1	1			

CGP; cold gel pack, mg; milligram

Discussion

In this study, we found that CGP effectively reduced postoperative pain intensity in patients who underwent benign gynecologic surgery under general anesthesia at 6 hours after surgery compare to pain intensity at 2 hours (baseline).

These result explained that CPG applied only one time at 2 hours after the procedure for 20 minutes were sufficiency to continue the mechanism of cryotherapy for reduce postoperative pain for 6 hours. Type of incisions did not influent postoperative pain, according to Habib et al[13], compared between vertical and Pfennenstiel incision in gynecologic surgery, there was no significant difference in postoperative pain and amount of opioid consumption. Their findings were comparable with ours. Koç et al[14], studied about the effectiveness of ice pack in patient who underwent inguinal hernia operation and found that ice pack could reduce postoperative pain at 2, 6 and 24 hours. This finding differed from ours by postoperative pain score at 2 and 24 hours, this might be due to different intervention, surgical incision. They also found no side effects of ice pack as in the present study.

In bigger incision and operation, Amari et al[15] studied efficacy of ice packs in reducing postoperative midline incision pain and narcotic usage. The result showed significant reduction in postoperative pain and narcotic used only in the first day after surgery, at 8.00 am and 4.00 pm. This finding was consistent with present study in the effectiveness of cryotherapy in reducing postoperative pain in first 24 hours as well as rate of wound infection and length of hospital stay. However, mode of pain measurement, duration of pain recorded and types of operations and intervention were different.

Michael et al[16] studied in patients who underwent exploratory laparotomy, who received postoperative pain relief using intravenous self-administered morphine sulfate infusion pump, they found that patients who received cryotherapy (ice thermal blanket) used more morphine sulfate on the first postoperative day than control group, which was inconsistent with our study. This might be due to different kind of cryotherapy and methods of pain management.

The morphine consumption in CGP group was lesser than control group by mean of 12 versus 15 milligrams, respectively. However there was no statistical significant difference (p = 0.09) probably due to the

sample size of this study calculated for the primary outcome (VAS) so it was insufficient to evaluate the morphine consumption.

The strength of our study was a randomized controlled trial. Our limitation was un-blinded intervention, and assessments of pain after discharge from hospital were done via phone interview.

Further study, the impact of the environment such as temperature, multiple or continuous place of CGP should be considered. Patient's satisfaction should be taken into account for further study.

Conclution

In conclusion, cold gel pack could effectively reduce postoperative pain at 6 hours in benign gynecological operation without complication.

Acknowledgements

We would like to show our sense of appreciation to the participants, the nurses of the operating room and the gynecologic ward for their assistance in data collection and the staffs of Department of Obstetrics and Gynecology, Khon Kaen Hospital for their assistance and support.

Potential conflicts of interest

The authors declare no conflict of interest.

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Thai Journal of Obstetrics and Gynaecology
January 2018, Vol. 26, pp. 59-67

GYNECOLOGY

Association Between Preoperative Serum Markers and Lymph Node Metastasis in Endometrioid Adenocarcinoma of Endometrium

Roseleeya Aleediman, M.D.*,
Kamaitorn Tientong, M.D.*,**.

** Department of Obstetrics and Gynecology, Rajavithi Hospital, Bangkok 10400, Thailand*
*** College of Medicine, Rangsit University, Bangkok, Thailand*

ABSTRACT

Objectives: Serum markers play many roles in various cancers. Our aim is to investigate whether preoperative serum markers can be used for predicting lymph node (LN) metastasis in endometrioid adenocarcinoma of endometrium.

