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#### **Chapter in a Book**

Phupong V. Management of PPROM AT 32 to 34 weeks. In: Desai SV, Tank P, eds. *Handbok on preterm prelabor rupture of membranes in a low source setting*. New Delhi: Jaypee Brothers Medical Publishers Ltd, 2012: 39-46.

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## EDITORIAL

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# Special Review and Topics in Fifth Issue of Thai Journal of Obstetrics and Gynaecology 2024

Vorapong Phupong, M.D., FRTCOG.\*

\* *Editor in Chief, Thai J Obstet Gynaecol, The Royal Thai College of Obstetricians and Gynaecologists*

This fifth issue of Thai Journal of Obstetrics and Gynaecology 2024 contains many interesting articles. The special article is “Practical initial evaluation of fetus and placenta of stillbirths for obstetricians in delivery room”. This article aimed to review the practical points and necessary assessment of fetal anomalies and placental examination in stillbirth. The contents showed how to examine the fetus and placenta in stillbirth<sup>(1)</sup>.

This issue also contains seven original articles and one case report. Jaishuen et al performed a retrospective cohort study to evaluate 5-year survival in patients with endometrioid endometrial carcinoma, who underwent evaluation of retroperitoneal pelvic lymph nodes alone, compared to evaluation of pelvic with paraaortic lymph nodes and found that the evaluation of pelvic and paraaortic lymph nodes was independently associated with enhanced survival outcomes<sup>(2)</sup>. Somjit et al performed a study to evaluate the efficacy of the ‘Si-Washer’ in cleansing gynaecological laparoscopic instruments. They found that the Si-Washer effectively decontaminated gynaecological laparoscopic instruments<sup>(3)</sup>. Terece et al performed a comparative study to evaluate the impact of delivery mode on sexual function in the first year after childbirth. They found that regardless of the mode of delivery, all participating women in the first year after childbirth had deficiencies in total female sexual function index scores and all subscale scores, indicating sexual dysfunction. This inadequacy in sexual dysfunction was more pronounced in women who gave birth vaginally than those who gave birth by cesarean section<sup>(4)</sup>. Chu et al performed a study to evaluate breast cancer susceptibility gene 1/2 (BRCA1/2) in cases of ovarian cancer among Vietnamese population. They found that found 5 pathogenic and likely pathogenic (P/LP) variants, including c.1801\_1808delCACAATTC, c.1016delA, c.1673\_1674delAA, c.928C>T in BRCA1 and c.2865delC in BRCA2 with a frequency of 3.03%, except for c.1801\_1808delCACAATTC (6.06%). The prevalence of BRCA1/2 P/LP variants in the high-risk group was 22.73%, while this figure for the low-risk group was 9.09%<sup>(5)</sup>. Wittayatanaseth et al performed a cross-sectional study to find the prevalence of iron-deficient erythropoiesis in Thai pregnant women during early pregnancy with the identification of associated factors. The results revealed the prevalence of iron-deficient erythropoiesis was 10.77% and multiparity was associated with iron-deficient erythropoiesis<sup>(6)</sup>. Sirisakpanich et al performed a retrospective study of women diagnosed with stage II-IV uterovaginal prolapse to evaluate surgical outcomes of laparoscopic high uterosacral ligament suspension for apical prolapse repair and the ability to perform concomitant bilateral salpingectomy/salpingo-oophorectomy. They found that high anatomical success of 90% was achieved at 12-month follow-up and bilateral salpingo-oophorectomy could be performed in all patients<sup>(7)</sup>. Sompratthana et al performed a retrospective study analyzed cases spanning from 2015 to 2019 of patients diagnosed with endometrial

intraepithelial neoplasia (EIN) to find the prevalence of concurrent endometrial cancer and to assess the characteristics and risk factors associated with occult endometrial cancer in EIN patients. The results demonstrated the prevalence of concurrent endometrial cancer in EIN patients was 23.1%. All patients diagnosed with concurrent endometrial cancer exhibited uterine-confined disease, with no diagnoses of stage III or IV disease<sup>(8)</sup>.

Regarding case report, Dasrihsyah et al reported an uncommon case of urethral caruncle in teenage girl presented with periurethral mass and successfully surgical management<sup>(9)</sup>.

Editor in Chief and managing staff of the Thai Journal of Obstetrics and Gynaecology were invited by the Thai-Journal Citation Index (TCI) Centre, financially supported by the Thailand Science Research and Innovation (TSRI) on August 26, 2024 at Eastin Grand Hotel Sathorn Bangkok, South Sathorn Road, Yannawa, Sathorn, Bangkok, Thailand. TCI has implemented the project "Development and improvement of the quality of Thai journals in the Scopus database Phase II". To raise the quartile of Thai journals in the Scopus machine, 48 journals remain in Q1-Q2, the organizational structure improves the system and the development of the quality of Thai journals in the Scopus process for the better, and reports the results of monitoring and evaluating the performance of journals in the project and provides knowledge about the development of journal websites according to the new criteria of Scopus, including new knowledge in issues related to AI and the ethics of journal editors, as well as guidelines for developing the quality of Thai journals in Scopus to have a higher Quartile.

The RCOG 39<sup>th</sup> annual meeting will be held during 29 October - 1 November 2024 at Dusit Thani, Pattaya, Chonburi, Thailand. The theme of the meeting is "Optimizing OBGYN". Wish to see you at RCOG Annual Meeting 2024 at Dusit Thani, Pattaya, Chonburi, Thailand.

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## SPECIAL ARTICLE

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# Practical Initial Evaluation of Fetus and Placenta of Stillbirths for Obstetricians in Delivery Room

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### ABSTRACT

Gross examination of fetus and placenta can simply be performed immediately after delivery in delivery room by the delivery attendant. The practical points and necessary assessment of fetal anomalies and placental examination have been summarized. The examination is not complicated and should be done in every stillbirth.

**Keywords:** stillbirths, gross examination, placenta.

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Stillbirth as defined by World Health Organization (WHO) is a baby born with no signs of life at 22 or more completed weeks of gestation or the birth of a stillborn that weighs at least 500 grams<sup>(1)</sup>. The “no signs of life” is indicated by the absence of breathing, heartbeats, pulsation of the umbilical cord, or definite movements of voluntary muscles. However, due to differences in capacity in measurement and ability of perinatal care, the definition is varied among institutes. For international comparisons, the definition is extended to include fetuses that are, by order of priority, of at least 1000 g birthweight, and/or at least 28 weeks gestation, and at least 35 cm long<sup>(2, 3)</sup>.

Stillbirths can be divided into two categories

based on the correlation of fetal death detection and the onset of labor as 1) antepartum stillbirths and 2) intrapartum stillbirths. Intrapartum stillbirths reflect the quality of care during labor, while antepartum stillbirths may represent the quality of antenatal care services and fetal surveillance. Determining a cause of stillbirth is very important as it may reveal an underlying maternal medical condition or pregnancy associated morbidity which affects further treatment and management of subsequent pregnancies. In general, the main components of evaluation of stillbirth are clinical history, perinatal autopsy, placental examination, and genetic testing. Further laboratory tests are rather time-sensitive and should be considered individually based on the

results of initial testing and relevant clinical setting<sup>(4)</sup>. Unfortunately, the autopsy rates are decreasing nowadays. This is owing to lack of parental consent, lack of skilled perinatal pathologists, cost containment, and technical limitations of conventional autopsy<sup>(5, 6)</sup>. Many families are reluctant to proceed with an evaluation for stillbirth, especially fetal autopsy and genetic testing. Besides of the increasing expenses, their perception is the workup will not bring their baby back and not guarantee of any direct benefits. Some misconceptions concern that the autopsy will disfigure the body which is prohibited and will affect with burial ceremony or rebirth in the next life by religious beliefs. The sending of placenta for pathologic examination is also refused by some personal beliefs. Although there are modern imaging techniques such as microfocus computed tomography (CT) and 3 Tesla magnetic resonance imaging (MRI) to perform virtual autopsy, but it is still limited by the resources and expenses<sup>(7)</sup>. The lack of histology is also a limitation of all postmortem imaging techniques, but it can be partially compensated by adding image-guided needle tissue biopsy.

In case that the complete fetal autopsy or placental pathologic examination is declined, and imaging is unavailable, the alternative method is only external fetal examination in which the attending obstetrician has to be the one who perform in delivery room. Many obstetricians lack the confidence to do and worry to misinterpret the findings. In fact, the examination is not complicated and should be done in every stillbirth, even in case that will be submitted for autopsy.

### Examination of the baby<sup>(8, 9)</sup>

The birth attendant should perform a complete examination of the baby shortly after birth before being sent to autopsy by pathologist. If possible, a chart should be available to guide the examiner to record all the required examined parts as follows:

1. Weighing the baby in grams
2. Measurements

Measurements consist of head circumference (HC), chest circumference (CC), abdominal circumference (AC), crown-rump (CR) length, crown

heel (CH) length, foot length (FL), inner and outer canthal distance, interpupillary distance, and measurements of the cranial fontanelles. However, if there are not available tools, the minimal measurement should be length and foot length. The CR length is usually two-thirds of the CH length. The CH length and HC usually not differ more than 1 cm and can be roughly used as indicator of macrocephaly or microcephaly. Fetal foot length is a reliable parameter for use in the assessment of gestational age and is particularly useful when other parameters do not accurately predict gestational age, for example, severely macerated fetuses, fragmented fetuses, and in fetuses with major malformations such as hydrocephalus, anencephaly, and short limb dysplasia<sup>(10, 11)</sup>.

3. Evaluate degree of maceration to determine the estimated time of fetal death<sup>(12, 13)</sup>

Degree of desquamation and cranial compression are capable in predicting the time interval from fetal death to delivery. However, the maceration rate may be influenced by maternal fever, fetal or placental infection, fetal hydration at the time of death, amniotic fluid volume, and delay from delivery to postmortem examination. The extent of maceration and estimated time of death is showed in Table 1.

**Table 1.** Time table of macroscopic features caused by maceration after intrauterine fetal death<sup>(13)</sup>.

Intrauterine duration of retention	Gross examination
6 hours	Desquamation of patches 1 cm; brown or red discoloration of umbilical stump
12 hours	Desquamation on face, back, or abdomen
18 hours	Desquamation of 5% body, or 2 or more body regions
24 hours	Brown or tan skin discoloration on abdomen Moderate desquamation
36 hours	Cranial compression
48 hours	Desquamation of > 50% of body
72 hours	Desquamation of > 75% of body
96 hours	Overlapping cranial sutures
1 week	Widely open mouth Collapse of calvarium Laxity and dislocation of joints
2 weeks	Mummification (dehydration, compression, tan color)

4. Systematic external examination includes

4.1. Head:

4.1.1. Shape, fontanelles, sutures, and skull bone abnormalities.

The skull deformation is proportional to the extent of the sutures involved. Early fusion of one or more cranial sutures (craniosynostosis) results in a characteristic dysmorphic head shape (Table 2). Nonsyndromic craniosynostosis can be classified into isolated and complex fusions. Isolated fusions involve only single cranial

suture and are more common than complex craniosynostosis.

4.1.2. Size.

Microcephaly is a small head and defined as head circumference smaller than 3 standard deviations (SD) below the mean for the gestational age<sup>(15)</sup>. In contrast, macrocephaly or large head is defined as occipitofrontal head circumference above the 98<sup>th</sup> percentile or greater than 2 SDs above the mean for gestational age<sup>(16)</sup>.

**Table 2.** Example of head deformities in craniosynostosis<sup>(14)</sup>.

Fused suture	Gross examination
<b>Isolated craniosynostosis</b>	
Sagittal	Scaphocephaly (meaning boat-like) or dolichocephaly (meaning long) <ul style="list-style-type: none"> <li>- Long, narrow head</li> <li>- Decreased posterior skull width and height</li> <li>- Frontal bossing</li> <li>- Bitemporal narrowing</li> </ul>
Metopic	Trigonocephaly (triangular-shaped head) <ul style="list-style-type: none"> <li>- Triangular-shaped forehead</li> <li>- Bitemporal narrowing</li> <li>- Supraorbital retrusion and deficient lateral orbital rims with hypotelorism</li> <li>- Occipitoparietal widening</li> </ul>
Coronal	Unilateral coronal synostosis creates an anterior plagiocephaly (asymmetric anterior head shape) <ul style="list-style-type: none"> <li>- Asymmetric anterior head shape</li> <li>- Ipsilateral forehead flattening</li> <li>- Ipsilateral supraorbital rim elevation and retrusion</li> <li>- Contralateral frontal bossing</li> <li>- Deviation of nasal root towards affected side</li> </ul>
Lambdoid	Unilateral lambdoid synostosis creates posterior plagiocephaly (asymmetric posterior head shape) <ul style="list-style-type: none"> <li>- Asymmetric posterior head shape</li> </ul>

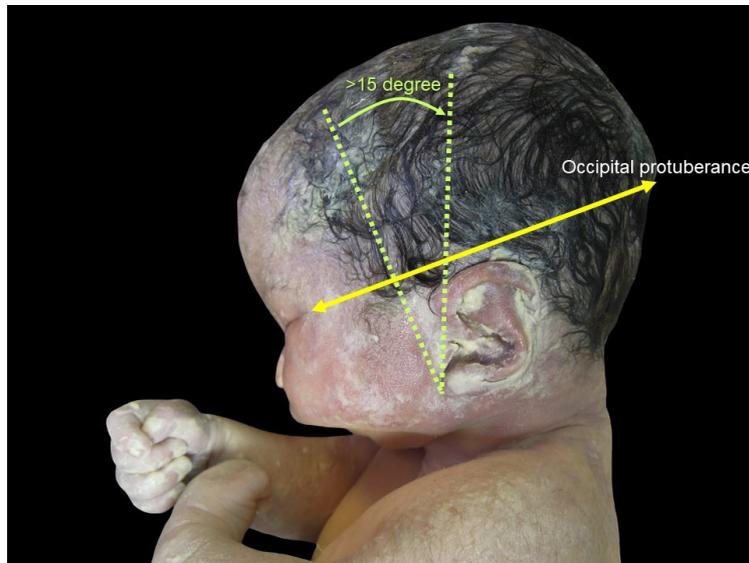
Fused suture	Gross examination
<ul style="list-style-type: none"> <li>- Ipsilateral occipital flattening</li> <li>- Ipsilateral occipitomastoid bulge</li> <li>- Reduction of ipsilateral skull height</li> <li>- Inferior positioning of ipsilateral skull base</li> <li>- Contralateral frontoparietal bossing</li> </ul>	
<b>Complex craniosynostosis</b>	
Bicoronal	Brachycephaly (short head shape) <ul style="list-style-type: none"> <li>- Short head in anteroposterior dimension and wide transverse diameter</li> <li>- Tall forehead (turricephaly)</li> <li>- Forehead flattening with elevated both eyebrows</li> <li>- Large or bulging eyes</li> </ul> <p>Some individuals develop acrocephaly (conical-shaped head) if the anterior fontanel is open</p>
Bilateral lambdoid	Deformational plagiocephaly <ul style="list-style-type: none"> <li>- Flattened occipital region</li> <li>- Widened occipitoparietal region</li> <li>- Elongation of the vertex</li> </ul>
Sagittal and both lambdoid sutures	Mercedes Benz pattern craniosynostosis <ul style="list-style-type: none"> <li>- Anterior turricephaly</li> <li>- Brachycephaly</li> <li>- Decreased posterior skull width</li> <li>- Flattened occiput with sublambdoidal indentation</li> </ul>

5. Facial features: ears, eyes, nose, mouth<sup>(17)</sup>

5.1. Ears: shape, position, and patency of ear canal.

Low-ser ears are present when the helix

(curved upper part of the outer ear) meets the cranium at a level below the horizontal plane with the corner of orbit. Slanted ears occur when the angle of the slope of the auricle exceeds 15 degrees from the perpendicular<sup>(18)</sup>. (Fig. 1)



**Fig. 1.** Low-set ears

5.2. Eyes: eyebrows, eyelashes, fusion of eyelids, abnormal folds (such as epicanthal folds), slanting of palpebral fissures, pupils (shape and size), iris (color and colobomas), sclera (color), conjunctiva, and corneal opacification.

The interocular distance and the binocular diameter are helpful in determining hypo- or hypertelorism. Hypertelorism is defined as an interocular distance of  $> 95^{\text{th}}$  percentile for gestational age, while hypotelorism is defined as an interocular distance of  $< 5^{\text{th}}$  percentile for gestational age<sup>(19, 20)</sup>.

5.3. Nose: shape, nasal bridge flatness or prominence, nares, and patency of choanae

5.4. Mouth: philtrum, lips, hard and soft palate, including the uvula, tongue, frenula, and teeth.

It is important to always examine the palate because cleft palate can occur as an isolated defect. In case of facial clefts, the examiner should state the

location of cleft (lip, alveolus, hard palate, or soft palate; including lateral (unilateral or bilateral) or median) and severity of the defect. The degree of clefting can vary from a notch to a full-thickness cleft<sup>(21)</sup>.

5.5. Chin: shape and size.

The chin and mandible are normally easily assessed subjectively and should be aligned with the upper lip and nose in the midsagittal view of the fetal face. The fetus with micrognathia or retrognathia will appear to have an overbite caused by the small and/or posteriorly displaced mandible<sup>(22)</sup>.

6. Neck: length of neck, mass, cystic hygroma, neck web, and abnormalities of nuchal skin.

7. Chest: shape, size, and symmetry.

Abnormal appearance of the chest can vary from mild to severe. Examples of chest wall deformities are shown in Table 3.

**Table 3.** Example of chest wall deformities<sup>(23, 24)</sup>.

Anomalies	Gross examination
Pectus Excavatum (sunken chest)	Central depression of the sternum, typically the lower half of the sternum, producing a funnel-shaped chest
Pectus Carinatum (pigeon chest)	Outward protrusion of the sternum and ribs
Pectus arcuatum (costomanubrial pectus carinatum (type 2), pouter pigeon breast, arcuate pigeon breast, Currarino-Silverman syndrome)	“Carinatum superiorly and excavatum inferiorly” - Anterior angulation of the superior ribs and cartilages (especially 1–3) - Foreshortened unusual sternum with anterior angulation of the manubrium - 90° angulation with posterior indentation
Jeune Syndrome	Small bell-shaped chest - Extremely small chest - Broad and short ribs with irregularly joined to the costal cartilages and sternum
Poland Syndrome	Spectrum of abnormalities of one side of the chest - Absence of the pectoralis muscle(s) - Under development of breast tissue and areola - Deformity of the costal cartilages of the upper ribs - Inadequate development of the arm, hand, and fingers of the same side
Sternal defects	Sternal defects are classified into three groups: 1) Sternal cleft - Failure of fusion of the two sternal halves, most commonly located superiorly - Heart position is usually normal - Intact skin integrity 2) Ectopia cordis - Abnormal position of the heart partially or totally outside the chest - 4 types based on the location of heart: cervical, thoracic, abdominal, and thoracoabdominal 3) Cantrell’s pentalogy (thoracoabdominal type of ectopia cordis) consists of 1) omphalocele, 2) anterior diaphragmatic hernia, 3) ectopia cordis, 4) cardiac anomalies, and 5) sternal cleft

8. Nipples: position and presence of palpable breast tissue and internipple distance

9. Abdomen: distension, defects, umbilicus, and umbilical stump.

The two most common congenital abdominal wall defects are gastroschisis and omphalocele. The main distinguishing features are that gastroschisis has no sac and the defect is located to the right of the midline and the umbilical cord, whereas an omphalocele typically has a sac and the defect is at

the base of umbilicus<sup>(25)</sup>. Size of the defect and protruding visceral organs should be recorded.

10. Genitalia:

10.1. Female: labia and clitoris (appearance and size), vagina (patency, hymenal ring, and duplication)

10.2. Male: penis (appearance, size, and position of meatus) and scrotum (appearance and position of testes)

11. Anus: position and patency

12. Back/vertebral column: alignment, neural tube defects, sacral dimples, and pigmented spots.

There are many different types of neural tube defects with varied deformities as shown in Table 4.

**Table 4.** Spectrum of neural tube defect<sup>(26)</sup>.

Anomalies	Gross examination
Anencephaly	Absence of most of the brain and most or all of the cranial vault <ul style="list-style-type: none"> <li>- Meroacrania: the defect is localized to the cranium</li> <li>- Holoacrania: the defect ends through the foramen magnum</li> </ul>
Exencephaly	Absence of the cranial vault, but with intact exposed brain tissue
Iniencephaly	Fixed fetal head retroflexion <ul style="list-style-type: none"> <li>- Head is extremely retroflexed and absent neck</li> <li>- The face skin is continuous with the chest skin</li> <li>- The posterior scalp is directly connected to the skin of the back</li> <li>- Occipital bone defect</li> </ul>
Spina bifida	Failure of the closure of the dorsal aspect of the vertebral foramen of one or a few vertebrae <ul style="list-style-type: none"> <li>- Spina bifida occulta (closed spinal dysraphism): involves a hidden vertebral defect and minimal neural involvement               <ul style="list-style-type: none"> <li>- No obvious deformity</li> <li>- Sometimes a hairy patch of skin or dimple at the expected spinal defect</li> </ul> </li> <li>- Spina bifida aperta (open spinal dysraphism): defect in which neural tissues communicate with the external environment               <ul style="list-style-type: none"> <li>- Meningocele: defect of the posterior elements of the spine with extrusion of meninges and cerebrospinal fluid (CSF) without the involvement of the neural elements</li> <li>- Myelomeningocele: clear meningeal cystic sac that contains CSF and nervous tissue</li> <li>- Myelocele: clear open defect with no meningeal sac is protruding</li> </ul> </li> </ul>
Rachischisis	Severe form of spina bifida <ul style="list-style-type: none"> <li>- Cleft through the entire spine</li> <li>- Spina bifida involving many adjacent vertebrae</li> </ul>
Encephalocele	Herniation such as a sac-like protrusion of the brain and/or the meninges through an opening in the skull <ul style="list-style-type: none"> <li>- Meningocele: herniation of meninges</li> <li>- Encephalomeningocele: herniation of meninges and brain</li> <li>- Encephalomeningocystocele: herniation of meninges, brain and ventricle</li> </ul>

13. Extremities: overall length, symmetry, contractures, pterygia, joint laxity, deformations, muscular mass, position and creases of digits (syndactyly, polydactyly, clinodactyly, and missing digits), nails (size,

curvature, and relation to tip of digits), and palmar and plantar creases.

Examination for hip dislocation or instability should be undertaken. Some extremity deformities are shown in Table 5.

**Table 5.** Terminology of limb deformities<sup>(27, 28)</sup>.

Anomalies	Gross examination
<b>Limb shortening</b>	
Rhizomelia	Shortening of the extremities, involving the proximal segment (humerus or femur)
Mesomelia	Shortening of the extremities, involving the middle segment (radius, ulna, tibia or fibula)
Acromelia	Shortening of the extremities, involving the distal segment (hands and feet)
Micromelia	Shortening of the extremities, involving the entire limb (proximal, middle, and distal segments)
Acromesomelia	Shortening of the extremities, involving the middle and distal segments
<b>Absence of extremities</b>	
Amelia	Absence of 1 or more limbs
Phacomelia	Shortening of limb with hand/foot arising near trunk (seal limb)
Hemimelia	Absence of distal limb
Acheiria	Absence of the hand
<b>Abnormalities of hands and feet</b>	
Polydactyly	Presence of more than five digits <ul style="list-style-type: none"> <li>- Postaxial: the extra digits are on the ulnar or fibular side</li> <li>- Preaxial: the extra digits are located on the radial or tibial side</li> </ul>
Syndactyly	Soft tissue or bony fusion of adjacent digits
Clinodactyly	Permanent curvature or deflection of one or more fingers
Symbrachydactyly	Fingers are short and webbed or joined together. Some or all of the fingers are typically underdeveloped or missing.
Macrodactyly	Abnormally large fingers
Clubfoot (talipes equinovarus)	Rigid inward turning of foot toward the midline of the body (forefoot and midfoot abductus, hindfoot varus, and ankle equinus)
Rocker bottom foot	Vertical talus, or eversion of the planar arch, produces a rocker-bottom (convex outward) appearance of the bottom of the foot
Clenched hands	The hands are clenched to fists <ul style="list-style-type: none"> <li>- All digits held completely flexed at the metacarpophalangeal and interphalangeal joints</li> <li>- Overlapping of index finger across other three clenched fingers.</li> </ul>
Ectrodactyly	Deficiency or absence of one or more central digits of the hand or foot (split hand, cleft hand, or lobster claw hand)

## Examination of the placenta

Many placental lesions can be diagnosed solely by gross examination which is very helpful in evaluation of stillbirth. Several conditions can be detected such as abruption and umbilical cord abnormalities. As the detail of placental examination in delivery room has been published<sup>(29)</sup>, so in this

section will discuss some specific points that related to stillbirths.

1. Weighing the placenta in grams
2. Umbilical cord examination: number of vessels at cut end of cord, length, diameter, coiling index, type of cord insertion, knots, and discoloration. Cord stricture can be seen at the fetal end, especially

in macerated stillbirth and misinterpret as the main cause of fetal demise. However, it is controversy and can be postmortem artifact. Some pathologists suggest that the diagnosis of “real” cord stricture as the cause of fetal death should be confirmed by histologic evidence of venous obstruction such as stenosis or obliteration of cord vessels at the narrow segment or intravascular cord thrombosis<sup>(30)</sup>.

3. Examination of membranes: type of insertion into the disk, completeness, color, opacity, and other lesions such as hemorrhage, cysts or nodules. Green brown discoloration reflects meconium-stained amniotic fluid, while red brown discoloration is seen in cases with old hemorrhage. Chorioamnionitis can be seen as opaque membranes with yellow white color.

4. Placental disk examination: assess the shape and extra lobes, disk size (3-dimensions), and evaluate the fetal and maternal surfaces. The fetal surface or chorionic plate is examined for color, vascular pattern and thrombosis, hemorrhage, abnormal nodules, and cysts. The maternal surface or basal plate should be inspected of color, completeness, discoloration, and adherent blood clot.

## Conclusion

Gross examination of fetus and placenta should be done in every stillbirth immediately after delivery. The evaluation should be done step by step. Examination of baby is starting from head to toe as well as placental examination that includes three main components: umbilical cord, membranes, and disk.

## Potential conflicts of interest

The author declares no conflicts of interest.

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## GYNAECOLOGY

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# Benefit of Paraaortic Lymph Node Evaluation in Endometrioid Endometrial Carcinoma; A 10-year retrospective study in Thailand

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### ABSTRACT

**Objectives:** To evaluate 5-year survival in patients with endometrioid endometrial carcinoma, who underwent evaluation of retroperitoneal pelvic lymph nodes alone, compared to evaluation of pelvic with paraaortic lymph nodes.

**Materials and Methods:** This retrospective cohort study enrolled 636 women who were diagnosed with endometrioid endometrial carcinoma and underwent surgical staging at Faculty of Medicine Siriraj Hospital, Mahidol University between January 2006 and December 2015. Patients who underwent pelvic lymph node evaluation (n = 257) and pelvic with paraaortic lymph node evaluation (n = 379) were included.

**Results:** The median follow-up time was 60 months. The 5-year overall survival rate (OS) in the pelvic lymph node (PLN) and pelvic with paraaortic lymph node (PPALN) groups was 81.6% and 87.7%, respectively (p = 0.073). However, the PPALN group had significantly longer survival than the PLN group after adjustment for other prognostic factors (adjusted hazard ratio (HR)) 1.63 (1.06-2.52, p = 0.028). There was a trend to improve 5-year disease-specific survival (DSS) for the European Society of Gynaecological Oncology (ESGO)/ European Society for Radiotherapy and Oncology (ESTRO)/ European Society of Pathology (ESP) high risk patients, who underwent PPALN evaluation (85.6%) compared with PLN evaluation (70.8%), p = 0.061.

**Conclusion:** In endometrioid endometrial carcinoma patients, the evaluation of pelvic and paraaortic lymph nodes is independently associated with enhanced survival outcomes.

Therefore, in situations when a sentinel lymph node biopsy is not feasible, we suggest evaluating the pelvic and paraaortic lymph nodes for all patients diagnosed with endometrioid endometrial cancer.