Materials and Methods: Clinical characteristics of all patients with pure endometrioid adenocarcinoma of endometrium who underwent complete surgical staging at Rajavithi hospital, between 1 January 2010 and 31 December 2014 were retrospectively reviewed. Preoperative serum markers including hemoglobin (Hb), neutrophil/lymphocyte ratio (NLR), platelet/lymphocyte ratio (PLR), multiplication of neutrophil and monocyte counts (MNM), platelet counts, mean platelet volume (MPV) and serum albumin levels were investigated. Sensitivity, specificity, positive predictive value, negative predictive value of serum markers to predict lymph node metastasis were evaluated.

Results: Two hundred eighty-three patients who meet the study inclusion criteria were included. There were 40/283 (14.3%) of LN metastasis. LN metastasis was significantly associated with lower Hb level ($p < 0.001$) and higher NLR, PLR, MNM and platelet count ($p = 0.018$, $p = 0.004$, $p = 0.008$ and $p = 0.03$ respectively). The best cut off values to identify lymph node metastasis were 11.85 for Hb (72.5% sensitivity, 59.7% specificity), 2.29 for NLR (60.0% sensitivity, 61.7% specificity), 10.37 for PLR (67.5% sensitivity, 56% specificity), 0.0068 for MNM (70% sensitivity, 56% specificity) and 297,500 for platelet count (62.5% sensitivity, 57% specificity).

Conclusion: Preoperative lower Hb level, higher NLR, PLR, MNM and platelet count were significantly related with lymph node metastasis. They could be used in clinical practice to rule in patients with low risk for LN metastasis without extra costs.

Keywords: Endometrioid adenocarcinoma of endometrium, lymph node metastasis, hemoglobin, neutrophil/lymphocyte ratio, platelet/lymphocyte ratio.

Correspondence to: *Kamaitorn Tientong, M.D., Department of Obstetrics and Gynecology, Rajavithi hospital Phyathai Road, Rajathevee, Bangkok 10400, Thailand, Telephone: (+66)813754048, E-mail: tulkamaitorn@gmail.com*

การศึกษาความสัมพันธ์ระหว่างค่าผลเลือดก่อนการผ่าตัดและภาวะการแพร่กระจายทางหลอดน้ำเหลืองในมะเร็งเยื่อบุโพรงมดลูกชนิด เนื้อเยื่อ endometrioid

โรสลียา อะลีดิมัน, กมัยธร เทียนทอง

บทคัดย่อ

วัตถุประสงค์: *เพื่อศึกษาประสิทธิภาพค่าความแม่นยำของการใช้ค่าผลเลือดก่อนการผ่าตัด ในการทำนายการแพร่กระจายทางหลอดน้ำเหลืองในมะเร็งเยื่อบุโพรงมดลูกชนิด endometrioid*

วัสดุและวิธีการ: *เป็นการศึกษาย้อนหลังโดยการคัดเลือกผู้ป่วยมะเร็งเยื่อบุโพรงมดลูกชนิด endometrioid ซึ่งได้รับการผ่าตัดและเลาะต่อมน้ำเหลืองเพื่อกำหนดระยะของโรค ในโรงพยาบาลราชวิถี ในช่วงวันที่ 1 มกราคม พ.ศ. 2553 จนถึงวันที่ 31 ธันวาคม พ.ศ. 2557 โดยค่าผลเลือดก่อนการผ่าตัดที่ใช้ในการวิเคราะห์ประกอบด้วย ความเข้มข้นของเม็ดเลือดแดง สัดส่วนของนิวโทรฟิลล์ต่อลิมโฟไซด์ สัดส่วนของเกร็ดเลือดต่อลิมโฟไซด์ การเพิ่มจำนวนเท่าตัวของนิวโทรฟิลล์และโมโนไซด์ จำนวนเกร็ดเลือด ค่าเฉลี่ยปริมาตรเกร็ดเลือด และระดับอัลบูมินในเลือด*

ผลการศึกษาวิจัย: *มีผู้เข้าเกณฑ์การวิจัยนี้ทั้งหมด 283คน โดยมี 40 คนมีการแพร่กระจายของโรคไปยังหลอดน้ำเหลือง พบว่าการแพร่กระจายไปยังหลอดน้ำเหลืองสัมพันธ์กับภาวะความเข้มข้นของเม็ดเลือดแดงต่ำ ($p<0.001$) สัดส่วนของนิวโทรฟิลล์ต่อลิมโฟไซด์ สัดส่วนของเกร็ดเลือดต่อลิมโฟไซด์ ค่าการเพิ่มจำนวนเท่าตัวของนิวโทรฟิลล์และโมโนไซด์ และจำนวนเกร็ดเลือด ($p=0.018$, $p=0.004$, $p=0.008$ and $p=0.03$ ตามลำดับ) ที่สูงขึ้นอย่างมีนัยสำคัญทางสถิติ โดยค่าที่เหมาะสมที่ใช้นำมาวิเคราะห์เพื่อหาค่าความแม่นยำของการแพร่กระจายไปหลอดน้ำเหลืองของตัวแปรต่างๆ มีดังนี้ ค่าความเข้มข้นเม็ดเลือดแดง คือ 11.85 โดยมีค่าความไวร้อยละ 72.5 และค่าความจำเพาะร้อยละ 59.7, สัดส่วนของนิวโทรฟิลล์ต่อลิมโฟไซด์ คือ 2.29 โดยมีค่าความไวร้อยละ 60.0 และค่าความจำเพาะร้อยละ 61.7, สัดส่วนของเกร็ดเลือดต่อลิมโฟไซด์คือ 10.37 โดยมีค่าความไวร้อยละ 67.5 และค่าความจำเพาะร้อยละ 56, ค่าการเพิ่มจำนวนเท่าตัวของนิวโทรฟิลล์และโมโนไซด์ คือ 0.0068 โดยมีค่าความไวร้อยละ 70 และค่าความจำเพาะร้อยละ 56 และจำนวนเกร็ดเลือด คือ 297,500 โดยมีค่าความไวร้อยละ 62.5 และค่าความจำเพาะร้อยละ 57*

สรุป: *ค่าความเข้มข้นเม็ดเลือดแดงที่ต่ำ และสัดส่วนของนิวโทรฟิลล์ต่อลิมโฟไซด์ สัดส่วนของเกร็ดเลือดต่อลิมโฟไซด์ การเพิ่มจำนวนเท่าตัวของนิวโทรฟิลล์และโมโนไซด์ รวมทั้งจำนวนเกร็ดเลือดที่สูง เป็นตัวบ่งชี้การแพร่กระจายทางหลอดน้ำเหลืองในมะเร็งเยื่อบุโพรงมดลูกชนิด เนื้อเยื่อ endometrioid ได้อย่างมีนัยสำคัญทางสถิติ*

คำสำคัญ: *มะเร็งเยื่อบุโพรงมดลูก, การแพร่กระจายของมะเร็งไปยังต่อมน้ำเหลือง, ความเข้มข้นของฮีโมโกลบิน, อัตราส่วนของเซลล์เม็ดเลือดขาว, อัตราส่วนของเกร็ดเลือดและเซลล์เม็ดเลือดขาว*

Introduction

Endometrial cancer (EMC) is the most common malignancy of female genital tract in more developed countries. In the United States, it was estimated that 54,870 new uterine cancers occurred in 2015[1]. On the contrary, incidence of this disease decreases in less developed countries. At Rajavithi hospital, EMC is the second most common cancer in gynecology. It was estimated that 167 new cases occurred in 2015.