**Keywords:** cancer of the endometrium, lymphadenectomy.

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## ประโยชน์ของการเลาะประเมนต่อมน้ำเหลืองรอบหลอดเลือดเอออร์ตา ในมะเร็งเยื่อบุมดลูกชนิด endometrioid; การศึกษาแบบย้อนหลัง 10 ปีในประเทศไทย

อรรถพล ใจชื่น, ภัทรา วิศาลศิริรักษ์, จันจิรา เพชรสุขศิริ, พีรพงศ์ อินทศร, สมภาพ กุลจรसनนท์, เขมณัญญ์ เขมวรพงศ์, วิชชา ปุณยกนก

### บทคัดย่อ

**วัตถุประสงค์:** เพื่อประเมินอัตราการรอดชีวิต 5 ปีในผู้ป่วยมะเร็งเยื่อบุมดลูกชนิด endometrioid ที่ได้รับการเลาะประเมนต่อมน้ำเหลืองเฉพาะในอุ้งเชิงกรานเทียบกับการเลาะประเมนทั้งต่อมน้ำเหลืองในอุ้งเชิงกรานและรอบหลอดเลือดเอออร์ตา

**วัสดุและวิธีการ:** การศึกษาแบบย้อนหลังนี้ได้รับรวมผู้ป่วยจำนวน 636 คน ที่ได้รับการวินิจฉัยว่าเป็นมะเร็งเยื่อบุมดลูกชนิด endometrioid และได้รับการผ่าตัดกำหนดระยะโรคที่คณะแพทยศาสตร์ศิริราชพยาบาล มหาวิทยาลัยมหิดล ระหว่างเดือนมกราคม 2549 ถึงธันวาคม 2558 โดยมีผู้ป่วยที่ได้รับการเลาะประเมนต่อมน้ำเหลืองเฉพาะในอุ้งเชิงกราน ( $n = 257$ ) และการเลาะประเมนต่อมน้ำเหลืองในอุ้งเชิงกรานและรอบหลอดเลือดเอออร์ตา ( $n = 379$ ) รวมอยู่ในการศึกษานี้

**ผลการศึกษา:** ผู้ป่วยได้รับการตรวจติดตามเฉลี่ยอยู่ที่ 60 เดือน พบว่าผู้ป่วย ในกลุ่มที่ได้รับการเลาะประเมนต่อมน้ำเหลืองในอุ้งเชิงกราน และกลุ่มที่ได้รับการเลาะประเมนต่อมน้ำเหลืองทั้งในอุ้งเชิงกรานและรอบหลอดเลือดเอออร์ตา อัตราการรอดชีวิตที่ 5 ปี เท่ากับร้อยละ 81.6 และร้อยละ 87.7 ตามลำดับ ( $p = 0.073$ ) อย่างไรก็ตามจากการวิเคราะห์หุ้พหุตัวแปร กลุ่มที่ได้รับการเลาะประเมนต่อมน้ำเหลืองทั้งในอุ้งเชิงกรานและรอบหลอดเลือดเอออร์ตามีอัตราการรอดชีวิตที่นานกว่าอีกกลุ่มอย่างมีนัยสำคัญ (adjusted HR 1.63 (1.06-2.52,  $p=0.028$ )) และ ผู้ป่วยที่มีความเสี่ยงสูงตามเกณฑ์ของ ESGO/ESTRO/ESP ที่ได้รับการเลาะประเมนต่อมน้ำเหลืองในอุ้งเชิงกรานและรอบหลอดเลือดเอออร์ตา มีแนวโน้มที่จะมีอัตราการรอดชีวิตเฉพาะโรคที่มากกว่าเมื่อเทียบกับอีกกลุ่ม (ร้อยละ 85.6 และร้อยละ 70.8,  $p=0.06$ )

**สรุป:** ในผู้ป่วยมะเร็งเยื่อบุมดลูกชนิด endometrioid การเลาะประเมนต่อมน้ำเหลืองในอุ้งเชิงกรานและรอบหลอดเลือดเอออร์ตา มีความสัมพันธ์กับอัตราการรอดชีวิตที่ดีขึ้น ดังนั้นคำแนะนำสำหรับการผ่าตัดรักษาผู้ป่วยมะเร็งเยื่อบุมดลูกชนิด endometrioid ในโรงพยาบาลที่ไม่สามารถตรวจประเมนต่อมน้ำเหลืองด้วยวิธี sentinel ได้ คือให้ทำการผ่าตัดเลาะประเมน

## Introduction

Endometrial carcinoma is the most common gynecologic cancer in developed countries<sup>(1, 2)</sup> and the third most common cancer in Thailand<sup>(3)</sup>. There are two types of endometrial carcinoma; endometrioid and non-endometrioid according to the Bokhman classification<sup>(4)</sup>. The endometrioid type accounts for 80% of endometrial carcinoma. In 1987, Gynecologic Oncology Group (GOG) 33 described the usefulness of surgical staging in patients with stage I clinical endometrial carcinoma<sup>(5)</sup>. In 1988, the International Federation of Gynecology and Obstetrics (FIGO) developed surgical staging for endometrial cancer instead of clinical staging. The surgical staging procedures include total hysterectomy, bilateral salpingo-oophorectomy, retroperitoneal pelvic, and paraaortic lymphadenectomy<sup>(6)</sup>. Although systematic lymphadenectomy is part of the surgical staging of endometrioid endometrial carcinoma, it is not performed universally. Patients with endometrial carcinoma are usually old, obese and have multiple comorbidities, for example, diabetes mellitus and hypertension. Furthermore, several studies report an increase in perioperative morbidity in lymphadenectomy patients, including increased blood loss, an increased risk of blood or blood component transfusion, an increased operative time, a longer hospital stay, surgical wound infection, lymphedema, and lymphocyst formation<sup>(7-10)</sup>. The survival benefits of lymphadenectomy are controversial. Two prospective randomized trials in patients with early endometrial carcinoma did not find therapeutic benefits for pelvic lymphadenectomy<sup>(11, 12)</sup>. However, several retrospective studies showed a survival benefit in patients with intermediate or high-risk endometrial carcinoma who underwent systematic

pelvic and paraaortic lymphadenectomy<sup>(13-15)</sup>. This study was designed to compare the survival benefit of pelvic lymph node evaluation with pelvic and paraaortic lymph node evaluation.

## Materials and Methods

### Study population

This retrospective cohort study was carried out at the Department of Obstetrics and Gynaecology, Faculty of Medicine Siriraj Hospital, Mahidol University. After institutional ethics board approval was obtained (CoA no.SI 025/2018 and 178/2023, the presence of two separate CoA numbers is attributed to our extended and more comprehensive review of the data), medical records were reviewed from January 2006 to December 2015. Inclusion criteria were patients who were diagnosed with endometrioid endometrial carcinoma and underwent surgical staging at Siriraj Hospital between January 2006 and December 2015. All patients must undergo evaluation of the pelvic lymph node with or without paraaortic lymph node. Patients with a history of other cancers within the previous 5 years or who had previously received chemotherapy or radiation therapy were excluded. Informed consent was not applicable due to retrospective design study.

Dissection, sampling or debulking of enlarged lymph nodes in internal iliac nodes, external iliac nodes, obturator nodes and common iliac nodes was defined as evaluation of pelvic lymph nodes. Evaluation of paraaortic lymph nodes was defined as dissecting, sampling or debulking of enlarged lymph nodes in the precaval, laterocaval, interaortocaval, preaortic, and lateroaortic areas up to the renal veins. Patients with high-risk features as MAYO criteria<sup>(16)</sup> underwent pelvic lymphadenectomy. Systematic/para-aortic

lymphadenectomy was performed at surgeon's discretion due to patients' characteristics. When a patient underwent an evaluation of the lymph nodes in both areas, we defined it as an evaluation of the pelvic and paraaortic lymph nodes. At our institution, preoperative imaging was not routinely performed as a guide for lymphadenectomy.

Adjuvant treatment is determined by the stage of the disease. In the early stage, patients undergo observation, brachytherapy, or whole pelvic radiation, depending on uterine risk factors. In advanced stages, patients receive six cycles or more of chemotherapy alone or in combination with whole pelvic radiation. For patients with paraaortic lymph node metastasis, extended field paraaortic radiation is administered.

Patient data were reviewed, including age, weight, height, body mass index, complete blood count, surgical approach, operative time, blood loss, tumor size, myometrial invasion, cervical involvement, ovarian or adnexal involvement, peritoneal washing for cytology, pelvic and paraaortic lymph node involvement, lymphovascular space invasion (LVSI), adjuvant postoperative treatment, and follow-up status. Patients were also divided into subgroup according to the European Society of Gynaecological Oncology (ESGO)/ European Society for Radiotherapy and Oncology (ESTRO)/ European Society of Pathology (ESP) guidelines<sup>(17)</sup> classified as low, intermediate, high-intermediate and high-risk groups.

"Recurrence-free survival" (RFS) was defined as the time from the diagnosis until the detection of recurrence or the latest follow-up. "Disease-specific survival" was defined as the time from diagnosis to death caused by endometrial carcinoma or the latest follow-up. "Overall survival" was defined as the time from the diagnosis to death of any causes or the last follow-up.

The primary outcome was to compare overall survival (OS) between patients in the pelvic lymph node (PLN) evaluation group and the pelvic with paraaortic lymph node (PPALN) evaluation group. Secondary outcomes were to evaluate recurrence-free survival (RFS) between the two groups.

## **Statistical analysis**

The sample size was calculated to compare 5-year OS according to the SEPAL study<sup>(15)</sup>. The 5-year OS in the pelvic lymph node evaluation groups and in the pelvic and paraaortic lymph nodes was 74.5% and 85.8%, respectively. We defined type I error rate ( $\alpha$ ) of 5% and power of test of 80%, the sample size by using nQuery Advisor was at least 199 patients in each group, which required 74 events of death. For the multivariable Cox regression model with 10 prognostic factors, the sample size was calculated according to the general rule of thumb. We defined events per variable (EPV) as 50 and then the required sample size was at least  $100+50(10) = 600$  patients.

Statistical analysis was performed using PASW (SPSS) version 18 (SPSS Inc., Chicago, IL, USA). Categorical data were reported as numbers and percentage, while mean and standard deviation (SD) or median and range (minimum, maximum) were used for continuous data, as appropriate. Independent t-test or Mann-Whitney U test was performed to compare mean or median between groups for continuous data. The Pearson chi-square test was used to compare the proportion between groups for categorical data. The Kaplan-Meier method was used to estimate survival time and survival rate, and the log-rank test was used to compare survival time between groups for the overall analysis and the subgroup analysis, classified as low, intermediate, high-intermediate and high-risk groups according to the ESGO / ESTRO / ESP guidelines<sup>(17)</sup>. Simple and multiple Cox regression analysis was performed to identify prognostic factors of survival and recurrence. The hazard ratio (HR) with the corresponding 95% confidence interval (95%CI) was used to determine the strength and direction of their association. The p value < 0.05 was considered statistically significant.

## **Results**

### **Patient Characteristics**

From January 2006 to December 2015, 1,300 medical records were reviewed, and 636 patients were included in this study. Two hundred and fifty-seven patients underwent only pelvic lymph node

evaluation, while 379 patients underwent pelvic with paraaortic lymph node evaluation. A comparison of

clinical and pathological characteristics is shown in Table 1.

**Table 1.** Comparison of clinical and pathological characteristics by extension of lymph node evaluation in 636 patients with endometrioid endometrial cancer.

	PLN evaluation (n = 257)	PPALN evaluation (n = 379)	p value
Age (years), mean ± SD	56.9 ± 10.9	57.7 ± 10.0	0.352
Body mass index (kg/m <sup>2</sup> ), mean ± SD	28.1 ± 6.5	26.1 ± 4.9	< 0.001
FIGO 2009 staging, n (%)			0.004
IA	159 (61.9)	204 (53.8)	
IB	40 (15.6)	72 (19.0)	
II	16 (6.2)	43 (11.3)	
IIIA	13 (5.1)	16 (4.2)	
IIIB	1 (0.4)	4 (1.1)	
IIIC1	23 (8.9)	21 (5.6)	
IIIC2	0 (0)	14 (3.7)	
IVB	5 (1.9)	5 (1.3)	
Histological grade, n (%)			0.005
Grade 1	134 (52.1)	151 (39.9)	
Grade 2	101 (39.3)	176 (46.4)	
Grade 3	22 (8.6)	52 (13.7)	
Risk groups			0.142
Low risk (LR)	144 (56.0)	179 (47.2)	
Intermediate risk (IR)	31 (12.1)	57 (15.1)	
High intermediate risk (HIR)	40 (15.6)	83 (21.9)	

	PLN evaluation (n = 257)	PPALN evaluation (n = 379)	p value
High risk (HR)	37 (14.4)	55 (14.5)	
Advanced/metastatic	5 (1.9)	5 (1.3)	
Surgical operation, n (%)			0.161
Laparotomy	234 (92.9)	335 (89.6)	
Laparoscopy	18 (7.1)	39 (10.4)	
Number of lymph nodes, median (min-max)			
Pelvic nodes	12 (2-61)	17 (2-72)	< 0.001
Paraaortic nodes	N/A	3 (1-21)	N/A
Operative time (minutes), median (min-max)	165 (60-355)	185 (60-400)	< 0.001
Estimate blood loss (mL), median (min-max)	200 (5-2000)	250 (10-2100)	0.599
Adjuvant treatment, n (%)		(n=378) <sup>†</sup>	0.006
None	133 (51.8)	143 (37.8)	
Chemotherapy	18 (7.0)	35 (9.3)	
Radiation	91 (35.4)	177 (46.8)	
Chemotherapy and radiation	15 (5.8)	23 (6.1)	

PPALN: Pelvic and paraaortic lymph node, PLN: Pelvic lymph node, FIGO: International Federation of Obstetricians and Gynecologists, SD: standard deviation

<sup>†</sup> No information of one patient who had adjuvant treatment at the other hospital

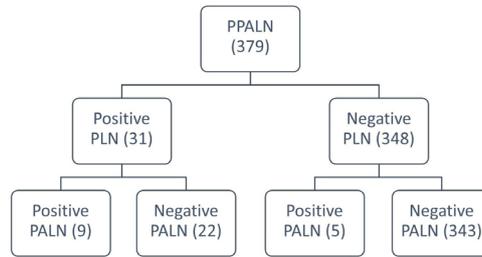
The median number of pelvic lymph node was 12 (2-61) in the PLN group and 17 (2-72) in the PPALN group. The median number of paraaortic lymph nodes was 3 (1-21). In the PLN group, pelvic lymph nodes were positive in 27 of 257 cases (10.5%). In the PPALN group, the isolated positive paraaortic lymph node was 1.3% (5/379), while 2.4% (9/379) had positivity for both the pelvic lymph node and the paraaortic lymph node and 8.2% (31/379) had positivity for the pelvic lymph node, as shown in Fig. 1. There were 57 cases (9%) that underwent laparoscopic surgery (18 in the PLN group and 39 in the PPALN group). The median number of pelvic lymph nodes and paraaortic lymph nodes in laparoscopic surgery was not different from that in laparotomy. In the PPALN group, operative time was

significantly longer than in the PLN group ( $p < 0.001$ ) but estimated blood loss was not different in both groups.

## Treatment outcomes: OS, DSS, RFS

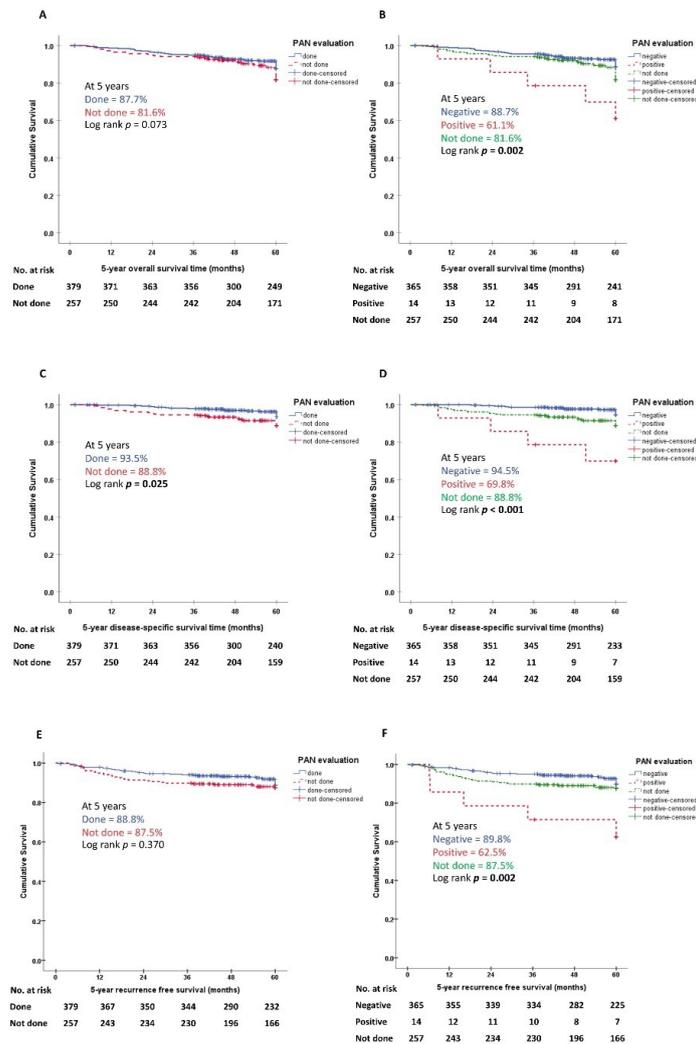
### Overall survival (OS)

The median follow-up time was 60 months (1.3-60.0). Forty-one patients (16%) died in the PLN group and 41 patients (10.8%) died in the PPALN group. The 5-year OS rate in the PLN and the PPALN groups were 81.6% and 87.7%, respectively ( $p = 0.073$ ) as shown in Fig. 2A. However, the PLN group had increased risk of 5-year death comparing to the PPALN group after adjustment for other prognostic factors (adjusted HR 1.63 (1.06-2.52,  $p = 0.028$ )) as shown in Table 2.



**Fig. 1.** Results from the pelvic and paraaortic lymph node evaluation groups.

\* PPALN: pelvic and paraaortic lymph node, PLN: pelvic lymph node, PALN: paraaortic lymph node



**Fig. 2.** Kaplan-Meier analysis of (A) 5-year OS of PLN and PPALN evaluation, (B) 5-year OS of PLN evaluation, PPALN evaluation with negative and positive PALN, (C) 5-year DSS of PLN and PPALN evaluation, (D) 5-year DSS of PLN evaluation, PPALN evaluation with negative and positive PALN, (E) 5-year RFS of PLN and PPALN evaluation, (F) 5-year RFS of PLN evaluation, PPALN evaluation with negative and positive PALN.

\* PPALN: pelvic and paraaortic lymph node, PLN: pelvic lymph node, PALN: paraaortic lymph node

**Table 2.** Prognostic factors for 5-year overall survival (OS) in 636 patients with endometrioid endometrial cancer patients.

Prognostic factors	5-year overall survival (OS)			
	Univariable		Multivariable	
	Unadjusted HR (95%CI)	p value	Adjusted HR <sup>†</sup> (95%CI)	p value
Age		0.002		0.003
< 55 years	Reference		Reference	
≥ 55 years	2.19 (1.32-3.62)		2.21 (1.32-3.70)	
Body mass index	0.99 (0.96-1.04)	0.970	-	-
Hemoglobin		0.053		0.042
≥ 13 g/dL	Reference		Reference	
< 13 g/dL	1.60 (0.99-2.57)		1.64 (1.02-2.65)	
Platelet	1.00 (1.00-1.00)	0.113	-	-
Tumor size		0.012		0.422
< 3 cm	Reference		Reference	
≥ 3 cm	2.09 (1.18-3.71)		1.30 (0.69-2.44)	
Risk groups		< 0.001		< 0.001
Low risk (LR)	Reference		Reference	
Intermediate risk (IR)	3.54 (1.81-6.94)	0.001	3.21 (1.63-6.32)	0.001
High intermediate risk (HIR)	3.51 (1.88-6.55)	< 0.001	3.26 (1.74-6.10)	< 0.001
High risk (HR)	4.79 (2.57-8.93)	< 0.001	4.89 (2.62-9.14)	< 0.001
Advanced/metastatic	9.25 (3.13-27.38)	< 0.001	10.35 (3.47-30.90)	< 0.001
Peritoneal washing for cytology		0.014		0.113
Negative	Reference		Reference	
Positive	2.29 (1.18-4.44)		1.73 (0.88-3.39)	
Number of pelvic lymph nodes	1.00 (0.97-1.02)	0.733	-	-
Lymph node evaluation		0.077		0.028
Pelvic and paraaortic lymph node (PPALN)	Reference		Reference	
Pelvic lymph node (PLN)	1.48 (0.96-2.28)		1.63 (1.06-2.52)	
Adjuvant treatment		0.001		0.387
No	Reference		Reference	
Yes	2.23 (1.37-3.64)		0.75 (0.39-1.44)	

<sup>†</sup> Analysis adjusted for all prognostic factors with p-value < 0.10 in univariable analysis with the main factor  
HR: hazard ratio, CI: confidence interval

In the PPALN group, 365 patients had a negative paraaortic lymph node (36 deaths), and 14 patients had a positive paraaortic lymph node (5 deaths). As shown in Fig. 2B, the 5-year OS rate in the PLN group, the negative paraaortic lymph node group and the positive paraaortic lymph node group were 81.6%, 88.7% and 61.1%, respectively (p = 0.002). Patients in the negative paraaortic lymph node group had statistically significantly longer survival than patients in the positive paraaortic lymph node group (p = 0.001) and the PLN group (p =

0.030).

### **Disease-specific survival (DSS)**

DSS was better for patients who underwent evaluation of the pelvic and paraaortic lymph nodes compared to patients who received evaluation of only the pelvic lymph nodes. The 5-year DSS rate was 88.8% in the PLN group and 93.5% in the PPALN group (p = 0.025) as shown in Fig. 2C. The PLN group had increased risk of 5-year disease specific death comparing to the PPALN

group after adjustment for other prognostic factors (adjusted HR 2.11 (1.17-3.79,  $p = 0.0213$ )) as shown in Table 3. As shown in Fig. 2D, the 5-year DSS rate in the PLN group, the negative paraaortic lymph node group, and the positive paraaortic lymph node group were

88.8%, 94.5%, and 69.8%, respectively ( $p < 0.001$ ). Patients with a negative paraaortic lymph node survived statistically significantly longer than patients with a positive paraaortic lymph node ( $p < 0.001$ ) and the PLN group ( $p = 0.006$ ).

**Table 3.** Prognostic factors for 5-year disease specific survival (DSS) in 636 patients with endometrioid endometrial cancer patients.

Prognostic factors	5-year disease specific survival (DSS)			
	Univariable		Multivariable	
	Unadjusted HR (95%CI)	p value	Adjusted HR <sup>†</sup> (95%CI)	p value
Age		0.079		0.047
< 55 years	Reference		Reference	
≥ 55 years	1.78 (0.94-3.38)		1.97 (1.01-3.83)	
Body mass index	1.00 (0.95-1.05)	0.889	-	-
Hemoglobin		0.194	-	-
≥ 13 g/dL	Reference			
< 13 g/dL	0.66 (0.35-1.24)			
Platelet	1.00 (1.00-1.00)	0.358	-	-
Tumor size		0.017		0.484
< 3 cm	Reference		Reference	
≥ 3 cm	2.85 (1.21-6.72)		1.39 (0.55-3.53)	
Risk groups		< 0.001		< 0.001
Low risk (LR)	Reference		Reference	
Intermediate risk (IR)	5.09 (1.89-13.66)	0.001	4.79 (1.77-12.91)	0.002
High intermediate risk (HIR)	3.69 (1.37-9.90)	0.010	3.46 (1.28-9.36)	0.014
High risk (HR)	9.45 (3.92-22.80)	< 0.001	9.37 (3.86-22.74)	< 0.001
Advanced/metastatic	22.94 (6.70-78.53)	< 0.001	23.56 (6.72-82.57)	< 0.001
Peritoneal washing for cytology		0.008		0.06
Negative	Reference		Reference	
Positive	2.95 (1.32-6.59)		2.19 (0.97-4.96)	
Number of pelvic lymph nodes	0.99 (0.96-1.02)	0.445	-	-
Lymph node evaluation		0.028		0.013
Pelvic and paraaortic lymph node (PPALN)	Reference		Reference	
Pelvic lymph node (PLN)	1.92 (1.07-3.44)		2.11 (1.17-3.79)	
Adjuvant treatment		0.001		0.810
No	Reference		Reference	
Yes	3.87 (1.80-8.23)		1.13 (0.42-3.07)	

<sup>†</sup> Analysis adjusted for all prognostic factors with p-value < 0.10 in univariable analysis with the main factor

HR: hazard ratio, CI: confidence interval

### Recurrent free survival (RFS)

Thirty-one patients (12.1%) had recurrence in the PLN group, and 37 patients (9.8%) recurred in the PPALN group. The 5-year RFS rate in the PLN

and PPALN groups was 87.5% and 88.8%, respectively ( $p = 0.370$ ) as shown in Fig. 2E. The PPALN group had still no different in recurrent comparing to the PLN group after adjustment for

other prognostic factors (adjusted HR 1.39 (0.86-2.25,  $p = 0.183$ )) as shown in Table 4.

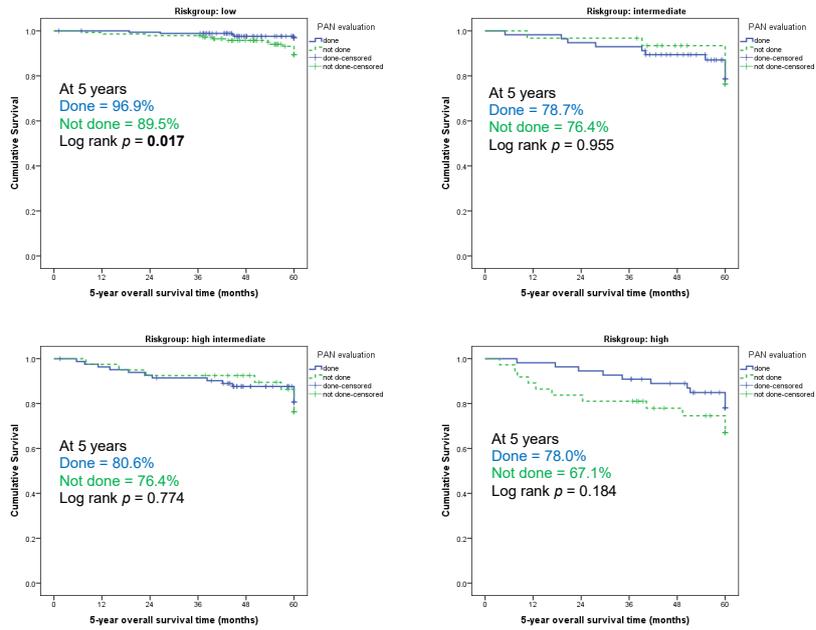
In the PPALN group, 365 patients had negative paraaortic lymph nodes (32 recurrences) and 14 patients had positive paraaortic lymph nodes (5 recurrences). As shown in Fig. 2F, the 5-year RFS rate in the PLN group, the negative paraaortic lymph

node group and the positive paraaortic lymph node group were 87.5%, 89.8% and 62.5%, respectively ( $p = 0.002$ ). Patients with a negative paraaortic lymph node had statistically significantly longer survival than patients with a positive paraaortic lymph node ( $p < 0.001$ ) but similar to the PLN group ( $p = 0.187$ ).

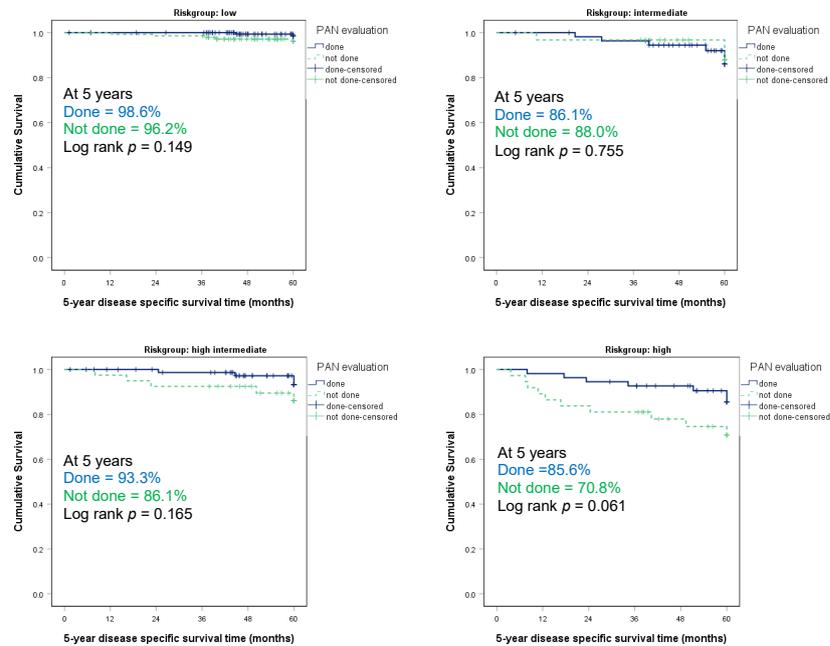
**Table 4.** Prognostic factors for 5-year recurrence free survival (RFS) in 636 patients with endometrioid endometrial cancer patients.

Prognostic factors	5-year recurrence-free survival (RFS)			
	Univariable		Multivariable	
	Unadjusted HR (95%CI)	p value	Adjusted HR† (95%CI)	p value
Age		0.098		0.065
< 55 years	Reference		Reference	
≥ 55 years	1.55 (0.92-2.59)		1.65 (0.97-2.79)	
Body mass index	1.00 (0.96-1.05)	0.920	-	-
Hemoglobin		0.616	-	-
≥ 13 g/dL	Reference			
< 13 g/dL	0.88 (0.54-1.44)			
Platelet	1.00 (1.00-1.00)	0.231	-	-
Tumor size		0.004		0.208
< 3 cm	Reference		Reference	
≥ 3 cm	2.78 (1.38-5.60)		1.62 (0.77-3.43)	
Risk groups		< 0.001		< 0.001
Low risk (LR)	Reference		Reference	
Intermediate risk (IR)	2.20 (0.97-4.97)	0.059	2.03 (0.89-4.61)	0.091
High intermediate risk (HIR)	2.85 (1.42-5.69)	0.003	2.66 (1.32-5.33)	0.006
High risk (HR)	5.48 (2.88-10.44)	< 0.001	5.55 (2.91-10.57)	< 0.001
Advanced/metastatic	13.43 (4.91-36.78)	< 0.001	15.34 (5.55-42.42)	< 0.001
Peritoneal washing for cytology		0.118	-	-
Negative	Reference			
Positive	1.87 (0.85-4.08)			
Number of pelvic lymph nodes	1.00 (0.98-1.03)	0.968		-
Lymph node evaluation		0.371		0.183
Pelvic and paraaortic lymph node (PPALN)	Reference		Reference	
Pelvic lymph node (PLN)	1.24 (0.77-2.00)		1.39 (0.86-2.25)	
Adjuvant treatment		0.003		0.382
No	Reference		Reference	
Yes	2.26 (1.32-3.88)		0.72 (0.34-1.51)	

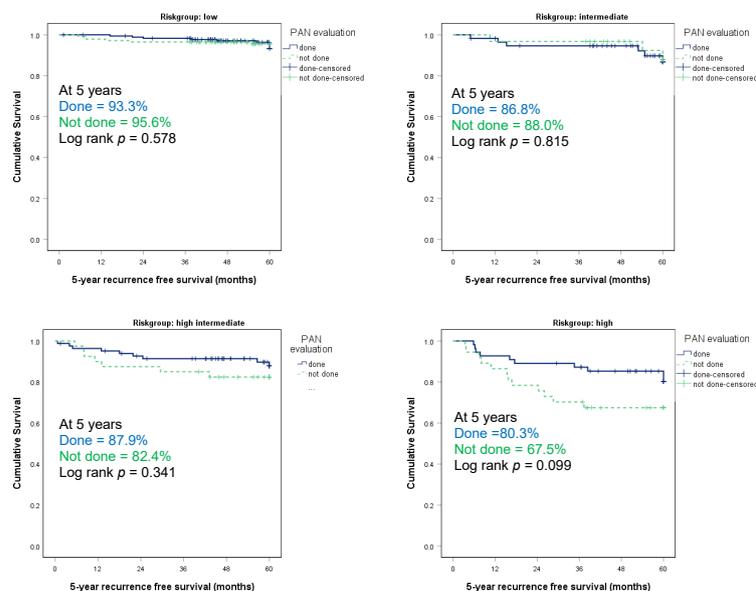
† Analysis adjusted for all prognostic factors with p-value < 0.10 in univariable analysis with the main factor  
HR: hazard ratio, CI: confidence interval



**Fig. 3.** Kaplan-Meier analysis of 5-year OS of PLN and PPALN evaluation stratified by ESGO/ESTRO/ESP risk groups (A) Low risk (B) Intermediate risk (C) High-intermediate risk (D) High risk



**Fig. 4.** Kaplan-Meier analysis of 5-year DSS of PLN and PPALN evaluation stratified by ESGO/ESTRO/ESP risk groups (A) Low risk (B) Intermediate risk (C) High-intermediate risk (D) High risk



**Fig. 5.** Kaplan-Meier analysis of 5-year RFS of PLN and PPALN evaluation stratified by ESGO/ESTRO/ESP risk groups (A) Low risk (B) Intermediate risk (C) High-intermediate risk (D) High risk

### Risk group analysis

We performed risk group analyses according to the ESGO / ESTRO / ESP guidelines<sup>(17)</sup>. For low-risk patients, the 5-year OS was higher for patients who received PPALN than the patients who received PLN evaluation (96.9% vs. 89.5%,  $p = 0.017$ ). However, there was no benefit of PPALN evaluation for 5-year OS in other groups. (Fig. 3) There was no difference in 5-year DSS and 5-year RFS between PPALN and PLN evaluation for all risk groups. However, there was a trend to improve 5-year DSS (85.6% vs 70.8%,  $p = 0.061$ ) and 5-year RFS (80.3% vs. 67.5%,  $p = 0.099$ ) in high-risk patients who received PPALN evaluation as shown in supplementary Fig. S2D and S3D. All Kaplan-Meier graph of risk group analyses were provided in the supplementary materials (Fig. 4, 5).