Patients with EMC have been treated with surgical staging since 1988 which includes hysterectomy, bilateral salpingo-oophorectomy, peritoneal washing for cytology, and pelvic and paraaortic lymph node (LN) sampling using explorative laparotomy or laparoscopy. However, there is a controversial issue about the role of lymphadenectomy in surgical management of EMC. Because some retrospective studies have suggested that systematic lymphadenectomy be beneficial, a full pelvic and paraaortic lymphadenectomy were previously done in all patients[2-4]. After that, two randomized clinical trials concluded that routine lymph node dissection did not improve the outcome of patients with early-stage EMC[5-6]. On the other hand, lymphadenectomy can identify true staging, guide the appropriate adjuvant treatment, and eradicate metastatic LN. To avoid systematic overtreatment, NCCN guideline recommends a more selective and tailored lymphadenectomy approach[7]. Computerized Tomography (CT) and Magnetic Resonance Imaging (MRI) do not have good sensitivity for detecting metastatic LN in EMC[8], but Positron Emission Tomography (PET)/CT scan has been used for preoperatively lymphatic spreading. In patients with deeply invasive grade 2 on preoperative MRI, grade 3, serous, and clear cell, preoperative PET/CT has moderate sensitivity (78%–79%) with good specificity (98%–99%), positive predictive value (91%–92%), and negative predictive value (95%–97%)[9-11]. Although PET/CT is moderate sensitivity for detecting metastatic LN, it is not used in routine practice in Thailand because of high expense. Therefore, we try to find the simple, inexpensive preoperative blood test to evaluate correlation with LN metastasis.

The host immune responses play important roles in the local environments of the tumors. Interactions between tumor cells and host immune responses cause tumor progression and growth[12]. Circulating cytokines and chemokines from tumor cells trigger systemic alterations of inflammatory responses, such as a low hemoglobin level, an increase in neutrophil counts, a slight increase in platelet counts, and a decline in lymphocyte counts[13]. Elevated neutrophil/lymphocyte ratio (NLR), platelet/lymphocyte ratio (PLR)] and multiplication of neutrophil and monocyte counts (MNM) have been investigated as prognostic and predictive markers in patients with various cancers[14-16]. Moreover, low-pretreatment hemoglobin (Hb) and thrombocytosis were associated with unfavorable prognosis in a number of epithelial malignancy including EMC[17]. Based on the theory that mean platelet volume (MPV) is an indicator of platelet activation, larger MPV was found to be a marker for predicting advanced-stage endometrial cancers in one study[18]. Therefore, this retrospective study was designed to investigate whether preoperative serum markers can be used for predicting LN metastasis in endometrioid adenocarcinoma of endometrium.

Materials and Methods

The study population consisted of patients with pathologically pure endometrioid adenocarcinoma of endometrium who underwent hysterectomy, salpingo-oophorectomy, pelvic and/or paraaortic LN sampling and peritoneal cytology at Rajavithi hospital, between 1 January 2010 and 31 December 2014. Patients with acute inflammatory disease, myeloproliferative disorders, autoimmune disease, hepatitis, splenectomy and synchronous malignancy were not included in the study. Patients using aspirin or clopidogrel which could affect platelet count and/or function were also excluded.

The study protocol was approved by the ethics committee of Rajavithi hospital, Thailand. Clinicopathological data, including age, body mass index (BMI), parity, menopausal status, diabetes, histopathological grade, surgical International Federation of Gynaecology and Obstetrics (FIGO) stage (revised in 2009), tumor diameter, myometrial invasion, lymphovascular space invasion (LVSI), lower uterine and cervical stromal involvement were retrospectively reviewed.

Preoperative hematological parameters were documented from medical records. Complete blood counts (CBCs) were obtained within one month before the operation using Coulterls780 and Xn1000 automatic analyzer in all patients. Serum albumin was collected as well within three months before the operation using Cobas8000 modular analyzer. The NLR was defined as the absolute neutrophil count divided by the absolute lymphocyte count, PLR was defined as the absolute platelet count divided by the absolute lymphocyte count and the MNM was defined as the multiplication of neutrophil counts and monocyte counts then divided by 10,000.

All statistical analyses were performed using the Statistical Package for Social Sciences version 16.0 software (SPSS Inc, Chicago, IL, USA). Categorical variables were analyzed using Pearson's chi-square test and Fisher's exact test. Continuous variables were shown as the mean ± standard deviation and compared using the student's t-test. The differences in each serum markers were compared using the Mann-Whitney U tests. Sensitivity and specificity for different serum markers cutoffs were calculated with receiver-operating curves. Receiver-operating curve analysis was plotted to investigate optimal cutoff values that maximized sensitivity and specificity. $P < 0.05$ was considered to be statistically significant.

Results

A total of 283 patients who meet the study inclusion criteria were included. Table 1 shows clinicopathological characteristics of the patients according to LN metastasis. Of the 283 patients, 213 patients (75.3%) underwent both pelvic and paraaortic LN sampling and 70 patients (24.7%) underwent only pelvic LN sampling. 40 patients (14.1%) had pelvic and/or paraaortic LN metastasis, and 243 patients (85.9%) did not have. Age, BMI, parity, menopausal status and diabetes were not different in group of LN positive and negative for malignancy. In the group of LN metastasis, there were significant higher in high grade of surgical specimen, FIGO staging, lower uterine and cervical involvement, depth of myometrial invasion, presence of LVSI and positive peritoneal cytology.

CBCs were collected in all patients, but data of serum albumin were missed in 50 patients (17.7%). Table 2 shows the comparison between each serum markers and LN metastasis. LN metastasis was significantly associated with lower Hb level ($p<0.001$) and higher NLR, PLR, MNM and platelet count ($p=0.018$, $p=0.004$, $p=0.008$ and $p=0.03$, respectively). MPV and serum albumin were quite higher in LN negative group, but there were no significant difference.

Receiver operating characteristics (ROC) curve findings, indicating the utility of the Hb, NLR, PLR, MNM and platelets count as predictive markers for lymph node metastasis in endometrioid adenocarcinoma of endometrium are shown in Fig. 1 and Table 3. Diagnostic sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy of preoperative serum markers by the ROC curve are shown in Table 4. The best cut off values to identify LN metastasis were 11.85 for Hb (72.5% sensitivity, 59.7% specificity, 22.8% PPV, 93.0% NPV), 2.29 for NLR (60.0% sensitivity, 61.7% specificity, 20.15% PPV, 90.4% NPV), 10.37 for PLR (67.5% sensitivity, 56% specificity, 20.15% PPV, 91.3% NPV), 0.0068 for MNM (70% sensitivity, 56% specificity, 20.74% NPV, 91.9% NPV) and 297,500 for platelet count (62.5% sensitivity, 57% specificity, 19.2% PPV, 90.2% NPV). Accuracy of Hb, NLR, PLR, MNM, platelet count are 61.5%, 61.5%, 57.6%, 58.0%, 57.6% respectively.

Table 1. Clinicopathological characteristics of patients with endometrioid adenocarcinoma of endometrium according to LN metastasis.