### Prognostic factors for 5-year OS, 5-year DSS and 5-year RFS

Table 2 shows the prognostic factors for 5-year OS of endometrial carcinoma by Cox regression analysis. The cutoff points for age, hemoglobin, and

tumor size were calculated based on the largest area under the receiver operating characteristic curve (ROC) curve (not demonstrated). In the univariable analysis, age  $\geq 55$  years, tumor size  $\geq 3$  cm, risk groups, positive peritoneal washing, and adjuvant treatment were statistically significant prognostic factors for 5-year OS. In multivariable analysis, age  $\geq 55$  years (adjusted HR 2.21 (1.32-3.70,  $p = 0.003$ )), hemoglobin  $< 13$  g/dL (adjusted HR 1.64 (1.02-2.65,  $p = 0.0422$ )), and risk groups ( $p < 0.001$ ) were independent negative prognostic factors for 5-year OS.

For secondary outcomes, the univariable analysis of 5-year RFS revealed that tumor size  $\geq 3$  cm, risk groups, and adjuvant treatment were significant risk factors. Only risk group ( $p < 0.001$ ) was significant risk factors for 5-year RFS in the multivariable analysis (Table 4). All cox regression analysis of 5-year OS, DSS, and RFS was provided in Table 2-4.

The data of Cox regression analysis for 5-year OS, DSS, and RFS using individual 15 risk factors were demonstrated in supplementary Table 5-7.

**Table 5.** Prognostic factors for 5-year overall survival (OS) in 636 patients with endometrioid endometrial cancer.

Prognostic factors	5-year overall survival (OS)			
	Univariable		Multivariable	
	Unadjusted HR (95%CI)	p value	Adjusted HR <sup>†</sup> (95%CI)	p value
Age		0.002		0.005
< 55 years	Reference		Reference	
≥ 55 years	2.19 (1.32-3.62)		2.10 (1.25-3.52)	
Body mass index	0.99 (0.96-1.04)	0.970	-	-
Hemoglobin		0.053		0.128
≥ 13 g/dL	Reference		Reference	
< 13 g/dL	1.60 (0.99-2.57)		1.45 (0.90-2.35)	
Platelet	1.00 (1.00-1.00)	0.113	-	-
Histological grade		< 0.001		0.001
Grades 1 and 2	Reference		Reference	
Grade 3	2.77 (1.67-4.58)		2.37 (1.41-3.96)	
Tumor size		0.012		0.06
< 3 cm	Reference		Reference	
≥ 3 cm	2.09 (1.18-3.71)		1.19 (0.62-2.30)	
Myometrial invasion		< 0.001		<0.001
< 50%	Reference		Reference	
≥ 50%	3.99 (2.55-6.24)		2.77 (1.71-4.46)	
Cervical involvement		< 0.001		0.019
Negative	Reference		Reference	
Positive	2.42 (1.51-3.90)		1.83 (1.11-3.02)	
Adnexal involvement		0.541	-	-
Negative	Reference			
Positive	1.30 (0.56-2.98)			
Peritoneal washing for cytology		0.014		0.214
Negative	Reference		Reference	
Positive	2.29 (1.18-4.44)		1.56 (0.78-3.13)	
Pelvic lymph node metastasis		< 0.001		0.005
Negative	Reference		Reference	
Positive	3.63 (2.17-6.06)		2.19 (1.27-3.76)	
Number of pelvic lymph nodes	1.00 (0.97-1.02)	0.733	-	-
Lymph node evaluation		0.077		0.004
Pelvic and paraaortic lymph node (PPALN)	Reference		Reference	
Pelvic lymph node (PLN)	1.48 (0.96-2.28)		1.94 (1.23-3.05)	
Adjuvant treatment		0.001		0.358
No	Reference		Reference	
Yes	2.23 (1.37-3.64)		0.75 (0.40-1.40)	
Lymphovascular space invasion (LVSI)		< 0.001		0.383
No	Reference		Reference	
Yes	2.56 (1.61-4.08)		1.25 (0.76-2.07)	

<sup>†</sup> Analysis adjusted for all prognostic factors with p-value < 0.10 in univariable analysis with the main factor  
HR = hazard ratio

**Table 6.** Prognostic factors for 5-year disease specific survival (DSS) in 636 endometrioid endometrial cancer patients.

Prognostic factors	5-year disease specific survival (DSS)			
	Univariable		Multivariable	
	Unadjusted HR (95%CI)	p value	Adjusted HR† (95%CI)	p value
Age		0.079		0.109
< 55 years	Reference		Reference	
≥ 55 years	1.78 (0.94-3.38)		1.72 (0.89-3.34)	
Body mass index	1.00 (0.95-1.05)	0.889	-	-
Hemoglobin		0.194	-	-
≥ 13 g/dL	Reference			
< 13 g/dL	0.66 (0.35-1.24)			
Platelet	1.00 (1.00-1.00)	0.358	-	-
Histological grade		0.016		0.079
Grades 1 and 2	Reference		Reference	
Grade 3	2.36 (1.17-4.76)		1.90 (0.93-3.88)	
Tumor size		0.017		0.968
< 3 cm	Reference		Reference	
≥ 3 cm	2.85 (1.21-6.72)		1.02 (0.38-2.76)	
Myometrial invasion		<0.001		< 0.001
< 50%	Reference		Reference	
≥ 50%	7.66 (3.89-15.09)		5.03 (2.46-10.3)	
Cervical involvement		<0.001		0.007
Negative	Reference		Reference	
Positive	3.42 (1.88-6.22)		2.39 (1.26-4.54)	
Adnexal involvement		0.140	-	-
Negative	Reference			
Positive	2.01 (0.80-5.09)			
Peritoneal washing for cytology		0.008		0.318
Negative	Reference		Reference	
Positive	2.95 (1.32-6.59)		1.54 (0.66-3.59)	
Pelvic lymph node metastasis		<0.001		0.001
Negative	Reference		Reference	
Positive	6.33 (3.45-11.62)		3.08 (1.61-5.88)	
Number of pelvic lymph nodes	0.99 (0.96-1.02)	0.445	-	-
Lymph node evaluation		0.028		0.001
Pelvic and paraaortic lymph node (PPALN)	Reference		Reference	
Pelvic lymph node (PLN)	1.92 (1.07-3.44)		2.76 (1.49-5.10)	
Adjuvant treatment		0.001		0.889
No	Reference		Reference	
Yes	3.87 (1.80-8.23)		0.94 (0.37-2.36)	
Lymphovascular space invasion (LVSI)		0.001		0.566
No	Reference		Reference	
Yes	2.93 (1.60-5.38)		1.21 (0.63-2.30)	

† Analysis adjusted for all prognostic factors with p-value < 0.10 in univariable analysis with the main factor  
HR = hazard ratio

**Table 7.** Prognostic factors for 5-year recurrence free survival (RFS) in 636 endometrioid endometrial cancer patients

Prognostic factors	5-year recurrence-free survival (RFS)			
	Univariable		Multivariable	
	Unadjusted HR (95%CI)	p value	Adjusted HR† (95%CI)	p value
Age		0.098		0.200
< 55 years	Reference		Reference	
≥ 55 years	1.55 (0.92-2.59)		1.41 (0.83-2.59)	
Body mass index	1.00 (0.96-1.05)	0.920	-	-
Hemoglobin		0.616	-	-
≥ 13 g/dL	Reference			
< 13 g/dL	0.88 (0.54-1.44)			
Platelet	1.00 (1.00-1.00)	0.231	-	-
Histological grade		0.026		0.153
Grades 1 and 2	Reference		Reference	
Grade 3	1.99 (1.09-3.64)		1.57 (0.85-2.92)	
Tumor size		0.004		0.232
< 3 cm	Reference		Reference	
≥ 3 cm	2.78 (1.38-5.60)		1.59 (0.74-3.40)	
Myometrial invasion		< 0.001		<0.001
< 50%	Reference		Reference	
≥ 50%	3.91 (2.40-6.38)		2.97 (1.76-5.00)	
Cervical involvement		< 0.001		0.028
Negative	Reference		Reference	
Positive	2.78 (1.68-4.63)		1.81 (1.07-3.08)	
Adnexal involvement		0.020		0.263
Negative	Reference		Reference	
Positive	2.40 (1.15-5.01)		1.55 (0.72-3.35)	
Peritoneal washing for cytology		0.118	-	-
Negative	Reference			
Positive	1.87 (0.85-4.08)			
Pelvic lymph node metastasis		< 0.001		0.08
Negative	Reference		Reference	
Positive	3.87 (2.23-6.70)		2.20 (1.23-3.95)	
Number of pelvic lymph nodes	1.00 (0.98-1.03)	0.968	-	-
Lymph node evaluation		0.371		0.103
Pelvic and paraaortic lymph node (PPALN)	Reference		Reference	
Pelvic lymph node (PLN)	1.24 (0.77-2.00)		1.50 (0.92-2.44)	
Adjuvant treatment		0.003		0.313
No	Reference		Reference	
Yes	2.26 (1.32-3.88)		0.70 (0.35-1.4)	
Lymphovascular space invasion (LVSI)		0.006		0.829
No	Reference		Reference	
Yes	2.09 (1.23-3.55)		0.94 (0.52-1.69)	

† Analysis adjusted for all prognostic factors with p-value < 0.10 in univariable analysis with the main factor  
HR = hazard ratio

## Discussion

Currently, the primary treatment for endometrial carcinoma is surgical staging, including lymphadenectomy. However, the extent and benefit of lymphadenectomy, especially in the paraaortic area, remains controversial. Our study showed that 5-year DSS was significantly better for patients who received evaluation of the pelvic with paraaortic lymph nodes compared to patients who underwent evaluation of only the pelvic lymph nodes. Additionally, PLN evaluation alone was also an independent risk factor for 5-year OS.

The characteristics of the patients were different due to the retrospective nature of the study. Patients in the PPALN group have a lower body mass index and higher grade of disease, suggesting a selection bias for surgeons to consider evaluation of pelvic with paraaortic lymph nodes which might impacts on outcomes.

The median number of pelvic lymph nodes was comparable to those in the other studies. Previous studies reported that the median number of evaluated pelvic lymph nodes was 10-19 lymph nodes<sup>(11-14, 18-20)</sup>. These numbers were comparable to our PLN group (12 lymph nodes). The median dissected pelvic lymph nodes of the PPALN groups in our study were higher than those of the other studies, at 17 lymph nodes. The median number of paraaortic lymph nodes has rarely been reported. The median number of paraaortic lymph node removals reported by May et al was 10 lymph nodes<sup>(19)</sup>. In our study, only 7.9% of the patients in the PPALN group had removed equal or more than 10 paraaortic lymph nodes. The median number of paraaortic lymph nodes removed in our study was 3 lymph nodes, since most patients received only a sampling of paraaortic lymph nodes. The SEPAL study demonstrated a survival benefit from pelvic and paraaortic lymph node dissection with a median removal of 23 paraaortic lymph nodes<sup>(15)</sup>. Although the median number of paraaortic lymph nodes in our study was less than the number in the SEPAL study, we measured a benefit from 5-year DSS and 5-year OS with the evaluation of both pelvic and

paraaortic lymph nodes. We found an isolated paraaortic lymph node metastasis of 1.3% in our study. This was comparable to other investigators who reported a low rate of paraaortic lymph node metastasis (1.2-5%) with or without pelvic lymph node metastases<sup>(18, 19)</sup>.

This study confirms the benefit of evaluation of both the pelvic and paraaortic lymph nodes. The positive paraaortic lymph node group showed the worst 5-year DSS outcomes compared to the other groups. Apparently, the negative paraaortic lymph node group had the best 5-year DSS among the groups. For overall survival, there was a significantly improved 5-year OS in patients who underwent pelvic with paraaortic lymph nodes compared to pelvic lymph nodes alone. Similarly, several studies found the survival benefit of paraaortic lymph node dissection<sup>(13-15)</sup>. This may be explained by more accurate staging and tumor debulking with paraaortic lymph node dissection. Consequently, the patient will receive more appropriate adjuvant treatments. In the secondary outcome, the RFS showed the same trend of nonsignificant improvement in the PPALN group. However, some studies showed that paraaortic lymph node dissection did not improve endometrial carcinoma survival<sup>(19, 21, 22)</sup>. This question will require future prospective studies to answer.

Improvement in 5-year OS and 5-year RFS may be confounded by many factors. Multivariable Cox regression analysis confirmed that extension of lymph node evaluation is an independent prognostic factor. This finding confirms another benefit of removal of paraaortic lymph nodes. Other factors, including age  $\geq 55$  years, hemoglobin  $< 13$  g/dL, and risk groups ( $p < 0.001$ ), are also independent factors in the multivariate analysis. Similarly, age  $\geq 55$  years, tumor size  $\geq 3$  cm, deep myometrial invasion, histological grade 3, and cervical involvement have been identified as prognostic factors for the survival of endometrial carcinoma<sup>(5, 23)</sup>. Risk groups are the only one prognostic factor for RFS. Chang et al also reported that the FIGO 2009 stage is an independent prognostic factor. They also reported that age and

extension of lymph node evaluation were prognostic factors for recurrence<sup>(18)</sup>. Different prognostic factors can be caused by different histological types. They included all histological subtypes, including endometrioid adenocarcinoma, papillary serous, and clear cell carcinoma.

We also performed a risk group analysis as the latest recommendation from ESGO / ESTRO / ESP guidelines<sup>(17)</sup>. There was no statistically significant difference in 5-year RFS between PPALN and the PLN group in all subgroups, but the data revealed the most relative reduction in the high-risk. For 5-year OS, patients in the low-risk subgroup who received PPALN evaluation were significantly better compared to only the PLN evaluation. This result was different from previous knowledge, which lymph node evaluation should be done in the intermediate and high-risk patients.

There might be bias in selecting healthy patients to undergo PPALN evaluation, resulting in better survival for these patients. In contrast, patients with co-morbidities may not be suitable for PPALN evaluation. These may explain the lack of difference in 5-year RFS and 5-year DSS between the PPALN evaluation and PLN evaluation in low-risk patients.

Our data demonstrated the most benefit from PPALN evaluation in the high-risk group. Given the small number of high-risk patients, the results did not reach statistical significance. There was no evidence to support PPALN evaluation in our study for intermediate-risk and high-intermediate-risk patients.

Another essential point of the paraaortic lymphadenectomy was its complications. The complications from PPALN evaluation were usually higher than those from PLN evaluation. Volpi et al reported that the addition of paraaortic lymphadenectomy was an independent predictor for both lymphedema and lymphocele<sup>(24)</sup>. Given the retrospective study design, we were not able to collect the complications data. We reported a non-significant difference in blood losses between the two procedural groups.

The strength of this study was the large sample size and the long follow-up time. This study reported almost 400 cases of evaluation of pelvic and paraaortic lymph nodes. However, there were several limitations due to the retrospective nature of the study. First, there might be some selection bias because a PPALN evaluation group tended to have lower BMI and higher histologic grade than a PLN evaluation group. Second, the pathological lymph node count was low in only median removal of 3 paraaortic lymph nodes. Third, we had a small proportion—only 9%—of laparoscopic approaches, which might be unusual in developing countries. Fourth, there was missing data regarding patients' co-morbidities, which might affect patients' survival. Finally, immunotherapy treatment and molecular profile-based treatment, which have been anticipated in recent years, were not available to us.

In the scope of this study, the utilization of sentinel lymph node sampling was notably absent from our institutional practices during the specified research period. However, in the present landscape, sentinel lymph node mapping plays a pivotal role in the surgical management of endometrioid endometrial carcinoma. The FIRES trial, conducted by Rossi et al, demonstrated a high sensitivity of 97.2% and a high negative predictive value of 99.6%. The study was stopped early according to the efficacy of the procedure. Interestingly, the results showed 17% isolated positive lymph nodes outside the standard dissection area of lymph nodes (internal iliac, presacral area)<sup>(25)</sup>. The recommendation from ESGO / ESTRO / ESP guidelines advocate that sentinel lymph node biopsy is an acceptable alternative to systematic lymphadenectomy for lymph node staging in stage I/II<sup>(17)</sup>. We are in the process of preparing to present a study employing sentinel lymph node techniques at our institution in the near future. However, it is essential to acknowledge that in instances where sentinel lymph node mapping encounters challenges, the necessity for systematic lymphadenectomy remains. Consequently, the potential implications of our study's findings are

substantial, offering invaluable insights into the scope of lymphadenectomy. Subsequent prospective research that compares pelvic lymph node dissection with combined pelvic and paraaortic lymph node dissection in endometrial carcinoma will provide further substantiation for this hypothesis (as demonstrated in the prospective SEPAL-P3 study)<sup>(26)</sup>.

## Conclusion

Our analysis of patients with endometrioid endometrial carcinoma revealed that the pelvic with paraaortic lymph node (PPALN) group exhibited an independent association with significantly prolonged survival when compared to the pelvic lymph node (PLN) group. These findings suggested a potential association between PPALN and improved survival outcomes.

Based on our findings, we suggest that all patients with endometrioid endometrial cancer, especially those in low- to middle-class settings or for whom sentinel lymph node biopsy was not feasible, should have their pelvic and paraaortic lymph nodes evaluated.

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## Potential conflicts of interest

The authors declare no conflicts of interest.

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## GYNAECOLOGY

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# Efficacy of the Si-Washer in Cleansing Gynaecological Laparoscopic Instruments: A randomized controlled trial

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### ABSTRACT

**Objectives:** This study aimed to evaluate the efficacy of the 'Si-Washer' in cleansing gynaecological laparoscopic instruments.

**Materials and Methods:** The instruments were randomized into either a control group or an experimental group using a block randomization method, with sequences securely concealed in sealed envelopes. The control group underwent traditional manual cleansing, whereas the experimental group utilized the Si-Washer.

**Results:** A total of 302 instruments were randomized, with 151 allocated to each group. The median operative duration was 135.0 minutes (interquartile range [IQR] 115.0–175.0), and the median blood loss was 50.0 mL (IQR 20.0–100.0). No significant differences were observed between the groups regarding diagnostic parameters, operator expertise, operation types, operative time, instrument types, surgical complications, irrigation volume or blood loss. The Si-Washer achieved 100% cleanliness at an adenosine triphosphate cut-off of <150 relative light units, markedly surpassing the manual method, which achieved 58.9% cleanliness at the same threshold.

**Conclusion:** The Si-Washer effectively decontaminated gynaecological laparoscopic instruments and alleviating the workload of operating theatre nursing staff.

**Keywords:** cleaning, washer, instrument, laparoscopy, Innovation.

## ประสิทธิภาพของการใช้เครื่องล้าง “the Si-Washer” สำหรับเครื่องมือผ่าตัดผ่านกล้องทางนรีเวช: การทดลองแบบสุ่มที่มีกลุ่มควบคุม

ศิริพร สมจิต, น้ำผึ้ง อินทโพธิ์, สุธาสินี เหลืองรัตนเจริญ, สาริศา ทะปะละ, กชกร ถึงกลาง, จิตรา เกิดเพชร, ไอริน เรืองขจร

### บทคัดย่อ

**วัตถุประสงค์:** การศึกษานี้มีวัตถุประสงค์เพื่อประเมินประสิทธิภาพของ Si-Washer ในการทำความสะอาดเครื่องมือผ่าตัดส่องกล้องทางนรีเวช

**วัสดุและวิธีการ:** เครื่องมือผ่าตัดถูกสุ่มแบ่งเป็นกลุ่มควบคุมหรือกลุ่มทดลองโดยใช้วิธีการสุ่มแบบบล็อก โดยลำดับการสุ่มถูกเก็บรักษาอย่างปลอดภัยในซองจดหมายที่ปิดสนิท กลุ่มควบคุมได้รับการทำความสะอาดด้วยวิธีการทำความสะอาดด้วยมือแบบดั้งเดิม ในขณะที่กลุ่มทดลองใช้ Si-Washer ในการทำความสะอาด

**ผลการศึกษา:** เครื่องมือทั้งหมด 302 ชิ้น ถูกแบ่งออกเป็นกลุ่มละ 151 ชิ้น ค่ากลางของเวลาในการทำผ่าตัดเท่ากับ 135.0 นาที (ช่วงระหว่างควอไทล์ [IQR] 115.0–175.0 นาที) ค่ากลางของการเสียเลือด 50.0 มิลลิลิตร (IQR 20.0–100.0 มิลลิลิตร) ไม่มีความแตกต่างกันระหว่างกลุ่ม ในด้านของโรคที่ได้รับการวินิจฉัย ความเชี่ยวชาญของแพทย์ ประเภทของการผ่าตัด ระยะเวลาการผ่าตัด ประเภทของเครื่องมือ ภาวะแทรกซ้อนจากการผ่าตัด ปริมาณสารน้ำที่ใช้ล้างขณะผ่าตัด หรือการสูญเสียเลือด เครื่องล้าง “Si-Washer” สามารถทำความสะอาดได้ร้อยละ 100 ที่ค่าอเดโนซีนไตรฟอสเฟต (adenosine triphosphate) ต่ำกว่า 150 หน่วยแสงสัมพัทธ์ ซึ่งสูงกว่าวิธีการทำความสะอาดด้วยมือที่ทำได้ร้อยละ 58.9 ที่ค่าเกณฑ์เดียวกันอย่างชัดเจน

**สรุป:** เครื่องล้าง Si-Washer มีประสิทธิภาพในการกำจัดสิ่งปนเปื้อนบนเครื่องมือผ่าตัดส่องกล้องทางนรีเวชและลดภาระงานของพยาบาลในห้องผ่าตัด

**คำสำคัญ:** การทำความสะอาด, เครื่องล้าง, เครื่องมือ, การส่องกล้อง, นวัตกรรม

## Introduction

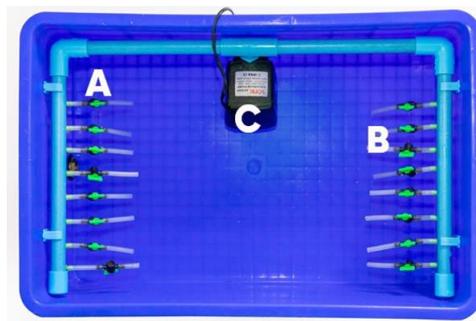
Laparoscopic surgery is increasingly recognized globally. The effective and prompt reprocessing of surgical instruments is vital for infection control, yet the complexity of laparoscopic tools presents significant challenges in achieving cleanliness. Post-cleaning, several rapid methods, including visual inspection, microscopy and adenosine triphosphate (ATP) bioluminescence assays, are utilized to ascertain cleanliness<sup>(1,2)</sup>. The ATP bioluminescence assay, particularly suited for instruments with narrow lumens, deems ATP levels  $\leq 150$  relative light units (RLUs) to be sufficiently clean<sup>(3-6)</sup>.

While manual cleaning is effective, it has certain constraints. Ling et al's study on the cleanliness of various surgical instruments, including hollow suction tubes and long forceps, revealed that 92% of

instruments with no visible contaminants achieved an ATP cleanliness threshold of  $\leq 150$  RLUs<sup>(6)</sup>. Other research has demonstrated comparable effectiveness between manual and automated methods for sterilizing endoscopic and laparoscopic instruments<sup>(7, 8)</sup>.

Despite their benefits, automated washers face barriers to their widespread adoption, primarily due to their high costs<sup>(9,10)</sup>. In response, the authors developed an economical, machine-assisted cleaning system named the 'Si-Washer' (Fig. 1). This system features a 60-watt electric pump with a flow rate of 2,800 litres per hour, and it uses polyvinyl chloride pipes with a diameter of 1.26 cm. The cost of the Si-Washer is approximately USD 55.

This study aimed to compare the effectiveness of the Si-Washer and manual flushing in cleaning laparoscopic instruments with narrow lumens.



**Fig. 1.** The Si-washer machine

*A: Water valve, B: Plastic tubing for connecting with instruments, C: Electrically powered pump*

## Materials and Methods

### ***Instrument selection and allocation***

The sample size was calculated based on a 92% effective cleaning rate for manual methods and an anticipated 99% effectiveness for the Si-Washer. Allowing for a 10% potential attrition rate and setting a power ( $1-\beta$ ) of 0.80, an  $\alpha$  level of 0.05 and a 1:1 randomization ratio, we determined that 151 instruments per group were needed.

In the Gynaecologic Surgical unit, instruments meeting the inclusion criteria were enrolled between July and September 2023. After surgery, laparoscopic

instruments such as graspers, Allis forceps and suction irrigation tubes that had been used for more than an hour were selected and randomized. These instruments underwent an initial rinse to remove surface debris. Randomization was facilitated by sequentially opening numbered envelopes. For both the manual and Si-Washer cleaning arms, a cleaning solution consisting of clean water and 3E-enzyme detergent at a 10:1 ratio was prepared. All study instruments were cleaned by N.I. and S.L. In the manual cleaning arm, the instruments were immersed for 15 minutes, followed by internal flushing with a 20

mL syringe until visibly clear. The duration of this flushing process was recorded with a stopwatch. In the Si-Washer arm, the instruments were connected to the machine tubing (Fig. 1.) and rinsed for 15 minutes. All 302 instruments were then dried. Cleanliness assessment was conducted using the 3M Clean-Trace™ water Test and the 3M Clean-Trace™ Luminometer LX 25, providing quantitative results in terms of RLUs.

**Data collection**

After submitting the protocol to the Siriraj Institutional Review Board (SiIRB) for ethical approval, the authors received a recommendation to exempt the requirement for informed consent. This exemption was due to the classification of the research as non-human in nature. Data on operative procedures and instrument contamination, operative duration, estimated blood loss, manual flushing duration and cleanliness levels, were meticulously gathered.

**Statistical analysis**

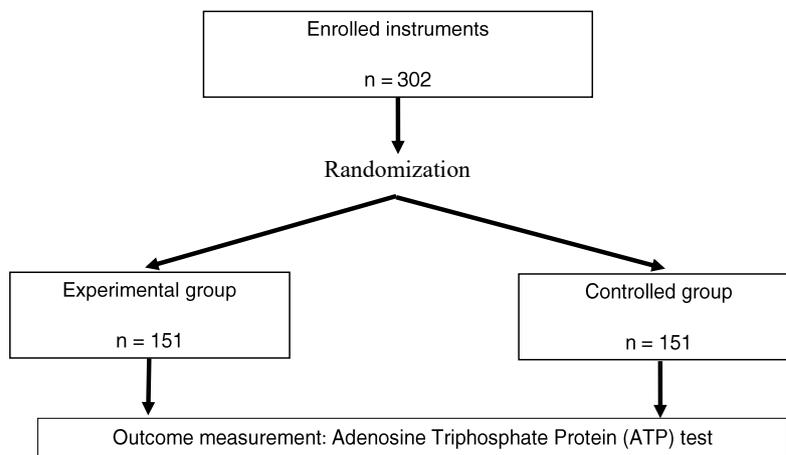
PASW Statistics, version 18 (SPSS Inc., Chicago, IL, USA), was utilized for all the statistical analyses. The data were presented as numbers and percentages or as medians and interquartile ranges (IQRs). Categorical variables were compared using

the chi-squared test or Fisher’s exact test. A p value < 0.05 was considered to indicate statistical significance.

**Results**

The Si-Washer is shown in Fig. 1, and The experimental setup flowchart is depicted in Fig. 2. Details of the operations, diseases, surgeon experience, operative times, complications and the types of the 302 instruments were enrolled. The median operative time was 135.0 minutes (IQR115.0-175.0). Fellows conducted surgery on one patient in the manual cleaning arm and three patients in the Si-Washer arm. All operations were completed without complications, with the sole exception of one instance of ureteric injury in the Si-Washer arm. The instrument components included in this study were suction irrigation tools and the outer sheaths of graspers, forceps and dissectors. The median time for manual flushing was 50.0 seconds (IQR 45.0–60.0).

Table 1 compares variables between the study arms. The Si-Washer arm exhibited significantly better cleaning outcomes than the manual cleaning arm, achieving 100% cleanliness. This outcome was evidenced by all of the instruments in the Si-Washer arm reaching the target cleanliness standard of an ATP level ≤ 150 RLUs.



**Fig. 2.** Flowchart of the study.

**Table 1.** Compared variables between two study arms.

Variables	Manual clean (n = 151)	Si-washer (n = 151)	p value
Operated by staffs, n = 298	150	148	0.314
Operations			0.158
Total laparoscopic hysterectomy, n = 192	96	96	
Myomectomy, n = 62	26	36	
Adnexal surgery, n = 48	29	19	
Instruments			0.741
Outer sheath of graspers, n = 155	76	79	
Outer sheath of forceps, n = 89	44	45	
Outer sheath of dissectors, n = 36	21	15	
Suction irrigation, n = 22	10	12	
Complication occurred, ureteric injury	0	1	0.317
Operative time (mins)	145.40 ± 49.499	152.42 ± 58.637	0.267
Intra-operative Irrigation volume (mL)	882.78 ± 443.924	884.11 ± 441.640	0.979
Estimated blood loss (mL)	82.91 ± 117.104	83.97 ± 121.532	0.939
ATP (RLU)	130.0 [72.0-180.0]	15.0 [8.25-30.0]	< 0.001
ATP < 150	89 (58.9%)	151 (100%)	
ATP ≥ 150	62 (41.1%)	0	

TLH: total laparoscopic hysterectomy, ATP: adenosine triphosphate protein; RLU: relative light units

## Discussion

Since its inception in 1957, the Spaulding classification system has categorized laparoscopic instruments used in gynaecological surgery as 'critical use items', highlighting the importance of comprehensive reprocessing to avert hospital-acquired infections<sup>(11)</sup>. Several steps are needed for effective reprocessing: cleaning, high-level disinfection, validation, drying, sterilization and storage<sup>(2,12)</sup>. The initial step, cleaning, employs chemical detergents and mechanical action, which can be manual or automated.

Research has consistently demonstrated the efficacy of manual cleaning<sup>(7,8,13)</sup>. For instance, a study examining the cleaning phase for flexible endoscopes (bronchoscopes, duodenoscopes and colonoscopes) revealed a reduction of more than 99.9% in

haemoglobin and protein contamination, with residual organic material below 6.4 µg/cm<sup>2</sup>. Additionally, there was no significant difference in viable organism counts between manual cleaning (ranging from 2.06 to 6.21 µg/cm<sup>2</sup>) and automated cleaning (ranging from 2.10 to 5.93 µg/cm<sup>2</sup>)<sup>(7)</sup>. Another investigation compared manual and automated cleaning methods for laparoscopic trocars and dissecting forceps, examining four distinct cleaning protocols: (1) manual cleaning followed by a tap water rinse, (2) manual cleaning, a tap water rinse and then ultrasonic cleaning, (3) manual cleaning, a tap water rinse, ultrasonic cleaning, and another tap water rinse, and (4) manual cleaning, a tap water rinse, ultrasonic cleaning, another tap water rinse, and a final rinse with sterile distilled water. The four approaches achieved protein contamination reductions of 95.1%, 100.0%, 98.3%

and 100.0%, respectively. The corresponding post-cleaning mean residual protein levels were 0.42, 0.16, 0.00 and 0.79  $\mu\text{g}/\text{cm}^2$  for trocars and 0.57, 0.24, 0.65 and 0.04  $\mu\text{g}/\text{cm}^2$  for laparoscopic instruments<sup>(6)</sup>. The current study demonstrated that the manual method achieved only 58.9% cleanliness. This discrepancy may be attributed to differences in cleanliness assessment methods and cut-off values compared to previous studies<sup>(7,8)</sup>. Additionally, the manual cleaning method in our study did not incorporate additional methods such as the use of an ultrasonic machine, as seen in the study by de Camargo et al.

A systematic review compared traditional manual cleaning to automated devices employing hydrogen peroxide or ultraviolet mycobactericidal light. The review concluded that there is limited value in conducting studies that directly compare the cost benefits of these methods. The review recommended that manual cleaning should continue to be used while cautioning against overreliance on novel technologies<sup>(13)</sup>. The various investigations collectively suggest that endoscopes and laparoscopic instruments can be effectively cleaned using either manual or automated methods, with both approaches demonstrating similar efficacy.

Automated washers outperform traditional manual cleaning of laparoscopic instruments due to their complex design and human factors that impair the effectiveness of manual methods<sup>(9,10)</sup>. A specific study on Crile forceps revealed the greater efficacy of ultrasonic automated cleaners compared to manual methods in removing *Staphylococcus epidermidis*, a biofilm-forming bacterium commonly isolated from surgical instruments during cleaning<sup>(10)</sup>. Another investigation evaluated manual versus automated cleaning of tubular laparoscopic instruments, such as scissors and forceps. Soil tests and bacterial cultures revealed that automated cleaners were markedly more efficient than manual cleaning for both ported and non-ported devices, achieving more than 99% reduction in soil tests. Additionally, only automated cleaning of ported devices accomplished a 103- to

104-fold reduction in bacterial counts<sup>(14)</sup>. Prominent medical organizations have established guidelines for cleaning gastrointestinal endoscopy instruments. The Asia Pacific Society of Infection Control approves of both effective manual and mechanical cleaning processes<sup>(15)</sup>. In contrast, the American Society of Gastrointestinal Endoscopy, the United States Food and Drug Administration and the United States Centers for Disease Control and Prevention strongly advocate for automated endoscope reprocessors to enhance consistency, reliability and efficiency. Nevertheless, the American Society of Gastrointestinal Endoscopy insists on supplementary manual cleaning, regardless of manufacturers' claims of its redundancy<sup>(9,16,17)</sup>.

This study demonstrated the high cleanliness efficacy of the Si-Washer. A noted limitation is the reliance on ATP bioluminescence for cleanliness validation, which may not directly indicate the presence of organisms. Future investigations should compare the cleaning efficacy of the Si-Washer against that of commercial ultrasonic washers, with microbiological cultures used as the primary evaluation criterion.

To further improve the effectiveness of the Si-Washer for cleaning, enhancements could involve increasing the motor's flushing power, maintaining a high-water quality, and using hotter water during the washing cycle. Safety improvements, such as fabricating the washing chamber from stainless steel, are also recommended. Consistent cleaning effectiveness requires establishing standardized international training for medical personnel in disinfection and sterilization, including competency assessments. Moreover, implementing systematic cleaning and validation protocols, such as daily or weekly monitoring, is essential.

## Conclusion

The study established that the Si-Washer was more efficient than manual cleaning, consistently achieving 100% cleanliness.

## Acknowledgments

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## Potential conflicts of interest

The authors declare no conflicts of interest.

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## OBSTETRICS

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# Impact of Delivery Mode on Sexual Function in the First Year after Childbirth: A comparative study of vaginal delivery and cesarean section

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### ABSTRACT

**Objectives:** Studies are showing that women's sexual functions are affected for a while after giving birth. However, there are relatively few studies showing which component of sexual function is affected by the mode of delivery. The aim of this study was to investigate the effect of the type of birth on sexual function.

**Materials and Methods:** This study was conducted with 228 participants who met the study criteria, 51 of whom had a vaginal delivery (VD), and 177 had a cesarean section (CS) delivery. The participants in the first year after childbirth, between 3-12 months, answered the Turkish version of the Female Sexual Function Index (FSFI) questionnaire. We compared the total FSFI and subscale scores of the groups.

**Results:** In both groups, weekly sexual intercourse frequency values after delivery were statistically lower than before delivery ( $p < 0.001$  and  $p < 0.001$ , respectively). Additionally, low FSFI scores indicating the risk of Female Sexual Dysfunction (FSD) were obtained in both groups. When the group giving birth with VD was compared with the CS group, the total FSFI and whole subscale scores were lower. It was determined that the risk of sexual dysfunction increased 20.8-fold in participants who gave birth vaginally.

**Conclusions:** Regardless of the mode of delivery, all participating women in the first year after childbirth had deficiencies in total FSFI scores and all subscale scores, indicating sexual dysfunction. This inadequacy in sexual dysfunction was more pronounced in women who gave birth VD than those who gave birth by CS. Considering the importance of sexual health for life quality standards, it would be appropriate for all women in the first year after childbirth (especially those who give birth with episiotomy) to receive sexual counseling in terms of FSD risk and monitoring of FSD symptoms.

**Keywords:** female sexual function index, normal birth, cesarean section, postpartum period, sexual dysfunction.

## Introduction

Female sexual dysfunction is defined as the person's dissatisfaction with the sexual act due to problems in desire, arousal, orgasm, or pain in the sexual cycle stage<sup>(1)</sup>. Sexual function may be affected by various reasons, such as psychological reasons, drugs used, previous surgical operations, or pregnancy<sup>(2, 3)</sup>.

It has been shown that there are adverse changes in sexual functions in women due to various factors during pregnancy and in the first year after childbirth<sup>(3, 4)</sup>. While some factors, such as physiological changes in the body, hormonal changes, and insomnia, are blamed for sexual dysfunctions occurring during pregnancy, it has been reported that the factors affecting sexual function in the postpartum period are different from these reasons<sup>(4-6)</sup>. Barrett et al<sup>(7)</sup> reported that postpartum bleeding, dyspareunia, and difficulty in reaching orgasm were the leading causes of sexual dysfunction in the postpartum period. Considering the importance of pelvic anatomy in sexual function, it has been suggested that vaginal delivery (VD) and significantly assisted vaginal delivery can cause various damage to the pelvic floor<sup>(8, 9)</sup>. In parallel with this idea, studies showed that less sexual dysfunction occurs after cesarean (CS) deliveries<sup>(8, 10)</sup>. However, some studies have stated that the mode of delivery does not affect sexual functions<sup>(11, 12)</sup>. The effect of the mode of delivery on sexual functions is a controversial issue.

Female Sexual Dysfunction (FSD) is defined as the deficiency of at least one component in the sexual response cycle (desire, arousal, ability to reach orgasm, and pain during sexual intercourse). One of the modern and convenient ways to evaluate FSD is to use validated questionnaires

and symptom scores. The Female Sexual Function Index (FSFI) is the gold standard method for screening female sexual function<sup>(13)</sup>. FSFI is a female sexual function index consisting of 19 questions and examining six sub-domains (desire, arousal, lubrication, orgasm, satisfaction, pain).

Our primary aim in this study was to investigate the effect of birth type on the total and subscale scores of FSFI. Our secondary objective was to determine the correlation of FSFI subscale scores with BMI, breastfeeding time (minutes) and time after birth depending on the type of birth. We also aimed to investigate factors that may have a negative impact on the sexual function of women in the first year after birth.

## Materials and Methods

This cross-sectional study was conducted between 01/09/2020 and 01/09/2021 at Gazi University Faculty of Medicine, Department of Obstetrics and Gynecology, Ankara, Turkey. Women aged 18 and over who gave birth in our hospital within a year after informed consent from all participants and approval from our hospital's ethics committee (ethics committee no: 2020/604.01.01-18) were included in the study. Women with postpartum hemorrhage, infection, gestational hypertension, preterm birth, preeclampsia, and other postpartum severe complications, previous pelvic surgery, sexual dysfunction with an underlying organic cause before pregnancy, patients who used drugs that would cause sexual dysfunction, and patients who stated that they did not want to participate in the study at any stage of the study were excluded from the study. In addition, we did not include births without episiotomy in our study since our rates of episiotomy in vaginal deliveries are very high. The

patients who gave birth were called for follow-ups between 3 and 12 months after delivery. FSFI was filled in after recording all participants' body mass index (BMI), age, parity, education level, mode of delivery, time elapsed after birth, breastfeeding status, and duration of breastfeeding (approximately how many minutes each feed lasts). All interviews were conducted face-to-face and in the company of a doctor, paying attention to patient privacy. All participants were divided into two groups, CS (n = 177) and VD (n = 51) deliveries, and the FSFI scores of the groups were compared.

### **Female Sexual Function Index (FSFI)**

Female sexual function was evaluated using the Turkish version of the FSFI index verified by the Turkish Society of Andrology<sup>(14, 15)</sup>. The FSFI questionnaire is a questionnaire containing six main headings and 19 questions. The distribution of these questions and titles is as follows. 1- desire (questions 1 and 2), 2- arousal (questions 3, 4, 5, 6), 3- Lubrication (questions 7, 8, 9, 10), 4- Orgasm (questions 11, 12, 13), 5- Satisfaction (questions 14, 15, 16), 6- Pain (questions 17, 18, 19). The scoring of the FSFI questionnaire is as follows: The scoring scale for questions 3 to 14 and 17 to 19 spans from 0 to 5, while for questions 1, 2, 15, and 16, it ranges from 1 to 5.

The total score is obtained by adding the scores in these six titles and multiplying their sums with the coefficients. These coefficients are 0.6 for desire, 0.3 for arousal and lubrication, and 0.4 for orgasm, pain, and satisfaction. Total scores range from 2 to 36. Patients with a total score of less than 26.5 will be considered risky for Female Sexual Dysfunction (FSD)<sup>(14)</sup>. Higher scores will be regarded as indicators of better sexual function. Furthermore, when evaluated separately for each sub-domains, scores below 4.28 for “desire,” below 5 for “arousal,” below 5.4 for “lubrication,” below 5 for orgasm, below 5 for “satisfaction,” and below 5.5 for “pain” will be considered insufficient in their respective fields<sup>(14, 15)</sup>.

### **Statistical analysis**

Continuous variables were expressed as mean  $\pm$  standard deviation and median (min-max), and categorical data were expressed as numbers and percentages. Independent samples t-test was used to analyze continuous variables with normal distribution between the two groups, and Mann-Whitney U test was used for those that did not. Intergroup comparisons were made with the Wilcoxon Signed Ranks test. The chi-square test was used for comparisons between discrete/categorical data. The linear relationship between continuous variables was tested with the Spearman Correlation test. The independent predictors for predicting the risk of sexual dysfunction were initially examined using univariate logistic regression analysis (enter method), utilizing potential factors identified in previous analyses. For the multivariate model, variables found to be significant at the level of significance ( $p < 0.250$ ) in the univariate analyses, including mode of delivery, time of birth, educational status, and BMI, were investigated through multivariate logistic regression analysis (backward LR). Model fit and significance were assessed using the Hosmer and Lemeshow test, Omnibus tests of model coefficient, and Nagelkerke R Square values. Analyzes were performed with IBM SPSS Package Program version 22.0 (IBM Corporation, Armonk, NY, USA). The statistical significance level was considered as  $p < 0.05$ . Power analysis was performed with the program G\*power (version 3.1.9.7, Universität Kiel, Kiel, Germany).

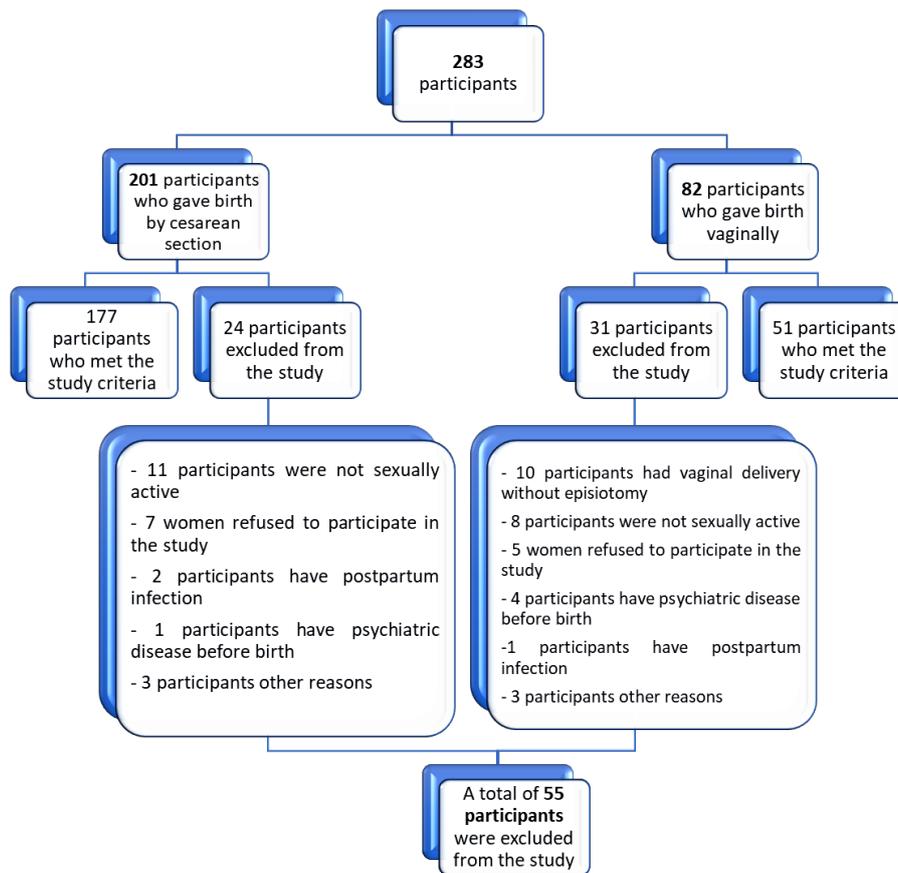
## **Results**

Between 01/09/2020 and 01/09/2021, 283 patients who applied to Gazi University Faculty of Medicine, Department of Gynecology and Obstetrics for gynecological examination were included in the study. Fifty-five patients were excluded from the study due to various reasons (Fig. 1). Participants were divided into two groups, CS and VD groups. The demographic data of the

participants are listed in Table 1. While no difference was observed between the groups in terms of age, fetal weight, duration of breastfeeding (approximately how many minutes breastfeeding lasts), and education period, statistically significant differences were found in terms of BMI, gravida, parity, monthly income level, time passed after birth, and breastfeeding status ( $p < 0.05$ ). There was no significant difference between the two groups in the frequency of weekly sexual intercourse after delivery ( $p = 0.117$ ). In both groups, weekly sexual intercourse frequency values after delivery

were statistically lower than before delivery ( $p < 0.001$  and  $p < 0.001$ , respectively).

The FSFI scores of the groups were compared in Table 2. We observed statistically significant decreased in desire, lubrication, orgasm, satisfaction, pain subscale scores, and FSFI scale total scores among women who delivered with VD compared to those who delivered CS ( $p < 0.001$ ,  $p < 0.001$ ,  $p = 0.049$ ,  $p = 0.001$ ,  $p = 0.012$ , and  $p = 0.001$ , respectively). In addition, while the total FSFI score was  $< 26.5$  in all participants in the VD group, this rate was 70.6% in the CS group.



**Fig. 1.** Study flow.

**Table 1.** Comparison of the groups according to sociodemographic characteristics of participants' and some fetal parameters

Characteristics	VD (n = 51)	Cesarean Section (n = 177)	p value
Age (years) (mean ± SD)	28.8 ± 7.3	27.2 ± 4.3	0.058*
BMI (kg/m <sup>2</sup> ) (mean ± SD)	27.2 ± 5.1	29.5 ± 4.8	0.003*
Gravidity [median (min-max)]	1 (1-4)	1 (1-3)	0.004**
Parity [median (min-max)]	1 (0-3)	1 (0-2)	0.027**
Fetal weight (g) [median (min-max)]	3300 (2850-4500)	3400 (2400-4050)	0.278**
Duration of breastfeeding (minute) [median (min-max)]	15 (10-20)	15 (0-20)	0.289**
Education, n (%)			
Primary	0 (0.0)	4 (2.3)	
Secondary	8 (15.7)	14 (7.9)	
High school	20 (39.2)	54 (30.5)	
Associate's degree	10 (19.6)	54 (30.5)	0.101***
University	13 (25.5)	41 (23.2)	
Master's degree	0 (0.0)	10 (5.6)	
Income (monthly), n (%)			
< 1600 TL (200\$ ↓)	8 (15.7)	18 (10.2)	0.020***
1600-2500 TL (200-312 \$)	6 (11.8)	55 (31.1)	
> 2500 TL (312\$ ↑)	37 (72.5)	104 (58.8)	
Period after birth, n (%)			
Between 3-6 months	33 (64.7)	96 (54.2)	0.007***
Between 6-9 months	0 (0.0)	29 (16.3)	
Between 9-12 months	18 (35.3)	52 (29.3)	
Breastfeeding, n (%)			
Yes	51 (100.0)	132 (74.6)	< 0.001***
No	0 (0.0)	45 (25.4)	
Frequency of postpartum sexual intercourse (weekly) (min-max)	1(0-3)	1(0-2)	0.117**
Frequency of sexual intercourse before pregnancy (per week) [median (min-max)]	3 (1-5)	2 (1-3)	< 0.001**

\* Independent samples t-test, \*\* Mann Whitney U test, \*\*\* Chi-square Test.  
BMI: body mass index, VD: vaginal delivery, SD: standard deviation. 1\$ = 8 Turkish Lira (TL).

**Table 2.** Comparison of the groups according to the total FSFI scores and its subscale scores.

Characteristics	VD (mean ± SD) (n = 51)	Cesarean Section (mean ± SD) (n = 177)	p value
Desire	2.69±1.02	3.46±1.02	< 0.001*
Arousal	3.20±1.15	3.33±1.20	0.487*
Lubrication	2.34±1.57	3.38±1.57	< 0.001*
Orgasm	2.80±1.62	3.34±1.92	0.049*
Satisfaction	3.01±1.49	3.93±1.77	0.001*
Pain	1.88±1.67	2.69±2.10	0.012*
Total FSFI score	15.94±6.70	20.16±8.21	0.001*

\* Independent samples t-test. FSFI: Female Sexual Function Index, VD: vaginal delivery, SD: standard deviation.

Table 3 shows the correlation between BMI, breastfeeding time (minutes), and time after birth in the VD group with the FSFI total and subscale scores. In the VD group, It was found that there were positive, powerful, statistically significant correlations between BMI and desire, orgasm, satisfaction, pain, and FSFI total scores ( $r = 0.747$ ,  $p < 0.001$ ,  $r = 0.756$ ,  $p < 0.001$ ,  $r = 0.775$ , respectively,  $p < 0.001$ ,  $r = 0.737$ ,  $p < 0.001$ , and  $r = 0.730$ ,  $p < 0.001$ ). In the VD group, a positive, moderately strong, and statistically significant correlation was found between breastfeeding duration and arousal and lubrication subscale scores ( $r = 0.474$ ,  $p < 0.001$  and  $r = 0.351$ ,  $p = 0.012$ , respectively). In contrast, a negative correlation between breastfeeding duration and desire and pain was found. It was determined that there were good, strong, statistically significant correlations in the direction ( $r = -0.868$ ,  $p < 0.001$  and  $r = -0.310$ ,  $p = 0.027$ , respectively). In the VD group, while a negative, moderate-good, statistically significant correlation was found between the time passed after delivery and arousal subscale scores ( $r = -0.597$ ,  $p < 0.001$ ), there was a positive, good and statistically significant correlation between the time passed after delivery and lubrication and orgasm. Very strong, statistically significant correlations were

determined ( $r = 0.571$ ,  $p < 0.001$  and  $r = 0.707$ ,  $p < 0.001$ , respectively).

Table 4 shows the correlation between BMI, breastfeeding time (minutes), and time after delivery in the CS group with the FSFI total and subscale scores. Within the CS group, it was found that the relationship between breastfeeding duration, lubrication, and pain subscale scores were negative

**Table 3.** Correlation of FSFI total score and subscale scores with BMI, breastfeeding time (minutes), and time after birth in the VD group.

		BMI	Duration of breastfeeding (minute)	Time passed after birth
Desire	r	0.747	-0.868	0.283
	p	< 0.001*	< 0.001*	0.044
	N	51	51	51
Arousal	r	0.155	0.474	-0.597
	p	0.278	< 0.001*	< 0.001*
	N	51	51	51
Lubrication	r	0.388	0.351	0.571
	p	0.005	0.012	< 0.001*
	N	51	51	51
Orgasm	r	0.756	-0.250	0.707
	p	< 0.001*	0.077	< 0.001*
	N	51	51	51
Satisfaction	r	0.775	-0.197	0.163
	p	< 0.001*	0.165	0.253
	N	51	51	51
Pain	r	0.737	-0.310	0.122
	p	< 0.001*	0.027	0.395
	N	51	51	51
FSFI	r	0.730	-0.153	0.229
	p	< 0.001*	0.283	0.106
	N	51	51	51

\* Spearman's rho (bold for p value < 0.05). FSFI: female sexual function index, BMI: body mass index, VD: vaginal delivery.

In the univariate analyses performed according to FSFI: the mode of delivery being VD increased the odds of sexual dysfunction risk by 20.8 times (OR 20.800, 95%CI 2.799-154.57,  $p = 0.003$ ). Additionally, having an educational status of high school or below was found to increase the odds of sexual dysfunction risk by 2.3 times (OR 2.393, 95%CI 1.228-4.662,  $p = 0.010$ ). Moreover, it was determined that each unit

with low and moderately statistical solid significance between them ( $r = -0.358$ ,  $p < 0.001$ ; and  $r = -0.444$ ,  $p < 0.001$ , respectively). In the same group, it was also determined that there were positive, low to moderately strong statistically significant correlations between the time passed after delivery, lubrication, and pain subscale scores ( $r = 0.377$ ,  $p < 0.001$ ; and  $r = 0.376$ ,  $p < 0.001$ , respectively).

**Table 4.** Correlation of FSFI total score and subscale scores with BMI, breastfeeding time (minutes), and time after delivery in the cesarean section group.

		BMI	Duration of breastfeeding (minute)	Time passed after birth
Desire	r	0.345	-0.058	-0.028
	p	< 0.001*	0.445	0.708
	N	177	177	177
Arousal	r	0.411	0.121	0.028
	p	< 0.001*	0.109	0.713
	N	177	177	177
Lubrication	r	-0.2	-0.358	0.377
	p	0.002	< 0.001*	< 0.001*
	N	177	177	177
Orgasm	r	0.017	-0.015	0.225
	p	0.824	0.839	0.003
	N	177	177	177
Satisfaction	r	0.208	-0.195	0.094
	p	0.006	0.009	0.216
	N	177	177	177
Pain	r	-0.027	-0.444	0.376
	p	0.719	< 0.001*	< 0.001*
	N	177	177	177
FSFI	r	0.132	-0.132	0.254
	p	0.080	0.079	0.001
	N	177	177	177

\* Spearman's rho (bold for p value < 0.05). FSFI: female sexual function index, BMI: body mass index.

increase in BMI acted as a protective factor for sexual dysfunction risk (OR 0.847, 95%CI 0.791-0.907,  $p < 0.001$ ) (Table 5).

In the multiple logistic regression analysis of the multivariate model, it was determined that the mode of delivery (VD), period after birth (< 6 months), educational status (high school or below), and BMI variables continued to be significant factors (Table 5).

**Table 5.** Examination of factors associated with sexual dysfunction in women giving birth within one year using univariate (binary) and multivariate logistic regression analysis.

	OR (95% CI)	p value		OR (95% CI)	p value
Age (years)	0.981 (0.925-1.040)	0.516			
BMI (kg/m <sup>2</sup> )	0.847 (0.791-0.907)	< 0.001	BMI (kg/m <sup>2</sup> )	0.724 (0.640-0.819)	< 0.001*
Duration of breastfeeding (minute)	0.977 (0.934-1.023)	0.328			
Education (high school and below)	2.393 (1.228-4.662)	0.010	Education (high school and below)	5.075 (2.119-12.152)	< 0.001*
Income (monthly) (\$200-\$312)	1009671790.23 (0.0-)	0.997			
Income (monthly) (> \$312)	1.424 (0.598-3.392)	0.424			
Mode of delivery (VD)	20.800 (2.799-154.57)	0.003	Mode of delivery (VD)	21.934 (2.310-208.314)	0.007*
Period after birth (< 6 months)	1.470 (0.778-2.781)	0.036	Period after birth (< 6 months)	7.146 (2.368-21.560)	< 0.001*
Breastfeeding status (no)	1.075 (0.492-2.349)	0.856			

Univariate (Binary) Logistic Regression analysis

\* Multivariate Logistic Regression Analysis (Backward: LR)  
 Omnibus Tests of Model Coefficients (p < 0.001).  
 Nagelkerke R Square (p = 0.416)  
 Hosmer and Lemeshow Test (p < 0.001)  
 The mode of delivery, period after birth, educational status, and BMI variables have been included in the model.

BMI: body mass index, OR: odds ratio, CI: confidence interval, VD: vaginal delivery.

## Discussion

We found the complete set of FSFI scores and subscale scores within the VD group to be significantly lower during the first year after childbirth than the CS group results. It was determined that the main factors that increased the risk of sexual dysfunction during the first year after birth were having a vaginal birth, having a low education level (high school or below) and less than 6 months after birth. In the CS group, it was observed that the income level, education level, and parity number were significantly higher in the participants with a total FSFI score above 26.5.

Some studies and related literature discussed whether the mode of delivery affects sexual functions. Griffiths et al<sup>(6)</sup> analyzed the sexual functions of women two years after giving birth. Their study found that dyspareunia and sexual dissatisfaction were more prevalent among women who gave birth through VD. In a prospective study involving 304 participants, it was determined that women who had a VD at the postpartum third month had lower FSFI scores compared to women who had a CS<sup>(16)</sup>. Another study looked into how the type of delivery affected sexual functions, where they found that dyspareunia was also more common in women who gave birth through VD<sup>(10)</sup>. A recent meta-analysis investigating the risk factors

for sexual dysfunction during the first year after childbirth and the sexual functions of those who gave birth through VD or CS was analyzed. Although there was no significant difference between the CS and VD groups when it came to sexual dysfunction (OR 1.14, 95%CI 0.89-1.46), it was found that dyspareunia was reported less in women who delivered through CS (OR 0.79, 95%CI 0.89–1.46)<sup>(17)</sup>. Other authors declare that the mode of delivery does not affect sexual functions in any significant way<sup>(11, 12, 18, 19)</sup>. Another recent meta-analysis did not find a meaningful relationship in terms of postpartum sexual dysfunction between patients with spontaneous/assisted delivery and patients with CS delivery. However, it is also emphasized that the regulatory factors in the studies that are the subject of meta-analysis may affect this relationship<sup>(20)</sup>. The effect of delivery method on sexual functions is quite controversial in the literature. In our study, we found that women who gave birth vaginally had an approximately 20-fold increased risk of developing sexual dysfunction compared to women who gave birth by CS. Both the FSFI scores, desire, lubrication, satisfaction, orgasm, and pain scores were found to be lower in women who delivered through VD than those who had given birth through CS. Additionally, some studies showed that dissatisfaction

during intercourse may cause a reduction in the frequency of sexual intercourse<sup>(21, 22)</sup>. Dyspareunia usually occurs during the first months after VD, increasing sexual dissatisfaction while also decreasing the frequency of sexual intercourse and affecting all other sexual functions negatively. Our study found that approximately 65% of participants who had given birth through normal delivery had lower sexual functions during the first 3-6 months after delivery compared to those who gave birth through CS. This may be due to sexual dissatisfaction experienced during the early stages of the postpartum period. Moreover, within the study, it was found that the frequency of postpartum sexual intercourse was lower than it was before delivery. At this point, it is necessary to discuss episiotomy, which has the potential to disrupt sexual function by affecting the perineum. Several studies also reported a positive relationship between the severity of dyspareunia experienced and painful experiences of perineal trauma and episiotomy<sup>(23)</sup>. Lagana et al found that sexual dysfunction in women led to significantly lower FSFI scores for patients who underwent episiotomy during childbirth<sup>(24)</sup>. Signorello et al<sup>(25)</sup> speculated that the degree of perineal trauma had a positive relation to the severity of postpartum dyspareunia, sexual sensation disorder, sexual satisfaction, and orgasm disorders. However, these findings were largely attributed by the authors to short-term changes in postpartum sexual functions. In contrast, other studies in the related literature have reported no such relationship between episiotomy and sexual dysfunction in women<sup>(26, 27)</sup>. A large prospective study found that the mode of delivery and episiotomy were unrelated to anorgasmia in primiparous women<sup>(28)</sup>. Furthermore, according to a systematic review evaluating the outcomes of routine episiotomy, no evidence suggests that episiotomy reduces impaired sexual function<sup>(9)</sup>. A randomized study compared the elective CS against VD in the case of a singleton fetus. Here, Hannah et al<sup>(29)</sup> suggested that there was no difference in sexual outcomes during a period of 3 months to 2 years after delivery. Episiotomy was

performed on all patients who delivered vaginally (VD). Additionally, the majority of patients in the VD group were within the first 6 months after birth. The high risk of sexual dysfunction observed in the VD group may be attributed to the cumulative effect of episiotomy-induced iatrogenic perineal trauma and the early period after birth in this patient cohort (VD). Our results showed that the lowest FSFI subscale scores in the CS and VD groups were attributed to "pain". Considering the related literature, we suppose that pain is the main factor behind the reduction in the frequency of sexual intercourse. However, the number of patients who did not have episiotomy in our hospital was minimal, so the entire VD group consisted of patients with episiotomy. Therefore, we could not compare the FSFI scores between vaginal delivery without episiotomy and cesarean birth groups. In other words, we could not distinguish to what extent episiotomy had a negative impact on FSFI scores, especially on the pain sub-domain, in women who gave birth vaginally.