Characteristics	Total (n = 283)		Pelvic + Paraaortic LN				p value
			Positive (n =40)		Negative (n = 243)		
	n	%	n	%	n	%	
Age (years)							0.113
< 40	15	5.3%	0	0%	15	6.2%	
40-60	178	62.9%	23	57.5%	155	63.8%	
> 60	90	31.8%	17	42.5%	73	30.0%	
BMI (kg/m^2)							0.782
Underweight (< 18.5)	9	3.2%	2	5.0%	7	2.9%	
Normal (≥ 18.5 – 24.99)	98	34.6%	15	37.5%	83	34.2%	
Overweight (≥ 25-29.99)	105	37.1%	15	37.5%	90	37.0%	
Obesity (≥ 30)	71	25.1%	8	20.0%	63	25.9%	
Parity							0.899
0	93	32.9%	14	35.0%	79	32.5%	
1	46	16.3%	7	17.5%	39	16.0%	
≥ 2	144	50.9%	19	47.5%	125	51.4%	
Menopausal status							0.248
premenopause	78	27.6%	8	20.0%	70	28.8%	
menopause	205	72.4%	32	80.0%	173	71.2%	
DM	89	31.4%	13	32.5%	76	31.3%	0.877
Grading of surgical specimen							< 0.001*
G1	135	47.7%	9	22.5%	126	51.9%	
G2	100	35.3%	14	35.0%	86	35.4%	
G3	48	17.0%	17	42.5%	31	12.8%	
Staging							< 0.001*[F]
1	193	68.2%	0	0%	193	79.4%	
2	33	11.7%	0	0%	33	13.6%	
3	53	18.7%	38	95.0%	15	6.2%	
4	4	1.4%	2	5.0%	2	0.8%	
Tumor diameter (cm)							< 0.001[M]
Mean ± S.D.	4.36 ± 2.39		6.63 ± 2.41		3.99 ± 2.18		
Median (min-max)	4 (0.1-13)		6.75 (0.5-13)		4 (0.1-12.5)		
Lower uterine involvement	93	32.9%	24	60.0%	69	28.4%	< 0.001*
Cervical involvement	56	19.8%	14	35.0%	42	17.3%	0.009*
Myometrial invasion							< 0.001*
≤ ½	171	60.4%	9	22.5%	162	66.7%	
> ½	112	39.6%	31	77.5%	81	33.3%	

Table 1. Clinicopathological characteristics of patients with endometrioid adenocarcinoma of endometrium according to LN metastasis. (Cont.)

Characteristics	Total (n = 283)		Pelvic + Paraaortic LN				p value
			Positive (n =40)		Negative (n = 243)		
	n	%	n	%	n	%	
LVSI							< 0.001*
Positive	95	33.6%	32	80.0%	63	25.9%	
Negative	188	66.4%	8	20.0%	180	74.1%	
Peritoneal washing							< 0.001*
positive	18	6.4%	9	22.5%	9	3.7%	
negative	224	79.2%	27	67.5%	197	81.1%	
no data	41	14.5%	4	10.0%	37	15.2%	

BMI: body mass index; DM; diabetes mellitus; LVSI: lymphovascular space invasion. p-value from Chi-Square test, F = p-value from Fisher's Exact Test, M = Mann-Whitney U test, * Significant at the 0.05 level

Table 2. the comparison between each serum markers and LN metastasis.

Serum markers	Pelvic + Paraaortic				p value
	Positive (n=40)		Negative (n=243)		
	Mean	S.D.	Mean	S.D.	
Hb	11.11	1.82	12.23	1.56	< 0.001*^{T}
NLR	3.36	2.20	2.55	1.62	0.018*^{M}
PLR	15.75	9.61	11.71	7.42	0.004*^{M}
MNM	0.00719	0.00113	0.00671	0.00106	0.008*^{M}
Plt count	322.40	77.61	294.81	92.49	0.030*^{M}
MPV	9.30	1.18	9.63	1.15	0.095^{T}
Albumin	4.14	0.44	6.32	29.41	0.293^{M}

Hb: hemoglobin; NLR: neutrophil/lymphocyte ratio; PLR: platelet/lymphocyte ratio; MNM: multiplication of neutrophil and monocyte counts; Plt: platelet; MPV:mean platelet volume. M = Mann-Whitney U test, T=p-value from Independent t-test, *Significant at the 0.05 level

Table 3. Area under the curve and cut-off value in the ROC curve for serum markers.

Variables	Cut-off value	AUC	p-value	95% CI
Hb	≥ 11.85	0.698	< 0.001	0.611-0.785
NLR	≥ 2.285	0.617	0.018	0.517-0.717
PLR	≥ 10.368	0.643	0.004	0.543-0.742
MNM	≥ 0.006815	0.632	0.008	0.533-0.730
Plt	≥ 297.5	0.607	0.030	0.513-0.701

Hb: hemoglobin; NLR: neutrophil/lymphocyte ratio; PLR: platelet/lymphocyte ratio; MNM: multiplication of neutrophil and monocyte counts; Plt: platelet; AUC: area under the curve.