In some studies, low educational levels have been indicated to be associated with the risk of female sexual dysfunction<sup>(30, 31)</sup>. Nappi et al<sup>(30)</sup> stated that women with lower educational levels experienced a reduction in their ability to cope with stress, leading to a higher prevalence of sexual dysfunction problems. Another study suggested that women with lower educational levels talked less about sexual dysfunction problems, and therefore this could be a negative factor<sup>(31)</sup>. In our study, we found that low educational levels (high school and below) increased the risk of sexual dysfunction several times. These results may be attributed to women with lower educational levels potentially perceiving their sexual dysfunction problems as normal postpartum problems (due to misinformation and misperceptions) and consequently speaking less about them. Undoubtedly, the reluctance of women experiencing sexual dysfunction problems to discuss them may hinder them from seeking appropriate medical support, potentially worsening their sexual function.

Breastfeeding may also negatively affect some

sexual functions in women. A decrease in sexual function may occur in lactating women after childbirth, usually attributed to the hypoestrogenic state that arises due to breastfeeding. During the process, high levels of prolactin and low levels of estrogen and progesterone can increase breast sensitivity, which may negatively affect one's sexual desire. In contrast, a decrease in vaginal lubrication may be caused by hormonal changes<sup>(7, 32)</sup>. Saleh et al<sup>(33)</sup> reported that lactating women had statistically lower sexual desires and FSFI scores when compared to non-lactating women. According to the results of our study, it was found that desire scores decreased as breastfeeding duration increased in both groups. Additionally, it was determined that lubrication decreased as breastfeeding duration increased in the CS group. Considering the physiology of sexual arousal, sexual arousal increases vaginal blood flow and allows for the secretion of fluids that act as lubrication for sexual intercourse<sup>(34)</sup>. In this study, we found that there were increasing lubrication scores in the VD group that were positively correlated with breastfeeding duration. Therefore, considering the physiology of lubrication explained above, increasing arousal scores may have affected lubrication scores positively.

Our study also found that as time passed after the delivery of the child, lubrication increased, and less dyspareunia was observed in both groups. Since breastfeeding periods are more extended during the early postpartum period, hormonal and psychological changes during the breastfeeding process<sup>(7)</sup> may result in decreased lubrication levels. It is also clear that as time passes during the postpartum period, breastfeeding durations will reduce, and feelings of pain, weakness, and fatigue observed in the first days after delivery will subside. Therefore, it is customary to expect that lubrication based on FSFI subscales will increase while pain decreases the more time passes after childbirth.

Related literature also states that women with a BMI score of above 25 have lower sexual performance<sup>(35, 36)</sup>. In contrast, other studies say that there is no significant relationship between BMI scores

and a woman's sexual performance<sup>(37, 38)</sup>. Although this issue is still widely debated, the results of our study found that all FSFI subscale scores were below typical levels for both VD and CS groups, with the disparity increasing as the participants' BMI scores increased. FSFI pain scores, arousal, and orgasm scores also seemed to increase as the participant's BMI score increased. Our results seem to contradict some data in the literature. First of all, it should be noted that studies indicating that obesity seriously negatively affects the FSFI score were conducted with a morbidly obese patient group<sup>(35, 39)</sup>. In another study conducted with 193 women with a mean BMI of  $24.5 \pm 3.75$ , BMI was not found to be related to FSFI total and subgroup scores<sup>(40)</sup>. Esposito et al<sup>(41)</sup> even stated that BMI may be positively correlated with desire, which is the FSFI subgroup. In our study, the mean BMI of participants was approximately 28. In other words, it was different from the study groups in the literature with women with very high BMI<sup>(35, 39)</sup>. Moreover, approximately 56% of the participants in our survey were in the first 3-6 months of their postpartum period. There is a societal pressure on women to rapidly lose weight and attain a desirable body shape immediately after childbirth, and this pressure may diminish gradually in the months following childbirth, potentially leading to weight regain after the initial months. Additionally, there is a documented relationship in the literature between being underweight and sexual dysfunction<sup>(42)</sup>. As an indirect result of this, an improvement in sexual functions can be expected, as in our study. Similar to our findings, O'Malley et al<sup>(43)</sup> and Szöllosi et al<sup>(42)</sup> stated that sexual dysfunction was less common in overweight mothers in the postpartum period.

There were several limitations in our study. Firstly, although the FSFI score is considered the gold standard in evaluating a woman's sexual functions, certain conditions such as psychological or subjective factors, childcare, body composition changes, and stress may have caused specific points to be missed while participants were evaluating the FSFI questionnaire. Another limitation of the study involved

the participants being uncomfortable and hesitating to talk about their sexual lives while filling out the questionnaire. This may have led to a decrease in the possibility of the participants answering the questionnaire correctly and honestly. Additionally, the educational status, socio-cultural, and socio-economical levels of the study participants may have affected their answers. One more limitation of the study was that the perineal traumas of the participants who gave birth through VD could not be properly evaluated. Therefore, the differences between 1st and 2nd perineal tears could not be differentiated as the number of CS patients was high. Our episiotomy rate was relatively high in our vaginal deliveries. Therefore, we did not include vaginal deliveries without episiotomy in our study. An evaluation could not be made regarding the sexual functions of the partners of the study's participants. However, we believe our study contributes to the body of knowledge surrounding this topic as it addresses critical issues that have not yet been given a clear conclusion.

The participants' sexual functions before pregnancy were not clearly known. Only the frequency of weekly sexual intercourse before pregnancy could be questioned.

Our study's participants were women within the first 12 months of postpartum. The data we gathered was mainly related to the sexual functions of women who were in their early postpartum periods. Another important limiting factor is the fact that we were not able to perform a medical interview by staying only on FSFI in the diagnosis of sexual dysfunction.

Despite some limitations, our study is essential in examining the relationship between delivery type and FSFI subscale scores. It also contributes to some controversial issues in the literature.

## Conclusion

Regardless of the mode of delivery, all participants who were in the first year after childbirth were found to have had deficiencies in their FSFI scores and across all subscale scores (desire, arousal, lubrication, orgasm, satisfaction, pain),

indicating sexual dysfunction risk within both groups. This increase in sexual dysfunction risk was found to be more pronounced in women who gave birth through VD than those who gave birth through CS. Considering the importance of sexual health for life quality standards, it would be appropriate for all women in the postpartum period (especially those who give birth vaginally) to receive sexual counseling in terms of FSD risk and monitoring of FSD symptoms.

## Potential conflicts of interest

The authors declare no conflicts of interest.

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## GYNAECOLOGY

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# NGS Analysis of the *BRCA1/BRCA2* Variants in the Ovarian Cancer Patients at High versus Low Risk of Mutation

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### ABSTRACT

**Objectives:** The pathogenic variant in breast cancer susceptibility gene 1/2 (*BRCA1/2*) is related to the risk of ovarian cancer, which is one of the most common cancers among women worldwide. Here, we aimed to understand the variants of these genes among Vietnamese population to improve early detection and diagnosis as well as treatment decisions.

**Materials and Methods:** *BRCA1/2* genes of tumor tissue from 33 ovarian cancer patients in high-risk and low-risk of mutation groups were sequenced by next-generation sequencing (NGS) using the *BRCA*accuTest™ PLUS kit.

**Results:** Among a total of 33 distinguished variants detected, we found 5 pathogenic and likely pathogenic (P/LP) variants, including c.1801\_1808delCACAATTC, c.1016delA, c.1673\_1674delAA, c.928C>T in *BRCA1* and c.2865delC in *BRCA2* with a frequency of 3.03%, except for c.1801\_1808delCACAATTC (6.06%). All variants led to defects in coding protein. c.1801\_1808delCACAATTC variant in *BRCA1* and c.2865delC in *BRCA2* was firstly introduced. The prevalence of *BRCA1/2* P/LP variants in the high-risk group was 22.73%, while this figure for the low-risk group was 9.09%. No statistically significant difference in other clinical features was found between P/LP variant-carried and non-P/LP variant-carried patients apart from age and comorbidities.

**Conclusions:** This study provided precious data for *BRCA1/2* variant mapping among Vietnamese ovarian cancer patients.

**Keywords:** ovarian cancer, NGS analysis, *BRCA1/BRCA2* variants, mutation.

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## Introduction

According to GLOBOCAN 2020, more than 300,000 new cases and more than 200,000 death were recorded to be related to ovarian cancer per year. That makes ovarian cancer become one of the most common cancers in women in both incidence and mortality rate<sup>(1)</sup>. In Vietnam, the GLOBOCAN 2020 report showed that the incidence rate is about 2.9 per 100,000 women in 2020<sup>(1)</sup>. Ovarian cancer includes 4 main stages: stage I - cancer cells limited to one or two ovaries, stage II - cancer cells spread to the uterus or some surrounding organs, stage III - metastatic cancer spreads to lymph nodes or abdominal mucosal areas, stage IV – the cancer has spread to more distant organs in the body<sup>(2)</sup>. Regarding the classification of ovarian cancer, there are 3 main types: germ cell, sex-cord-stromal and epithelial. The most common currently is epithelial (about 95%), while the other two are about 5%<sup>(2)</sup>.

One difficulty in ovarian cancer treatment is that patients are usually at the advanced stages at the first time of diagnosis. Due to its complex nature, the detection and treatment of ovarian cancer have been a major challenge for global health. In particular, the source of all challenges has been attributed to be the vague early symptoms of ovarian cancer. Women with ovarian cancers have often been detected postmenopausal; thus, the development of ovarian tumors usually does not cause pronounced symptoms until they reach a large size and spread<sup>(3)</sup>. That has significantly

hindered the development of ovarian cancer screening and early detection measures. Therefore, most patients were detected at a late stage and had low therapeutic efficacy. Up to 90% of cases were diagnosed at a late stage when tumors have spread to the pelvic or abdominal area<sup>(3)</sup>. The 5-year survival rate of ovarian cancer patients who have been detected at an early stage is 70%; however, it is disturbing that it is only 35% at a late stage<sup>(4)</sup>. That is the urgent need to develop comprehensive ovarian cancer early detection measures and treatment.

There are many causes leading to ovarian cancer, including genetic variants, and one of the most well-known genetic causes was germline variants in the breast cancer susceptibility gene 1/2 (*BRCA1/2*) genes<sup>(5, 6)</sup>. A study of Kast et al indicated that germline *BRCA1/2* variants contribute to about 25% of the heredity ovarian cancer and breast cancer across the world<sup>(7)</sup>. Moreover, since *BRCA1* and *BRCA2* genes play roles in producing proteins for the purpose of repairing damaged deoxyribonucleic acid (DNA), pathogenic variants in the *BRCA1/2* genes are considered as one of the strong predictors of patients' response to a drug class called poly adenosine diphosphate (ADP)-ribose polymerase inhibitor (PARPi). While previous studies provided low evidence about the relationship between genetic variants and the risk of non-epithelial ovarian cancer like germ cell or sex-cord-stromal<sup>(8)</sup>, according to Candido-dos-Reis et al, mutation in

*BRCA1/2* is responsible for about 5% to 15% of epithelial ovarian cancer cases, which is the most common type of ovarian cancers. However, epithelial ovarian cancer patients having *BRCA1/2* mutations have more advantage than non-carrier patients in short-term (5 years) survival<sup>(9)</sup>. Another study reported that mutation in *BRCA1/2* gene increase the lifetime risk of epithelial ovarian cancer into more than 10 times compared to general population<sup>(10)</sup>.

Nowadays, the potential of personalized therapies has increasingly unraveled, ushering in a new era of precision medicine. However, the researchers could not approach this therapy if the ovarian cancer genetic information had not been elucidated. Next-generation sequencing (NGS) is a new method with outstanding advancement in the molecular profiling of tumors, including ovarian tumors. Through the ability to analyze multiple genes in one assay, NGS can identify the variant quickly and guide treatment decisions<sup>(11)</sup>. The technology has many advantages over traditional gene sequencing, such as faster reading times, extensive output data, and cost savings<sup>(11)</sup>. Studies in ovarian have often used NGS to identify *BRCA1/2* gene variants. For example, NGS combined with MyChoice CDx testing is able to detect *BRCA1/2* pathogenic large arrangements in ovarian tumors, which is commonly challenging<sup>(12)</sup>. A study in Italy by Paola Concolino et al was conducted using NGS in 132 ovarian cancer patients<sup>(13)</sup>. The results detected 37 *BRCA1/2* variants, including 7 new ones that had never been reported. Another study in ovarian cancer patients from the North Caucasus also used NGS to provide founder *BRCA1/2* alleles in all ethnic groups in this area<sup>(14)</sup>.

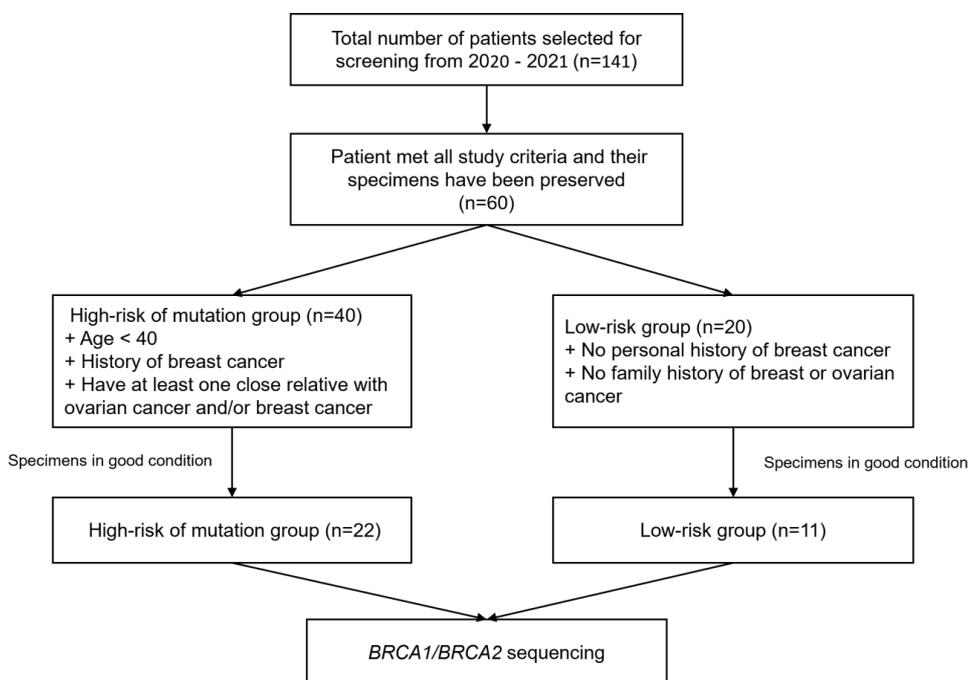
For countries with limited resources, ovarian cancer management has been seen as a significant challenge. In particular, the genetic characteristics

of the disease can differ significantly between ethnic groups and races<sup>(15)</sup>. Therefore, it is essential to have a comprehensive view of the genetic characteristics of ovarian cancer in Vietnamese people. Moreover, identifying high-risk groups based on genetic history should also be emphasized to make screening more convenient. This study was conducted to study *BRCA1* and *BRCA2* gene variants characteristics in ovarian cancer patients in Vietnam using NGS. The evidence can be valuable documents to build a standard ovarian genetic panel for Vietnamese people to develop effective screening and treatment strategies, which aligns with the current precision medicine era.

## Materials and Methods

### *Selection of study patients*

Among 141 patients diagnosed with ovarian cancer and treated at the Vietnam National Cancer Hospital (Hanoi, Vietnam), we selected a total of 60 patients that their tissue specimens after surgery have still been stored at the hospital. We divided 60 patients into high-risk and low-risk group of having genetic variants according to the following criteria: i) High-risk group included 40 patients with a confirmed diagnosis of ovarian cancer by histopathological examination results, and had one or both criteria of cancer syndrome breast – hereditary ovarian cancer: 1) age under 40; 2) patients with a history of breast cancer; 3) there was at least one family member with ovarian cancer and/or breast cancer; 4) higher stage of the disease. ii) The low-risk group included the remaining. There were 22 patients over 40 patients in the high-risk group and 11 patients over 20 patients in the low-risk group whose specimens were qualified for DNA extraction and sequencing (Fig. 1). Their medical information were collected from their medical records.



**Fig. 1.** Selection of study objects.

*BRCA*: breast cancer susceptibility gene

### Collection of tumor tissue samples

In this study, the 33 clinical samples of tumor tissue were obtained from 33 ovarian cancer patients after their surgery and were stored under formalin-fixed paraffin-embedded (FFPE) tumor tissue conditions at the Vietnam National Cancer Hospital (Hanoi, Vietnam). All FFPE tissue specimens in the research were collected under institutional review board approval by certified medical pathologists. Sample tissues were fixed in 10% neutral-buffered formalin (NBF) within 30 to 60 minutes of surgery excision. The samples were fixed for 18 – 24 hours at room temperature, subsequent embedded in Immunohistochemistry (IHC) - grade paraffin. Standard tissue sample size was 1x1x0.5cm, but can vary based on the nature of the disease or tissue type. FFPE tissues were stored at room temperature. FFPE blocks were cut to slices (5µm thick) and stained with hematoxylin & eosin (H&E) using the Dako Coverstainer system (Agilent, CA 95051, USA) for histopathological evaluation. Diagnostic pathology was made by more

than two pathologists based on the American Joint Committee on Cancer (AJCC, 2009) guidelines.

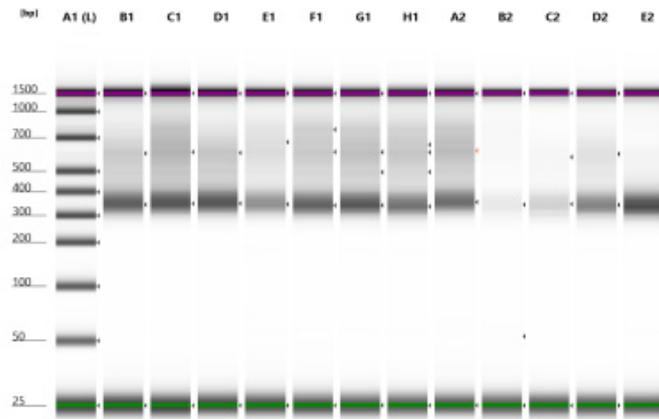
### DNA extraction and next generation sequencing

Genomic DNA was extracted from 5 to 10 sections of 10 µm thickness of macro-dissected ovarian tumor tissues using QIAamp DNA FFPE Tissue Kit (Qiagen, Valencia, CA, USA). The extracted samples contain at least 30% tumor cells. Quality and quantity of extracted DNA was measured using BioDrop UV-Visible spectrophotometer (Biochrom, United Kingdom). Furthermore, the double strand DNA was assessed by Qubit 4 Fluorometer (Invitrogen, Thermo Fisher Scientific Inc, MA, USA). Sample met the inclusion criteria was involved in this study.

BRCAaccuTest™ PLUS kit (NGeneBio Co., Ltd, South Korea) was utilized for library preparation of *BRCA1* and *BRCA2* (transcript accession of NM\_007294.3 and NM\_000059.3, respectively). A total of 100ng of high-quality genomic DNA was used for two separate library preparation reactions

following the manufacturer's protocol. DNA library was validated by Agilent 4200 TapeStation D1000

ScreenTape Assay. The size of the prepared DNA library was checked by size marker (Fig. 2).



**Fig. 2.** TapeStation gel image of DNA library preparation for sequencing.

*DNA: damaged deoxyribonucleic acid*

The length DNA library segments must be between 300 to 500 bp. The size of the prepared DNA library was confirmed through the horizontal axis of the graph. If there was an abnormal peak of small size (under 200 bp, dimer of primer), the library had been re-prepared since it may affect the analysis result even though the size of the main peak appeared within the normal range (300 ~ 500 bp).

MiSeqDx instrument (Illumina, Inc. USA) and MiSeq Reagent Micro v2 (300 cycles) (Illumina, San Diego, CA, USA) were used for NGS. 3 sequencing runs were performed, each run included 1 control DNA and 11 somatic samples. More detail was provided in our previous work<sup>(16)</sup>.

### **Data analysis**

Raw sequencing data under FASTQ format were experienced the following pipeline workflow of bioinformatics: quality control of data, trimming adapter, alignment, variant calling and annotation. Raw data was firstly checked by FASTQC. The Sickle v1.33 tool was used to trim sequencing adapters and low Q-score base (the cut-off threshold of Q-score was  $Q\text{-score} \geq 30$ ), then the Burrows–Wheeler

aligner-maximum exact matches (BWA-MEM) v0.7.10 aligned processed sequences to the Human hg19 genome as reference genome. The unmapped reads were extracted (mapping quality (MAPQ) < 60). Both single nucleotide variants and short indels (insertion/deletion) were identified by FreeBayes v9.9.2 and GATK v2.3. Variant annotation was performed by snpEff v4.2, which all were included in NGeneAnalySys™ software (NGeneBio Co., Ltd, South Korea). MEGA v.11.0.13 was also utilized for visualization and alignment. Variants were categorized based on the five-tier criteria of the American College of Medical Genetics and Genomics (ACMG) standards and guidelines. In this study, pathogenic and likely pathogenic variants were mostly focused on. Copy number variant (CNV) detection was not recommended by the manufacturer.

Statistics were performed by SPSS v.22.0 (SPSS Inc., Chicago, Illinois). Quantitative data were presented in mean and standard deviation, while qualitative data were presented in frequency and percentage. Fisher's exact test and Wilcoxon test were performed to compare several features between two groups.

# Results

## Characteristics of selected subjects

Our study involved 33 patients divided into the high-risk group (n = 22) and the low-risk group (n = 11) (Table 1). The mean age of diagnosis among studied patients was 49.06 ± 14.79 years, and the mean value of body mass index (BMI) was 21.15 ± 2.45 kg/m<sup>2</sup>. Only 2 patients whose families had cancer patients, and both of them were in the high-risk group (accounted for 6.06%). No statistically significant difference was found between the high-risk group and low-risk group in age, BMI, patient's medical history as well as their family history, and patient's comorbidities. Regarding pathological classification, our results showed that most of the studied patients had epithelial cancer, which accounted for at least 76.00% of all patients. The prevalence of non-epithelial ovarian cancer patients

among the low-risk group was significantly higher than in the high-risk group. Statistics also indicated that patients in the low-risk group were diagnosed to be at an earlier stage than those in the high-risk group (p = 0.003). Most of the patients in the high-risk group were at advanced stages (stage III and stage IV) when diagnosed with a prevalence of 63.64%, while most of the patients in the low-risk group were at stage I of diagnosis with the same prevalence (63.64%).

## BRCA1 and BRCA2 variants

The variant calling results showed that a total of 323 variants were recorded in 33 patients. After removing overlapped variants and several variants that are ubiquitous variants of the Asian population, including 4563A>G, c.6513G>C, and c.7397T>C in BRCA2<sup>(17)</sup>, 33 distinguished variants were found in BRCA1/2 (Table 2).

**Table 1.** Characteristics of selected subjects.

Features		High risk group		Low risk group		Total		p value
	Age*	47.09	3.67	53.00	2.21	49.06	14.79	0.349
	BMI*	21.44	0.52	20.64	1.03	21.15	2.45	0.571
Tumor pathological classification**	Epithelial cancer	17	77.27%	8	72.73%			0.041
	Non-epithelial cancer	2	9.09%	3	27.27%			
	Not mentioned	3	13.64%	0	0.00%			
Stage**	I	3	13.64%	7	63.64%	10	30.30%	0.003
	II	1	4.55%	2	18.18%	3	9.09%	
	III	13	59.09%	1	9.09%	14	42.42%	
	IV	1	4.55%	0	0.00%	1	3.03%	
	Not mentioned	4	18.18%	1	9.09%	5	15.15%	
Family history**	Having cancer patient	2	9.09%	0	0.00%	2	6.06%	0.542
	Not having cancer patient	20	90.91%	11	100.00%	31	75.76%	
Comorbidities**	Diabetes	0	0.00%	1	9.09%	1	3.03%	0.261
	Hypertension	2	9.09%	2	18.18%	4	12.12%	
	None	20	90.91%	8	72.73%	28	84.85%	
Medical history**	Ovarian cyst	1	4.55%	1	9.09%	2	6.06%	0.592
	Cancer	1	4.55%	0	0.00%	2	6.06%	
	None	20	90.91%	10	90.91%	29	87.88%	
BRCA1/BRCA2 variants**	Benign	22	100.00%	11	100.00%	6	18.18%	0.637
	Uncertain significance	9	40.91%	6	54.55%	15	45.45%	
	Pathogenic	2	9.09%	1	9.09%	3	9.09%	
	Likely pathogenic	3	13.64%	0	0.00%	3	9.09%	

\*Data are presented as mean ± standard deviation and %; and \*\*Data are presented as n and %  
BMI: body mass index, BRCA: breast cancer susceptibility gene

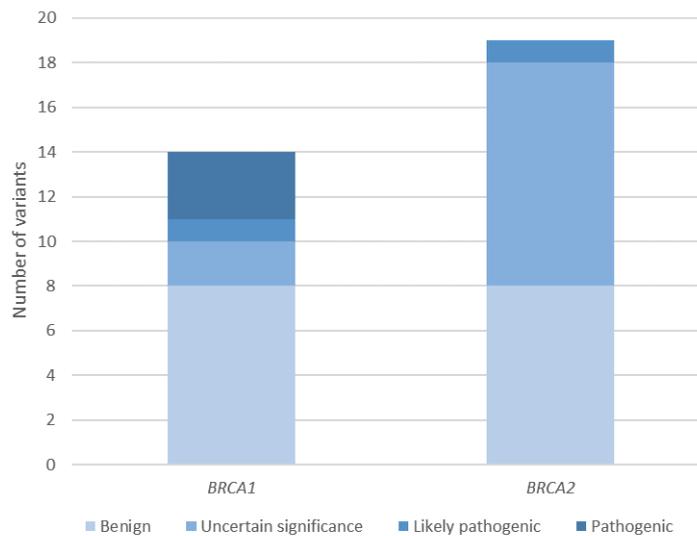
**Table 2.** Characteristics of *BRCA1* and *BRCA2* variants in ovarian cancer patients.

No.	NT Change	Frequency	Pathogenicity	Gene	Type	Consequence	Exon	dbSNP ID	Zygosity	AA Change
1	c.4837A>G	48.48%	B	BRCA1	snv	missense	15/23	rs1799966	heterozygote	p.Ser1613Gly
2	c.4308T>C	48.48%	B	BRCA1	snv	synonymous	12/23	rs1060915	heterozygote	p.Ser1436Ser
3	c.3548A>G	48.48%	B	BRCA1	snv	missense	10/23	rs16942	heterozygote	p.Lys1183Arg
4	c.3113A>G	48.48%	B	BRCA1	snv	missense	10/23	rs16941	heterozygote	p.Glu1038Gly
5	c.2612C>T	48.48%	B	BRCA1	snv	missense	10/23	rs799917	heterozygote	p.Pro871Leu
6	c.2311T>C	48.48%	B	BRCA1	snv	synonymous	10/23	rs16940	heterozygote	p.Leu771Leu
7	c.2082C>T	48.48%	B	BRCA1	snv	synonymous	10/23	rs1799949	heterozygote	p.Ser694Ser
8	c.114G>A	6.06%	B	BRCA1	snv	synonymous	3/23	rs1800062	heterozygote	p.Lys38Lys
9	c.1114A>C	42.42%	B	BRCA2	snv	missense	10/27	rs144848	heterozygote	p.Asn372His
10	c.3396A>G	60.61%	B	BRCA2	snv	synonymous	11/27	rs1801406	heterozygote	p.Lys1132Lys
11	c.7242A>G	60.61%	B	BRCA2	snv	synonymous	14/27	rs1799955	heterozygote	p.Ser2414Ser
12	c.865A>C	18.18%	B	BRCA2	snv	missense	10/27	rs766173	heterozygote	p.Asn289His
13	c.1365A>G	18.18%	B	BRCA2	snv	synonymous	10/27	rs1801439	heterozygote	p.Ser455Ser
14	c.2229T>C	18.18%	B	BRCA2	snv	synonymous	11/27	rs1801499	heterozygote	p.His743His
15	c.2971A>G	18.18%	B	BRCA2	snv	missense	11/27	rs1799944	heterozygote	p.Asn991Asp
16	c.3807T>C	27.27%	B	BRCA2	snv	synonymous	11/27	rs543304	heterozygote	p.Val1269Val
17	c.1801_1808delCACAATTC	6.06%	LP	BRCA1	del	frameshift	10/23	New	homozygote	p.His601fs
18	c.2865delC	3.03%	LP	BRCA2	del	frameshift	11/27	New	heterozygote	p.Asn955fs
19	c.1016delA	3.03%	P	BRCA1	del	frameshift	10/23	rs80357569	heterozygote	p.Lys339fs
20	c.1673_1674delAA	3.03%	P	BRCA1	del	frameshift	10/23	rs80357600	heterozygote	p.Lys558fs
21	c.928C>T	3.03%	P	BRCA1	snv	stop_gained	10/23	rs397509338	heterozygote	p.Gln310*
22	c.825C>T	3.03%	US	BRCA1	snv	synonymous	10/23	rs397509328	heterozygote	p.Gly275Gly
23	c.2566T>C	6.06%	US	BRCA1	snv	missense	10/23	rs80356892	heterozygote	p.Tyr856His
24	c.1362A>G	6.06%	US	BRCA2	snv	synonymous	10/27	rs55919657	heterozygote	p.Lys454Lys
25	c.6322C>T	3.03%	US	BRCA2	snv	missense	11/27	rs55794205	heterozygote	p.Arg2108Cys
26	c.8187G>T	6.06%	US	BRCA2	snv	missense	18/27	rs80359065	heterozygote	p.Lys2729Asn
27	c.5785A>G	3.03%	US	BRCA2	snv	missense	11/27	rs79538375	homozygote	p.Ile1929Val
28	c.943T>A	3.03%	US	BRCA2	snv	missense	10/27	rs79483201	heterozygote	p.Cys315Ser
29	c.3445A>G	3.03%	US	BRCA2	snv	missense	11/27	rs80358589	heterozygote	p.Met1149Val
30	c.2133C>T	3.03%	US	BRCA2	snv	synonymous	11/27	rs535547513	heterozygote	p.Cys711Cys
31	c.2920G>A	3.03%	US	BRCA2	snv	missense	11/27	rs539613324	heterozygote	p.Asp974Asn
32	c.3420T>C	3.03%	US	BRCA2	snv	synonymous	11/27	rs118093942	heterozygote	p.Ser1140Ser
33	c.7052C>G	3.03%	US	BRCA2	snv	missense	14/27	rs80358932	heterozygote	p.Ala2351Gly

\*B: Benign, US: Uncertain significance, LP: Likely pathogenic, P: Pathogenic

Of the 33 above variants, 14 variants were detected in the *BRCA1* gene (accounted for 38.8%), while this figure for *BRCA2* was 61.1%. Only 4 variants were deletions, which accounted for 12.12%, while the remaining were single nucleotide variants. About 45.45% of variants were missense, followed by synonymous variants with 39.39%. Only 4 variants were frameshift, and 1 variant was a stop variant. Most of the *BRCA1* variants were detected on exon 10 over 23 exons of *BRCA1*, while each of exons 3, 12, and

15 had 1 variant. Variants in *BRCA2* were found in exons 10, 11, 14, and 18 over 27 exons of *BRCA2*. However, almost 60% of them were on exon 11. Except for c.5785A>G in *BRCA2* and c.1801\_1808delCACAATTC in *BRCA1*, all variants were heterozygote (Table 2). According to ACMG classification, the percentage of pathogenic and likely pathogenic was 15.15%, while this figure for benign was 48.48%, followed by uncertain significance with 36.36% (Fig. 3).



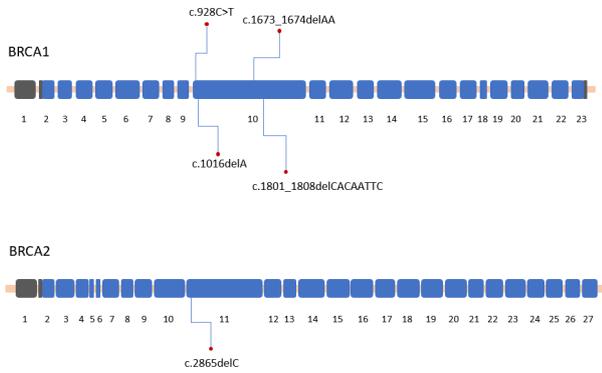
**Fig. 3.** The distribution of 4 variant types among *BRCA1* and *BRCA2* detected variants.  
*BRCA*: breast cancer susceptibility gene

Regarding the high-risk and low-risk group, although we found *BRCA1* and *BRCA2* benign variants in all 33 patients (4563A>G, c.6513G>C, and c.7397T>C variants in *BRCA2* were not taken into account), uncertain significance variants were detected in 9 patients of the high-risk group (40.91%) and 6 patients of the low-risk group (54.55%) (Table 1). Pathogenic and likely pathogenic (P/LP) variants were found in more than 20% of the high-risk group while this figure for the low-risk group was 9.09%. However, statistical analysis found no significant difference

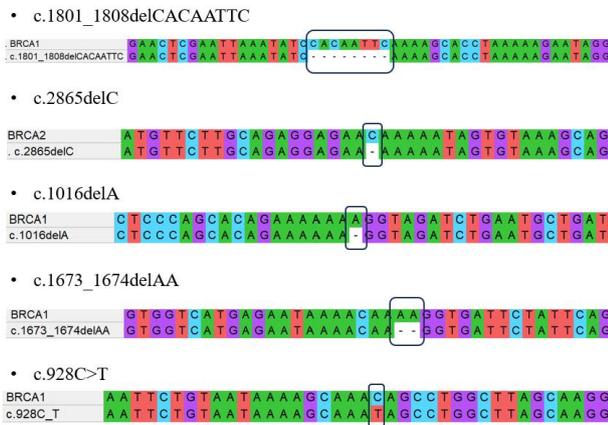
in the prevalence of variants between the high-risk and low-risk groups.

Pathogenic and likely pathogenic variants of *BRCA1* and *BRCA2* genes

Of 33 variants detected in *BRCA1* and *BRCA2* genes among 33 ovarian cancer patients involved in this study, 5 variants were classified as P/LP variants in the *BRCA1/BRCA2* genes in 6 out of 33 patients (18.18%), including c.1801\_1808delCACAATTC, c.1016delA, c.1673\_1674delAA, c.928C>T in *BRCA1* and c.2865delC in *BRCA2* (Fig. 4 and Fig. 5).



**Fig. 4.** Pathogenic and likely pathogenic variants of BRCA1 and BRCA2.



**Fig. 5.** Sequence data of pathogenic and likely pathogenic variants in BRCA1 and BRCA2 genes.

Among them, four were frameshift and 1 was stop-gained. All detected pathogenic variants were presented in the coding regions, particularly, in *BRCA1* exons 10 and in *BRCA2* exon 11. The c.1801\_1808delCACAATTC was detected in 2 patients with a frequency of 6.06%, while others was detected in one patient with a frequency of 3.03%. c.1016delA, c.1673\_1674delAA, and c.928C>T variants were reported in previous publication, while c.1801\_1808delCACAATTC variant in *BRCA1* and c.2865delC in *BRCA2* were firstly recorded (Table 3). c.1801\_1808delCACAATTC was also the unique homozygote variant among 33 detected variants.

**Table 3.** Characteristics of BRCA1 and BRCA2 pathogenic and likely pathogenic variants in ovarian cancer patients.

Patient ID	NT Change	AA Change	Frequency	Pathogenicity	Gene	Exon	Type	Consequence	Start Position	Fraction	Depth	Zygoty	Note
OC13, OC26	c.1801_1808delCACAATTC	p.His601fs	6.06%	LP	BRCA1	10/23	del	FS	41245739	2.18	3984	homozygote	New
OC27	c.2865delC	p.Asn955fs	3.03%	LP	BRCA2	11/27	del	FS	32911356	45.79	439	heterozygote	New
OC29	c.1016delA	p.Lys339fs	3.03%	P	BRCA1	10/23	del	FS	41246531	55.99	1704	heterozygote	rs80357569
OC36	c.1673_1674delAA	p.Lys558fs	3.03%	P	BRCA1	10/23	del	FS	41245873	49.21	1331	heterozygote	rs80357600
OC48	c.928C>T	p.Gln310*	3.03%	P	BRCA1	10/23	snv	SG	41246620	56.15	1560	heterozygote	rs397509338
31	c.2920G>A	3.03%	US	BRCA2	snv	missense	11/27	rs539613324	heterozygote	p.Asp974Asn			
32	c.3420T>C	3.03%	US	BRCA2	snv	synonymous	11/27	rs118093942	heterozygote	p.Ser1140Ser			
33	c.7052C>G	3.03%	US	BRCA2	snv	missense	14/27	rs80358932	heterozygote	p.Ala2351Gly			

\*FS: Frameshift; SG: Stop-gained

### Clinical features and BRCA1/BRCA2 variants

The mean age of patients who did not carry P/LP variants in *BRCA1/2* was significantly lower than those having P/LP variants, which was  $46.44 \pm 2.72$  versus  $60.83 \pm 5.17$  years (Table 4). Although all patients having P/LP variants were classified as epithelial cancer patients, no statistical relationship was found between the tumor pathological features and the status of variant carrying. There are 5 over 6 P/LP variant-

carried patients (accounting for 83.34%) who were diagnosed to be in the advanced stage and only 1 patient in stage II. No P/LP variants in *BRCA1* and *BRCA2* genes were found in stage I patients. Of 33 patients, we did not find any P/LP variants in the 2 patients whose families had cancer patients. No statistically significant difference was found between non-variant and variant patients in BMI, pathological classification, diagnosed stage, their families, and their medical history (Table 4).

**Table 4.** Clinical features between non-P/LP variant-carried patients and P/LP variant-carried patients.

Features		Non-P/LP variant carrying		P/LP variant carrying		p value
	Age*	46.44	2.72	60.83	5.17	0.019
	BMI*	21.38	0.56	19.94	0.8	0.289
Tumor pathological classification**	Epithelial cancer	19	70.37%	6	100.00%	0.167
	Non-epithelial cancer	5	18.52%	0	0.00%	
	Not mentioned	3	11.11%	0	0.00%	
Stage**	I	10	37.04%	0	0.00%	0.066
	II	2	7.41%	1	16.67%	
	III	10	37.04%	4	66.67%	
	IV	0	0.00%	1	16.67%	
	Not mentioned	5	18.52%	0	0.00%	
Family history**	Having cancer patient	2	7.41%	0	0.00%	1
	Not having cancer patient	25	92.59%	6	100.00%	
Comorbidities**	Diabetes	1	3.70%	0	0.00%	0.017
	Hypertension	3	11.11%	1	16.67%	
	None	23	85.19%	5	83.33%	
Medical history**	Ovarian cyst	2	7.41%	0	0.00%	0.1
	Cancer	0	0.00%	2	33.33%	
	None	25	92.59%	4	66.67%	

\* Data are presented as mean  $\pm$  standard deviation and %, \*\*Data are presented as n and %  
BMI: body mass index, P/LP: pathogenic and likely pathogenic

## Discussion

Among a total of 33 ovarian cancer recruited in our study, the *BRCA1/2* variant frequency was 18.18%, which was within the range of variant frequency in ovarian patients of different ethnicities (5-30%)<sup>(18)</sup>. Although more variants were found in patients with high risk of variants compared to low

risk group (5/22 versus 1/11), this difference was not statistically significant ( $p = 0.076$ ). This result was corresponding to our classifications of patients into high-risk and low-risk of variants.

Using NGS, five *BRCA1/2* deleterious variants have been identified, including two new variants. All four *BRCA1* variants are located in coding regions of

exon 10. Mutations of the central portion of this exon are widely known for their association with an increased risk of ovarian cancer<sup>(19)</sup>. The remaining variant is in coding regions of exon 11 of *BRCA2* gene which is also associated with higher ovarian cancer risk than other regions of the gene<sup>(19)</sup>. Two variants found, c.1673\_1674delAA and the novel variant c.1801\_1808delCACAATTC are located in the ovarian cancer cluster region (OCCR) of *BRCA1* gene, which is understandable since the incidence of ovarian cancer is significantly high in patients with a *BRCA* variant in the OCCR<sup>(20)</sup>. It also means that these variants have higher risk of ovarian cancer than those in other regions<sup>(21)</sup>. Among five variants, c.1016delA was also detected in another study on Vietnamese patients with ovarian cancer<sup>(21)</sup>. Additionally, such variant was presented in other populations including Canada, the USA, Norway and Czech Republic according to the database of NCBI (dbSNP). This database also showed that besides Vietnam, variant named c.928>T was described in Taiwanese people, another Asian population.

The interaction between germline and somatic variants in the *BRCA1* gene was reported in a study by Kanchi et al (2014), the authors also suggested that analyzing both germline and somatic variants could identify notably altered pathways in ovarian cancer<sup>(22)</sup>. Although the sequencing was performed using FFPE samples instead of blood samples, we did not detect any somatic variants (data not shown). All detected variants were germline. This result was consistent with the findings in a study by Mafficini et al (2016), in which 11/13 pathogenic variants were germline<sup>(23)</sup>. According to Alsop et al (2012), having a *BRCA1/BRCA2* germline mutation even without any family history of ovarian cancer was still alert for ovarian cancer risk<sup>(24)</sup>.

Two novel variants, c.1801\_1808delCACAATTC and c.2865delC variants, have not been described in public databases. The prior variant located in exon 10 of the OCCR causes a sequence frameshift involving amino acid number 601, histamine. Meanwhile, c.2865delC was the only identified

*BRCA2* variant in our study. The deletion of nucleotide cytosine leads to the alteration of asparagine at the position of 955 in the polypeptide chain. The pathogenesis of this variant is remaining unknown since it is not located in *BRCA2* OCCR or any protein binding sites.

We observed that all likely pathogenic and pathogenic variants were found in patients diagnosed with epithelial cancer. Such finding is reasonable since germline variants in *BRCA1/2* genes accounted for up to 18% of epithelial cancer cases and somatic alterations are observed in another 7%<sup>(20)</sup>. It has raised a new hope for patients with epithelial cancer because *BRCA1/2* variants are related to higher sensitivity to platinum regimens and other chemotherapeutic agents and therefore, better prognosis. Patients with *BRCA1/2* variants, especially *BRCA1/2* somatic variants are particularly sensitive to PARPi and DNA damaging agents such as platinum-based compounds possibly due to the precedence of error-prone DNA-repair pathways of *BRCA*-altered cells and the presence of homologous recombination deficiency (HRD)<sup>(25)</sup>. Therefore, HRD testing is currently crucial for ovarian cancer patient management, especially when solid evidence has shown ovarian patients with HRD tumors gain greater benefit from PARPi treatment than patients with non-HRD tumors<sup>(26)</sup>.

Our study results also revealed that *BRCA*-variant carriers were older than non-variant carriers at the time of diagnosis ( $p < 0.05$ ). However, Hamdi et al stated that early age at onset was significantly associated with *BRCA* status in a study on breast and ovarian cancer patients in Tunisia<sup>(27)</sup>. Another study in China showed that no significant differences were found in onset age<sup>(28)</sup>. The discrepancy in the results of such papers might be because of differences in ethnicity and most of variants in our study being germline mutations. The mean age of cancer diagnosis for the variant carrier group in our study was  $60.83 \pm 5.17$  years. It was in agreement with another study in the Denmark population in which the mean age of diagnosis for *BRCA1* and *BRCA2*-

variant carriers were  $61 \pm 1.59$  and  $60 \pm 0.54$  years, respectively<sup>(29)</sup>. Besides, other clinical features including tumor pathology, FIGO stage, family history, BMI, comorbidities and medical history had no association with the status of variants.

There were several limitations in our study. Firstly, the sample size of our study was relatively small, so we could not find a large number of variants as well as founder variants for Vietnamese population. However, to date, there have been a few studies on *BRCA1/2* variants in Vietnam and our study was among those with adequate sample size. We also detected variants in tumors of high-risk of mutation patients to maximize the number of possible variants detected. Our study met the same challenge as several previous studies in CNV analysis using NGS<sup>(30)</sup>, moreover, the FFPE samples used in our study are not recommended by manufacturers in CNV detection. Besides, NGS results are commonly confirmed by using Sanger sequencing afterwards. Nevertheless, Kim et al proved that NGS applied to analyze data from *BRCA*accuTest™ (NGeneBio), which we used as an in vitro diagnostic reagent, provided 100% overall diagnostic consistency with Sanger sequencing<sup>(17)</sup>.

## Conclusion

Our study provides new insights into the genetic landscape of ovarian cancer patients in Vietnamese population. 18.18% of our Vietnamese cohort carried *BRCA1/2* variants, which was comparable to global statistics. Five *BRCA1/2* P/LP variants had been detected, including two novel variants that have not been described in public databases. All five variants are in coding regions and cause premature termination of translation. One of the newly detected variants is located in OCCR of *BRCA1* gene, which produces more evidence to support the role of these regions in the pathogenesis of ovarian cancer. Age of onset was significantly associated with the status of *BRCA1/2* variants, with *BRCA*-variant carriers being older than non-variant group, which was different from other previous work. All five deleterious *BRCA1/2* variants

were detected in patients with epithelial cancer, which benefits the results of chemotherapeutics in these patients. Research in *BRCA1/2* mutational spectrum in Vietnamese population helps to not only support early detection of the disease, but only develop personalized treatments for *BRCA*-related diseases, especially for ovarian cancer.

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## Ethics approval

The research was approved by the Institute of Genome Research Institutional Review Board according to the decision number: 02-2022/NCHG-HĐĐĐ on March 09, 2022.

## Potential conflicts of interest

The authors declare no conflicts of interest.

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## GYNAECOLOGY

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# Prevalence and Associated Factors of Iron- Deficient Erythropoiesis in Thai Pregnant Women

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### ABSTRACT

**Objectives:** The primary objective was the prevalence of iron-deficient erythropoiesis (IDE) in Thai pregnant women during early pregnancy, with the identification of associated factors as the secondary goal.

**Materials and Methods:** A cross-sectional survey was conducted on pregnant women aged  $\geq 20$  years, who visited the antenatal clinic during the first 14 weeks of gestation without anemia. IDE was defined as hemoglobin level  $\geq 11$  g/dL and serum ferritin  $< 30$  ng/mL.

**Results:** A total of 130 pregnant women were enrolled. IDE was detected in 10.77% (95% confidence interval 6.26 - 16.83). In univariate analysis, various factors with p value of  $\leq 0.2$  were identified. From multivariate analysis of these factors, the number of pregnancies (parity  $\geq 2$ ) was significantly associated with IDE (adjusted odds ratio 5.728, 95% confidence interval 1.019 - 32.210,  $p = 0.048$ ).

**Conclusion:** Out of every 100 pregnant women without anemia, 10 had IDE in early pregnancy. Multiparity emerged as the most significant factor for IDE.

**Keywords:** Iron-deficient erythropoiesis, iron deficiency anemia, serum ferritin, pregnant women.

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# ความชุกและปัจจัยที่เกี่ยวข้องของการมีธาตุเหล็กสะสมในร่างกายต่ำแต่ยังไม่มีภาวะโลหิตจางในหญิงตั้งครรภ์ชาวไทย

ภาลินรัตน์ วิทยานเศรษฐ์, ชลลดา เหล่าเรืองโรจน์, ศักดิ์ชัย พานิชวงษ์

## บทคัดย่อ

**วัตถุประสงค์:** วัตถุประสงค์หลักเพื่อหาความชุกของการมีธาตุเหล็กสะสมในร่างกายต่ำแต่ยังไม่มีภาวะโลหิตจางในช่วงแรกของการตั้งครรภ์ของหญิงตั้งครรภ์ชาวไทย และปัจจัยที่เกี่ยวข้องเป็นวัตถุประสงค์รอง

**วัสดุและวิธีการ:** การศึกษาภาคตัดขวางในหญิงตั้งครรภ์ที่อายุมากกว่าหรือเท่ากับ 20 ปี ที่มาเข้ารับการฝากครรภ์ในช่วงอายุครรภ์ 14 สัปดาห์แรกที่ไม่มีการใช้ยา โดยนิยามของการมีธาตุเหล็กสะสมในร่างกายต่ำแต่ยังไม่มีภาวะโลหิตจางคือระดับฮีโมโกลบินในเลือดมากกว่าหรือเท่ากับ 11 กรัม/ดล. และค่าซีรีรัมเฟอริตินน้อยกว่า 30 นาโนกรัม/มล.

**ผลการศึกษา:** ผู้เข้าร่วมการวิจัยทั้งสิ้น 130 ราย พบภาวะธาตุเหล็กสะสมในร่างกายต่ำแต่ยังไม่มีภาวะโลหิตจางคิดเป็นร้อยละ 10.77 (95% confidence interval 6.26 - 16.83) จากการวิเคราะห์ข้อมูลแบบตัวแปรเดียวพบปัจจัยที่มีค่า  $p < 0.2$  จำนวนหลายปัจจัย และเมื่อนำปัจจัย เหล่านั้น มาวิเคราะห์ข้อมูลแบบหลายตัวแปร พบว่าการมีบุตรมากกว่าหรือเท่ากับสองคน มีความสัมพันธ์ต่อการเกิดธาตุเหล็กสะสมในร่างกายต่ำอย่างมีนัยสำคัญทางสถิติ (adjusted odds ratio 5.728, 95% CI 1.019 -32.210,  $p = 0.048$ )

**สรุป:** ในหญิงตั้งครรภ์จำนวน 100 รายที่ไม่มีการใช้ยา มีโอกาสพบธาตุเหล็กสะสมในร่างกายต่ำแต่ยังไม่มีภาวะโลหิตจางประมาณ 10 ราย ในช่วงแรกของการตั้งครรภ์ ซึ่งปัจจัยเสี่ยงที่สำคัญคือ การตั้งครรภ์มากกว่าหรือเท่ากับ 2 ครั้ง

**คำสำคัญ:** ธาตุเหล็กสะสมในร่างกายต่ำแต่ยังไม่มีภาวะโลหิตจาง, โลหิตจางจากการขาดธาตุเหล็ก, ค่าซีรีรัมเฟอริติน, หญิงตั้งครรภ์

## Introduction

Iron deficiency is the most global nutritional deficiency disorder, impacting more than 2 billion individuals<sup>(1)</sup>. Although pregnant women and infants in developing countries are at heightened risk, comparable vulnerabilities are evident in developed nations as well. The World Health Organization (WHO) defines anemia in pregnant women as hemoglobin levels < 11 g/dL during the first trimester<sup>(2)</sup>. Adverse pregnancy outcomes, such as preeclampsia, preterm birth, and neurocognitive developmental issues are associated with iron deficiency anemia (IDA)<sup>(1)</sup>. Furthermore, infants born to mothers with IDA often have low birth weight<sup>(3)</sup>. Beyond its impact on pregnancy, IDA can lead to symptoms like fatigue, and impaired cognitive function in the women.

Iron-deficient erythropoiesis (IDE), also known as latent iron deficiency (LID) is a medical condition characterized by the presence of iron deficiency without concurrent anemia, as evidenced by normal hemoglobin level. Maintaining a balanced iron status is a crucial protective measure against IDA during pregnancy<sup>(4)</sup>. Individuals with negative iron status may progress to IDA due to an inability to support red blood cell synthesis during menstruation, pregnancy, and lactation<sup>(5)</sup>. Without inflammation, serum ferritin can be a key indicator for assessing iron status and IDE can be diagnosed when the ferritin level is below 30 ng/mL without anemia. It is also important to note that serum iron levels fluctuate throughout the day and should not be solely relied upon for diagnosis<sup>(6)</sup>.

A study conducted at Maharaj Nakorn Chiangmai Hospital in 648 pregnant women across all gestational age revealed a significant prevalence of IDA of 51.8%, with 43.8% of cases exhibiting serum ferritin levels of < 30 ng/mL<sup>(3)</sup>. Unfortunately, specific data on IDE was not provided. Another study from Naresuan University focusing on the prevalence and risk factors of IDA, which included 401 pregnant women, with and without anemia in

all gestational age, revealed 15.2% of IDE and serum ferritin levels < 15 ng/mL<sup>(7)</sup>. Additionally, a study from China with 4,000 participants included across all gestational ages reported a prevalence of 42.6% for IDE<sup>(8)</sup>. Notably, none of these studies identified the associated factors of IDE. Conversely, a study conducted in European Mediterranean countries focusing on 791 pregnant women before 12 weeks gestation with a hemoglobin level  $\geq$  11 g/dL reported a prevalence of 13.9% for iron deficiency with serum ferritin < 15 ng/mL. The associated factors included being underweight, multiparous, or a vegetarian diet<sup>(9)</sup>.

Despite its high prevalence, IDE is often unrecognized by clinicians due to suboptimal screening recommendations<sup>(6)</sup>. This research emphasizes the important aspect of detecting IDE before it progresses to IDA. Effective prevention of IDA can be achieved through iron supplementation. While many studies primarily focus on populations with established IDA, there is limited attention given to individuals at risk of developing IDA.

This research aimed to identify the prevalence and risk factors of IDE in early pregnancy. The results could be used to guide and generate a strategy for primary prevention of IDA in order to alleviate its consequences during the pregnancy period. Insights from this study can also suggest future investigations into appropriate iron therapy and its impact on newborn iron storage, promoting optimal care throughout pregnancy, post-gestation, and the postnatal periods.

## Materials and Methods

A cross-sectional study conducted at Phramongkutklo Hospital, approved by the Institutional Review Board of the Royal Thai Army Medical Department (R171h/65), enrolled 130 pregnant women aged  $\geq$  20 years in the first 14 weeks of gestation without anemia, no prior iron supplements, and without active systemic diseases. Anemia was defined as hemoglobin level < 11 g/

dL during the first trimester according to WHO criteria<sup>(2)</sup>. The sample size was calculated based on 42.6% prevalence of IDE in pregnant women from The Chinese Children, Pregnant Women & Premenopausal Women Iron Deficiency Epidemiological Survey Group (November 2004)<sup>(8)</sup> with tolerance of 20% of the prevalence. In this study, we utilized a serum ferritin threshold of < 30 ng/mL, based on a sensitivity of 92% and specificity of 98% for detecting iron deficiency<sup>(10)</sup>. Blood samples (7 ml from each woman) were collected for iron studies, accompanied by a questionnaire covering demographic data, menstruation details, dietary habits, and lifestyle factors. Menstruation regularity was categorized as monthly, tri-monthly and once in 5-6 months. Menstruation amount was evaluated by average number of menstrual pads used per day; unfortunately, menstrual duration data was not collected. The primary outcome was the prevalence of IDE, and the secondary outcome was the associated risk factors. Descriptive statistics included age, body mass index (BMI), education, residency, career, marital status, parity and income. Continuous data was categorized in mean  $\pm$  standard deviation (SD). A chi square was used to find associated factors. Statistical significance was set at p values < 0.05. The related factors and factors with p value < 0.2 were selected to analyze in multiple logistic regression with a confidence interval (CI) of 95%. All statistical analyses were performed using SPSS version 26.

## Results

Between February 2023 and May 2023, a total of 130 eligible pregnant women were enrolled

and analyzed. The mean age was  $29.89 \pm 5.73$  years, and the mean gestational age was  $9 \pm 2.23$  weeks. The majority held a bachelor's degree (56.92%). Most women resided in Bangkok (96.15%), and most worked as company employees (72.31%). Over half were primiparous (56.92%), and about half had an average monthly income ranging from 15,000 to 29,999 baht. The mean age of menarche of the women was  $13.08 \pm 1.55$  years with most having regular monthly menstruation, and over half using on average of  $\geq 3$  menstrual pads per day. Fourteen participants (10.77%) had a history of gastrointestinal tract bleeding and 10% of the women had a history of regular, monthly non-steroidal anti-inflammatory drugs (NSAIDs) use. Among the participants, one woman was a vegetarian (0.77%) and eight were weekly alcohol drinkers prior to pregnancy. Seventy percent had never donated blood, while 6.92% were regular blood donors, contributing up to four times a year (Table 1).

The mean hemoglobin level was  $12.40 \pm 0.79$  g/dL. The prevalence of IDE in the study population was 10.77% (95%CI 6.26 - 16.83). Potential associated factors were systematically organized in the questionnaire, as outlined in Table 2.

Univariate logistic regression analysis showed potential associated factors with p value of < 0.2 as follows: number of parities, income, average menstrual pad usage of  $\geq 3$ , and blood donation. Following the adjustment of odds ratios through multiple logistic regression, having had  $\geq 2$  deliveries emerged as a significant predictor (adjusted odds ratios (OR) 5.728, 95%CI 1.019 - 32.210, p = 0.048) (Table 3).

**Table 1.** Baseline Characteristics (n = 130).

	n	%		n	%
Age (years)			Menarche (years)		
Mean ± SD	29.89 ± 5.73		mean ± SD	13.08 ± 1.55	
Education			Menstrual regularity		
Below Bachelor's degree	56	43.08	Every month	113	86.92
Bachelor's degree	74	56.92	Every 3-4 months	16	12.31
Residence			Every 5-6 months	1	0.77
Bangkok	125	96.15	Average menstrual pads per day (pads)		
Other Provinces	5	3.85	1-2	53	40.77
Career			≥ 3	77	59.23
Government officer	20	15.38	History of gastrointestinal bleeding		
Employee	94	72.31	Yes	14	10.77
Others	16	12.31	No	116	89.23
Marital status			Frequency of NSAIDs use/**		
Single	36	27.69	Once a month	13	10.08
Married	93	71.54	More than a month	51	39.53
Divorced	1	0.77	More than a year	65	50.39
Number of parities			Dietary style		
0	74	56.92	Normal diet	129	99.23
1	46	35.38	Vegetarian	1	0.77
≥ 2	10	7.69	Alcohol consumption		
Income (Baht per month)			Weekly	8	6.15
< 15,000	19	14.62	Monthly	25	19.23
15,000 – 29,999	64	49.23	> Monthly	21	16.15
30,000 – 39,999	25	19.23	Never	76	58.46
40,000 – 49,999	7	5.38	History of blood donation		
≥ 50,000	15	11.54	Every 3 months	9	6.92
BMI (kg/m <sup>2</sup> )			Yearly	29	22.31
mean ± SD	22.77 ± 4.76		Never	92	70.77

\*\* n = 129

SD: standard deviation, BMI: body mass index, NSAIDs: non-steroidal anti-inflammatory drugs

**Table 2.** Univariable Analysis for Iron Deficiency (n = 130).

	No iron-deficient erythropoiesis (n = 116)	Iron-deficient erythropoiesis (n = 14)	p value
	n (%)	n (%)	
Age (years)			0.499
< 35	92 (90.20)	10 (9.80)	
≥ 35	24 (85.71)	4 (14.29)	
Education			0.986
Below bachelor's degree	50 (89.29)	6 (10.71)	
Bachelor's degree	66 (89.19)	8 (10.81)	
Number of parities			0.108
0	68 (91.89)	6 (8.11)	
1	41 (89.13)	5 (10.87)	
≥ 2	7 (70.00)	3 (30.00)	
Income (Baht per month)			0.003
< 15,000	15 (78.95)	4 (21.05)	
15,000 – 29,999	60 (93.75)	4 (6.25)	
30,000 – 39,999	24 (96)	1 (4)	
40,000 – 49,999	3 (42.86)	4 (57.14)	
≥ 50,000	14 (93.33)	1 (6.67)	
BMI (kg/m <sup>2</sup> )			0.722
≤ 18.5	21 (87.5)	3 (12.5)	
> 18.5	95 (89.62)	11 (10.38)	
Menstrual regularity			0.719
Every month	101 (89.38)	12 (10.62)	
Every 3-4 months	14 (87.5)	2 (12.5)	
Every 5-6 months	1 (100.00)	-	
Average menstrual pads per day (pads)			0.038*
1-2	50 (96.15)	2 (3.85)	
≥ 3	66 (84.62)	12 (15.38)	
History of gastrointestinal bleeding			0.648
Yes	12 (85.71)	2 (14.29)	
No	104 (89.66)	12 (10.34)	
Frequency of NSAIDs use**			0.245
Multiple times per year	55 (85.94)	9 (14.06)	
Less than once a year	60 (92.31)	5 (7.69)	
Blood donation			0.115
Yes	31 (81.58)	7 (18.42)	
No	85 (92.39)	7 (7.61)	

BMI: body mass index, NSAIDs: non-steroidal anti-inflammatory drugs

\*\*n = 129

**Table 3.** Multivariable analysis for iron deficiency (n = 130).

	Crude odds ratio (95%CI)	p value	Adjusted odds ratio (95%CI)	p value
Number of parities				
1	1.382 (0.397 - 4.817)	0.611	1.279 (0.348 - 4.701)	0.711
≥ 2	4.857 (0.991 - 23.802)	0.051	5.728 (1.019 - 32.210)	0.048
Income < 15,000 Baht/month	2.693 (0.749 - 9.688)	0.129	2.792 (0.712 - 10.954)	0.141
Average menstrual pads/day ≥ 3	4.545 (0.973 - 21.232)	0.054	3.358 (0.684 - 16.478)	0.136
History of blood donation	2.742 (0.890 - 8.450)	0.079	2.626 (0.780 - 8.838)	0.119

CI: confidence interval

## Discussion

This study aimed to identify the prevalence and associated factors of IDE in early pregnancy. The findings revealed a prevalence of 10.77%, aligning with similar studies reporting 15.2% in northern regions of Thailand<sup>(7)</sup> and 13.9% in Europe<sup>(9)</sup>.

In contrast, a study from China reported a notably higher prevalence of IDE at 42.6%. However, their extensive sample size of nearly 4,000 participants included pregnant women across all gestational ages<sup>(8)</sup>. It is noteworthy that half of the IDE cases in their study were observed in late pregnancy. Presumably, as pregnancy progresses, a growing number of women may become iron-deficient if not adequately supplemented. Further studies should dive into the incidence of IDA in pregnant women with IDE, exploring the efficacy of routine iron supplementation as a preventive measure for IDA in this population. These data could significantly contribute to refining guidelines for iron supplementation during pregnancy, particularly as a primary prevention strategy for IDA. Additionally, investigating children born to mothers with IDE/IDA could unveil potential long-term effects, including physical and cognitive developmental delays.