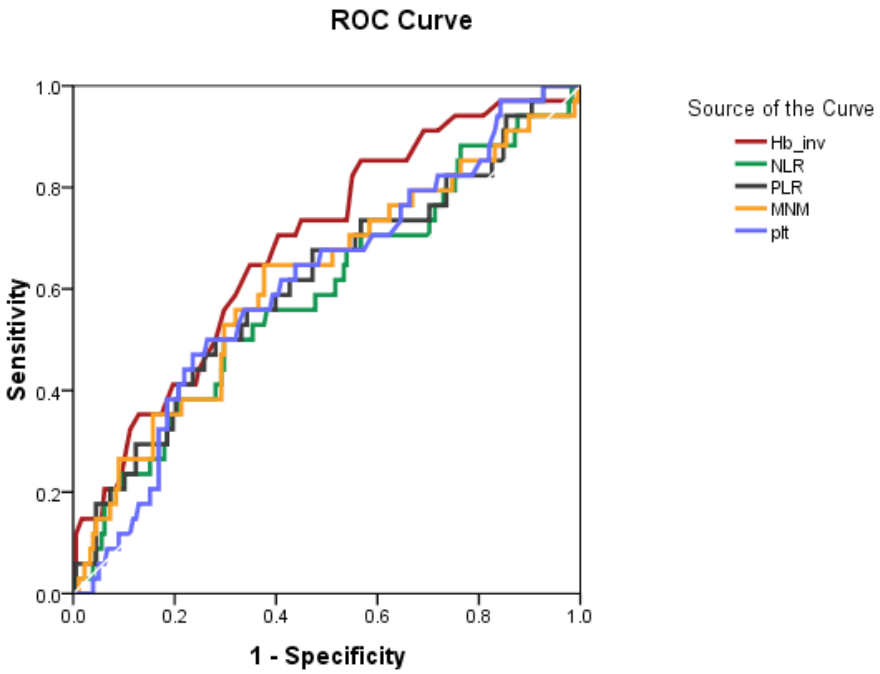

Fig. 1. ROC curve for the relationship between Hb level, NLR, PLR, MNM and platelet count and LN metastasis

Table 4. Diagnostic Sensitivity, specificity, PPV, NPV and accuracy of Serum markers.

serum markers	Cut-off value	Sensitivity (95%CI)	Specificity (95%CI)	PPV (95%CI)	NPV (95%CI)	Accuracy
Hb	< 11.85	72.50%	59.67%	22.83%	92.95%	61.48%
	≥ 11.85	56.11-85.40	53.21-65.89	15.86-31.12	87.73-96.43%	
NLR	≥ 2.285	60.00%	61.73%	20.51%	90.36%	61.48%
	< 2.285	43.33%-75.14%	55.30%-67.87%	13.61%-28.97%	84.82%-94.39%	
PLR	≥ 10.368	67.50%	55.97%	20.15%	91.28%	57.60%
	< 10.368	50.87%-81.43	49.48%-62.31	13.72%-27.95%	85.54%-95.27%	
MNM	≥ 0.006815	70.00%	55.97%	20.74%	91.89%	57.95%
	< 0.006815	53.47%-83.44%	49.48%-62.31%	14.25%-28.56%	86.27%-95.74%	
Plt	≥ 297.5	62.50%	56.79%	19.23%	90.20%	57.60%
	< 297.5	45.80%-77.27%	50.13%-63.11%	12.85%-27.07%	84.35%-94.41%	

Hb: hemoglobin; NLR: neutrophil/lymphocyte ratio; PLR: platelet/lymphocyte ratio; MNM: multiplication of neutrophil and monocyte counts; Plt: platelet; PPV: positive predictive value; NPV: negative predictive value.