Among the analyzed maternal biomarkers, baseline demographics, and lifestyle conditions, multiparity (≥ 2 pregnancies) emerged as the most significant factor for IDE in early pregnancy, illustrating almost six times higher odds compared to women without or with only one prior delivery. However, it is worth recognizing that the 95%CI almost crosses 1,

indicating potential imprecision in the analysis. This finding aligned with a prior study conducted in Singapore, which examined the iron status of 985 Asian pregnant women during the first 14 weeks of gestation (cut-off ferritin level of < 30 ng/mL). Multiparity was linked to an increased risk of iron depletion (OR 1.73, 95%CI 1.23–2.44)<sup>(11)</sup>. A similar result was also observed in The United States National Health and Nutrition Examination Survey which involved 6,463 pregnant women of unknown gestational age, with a cut-off ferritin level of < 15 ng/mL (OR 7.39, 95%CI 3.10-17.59)<sup>(12)</sup>. It is explained by the high iron requirement of pregnancy, putting multiparous women at risk of not recovering iron stores between pregnancies<sup>(10,12,13)</sup>. Another statistically relevant factor was income, but the trend of association could not be demonstrated due to the uneven number of women in each income level. In contrast to other studies<sup>(9)</sup> where being underweight was a related factor, we found no association with IDE, likely due to the majority of participants being within the normal weight range. Vegetarian diet has been shown to play a significant role in recent studies affecting IDE<sup>(6, 9)</sup> by stating that iron absorption from meat diet is higher than from vegetarian diet<sup>(14,15)</sup>. However, as there was only one vegetarian in the current study, this issue could not be analyzed.

A significant proportion of women with IDE in early pregnancy are underdiagnosed, allowing their low iron stores to go undetected and untreated, given that IDE is asymptomatic. This is primarily because

hemoglobin, often the sole biomarker assessed for iron status in routine practice, is altered only in the late stages of iron deficiency when stores are further depleted<sup>(5,16)</sup>. Consequently, hemoglobin proves to be an ineffective biomarker for preventing iron deficiency in pregnancy. Although, serum ferritin is not commonly measured in clinical practice due to cost considerations, it is internationally recognized as a robust marker for assessing iron storage<sup>(6)</sup>. From this study, we propose adding a test for serum ferritin level in women without anemia in early pregnancy who had  $\geq 2$  prior deliveries.

The strength of this study lays in its contribution to bridging a gap in the limited available data by revealing the prevalence of IDE in early pregnancy in Thailand. This study emphasizes the need for assessment of iron reserves and proactive prevention especially in multiparous women to alleviate the consequences of IDE during pregnancy. Certain limitations should be considered, primarily the small sample size due to financial constraints, potentially limiting the generalizability of our findings. Future research should examine the long-term outcomes of IDE and appropriate iron supplement therapies.

## Conclusion

This research shed light on the prevalence and risk factors of IDE in early stages of pregnancy, revealing a rate of 10.77% and multiparity was the significantly associated factor for IDE.

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## Potential conflicts of interest

The authors declare no conflicts of interest.

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## GYNAECOLOGY

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# Prevalence of Endometrial Cancer in Patients with Endometrioid Intraepithelial Neoplasia Histology

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### ABSTRACT

**Objectives:** The primary objective was to ascertain the prevalence of concurrent endometrial cancer among patients initially diagnosed with endometrial intraepithelial neoplasia (EIN). The secondary objective was to assess the characteristics and risk factors associated with occult endometrial cancer in EIN patients.

**Materials and Methods:** This retrospective study analyzed cases spanning from 2015 to 2019 of patients diagnosed with EIN through various endometrial biopsy methods, including endometrial sampling, fractional curettage, or hysteroscopic biopsy. Demographic information and histological findings from hysterectomy specimens were collected to determine the prevalence of concurrent endometrial cancer. In the conservative group, patients were categorized as not having concurrent cancer if malignancy was not found in the 12-month follow-up period. Descriptive statistics were employed to evaluate the prevalence of concurrent endometrial cancer in EIN patients, alongside demographic and clinical characteristics. Multivariable logistic regression analysis was utilized to explore the association between demographics and clinical factors with concurrent endometrial cancer.

**Results:** A total of 130 EIN patients were included. The mean age of patients diagnosed with concurrent endometrial cancer was 47.02 (standard deviation (SD) 13.2) years and 52.23 (SD 10.9) years for those without endometrial cancer. The prevalence of concurrent endometrial cancer in EIN patients was 23.1%, (95% confidence interval 16.1 - 31.2). The prevalence of concurrent endometrial cancer in the surgical group was 32.5% and in the conservative group was 8%. All patients diagnosed with concurrent endometrial cancer exhibited uterine-confined disease, with no diagnoses of stage III or IV disease.

**Conclusion:** Approximately one-third to one-fourth of EIN patients diagnosed via endometrial sampling were found to have concurrent endometrial cancer. Notably, all patients diagnosed with concurrent endometrial cancer exhibited uterine-confined disease. We recommend using this data for counseling patients who have EIN in both surgical and conservative cases.

**Keywords:** atypical endometrial hyperplasia, concurrent endometrial cancer, endometrial carcinoma, endometrioid intraepithelial neoplasia (EIN), occult cancer.

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## ความชุกของมะเร็งเยื่อบุโพรงมดลูก ในผู้ป่วยที่มีผลตรวจเยื่อบุโพรงมดลูกเป็น Endometrioid Intraepithelial Neoplasia (EIN)

ธัญชนก สมปรารถนา, ญัฐฤกษ์ฤตา โพธิ์พรวัฒน์, สมสุข สันติเบญจกุล, ชินา โอฟารัตนพันธ์

### บทคัดย่อ

**วัตถุประสงค์:** วัตถุประสงค์หลักเพื่อศึกษาความชุกของมะเร็งเยื่อบุโพรงมดลูกในผู้ป่วยที่มีผลการตรวจชิ้นเนื้อจากโพรงมดลูกเป็น Endometrioid Intraepithelial Neoplasia (EIN) วัตถุประสงค์รอง เพื่อประเมินลักษณะและปัจจัยเสี่ยงในผู้ป่วยมะเร็งเยื่อบุโพรงมดลูกในผู้ป่วยที่มีผลการตรวจชิ้นเนื้อจากโพรงมดลูกเป็น EIN

**วัสดุและวิธีการ:** เป็นการศึกษาแบบย้อนหลัง ตั้งแต่ปี พ.ศ.2558 ถึง 2562 โดยรวบรวมผู้ป่วยที่มีผลการตรวจชิ้นเนื้อจากโพรงมดลูกเป็น EIN ซึ่งจะเก็บทั้งการส่องกล้องตัดชิ้นเนื้อจากโพรงมดลูก (endometrial biopsy) การขูดมดลูกแบบแยกส่วน (Fractional curettage) และการส่องกล้องตัดชิ้นเนื้อในโพรงมดลูก (Hysteroscopic biopsy) โดยเก็บข้อมูลพื้นฐานของผู้ป่วยและใช้ผลพยาธิวิทยาของชิ้นเนื้อที่ได้จากการตัดมดลูกมาวิเคราะห์ว่ามีมะเร็งซ่อนอยู่หรือไม่ ในกลุ่มที่ได้รับการรักษาแบบรังภาวะเจริญพันธุ์ จะถือว่าไม่มีมะเร็งซ่อนอยู่เมื่อตรวจติดตามไปมากกว่า 1 ปี แล้วไม่พบหลักฐานว่ามีมะเร็งซ่อนอยู่ ข้อมูลพื้นฐานของผู้ป่วย รวมทั้งข้อมูลทางคลินิก และผลทางพยาธิวิทยาจะถูกรวบรวมและนำมาวิเคราะห์หาความชุกของการมีมะเร็งเยื่อบุโพรงมดลูกซ่อนอยู่ และความสัมพันธ์ของปัจจัยกับการมีมะเร็งเยื่อบุโพรงมดลูกซ่อนอยู่

**ผลการศึกษา:** การศึกษานี้รวบรวมผู้ป่วยที่มีผลการตรวจโพรงมดลูกเป็น EIN จำนวน 130 ราย อายุเฉลี่ยของผู้ป่วยกลุ่มที่มีมะเร็งซ่อนอยู่คือ 47.02 ปี (SD 13.2) และกลุ่มที่ไม่มีมะเร็งซ่อนอยู่คือ 52.23 ปี (SD 10.9) ความชุกของการมีมะเร็งซ่อนอยู่คือ ร้อยละ 23.1 (95%CI : 16.1-31.2) โดยความชุกของการมีมะเร็งซ่อนอยู่ในกลุ่มที่ได้รับการตัดมดลูกคือ ร้อยละ 32.5 และกลุ่มที่ได้รับการรักษาแบบรังภาวะเจริญพันธุ์ คือ ร้อยละ 8 ผู้ป่วยที่มีมะเร็งซ่อนอยู่ทั้งหมดนั้นยังไม่มีกระจายออกนอกตัวมดลูก และไม่พบผู้ป่วยมะเร็งเยื่อบุโพรงมดลูกระยะที่ 3-4 ในการศึกษา

**สรุป:** หนึ่งในสาม ถึง หนึ่งในสี่ของผู้ป่วยที่มีผลการตรวจเยื่อบุโพรงมดลูกเป็น EIN จะมีมะเร็งเยื่อบุโพรงมดลูกซ่อนอยู่ และในกลุ่มนั้น รอยโรคจะยังอยู่ในตัวมดลูก ข้อมูลดังกล่าวเป็นข้อมูลสำคัญที่ควรให้แก่ผู้ป่วยก่อนเริ่มรักษาทั้งในกลุ่มที่ตัดมดลูก

**คำสำคัญ:** ภาวะเยื่อบุโพรงมดลูกหนาตัวชนิด Atypical endometrial hyperplasia, การพบมะเร็งร่วม, มะเร็งเยื่อบุโพรงมดลูก, ภาวะเยื่อบุโพรงมดลูกหนาตัวชนิด Endometrioid intraepithelial neoplasia (EIN), การมีมะเร็งซ่อนอยู่

## Introduction

Endometrial cancer is the most common gynecologic cancer in developed countries. The incidence of endometrial cancer has continuously increased<sup>(1)</sup>. Endometrioid intraepithelial neoplasia (EIN) in the World Health Organization (WHO) classification of endometrial hyperplasia, is considered a precursor of endometrial cancer. Endometrial hyperplasia is a disorder induced by prolonged unopposed endogenous or exogenous estrogen exposure<sup>(2,3)</sup>.

The pathology of endometrial hyperplasia is defined by an increase in gland and stroma ratio that resulting in abnormal architecture and cytologic features of the endometrial gland. Endometrial hyperplasia is diagnosed using various criteria. According to the WHO 1994 classification, endometrial hyperplasia is classified as simple hyperplasia without atypia, complex hyperplasia without atypia, simple hyperplasia with atypia, and complex hyperplasia with atypia. In 2000, the International Endometrial Collaborative Group proposed the EIN classification, which the International Society of Gynecologic Pathologists later accepted in 2014. The WHO 2014 classification later divided hyperplasia into benign hyperplasia and atypical hyperplasia (AH)/EIN. Therefore, the EIN classification is becoming more popular because it is easier to use and has more defined criteria for interpreting data<sup>(3-5)</sup>.

Occult endometrial cancer has been detected in several patients following curettage, endometrial sampling, or hysterectomy subsequently to the EIN diagnosis. Previous studies have been reported

variable rates of occult endometrial cancer in patients with EIN, ranging from 17 to 52%<sup>(6,7)</sup>. EIN can be managed with hormonal therapy or hysterectomy, which is less aggressive than the treatment of endometrial cancer. Standard treatment of endometrial cancer typically involves surgical staging for determining cancer stage and may include radiation or chemotherapy<sup>(8,9)</sup>.

In Thailand, endometrial hyperplasia has been characterized utilizing diverse classification due to transition in terminology from the WHO 1994 classification to the EIN classification. Furthermore, variations in classification utilization among pathologists have been observed due to individual preferences. Research endeavors exploring the prevalence of occult endometrial cancer in endometrial hyperplasia or EIN biopsies in Asia have been limited, with most focusing solely on endometrial hyperplasia classification<sup>(10-12)</sup>. Therefore, the primary objective of this study was to ascertain the prevalence of concurrent endometrial cancer in patients initially diagnosed with EIN, while the secondary objective was to evaluate the characteristics and risk factors associated with occult endometrial cancer in EIN patients.

## Materials and Methods

This retrospective cohort study was conducted at King Chulalongkorn Memorial Hospital (KCMH), a university hospital in Bangkok, Thailand. The study protocol was approved by the Research Ethics Committee of the Faculty of Medicine, Chulalongkorn University. Data collection commenced following the

institutional review board approved the protocol (IRB No. 557/64).

Data spanning from 2015 to 2019 were gathered. All specimens obtained through endometrial sampling including endometrial biopsy, fractional curettage or hysteroscopic biopsy, were reviewed. Pathological results indicative of any type of endometrial hyperplasia or EIN were included in the study.

All patients diagnosed with endometrial hyperplasia and EIN at KCMH between 2015 and 2019 were recruited. In our institution, most of the endometrial hyperplasia cases were reported according to the WHO 1994 classification. EIN classification has been used since 2018. Endometrial hyperplasia included simple hyperplasia without atypia, complex hyperplasia without atypia, simple hyperplasia with atypia, and complex hyperplasia with atypia from WHO 1994 classification. Pathological slides initially classified under the WHO 1994 classification were re-classified as per the EIN classification by two pathologists (N.P. and C.A.). Any discordant results were jointly reviewed, and a consensus diagnosis was reached. Slides initially classified under the EIN classification were also reviewed to confirm the diagnosis of EIN. The pathologists were blinded to the initial diagnosis and all slides were re-categorized as less than EIN, EIN, and malignancy.

The study population comprised patients identified with EIN following slide review by pathologists. Data collected included demographic information, risk factors, treatment options, pathological diagnosis following hysterectomy, and details of follow-up visits.

Final diagnosis was determined based on the final pathological findings of hysterectomy specimens in surgical cases. Conservative cases encompassed patients requiring fertility preservation or those who declined surgical interventions for other reasons. For conservative cases, treatment included oral progestin or levonorgestrel intrauterine devices (IUDs) were offered. Patients declining surgery, oral progestin and levonorgestrel IUDs were offer closed follow-up option.

Endometrial sampling was conducted every 3 to 6 months for conservative cases, with the absence of malignancy in consecutive samples over a minimum 12-month follow-up period indicative of no occult malignancy. Patients with insufficient information or unavailable slides for review were excluded from the study.

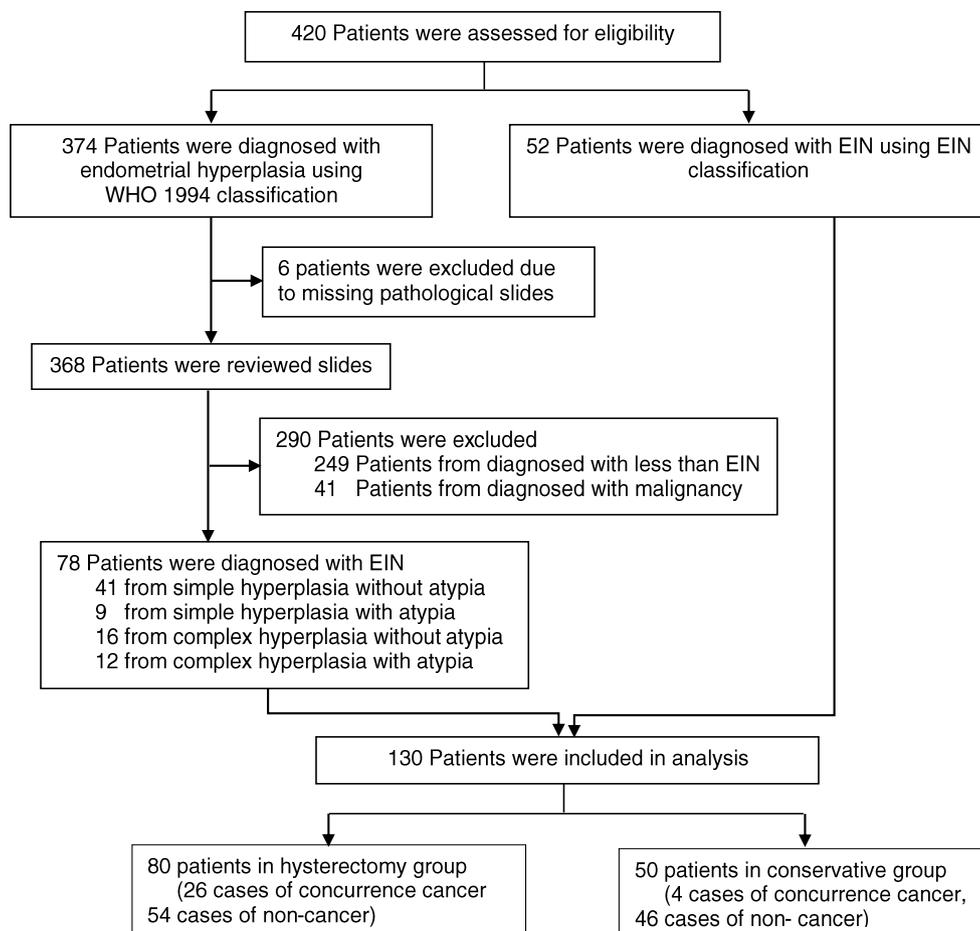
Statistical analysis was performed using SPSS version 22 (SPSS Inc, Chicago, IL, USA). Descriptive analyses were employed to assess the prevalence of concurrent endometrial cancer in EIN patients. Demographic data were presented as frequency, percentage, mean, and standard deviation (SD) as appropriate. Between groups comparisons (with and without occult malignancy) utilized independent t-tests for continuous variables and a chi-square tests or Fisher's exact tests for categorical variables. Logistic regression analyses were conducted to evaluate the association between demographics and clinical factors with concurrent endometrial cancer, presenting odds ratios (ORs) and 95% confidence intervals (95% CIs). Parameters for multivariable analysis were derived from the literature review, incorporating demographics and clinical factors exhibiting statistical significance ( $p < 0.05$ ) in the univariable model. Statistical significance was set at  $p$  value  $< 0.05$ . Sample size calculation was based on data from a previous study<sup>(7)</sup>, with 94 participants deemed necessary to provide adequate power for addressing the primary research question.

## Results

Between 2015 and 2019, a total of 374 patients were diagnosed with endometrial hyperplasia according to the WHO 1994 classification, while 52 patients were diagnosed with EIN using the EIN classification from endometrial sampling, fractional curettage and hysteroscopic biopsy. Six patients from the WHO classification were excluded due to missing pathological slides. For the remaining 368 patients who were previously classified with the WHO classification, their slides were reviewed and re-

categorized into three categories: less than EIN, EIN, and malignancy. Upon evaluation, 21.1% (78/368 patients) were diagnosed with EIN, while 67.7% (249/368 patients) were categorized as less than EIN, and 11.1% (41/368 patients) were diagnosed with endometrial cancer (Fig. 1). Among the 78 patients reclassified as EIN, 52.6% (41/78 patients) had previously been diagnosed with simple

hyperplasia without atypia, 11.5% (9/78 patients) with simple hyperplasia with atypia, 20.5% (16/78 patients) with complex hyperplasia without atypia, and 15.4% (12/78 patients) with complex hyperplasia with atypia. Of the 52 patients previously diagnosed with EIN, all were confirmed as EIN. Consequently, 130 patients with EIN were included in the study (Fig. 1).



**Fig. 1.** Study Flow.

*EIN: Endometrioid Intraepithelial Neoplasia*

Among the 130 EIN patients, 80 underwent hysterectomy while 50 received conservative treatment. Following the final results, 30 out of 130 patients (23.1% (95%CI 16.1-31.2) were diagnosed with concurrent endometrial carcinoma, with the remaining 100 patients showing no concurrent

endometrial carcinoma. Of the 80 patients in the surgical group, 26 had concurrent cancer, compared to 4 out of 50 in the conservative group. The mean age of the patients in the concurrent endometrial cancer group was 47.02 years (SD 13.2) compared to 52.23 years (SD 10.9) for those without endometrial

cancer. While body mass index (BMI) did not statistically significant differences between the cancer and non-cancer groups, there was a tendency towards higher proportions of overweight and obesity individuals in the former group. Most patients in both groups were premenopausal women (60% and 77.0%, respectively). The majority of the patients had no

underlying medical conditions. Patients with underlying medical conditions included hypertension, diabetes, dyslipidemia, breast cancer, and polycystic ovarian syndrome (PCOS) were not different between the two groups. Three patients with underlying breast cancer were taking tamoxifen at the time of EIN diagnosis (Table 1).

**Table 1.** Baseline characteristics of the study participants (n = 130).

Characteristics		Concurrent endometrial cancer		p value
		Yes n (%) = 30 (23.1)	No n (%) = 100 (76.9)	
Age (mean (SD), years)		47.02 (13.2)	52.23 (10.9)	0.03**
BMI	Underweight (< 18.5 kg/m <sup>2</sup> )	1 (3.3)	4 (4.0)	0.18
	Normal (18.5-24.9 kg/m <sup>2</sup> )	8 (26.7)	38 (38.0)	
	Overweight (25-29.9 kg/m <sup>2</sup> )	15 (50.0)	26 (26.0)	
	Obese class I (30-34.9 kg/m <sup>2</sup> )	2 (6.7)	18 (18.0)	
	Obese class II (35-39.9 kg/m <sup>2</sup> )	3 (10.0)	8 (8.0)	
	Obese class III (≥ 40 kg/m <sup>2</sup> )	1 (3.3)	6 (6.0)	
Parity	Nulliparous	17 (56.7)	55 (55.0)	0.87
	Multiparous	13 (43.3)	45 (45.0)	
Menopausal status	Premenopause	18 (60.0)	77 (77.0)	0.06
	Postmenopause	12 (40.0)	23 (23.0)	
Underlying disease	No	20 (66.7)	54 (54.0)	0.96
	Hypertension	2 (6.7)	12 (12.0)	
	Diabetes	3 (10.0)	7 (7.0)	
	Dyslipidemia	2 (6.7)	9 (9.0)	
	Breast cancer	2 (6.7)	5 (5.0)	
	Polycystic ovarian syndrome	0 (0.0)	2 (2.0)	
Medication	No	20 (66.7)	70 (70.0)	0.76
	Tamoxifen	1 (3.3)	2 (2.0)	
	non-hormonal medication†	9 (30.0)	28 (28.0)	
Treatment	Hysterectomy	26 (86.7)	53 (53.0)	< 0.01
	Conservative treatment	4 (13.3)	47 (47.0)	
Methods of tissue collection	Endometrial sampling	16 (53.3)	41 (41.0)	0.30
	Fractional curettage	12 (40.0)	55 (55.0)	
	Hysteroscopic biopsy	2 (6.7)	4 (4.0)	

\* Chi-square or Fisher Exact test, \*\* Independent t-test, † non-hormonal medication was a current medication that patients were taken regularly without the hormone estrogen  
SD: standard deviation, BMI: body mass index

Most tissues were obtained via fractional curettage and endometrial biopsy. Demographic data of surgical cases and conservative cases are presented in Table 2, indicating that the patients in the conservative group were younger than those in the surgical group (43.66 vs 51.07 years old). Additionally, the percentage of menopausal status was lower in the conservative group compared to the surgical group (14% vs 35%).

All concurrent endometrial cancer cases were diagnosed as uterine-confined disease, with no diagnoses of stage III or IV. Ten concurrent endometrial cancer cases were associated with high-risk features, including stage IB, stage II, and pathological grade 2 and 3 (Table 3).

Age, menopausal status, parity, underlying medical conditions, and BMI were considered potential confounders in multivariable analysis. Patients over the age of 60 were independently associated with concurrent endometrial cancer, adjusted OR 4.37 (95%CI 1.04-18.29) ( $p = 0.04$ ), when compared to patients under 40 years old. Association between menopausal status, parity, underlying disease, and BMI with concurrent endometrial cancer was not found (Table 4).

**Table 2.** Demographic data of the surgical group and the conservative group.

Demographic data	Surgical group n = 80	Conservative group n = 50
Age (mean (SD))	51.07 (10.25)	43.66 (12.01)
BMI (mean (SD))	27.23 (6.08)	29.23 (7.09)
Underlying disease (n (%))		
Yes	44 (55)	30 (60)
No	36 (45)	20 (40)
Menopause (n (%))	28 (35)	7 (14)
Concurrent malignancy (n (%))	26 (32.50)	4* (8)

In the conservative group, concurrent malignancy was found in 3 out of 43 oral progestin cases, 0 out of 2 levonorgestrel/levonorgestrel intrauterine devices (IUDs) cases and 1 out of 5 closed observe cases.

SD: standard deviation, BMI: body mass index

**Table 3.** Uterine cancer staging and FIGO grade in patients with concurrent endometrial cancer (n = 30).

Stage	FIGO grade			Total n (%)
	Grade 1	Grade 2	Grade 3	
IA	20	6	0	26 (86.7)
IB	3	0	0	3 (10.0)
II	0	0	1	1 (3.3)
Total n (%)	23 (76.7)	6 (20.0)	1 (3.3)	

FIGO: International Federation of Gynecology and Obstetrics

**Table 4.** Multivariable logistic regression analysis of the association between demographics and clinical factors with concurrent endometrial cancer (n = 130).

Variable	Adjusted odds ratio (95% confidence interval)	p value	
Age (mean (SD))	< 40 years	Reference	-
	40-59 years	1.82 (0.56-5.90)	0.31
	≥ 60 years	4.37 (1.04-18.29)	0.04
Menopausal status	2.23 (0.93-5.30)	0.06	
Multiparity	0.93 (0.41-2.12)	0.87	
Underlying disease	No	Reference	-
	Yes	0.58 (0.25-1.38)	0.22
BMI	Normal (18.5-24.9 kg/m <sup>2</sup> )	Reference	-
	Underweight (< 18.5 kg/m <sup>2</sup> )	1.18 (0.11-12.08)	0.88
	Overweight and Obese (≥ 25 kg/m <sup>2</sup> )	1.72 (0.69-4.27)	0.24

BMI: body mass index

## Discussion

The prevalence of concurrent endometrial cancer among patients with EIN histology in our study was 23.1% (95%CI 16.1-31.2), which was lower than the reported in European country and the United States<sup>(6, 7, 13)</sup>, yet higher than the research published

between 1992-2005, which reported rate of approximately 9-18%<sup>(14, 15)</sup>. A meta-analysis encompassing 15 trials, 1496 patients, found the prevalence of concurrent endometrial cancer in patients diagnosed with endometrial hyperplasia was 32.1%, although the data exhibited high heterogeneity ( $I^2 = 87.6\%$ )<sup>(13)</sup>. Notably, our study comprised two distinct subgroups: surgical and conservative groups. In the surgical group, the prevalence of concurrent endometrial cancer was 32.5%, mirroring the findings from papers in Europe and the United States<sup>(6, 7, 13)</sup>. Conversely, the prevalence of concurrent malignancy in the conservative group was markedly lower at 8% (4 out of 50), suggesting potential impacts of patient selection and treatment intervention. Patients in the conservative group tended to be younger than those in the surgical group. Additionally, treatment interventions such as oral progestin or levonorgestrel IUDs may influence pathologic findings in final histology. Furthermore, the follow-up endometrial sampling technique at the 12-months follow-up period, utilizing endometrial biopsy or curettage, may present a reduced chance of detecting concurrent malignancy compared to histologic results from hysterectomy specimens. Therefore, caution is warranted in interpreting this result.

In our study, some patients received conservative treatment. The reasons for choosing conservative treatment include young age and still have fertility desire. In the conservative group follow-up endometrial biopsy was performed every 3-6 months. The patient was considered to have no occult malignancy if no malignancy was found from endometrial sampling in at least 12 months in follow-up. This follow-up scheme aligned with the approach in the study by Baak et al<sup>(14)</sup>, where endometrial curettage or biopsy was utilized for diagnosing concurrent endometrial cancer in conservative cases. Patients with endometrial hyperplasia who underwent conservative treatment in the study by Baak et al were monitored for 12 months, similar to our study<sup>(14)</sup>.

We analyzed age, menopausal status, parity, underlying disease, and BMI to assess their

association with concurrent endometrial cancer. Patients aged over 60 years were independently associated with concurrent cancer (adjusted OR 4.37 (95%CI 1.04-18.29),  $p = 0.04$ ) compared to patients under 40 years old. However, menopausal status, parity, underlying disease, and BMI were not independently associated with concurrent endometrial cancer. These findings were partly different from the previous research by Kender et al and Trimble et al that reported age and atypia histology have a higher risk of concurrent endometrial cancer and high-risk cancer<sup>(7, 16)</sup>. However, our sample size was not calculated for detecting all the aspects of risk factors and associations in secondary objective, necessitating further research with adequate sample size to address these associations comprehensively.

Given the retrospective nature of our study, limitations exist, such as potential limitations in data quality, including slide degradation due to storage techniques. Missing data also poses a limitation. However, we made efforts to mitigate these limitations by meticulously evaluating slides from the WHO 1994 classification to establish a new EIN classification. The pathologists were blinded to the prior pathologic results to ensure the accuracy of the information. This study contributed to the limited body of research on the Asian population's prevalence of cancer in patients with EIN histology.

## Conclusion

Approximately one-third to one-fourth of patients diagnosed with EIN via endometrial sampling had concurrent endometrial cancer. EIN in patients over 60 years old was independently associated with concurrent cancer. Notably, all patients diagnosed with concurrent endometrial cancer exhibited uterine-confined disease. We recommend counseling patients with EIN in both surgical and conservative cases using these data.

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## Potential conflicts of interest

The authors declare no conflicts of interest.

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## GYNAECOLOGY

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# Surgical Outcomes of Laparoscopic High Uterosacral Vaginal Vault Suspension for Apical Prolapse Repair

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### ABSTRACT

**Objectives:** To evaluate surgical outcomes of laparoscopic high uterosacral ligament suspension (HUSLS) for apical prolapse repair in terms of success rates, perioperative complications, and the ability to perform concomitant bilateral salpingectomy/salpingo-oophorectomy.

**Materials and Methods:** This was a retrospective study of women diagnosed with stage II-IV uterovaginal prolapse who had undergone laparoscopic HUSLS for apical prolapse repair at Department of Obstetrics and Gynecology, Phramongkutklao Hospital between January 2017 and September 2020. Patients' baseline characteristics, clinical presentation, pre- and post-operative prolapse stage and location, pre- and post-operative pelvic organ prolapse quantification (POP-Q) measurements, perioperative complications, and feasibility of opportunistic salpingectomy/salpingo-oophorectomy were collected and analyzed.

**Results:** Of 40 patients enrolled, mean age was  $62.3 \pm 7.5$  years, whereas mean body mass index was  $24.7 \pm 3.2$  kg/m<sup>2</sup>. Most were postmenopausal (92.5%) and presented with bulge symptoms (97.5%). Thirty-five (87.5%) patients were diagnosed with advanced stage prolapse. All underwent hysterectomy prior to laparoscopic HUSLS. Additional procedures comprised anterior colporrhaphy (42.5%), posterior colporrhaphy (70%), perineorrhaphy (50%), and midurethral sling (5%). Bilateral salpingo-oophorectomy was performed in all patients. Mean operative time for all procedures was  $3.1 \pm 0.8$  hours, whereas median blood loss was 50 milliliters. No major perioperative complications, including ureteric injury, were encountered. High anatomical success of 90% was achieved at 12-month follow-up with significant improvement in all POP-Q measurements, except perineal body (PB) and total vaginal length (TVL). Of 4 patients with recurrent prolapse, 3 later underwent total colpocleisis, whereas 1 required anterior vaginal mesh repair.

**Conclusion:** Laparoscopic HUSLS was a safe and effective procedure for apical prolapse repair without major perioperative complications when performed in the hands of experts.

**Keywords:** laparoscopic high uterosacral ligament suspension, apical prolapse repair, success rate, perioperative complications.

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## ผลลัพธ์ของการผ่าตัดส่องกล้องทางหน้าท้องในการรักษาอวัยวะอุ้งเชิงกรานหย่อนบริเวณส่วนยอดด้วยการยึดพยุงยอดช่องคลอดกับเอ็นยึดมดลูก uterosacral

เกรียงศักดิ์ ศิริศักดิ์พานิชย์, เศรษฐัญญบงกร เรืองสุวรรณ, พลอยวรรณค์ เรืองเกตุ

### บทคัดย่อ

**วัตถุประสงค์:** เพื่อศึกษาอัตราความสำเร็จของการผ่าตัดส่องกล้องทางหน้าท้องในการรักษาอวัยวะอุ้งเชิงกรานหย่อนบริเวณส่วนยอดด้วยการยึดพยุงยอดช่องคลอดกับเอ็นยึดมดลูก uterosacral ได้แก่ อัตราความสำเร็จในเชิงกายวิภาคและอาการทางคลินิก อัตราการเกิดภาวะแทรกซ้อนในขณะผ่าตัดและภายหลังการผ่าตัด และอัตราการกลับเป็นซ้ำของภาวะกระบังลมหย่อนหลังจากการผ่าตัด

**วัสดุและวิธีการ:** ศึกษาย้อนหลังจากเวชระเบียนของผู้ป่วยที่ได้รับการวินิจฉัยว่ามีภาวะอวัยวะอุ้งเชิงกรานหย่อนอย่างน้อยระดับ 2 และได้รับการรักษาอวัยวะอุ้งเชิงกรานหย่อนบริเวณส่วนยอด (apical compartment prolapse) ด้วยการยึดพยุงยอดช่องคลอดกับเอ็นยึดมดลูก uterosacral (High uterosacral ligament suspension : HUSLS) โดยวิธีผ่าตัดส่องกล้องทางหน้าท้อง ณ อนุสาขานรีเวชทางเดินปัสสาวะและผ่าตัดซ่อมเสริมอุ้งเชิงกราน โรงพยาบาลพระมงกุฎเกล้า ตั้งแต่เดือนมกราคม 2560 ถึง เดือน สิงหาคม 2564 โดยศึกษาและวิเคราะห์ข้อมูลทั่วไป อาการแสดง ค่า พอปคิว (POP-Q) ก่อนและหลังผ่าตัด ตำแหน่งที่มีภาวะอุ้งเชิงกรานหย่อน รวมถึงภาวะแทรกซ้อนระหว่างและหลังการผ่าตัด

**ผลการศึกษา:** เก็บข้อมูลผู้ป่วย 40 รายที่ได้รับการผ่าตัด HUSLS อายุเฉลี่ยคือ  $62.3 \pm 7.5$  ปี โดยผู้ป่วยได้รับการผ่าตัดส่องกล้องทางหน้าท้องเพื่อตัดมดลูกร้อยละ 95 และทำการผ่าตัดมดลูกทางช่องคลอดร้อยละ 5 ผู้ป่วยได้รับการผ่าตัดครั้งไขทั้งสองข้างร้อยละ 100 นอกจากนี้เหตุการณ์ที่ร่วมกันในระหว่างผ่าตัดได้แก่ การผ่าตัดซ่อมเสริมผนังช่องคลอดด้านหน้า ร้อยละ 42.5, การผ่าตัดซ่อมเสริมผนังช่องคลอดด้านหลัง ร้อยละ 70, การผ่าตัดใส่สายคล้องใต้ท่อปัสสาวะส่วนกลาง ร้อยละ 5 โดยทั้งหมดทำตามข้อบ่งชี้ ค่าพอปคิวหลังการผ่าตัดมีค่าน้อยกว่าก่อนผ่าตัดอย่างมีนัยสำคัญทางสถิติ ในจุด Aa, Ba, C, Ap, Bp and Gh พบว่าอัตราความสำเร็จของการผ่าตัดในการศึกษานี้อยู่ที่ร้อยละ 90 อัตราการกลับเป็นซ้ำอยู่ที่ร้อยละ 10

**สรุป:** การผ่าตัดส่องกล้องทางหน้าท้องด้วยการยึดพยุงยอดช่องคลอดกับเอ็นยึดมดลูก uterosacral มีประสิทธิภาพที่ดีและปลอดภัยสำหรับการแก้ไขภาวะอุ้งเชิงกรานหย่อนบริเวณส่วนยอด โดยมีอัตราการเกิดภาวะแทรกซ้อนต่ำทั้งระหว่างและหลังผ่าตัด รวมทั้งมีอัตราการกลับเป็นซ้ำอยู่ในระดับที่ต่ำ

**คำสำคัญ:** อวัยวะอุ้งเชิงกรานหย่อน, การยึดพยุงยอดช่องคลอด, มาตรฐานพอปคิว, การผ่าตัดส่องกล้องยึดพยุงยอดช่องคลอดกับเอ็นยึดมดลูก

## Introduction

According to the definition by the International Urogynecological Association (IUGA)/International Continence Society (ICS) joint report on the terminology, pelvic organ prolapse (POP) is the descent of one or more of the anterior vaginal walls, the posterior vaginal wall, the uterus (cervix) or the apex of the vagina after hysterectomy<sup>(1)</sup>. With Thailand becoming an aging society, POP has become highly prevalent among women of advanced age, causing a negative impact on their quality of life. Due to increasing age, the pelvic floor gradually deteriorates leading to weakness in all or multiple coexisting compartments<sup>(2)</sup>. With the development of surgical techniques and perioperative care, most women are rather inclined towards surgical repair rather than conservative treatment. Unfortunately, nearly one-third of the women undergoing surgery for POP later required a second operation for POP recurrence<sup>(3)</sup>. Hence, to achieve anatomical success in POP repair and prevent prolapse recurrence, all pelvic supportive defects must be thoroughly identified and effectively corrected at the time of the index surgery, specifically apical support restoration which is the key to a successful prolapse repair<sup>(4, 5)</sup>. Different surgical treatments for apical prolapse repair can be performed via vaginal, abdominal, or laparoscopic route with the mutual aim of restoring the vaginal apex to its usual position using ligament fixation, with or without mesh augmentation. Examples include (1) sacrospinous ligament (sacrospinous fixation), (2) presacral ligament (sacrocolpopexy), (3) Cooper's ligament (pectopexy), (4) round ligament (round ligament suspension), and (5) uterosacral ligament (uterosacral ligament suspension).

According to Delancey's fascial support theory<sup>(6)</sup>, re-suspending of the apical compartment to the intact cardinal/uterosacral ligament complex at the level of ischial spine, called high uterosacral ligament suspension (HUSLS), can provide a strong support to the vaginal apex without distorting its axis and no additional need for mesh augmentation. This is often performed after hysterectomy procedure, either

vaginally, abdominally, or laparoscopically, with high success rates<sup>(7)</sup>. However, an important disadvantage of the transvaginal HUSLS is the possible risk of ureteric injury, approximately 1-11%<sup>(8)</sup>. To avoid ureteric complication, HUSLS can be conducted via laparoscopic approach which can aid in clear visualization and identification of both ureters and uterosacral ligaments, therefore providing solid and safe apical suspension. Moreover, opportunistic bilateral salpingectomy and/or oophorectomy can be readily achieved to eliminate the risk of future tubo-ovarian cancer<sup>(9)</sup>. Nevertheless, there are unavoidable drawbacks of laparoscopy, especially high cost and the need for advanced skills in minimally invasive surgery. As one of The Royal Thai College of Obstetricians and Gynaecologists (RTCOCG) affiliated training center, Phramongkutklao Minimally Invasive Gynecologic Surgery (MIGS) Unit is responsible for providing and offering various and standardized minimally invasive procedures, including pelvic floor reconstruction, to our patients and trainees. Laparoscopic HUSLS is one of the common urogynecological procedures performed to correct apical descent at our center. Therefore, this study primarily aimed to evaluate the surgical outcomes of laparoscopic HUSLS for apical prolapse repair in terms of success rates and perioperative complications, as well as to assess the ability in performing concomitant bilateral salpingo-oophorectomy procedure.

## Materials and Methods

After receiving an ethical approval from the Institutional Review Board of Phramongkutklao Hospital, the medical records of the patients diagnosed with at least stage II uterovaginal prolapse who had undergone laparoscopic high uterosacral vaginal vault suspension at the Minimally Invasive Gynecologic Surgery Unit, Department of Obstetrics and Gynecology, Phramongkutklao Hospital between January 2017 and September 2020 were retrospectively reviewed. Information regarding patients' baseline characteristics, clinical presentation, pre- and post-

operative POP staging and location, pre- and post-operative POP-Q measurements, operative outcomes, and perioperative adverse events were collected and analyzed. Cases with incomplete medical data and follow-up duration of less than 1 year were excluded from the study.

### **Sample size calculation**

According to the literature review, the anatomical cure rate of laparoscopic HUSLS was 76-100%<sup>(10, 11)</sup>. The expected success rate for this study was then assumed to be 90%. With the pre-defined values of (1) Z-alpha/2 score at the confidence level of 95% (1.96), (2) 90% expected success rate of laparoscopic HUSLS (0.90), and (3) 10% discrepancy (0.09), the sample size was finalized at 43 patients for this study.

### **Surgical techniques**

Only one experienced urogynecologist (KS) was responsible for all carried-out surgical procedures. Hysterectomy, either vaginal or laparoscopic, was performed prior to laparoscopic HUSLS procedure. For laparoscopic port entry, a primary 10-mm trocar was introduced at umbilicus for camera port, followed by three additional 5-mm trocars placed at left lower, suprapubic and right lower abdominal areas for ancillary ports. After either vaginal or laparoscopic hysterectomy, bilateral salpingoophorectomy was performed for the patients who had already approached menopause. Regarding the details for laparoscopic HUSLS procedure, the initial step was to enter the retroperitoneal space to clearly visualize and lateralize both ureters away from the peritoneal fold covering uterosacral ligaments. Retroperitoneal dissection was continued into pararectal space to medialize the rectum and clearly identify the fibers of both uterosacral ligaments at the level of ischial spine. The long-term absorbable polydioxanone suture material (1-0 Monomax<sup>®</sup>) which was inserted from the vagina was used to make a suspensory suture between each uterosacral ligament and the ipsilateral vaginal cuff corner. The suture ends were then retrieved back into

the vagina. After closure of the vaginal cuff with the mid-term absorbable braided and coated synthetic suture material (1-0 Novosyn<sup>®</sup>), both uterosacral suspensory sutures were then tied vaginally. Additional procedures including anterior colporrhaphy, posterior colporrhaphy, perineorrhaphy, and midurethral sling were also carried out if needed. The Foley's catheter was removed on postoperative day 1.

The patients were scheduled for postoperative follow-up at 1 month, 3 months, 6 months, 12 months and annually thereafter. POP stage and location, as well as POP-Q measurements were re-evaluated at each follow-up visit. With the success rate being the primary objective of this study, we defined the anatomical success at one-year follow-up according to the NICHD (National Institute of Child Health and Human Development) Pelvic Floor Disorders Network recommendations<sup>(12)</sup> as "no descent of the vaginal walls beyond the hymen" or POP-Q measurements Aa, Ap, Ba, Bp and C less than or equal to 0. Moreover, we secondarily evaluated the perioperative complications and assessed the feasibility of opportunistic bilateral salpingoophorectomy among our patients.

### **Statistical analysis**

The statistical analysis was performed using the Statistical Packages for the Social Sciences version 23.0 for Windows (SPSS Inc, Chicago, IL, USA). Continuous data were described as mean  $\pm$  standard deviation (SD), median (range), or median with interquartile range (IQR) whereas categorical variables were expressed as number and percentage. The paired Student t-test or Wilcoxon signed-rank test was used to assess the significance of the difference between pre- and post-operative POP-Q measurements. The p value of less than 0.05 was used to determine statistical significance.

## **Results**

A total of 40 patients having undergone laparoscopic HUSLS procedure during the study

period were recruited. Among these, 92.5% were postmenopausal with the mean age of  $62.3 \pm 7.5$  years and the mean body mass index (BMI) of  $24.7 \pm 3.2$  kg/m<sup>2</sup>. With the median parity of 2, 95% of the patients had vaginal deliveries. “Feeling of a bulge” was the most common presenting symptom among our patients (97.5%). Regarding POP-related lower

urinary tract symptoms, both voiding difficulty and stress urinary incontinence (SUI) were reported in one-third of the patients. Only a few patients complained of defecatory symptoms. Regarding preoperative POP stage, 35 out of 40 patients (87.5%) initially presented with advanced stage (stage 3-4) prolapse (Table 1).

**Table 1.** Baseline characteristics.

Characteristics	Values (n = 40)
Age (years)	62.3 ± 7.5
BMI (kg/m <sup>2</sup> )	24.7 ± 3.2
Parity	2 (0-6)
Mode of delivery	
NL	38 (95.0)
V/E	1 (2.5)
F/E	1 (2.5)
C/S	2 (5.0)
Menopause	37 (92.5)
POP symptom	
Bulge	39 (97.5)
Dragging	2 (5.0)
LUT symptom	
Voiding difficulty	13 (32.5)
OAB	8 (20.0)
SUI	12 (30.0)
Digitation	2 (5.0)
Bowel symptom	
Constipation	1 (2.5)
Incomplete evacuation	2 (5.0)
Straining	1 (2.5)
Digitation	3 (7.5)
POP Stage	
Stage 0-1	0 (0.0)
Stage 2	5 (12.5)
Stage 3-4	35 (87.5)

Data presented as mean ± standard deviation, median (min, max) or number (%).

BMI: body mass index, POP: pelvic organ prolapse, LUT: lower urinary tract, OAB: overactive bladder, SUI: stress urinary incontinence, NL: normal labour, V/E: vacuum extraction, F/E: forceps extraction, C/S: cesarean section.

All patients underwent hysterectomy prior to laparoscopic high uterosacral vault suspension procedure, 38 laparoscopically and 2 vaginally. The mean operative time for all conducted procedures was  $3.1 \pm 0.8$  hours, whereas the median intraoperative blood loss was 50 milliliters, ranging from 10 to 350 milliliters. No major intraoperative adverse events, especially ureteric injury, were encountered. Bilateral salpingoophorectomy was successfully performed in every patient. Other concomitant procedures comprised anterior colporrhaphy (42.5%), posterior

colporrhaphy (70%), perineorrhaphy (50%), and midurethral sling (5%). Fever was found to be the most common early postoperative complication which could spontaneously resolve after close monitoring. For one patient having acute urinary retention postoperatively, this occurred after midurethral sling placement and subsequently required loosening of the tape. De novo stress incontinence was discovered in 5 out of 40 patients (12.5%). Fortunately, this could be conservatively managed without the need to undergo anti-incontinence surgery (Table 2).

**Table 2.** Operative outcomes and perioperative complications.

Perioperative variables	Values (n = 40)
Operative outcomes	
Operative time (hour)	3.1 ± 0.8
Blood loss (ml)	50 (10 – 350)
V-hysterectomy	2 (5.0)
TLH	38 (95.0)
Bilateral S/SO	40 (100.0)
Midurethral sling	2 (5.0)
Anterior colporrhaphy	17 (42.5)
Posterior colporrhaphy	28 (70.0)
Perineorrhaphy	20 (50.0)
Perioperative complications	
Fever	4 (10)
Urinary retention	1 (2.5)
De novo SUI	5 (12.5)

Data presented as mean ± standard deviation, number (%), median (min, max)

TLH: total laparoscopic hysterectomy, S: salpingectomy, SO: salpingoophorectomy, SUI: stress urinary incontinence, V-hysterectomy: vaginal hysterectomy.

At 12-month follow-up, substantial improvement in bulge symptom and POP-Q measurements were readily observed. The comparative outcome of pre- and post-operative POP-Q measurements revealed statistically significant improvement in all compartments, including anterior (point Aa and Ba), posterior (point Ap and Bp), and apical (point C), as well as smaller genital hiatus (Gh) (all with p value < 0.001). However, no significant changes were

detected in the perineal support (p = 0.520) and total vaginal length (p = 0.124) (Table 3). These comparative outcomes are also illustrated for easy understanding in Fig. 1.

With the hymen being referenced as the cut-off point to objectively define POP recurrence, 4 patients (10%) were found to have recurrent prolapse. When specifically investigating for the location of POP recurrence, 3 patients presented

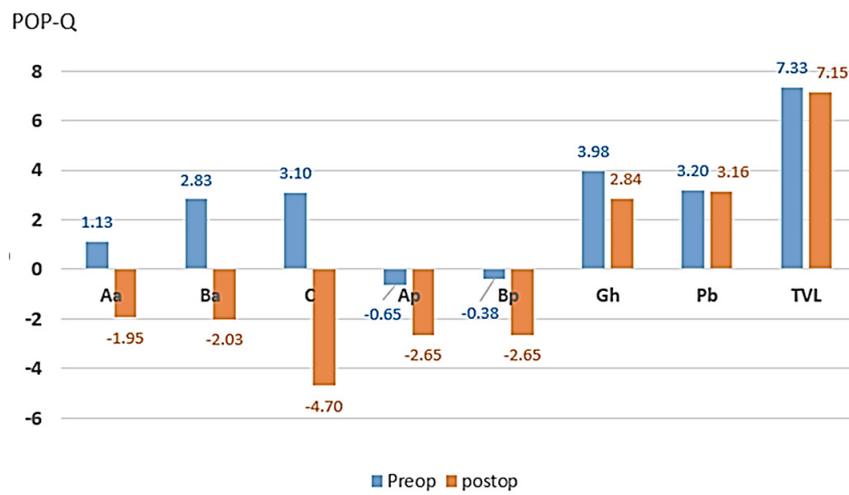
with advanced stage anterior and apical prolapse, whereas 1 patient demonstrated only stage 2 apical descent. Thus, the anatomical success rate of laparoscopic HUSLS for apical prolapse repair was

90%. Finally, of 4 patients diagnosed with recurrent prolapse, three later underwent total colpocleisis while one eventually required anterior vaginal mesh repair.

**Table 3.** Pre- and post-operative POP-Q measurements.

POP-Q	Preoperative	Postoperative	Mean difference		95% CI		p value
			Lower	Upper	Lower	Upper	
Aa	+1.1 ± 1.6	-2.0 ± 1.0	3.1	2.5	3.7	< 0.001	
Ba	+2.8 ± 1.7	-2.0 ± 1.4	4.9	4.2	5.5	< 0.001	
C	+3.1 ± 3.0	-4.7 ± 2.1	7.8	6.6	9.0	< 0.001	
Ap	-0.7 ± 2.0	-2.7 ± 0.7	2.0	1.4	2.6	< 0.001	
Bp	-0.4 ± 2.5	-2.7 ± 0.7	2.3	1.5	3.0	< 0.001	
Gh	4.0 ± 1.0	2.8 ± 0.6	1.1	0.9	1.4	< 0.001	
Pb	3.2 ± 0.7	3.2 ± 0.6	0.0	-0.2	0.3	0.520	
TVL	7.3 ± 1.0	7.2 ± 0.7	0.2	-0.1	0.4	0.124	

Data presented as mean ± standard deviation, Statistical analysis: Wilcoxon signed ranks test. POP-Q: pelvic organ prolapse quantification, CI: confidence interval.



**Fig. 1.** Comparative outcome of pre- and post-operative POP-Q measurements. POP-Q: pelvic organ prolapse quantification

## Discussion

The favorable anatomical success rate of 90% from this study has confirmed the effectiveness of laparoscopic HUSLS procedure in the apical prolapse repair. The result was similar to those of several

previous research works<sup>(10,11)</sup>. Apart from laparoscopic re-suspension of the vaginal apex, concomitant vaginal procedures, including anterior and posterior colporrhaphy, were also conducted in order to correct the supportive defect in the anterior and posterior

compartment, respectively. This definitely led to remarkable improvement of POP-Q measurements in all three compartments, except the perineal body (Pb) and the total vaginal length (TVL). Since perineorrhaphy was less likely performed among our patients, this could contribute to the non-significant change in the thickness of the perineal body. Although the descending vaginal apex (point C) could be significantly lifted up and brought back to its usual position, the vaginal length remained the same, resulting in unchanged TVL value.

Results from our study have also proved the benefits of laparoscopy. With the advantage of better visualization and lateralization of the ureter, no ureteric injury was encountered. Moreover, laparoscopic approach could undoubtedly facilitate the safe removal of both adnexa (bilateral salpingo-oophorectomy) in all patients. This could be beneficial for postmenopausal women in terms of eliminating the risk of future tuboovarian cancer<sup>(9)</sup>. Although no major intraoperative complications were encountered, the late postoperative stress incontinence or 'de novo SUI' seemed to be an unavoidable incident. Our 12.5% occurrence of de novo SUI reflects the results of several previous studies<sup>(13-15)</sup>. It was suggested that POP could cause urethral kinking and POP repair could subsequently restore the normal urethral anatomy, unmasking the symptom of SUI that was covertly present before the operation.

Previous literatures have determined the strong association between anterior and apical compartment prolapse<sup>(4, 16-17)</sup>. Our findings have also highlighted this relationship. Among 4 patients diagnosed with recurrent POP, 3 (75%) were found to have recurrent anterior prolapse together with apical descent. The nature of unsustainable native tissue repair and inadequate endopelvic fascial plication were the possible causes of POP recurrence.

### **Strength and limitation**

With only one urogynecologist who was highly experienced in both laparoscopic and transvaginal

pelvic floor reconstruction, the strength in terms of uniform and standardized surgical techniques could be ensured. Furthermore, expertise skills in laparoscopic retroperitoneal dissection could facilitate clear identification of both ureters and cardinal-uterosacral complex, thus providing a safe and sound fixation on the ligaments without any doubt of ureteric injury. All of these could, therefore, result in favorable surgical outcomes. However, the small sample size and the retrospective nature of this study were unavoidable limitations causing measurement and patient selection bias.

### **Implementation**

With expertise skills in laparoscopic retroperitoneal dissection, our study has provided an effective technique in identification of both ureters and the exact location of the uterosacral ligaments. Hence, this confirmed a safe and definitive placement of the suspensory sutures onto the ligaments, ensuing a secure apical suspension and a high anatomical success. Our surgical technique was different from the intraperitoneal plication of peritoneal fold covering uterosacral ligaments which was the technique utilized by most researchers. Therefore, this retroperitoneal dissection technique can be implemented or added to the established knowledge of laparoscopic high uterosacral vault suspension.

### **Conclusion**

Laparoscopic high uterosacral vaginal vault suspension was a safe and highly effective procedure for apical prolapse repair with minimal complications, especially when performed in the hands of experts.

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### **Potential conflicts of interest**

The authors declare no conflicts of interest.

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## CASE REPORT

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# Urethral Caruncle: A Diagnosis Conundrum in Teenage Girl: A case report

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### ABSTRACT

**Background:** Urethral caruncle in young female is uncommon. Here we reported a case of it seen in our urogynaecology outpatient clinic which was surgically managed.

**Case:** An 18-year-old teenage girl presented with periurethral mass for few months. Suspicion of the diagnosis of urethral caruncle was made clinically and we proceeded with surgical excision of the mass under local anaesthesia. Histopathological examination confirms the diagnosis. This case showed that although the presentation was atypical and is rarely seen in teenagers, it should be considered as one of the differentials when dealing with vaginal mass besides urethral prolapse, urethral diverticulum, urethrocele or even malignancy.

**Conclusions:** Although urethral caruncle is more commonly associated with postmenopausal due to the hypoestrogenic state, it is important to integrate a clinical suspicion and decision for excision based on presenting symptoms.

**Keywords:** urethral, caruncle, female, young, adolescent.

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## Introduction

Urethral caruncle being the most common benign mass of the female urethra and is usually asymptomatic. It can sometimes present with bleeding, pain or urinary symptoms. Conservative or surgical management will depend on individual cases. It is commonly seen in postmenopausal women due to the hypoestrogenic state and rarely in young women or children. Here, we reported a case of urethral caruncle in an adolescent girl who underwent surgical excision of the mass.

## Case report

An 18-year-old, sexually inexperienced teenage girl complained of perineal mass that has been progressively increasing in size for three months. Other than discomfort while riding a motorcycle, she did not report any vaginal discharge or bleeding. She had no pain or difficulty on passing urine. Our further assessment revealed a 4 cm x 4 cm broad base pedunculated, firm, fleshy reddish mass arising from the left lateral urethral meatus (Fig. 1).

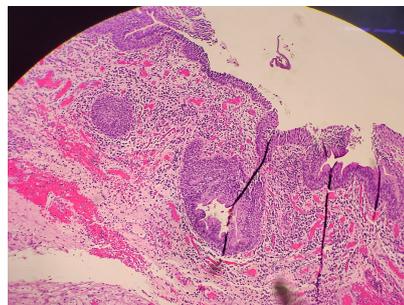


**Fig. 1.** Image showing mass arising from the left lateral urethral meatus.

It was non tender with no contact bleeding. The surface was smooth. Vaginal hymen was intact. No similar swelling seen elsewhere. No further investigation done due to its obvious nature. We directly proceeded with excision under local anaesthesia with 2% lignocaine local infiltration in the

outpatient clinic. Continuous bladder drainage was inserted prior to the procedure. The mass was completely excised using diathermy and its base sutured using absorbable polyglactine suture size 3/0. Patient was discharged home with bladder catheter in situ, antibiotics and analgesia. Urinary catheter was removed after a week. Histopathological examination (HPE) result reported the lesion demonstrated a benign reactive polypoidal lesion, covered by reactive epithelium and was composed of inflamed granulation tissue accompanied by mixed inflammatory cell infiltrate along with many congested and dilated vessel in which features were in keeping with urethral caruncle (Fig. 2).

She was seen back after 5 months, there was no recurrent of mass, she was asymptomatic and well (Fig. 3).



**Fig. 2.** Image showing the microscopic examination findings.



**Fig. 3.** Image showing no recurrent mass on follow-up

## Discussion

Typically, urethral caruncles- described as fleshy outgrowth at urethral meatus only affect the female urethra. They are infrequently observed in young girls and most frequently affect middle-aged and elderly women<sup>(1)</sup>. It is a vascular lesion of various sizes, sessile or pedunculated and is usually seen on the posterior lip of distal urethral meatus.

Urethral caruncles are usually asymptomatic and are typically discovered incidentally during pelvic examinations. Common symptoms include bleeding or observing blood on undergarments, bloody discharge, hematuria, pain, dysuria, or the presence of a lump<sup>(2, 3)</sup>.

As for our case, her only presenting symptoms were discomfort and the presence of a mass. A PubMed search (<https://pubmed.ncbi.nlm.nih.gov>) was performed on October 17, 2023, using search terms "urethral caruncle female" retrieved 68 results and none reported on caruncle in teenager. Miriam et al reported on 41 histopathologic characters of urethral caruncle in women between 28-87 years (mean age 68years) with 32% of them being asymptomatic, 37% pain symptom followed by hematuria (27%) and dysuria (20%)<sup>(2, 4)</sup>.

Urethral masses pose a diagnosis conundrum as management is dependent on the diagnosis. Its fleshy character can be mistaken as urethral prolapse, diverticulum, urethroceles, urethral varicosities, periurethral gland abscess, condyloma, cysts of Skene's glands and rarely, urethral carcinoma- primary urethral melanoma or transitional cell carcinoma. The latter will require more aggressive treatment approach for best chance of cure.

No single theory has been proposed that provides an adequate explanation for the exact aetiology and pathophysiology of the urethral caruncle. As this condition is almost exclusively seen in the extreme end of either postmenopausal or younger infants, it has been linked to estrogen insufficiency. Between birth and puberty, estrogen levels transition from being non-existent to reaching their maximum level, which remains relatively stable

until gradually decreasing towards the menopausal state which explained why urethral caruncle happen more common in these two age groups<sup>(5)</sup>.

Other possibilities of urethral irritation, trauma, and congestion of the urethra have all been identified as potential cause. It has also been linked to possible autoimmune phenomenon<sup>(3, 4, 6, 7)</sup>. In our case, the possible potential trigger might be chronic inflammation as young female urethra is prone to infection and inflammation.

Although clinical suspicion alone is sufficient, imaging modalities may have a role to assist in diagnosis. Transvaginal or tranperineal ultrasound of hypoechoic or heterogeneous solid masses located at periurethral area and exhibit blood flow signals on colour Doppler imaging is seen in urethral caruncle<sup>(8, 9)</sup>. It can be used as a valid non-invasive tool to differentiate various urethral and periurethral masses.

Asymptomatic urethral caruncle can be reassured. Symptomatic woman can be treated conservatively with warm sitz baths, topical oestrogen, and topical anti-inflammatory medications<sup>(2)</sup>. Surgery is reserved for large symptomatic lesions or not responding to conservative treatment, unclear diagnosis, or with atypical features.

Surgical excision was performed as she was symptomatic and affected her daily living activity-from constant rubbing and pressure when riding her motorcycle, which serves as her primary mode of transportation. This led her to opt for a swift and definitive approach to treatment.

Various surgical techniques have been described in the literature - pinching, snaring, ligating, cutting, cautery by heat, destruction with chemicals and fulguration. Although some of the techniques have been discontinued or not recommended as it carries complications such as extensive scarring leading to urethral stricture or no tissue biopsy provided, most of the patient treated with excision method have good outcome with low recurrence rate (3.5-7%)<sup>(2, 4)</sup>.

Cystourethroscopy is recommended prior to surgical intervention to rule out serious bladder and urethral abnormalities. There was also recommendation use of urethral catheter for few days to allow proper healing of the urethral mucosa to minimise complications<sup>(2)</sup>.

Other simple method reported in literature includes ligation of the base of a urethral caruncle with 1-0 silk, allowing it to shed off after 7–14 days and minimally invasive technique of transurethral plasmakinetic resection of urethral caruncle. Both techniques well tolerated by patient with shorter recovery and fewer complication<sup>(10, 11)</sup>.

## Conclusion

Although it is rare, urethral caruncle should be included in differential diagnosis when managing periurethral mass in young female. Mainstay of treatment is surgical in which carry good satisfaction and outcome.

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## Potential conflicts of interest

The authors declare no conflicts of interest.

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