Discussion

LN sampling is the necessary procedure of surgical staging in EMC because it can make true staging, guide the appropriate adjuvant treatment, and eradicate metastatic LN. This procedure causes complications such as vessel injury, postoperative lymphedema. To avoid systematic overtreatment, NCCN guideline recommends a more selective and tailored lymphadenectomy approach according to intraoperative finding[19]. However, risk factors for

predicting LN metastasis are cell type, grade, depth of myometrial invasion, LVSI and cervical involvement which are clearly detected after surgical procedure[20]. Thus, many studies have investigated about noninvasive preoperative methods with high accuracy and sensitivity to detect LN metastasis. To date, no preoperative test whether it is PET/CT scan, MRI or any preoperative blood tests is able to detect LN micrometastasis.

Tumor cells can produce cytokines, such as interleukins, interferon gamma and tumor necrosis factor which induce hemolysis, suppress erythropoiesis and inhibit the response of erythroid progenitor cells to erythropoietin[21]. Platelets have been associated with malignant diseases, based on alteration of coagulation and abnormal hemostasis in cancer patients[18]. The presence of neutrophilia, leukocytosis and thrombocytosis represents a nonspecific response to cancer-related inflammation[22]. Neutrophilia provides a suitable environment and secrets most circulating vascular endothelial growth factors, and finally it causes cancer progression[23].

At present, a few studies have investigated the accuracy of systemic inflammatory response (SIR) markers for LN metastasis in endometrial cancer. One retrospective study reviewed the correlation of SIR markers with LN metastasis compared with serum CA-125 in endometrioid adenocarcinoma of endometrium after surgical staging. Preoperative SIR markers including CBCs, CRP, fibrinogen and serum albumin were obtained. This study found that preoperative NLR, PLR, CRP, albumin, and fibrinogen levels were not superior to CA-125 for the prediction of LN metastasis, but that among the markers, only NLR and PLR reached comparable sensitivities (63.3% and 64.5%, respectively) (24). Serum CRP, fibrinogen and CA 125 were not used in routine preoperative blood tests in our country. This study has some limitations. First, only 26% of patients underwent both pelvic and paraaortic lymphadenectomy and 8.8% of patients did not undergo pelvic and paraaortic lymphadenectomy. Second, CRP and fibrinogen values were missing for several patients.

In the present study, a total of 283 patients were included. All of our patients were surgical staging with pelvic and/or paraaortic LN sampling. Of the 283 patients, 213 patients (75.3%) underwent both pelvic and paraaortic LN sampling and 70 patients (24.7%) underwent only pelvic LN sampling. Serum markers were moderate sensitivity to detect LN metastasis in endometrioid adenocarcinoma of endometrium especially Hb, PLR and MNM. Furthermore, negative predictive values (NPV) of Hb, NLR, PLR, MNM and platelet count to predict LN metastasis were more than 90%. These serum markers were acceptable, simple to obtain and calculate. They could be used in clinical practice to rule in patients with low risk for LN metastasis without extra costs. If using these serum markers with other blood test (CA125) or imaging (PET/CT, MRI), it will improve the good performance to rule in patients with low risk for LN metastasis.

Our study has some limitations. First, it was retrospective in nature based on a single institution. Second, there was difference in timing of collecting preoperative hematological parameters in each patient. Lastly, data of serum albumin were missing up to 17.7% of patients. Although the association of these serum markers between the patients with positive or negative lymph nodes was statistically significant, the differences of these values were only small and may be not clinically important. If we had been able to collect all data, the test value would have been improved for using in clinical practice.

Conclusion

Preoperative lower Hb level, higher NLR, PLR, MNM and platelet count were significantly related with lymph node metastasis. They could be used in clinical practice to rule in patients with low risk for LN metastasis without extra costs.

Potential conflicts of interest

The authors declare no conflict of interest.

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