UJOD
Uluslararası Jinekoloji ve Obstetrik Derneği

ANATOLIAN JOURNAL OF OBSTETRICS & GYNECOLOGY RESEARCH

Years | **2024** | Issue | **2** | Month | **August**

galenos yayınevi

www.anajog.com

ANATOLIAN JOURNAL OF
OBSTETRICS & GYNECOLOGY
RESEARCH

Years | 2024 | Issue | 2 | Month | August

ANATOLIAN JOURNAL OF
OBSTETRICS & GYNECOLOGY
RESEARCH

Years | **2024** | Issue | **2** | Month | **August**

Please refer to the journal's webpage (**https://www.anajog.com/**) for "Aims and Scope", "Instructions to Authors" and "Ethical Policy".

The editorial and publication process of the **Anatolian Journal of Obstetrics and Gynecology Research** are shaped in accordance with the guidelines of **ICMJE, WAME, CSE, COPE, EASE**, and **NISO**. The journal is in conformity with the Principles of Transparency and Best Practice in Scholarly Publishing.

The journal is published online.

Owner: International Society of Gynecology and Obstetrics

Responsible Manager: Eray ÇALIŞKAN

Contact
Ömerağa Mahallesi, Alemdar Cad. Soydan İş Merkezi Kat: 4 Nasıl: 122/A İzmit, Kocaeli/Turkey
Phone: +90 530 339 50 55 x E-mail: ujod@ujod.org

Publisher Contact
Address: Molla Gürani Mah. Kaçamak Sk. No: 21/1 34093 İstanbul, Turkey
Phone: +90 (530) 177 30 97 / +90 (212) 621 99 25
E-mail: info@galenos.com.tr/yayin@galenos.com.tr
Web: www.galenos.com.tr

Publisher Certificate Number: 14521
Online Publication Date: October 2024 **E-ISSN:**
International periodical journal published three times in a year.

ANATOLIAN JOURNAL OF
OBSTETRICS & GYNECOLOGY
RESEARCH

Years | 2024 | Issue | 2 | Month | August

CONTENTS

ANATOLIAN JOURNAL OF
OBSTETRICS & GYNECOLOGY
RESEARCH

Years | 2024 | Issue | 2 | Month | August

EDITORIAL

Obstetrics and Gynecology, cornerstones of women's health, continuously evolve as new research broadens our understanding and refines clinical practices. This collection of studies covers various topics, each contributing unique insights and addressing key challenges in the field. By focusing on current issues from thrombocyte-rich plasma (PRP) therapies to cesarean birth rates, these articles exemplify the commitment to enhancing patient care, reducing adverse outcomes, and exploring novel therapeutic approaches.

The first article, *"Thrombocyte-rich Plasma in Gynecology: A Review"*, explores a promising therapeutic avenue for several gynecological conditions. PRP therapy has emerged as an innovative option, aiming to enhance tissue repair and regeneration due to its high concentration of growth factors. The potential benefits of PRP therapy include applications in infertility treatments and management of endometrial pathologies. However, further research is necessary to refine treatment protocols, assess long-term efficacy, and establish standardized guidelines for its application in clinical practice.

In *"The Role of Matrix Metalloproteinases in Copper IUD-Induced Dysmenorrhea and Prolonged Menstruation"*, the authors shed light on the involvement of matrix metalloproteinases (MMPs) in copper intrauterine device (IUD) side effects. By examining the upregulation of MMPs, this study provides insight into the molecular mechanisms underlying dysmenorrhea and prolonged bleeding in IUD users. Identifying these pathways could ultimately guide the development of adjunct therapies to alleviate side effects and improve patient compliance with this highly effective contraceptive method.

Maternal health remains a critical concern, as seen in *"Decreasing and Maintaining Low Maternal Mortality Rate and Near Miss in Kocaeli District"*. This article highlights the success of targeted interventions to reduce maternal mortality rates in the Kocaeli district. Through comprehensive maternal healthcare programs and timely interventions, the district has demonstrated how systematic approaches can significantly impact maternal outcomes. Such studies are invaluable as models for broader implementation, particularly in regions still struggling with high maternal mortality rates.

Another compelling study, *"Could Clinically Suspicious Cervix Predict Cervical Premalignant and Malignant Lesions in Postmenopausal Women?"* tackles a critical issue in cervical cancer screening. The findings suggest that a visually suspicious cervix might serve as an early warning sign for premalignant and malignant lesions in postmenopausal women, emphasizing the importance of vigilant screening. This study supports the need for gynecologists to consider visual assessments as a supplementary tool in cervical cancer prevention.

The study on the differential expression of MMPs and other markers in endometrial polyps, *"MMP-1, MMP-2, MMP-9, TIMP-1, TIMP-2, MUC1, and CD29 Expression in Endometrial Polyps at Implantation Window Compared to Neighboring Normal Endometrium"*, provides insights into molecular distinctions between polyp tissues and normal endometrial tissue during the implantation window. The findings may have implications for understanding infertility in patients with endometrial polyps and aid in developing targeted treatments to enhance implantation success.

Cesarean section rates are a topic of debate worldwide, with the study *"What is an Ideal Cesarean Birth Rate? The Use of the C-Model and Further Interpretation with the Robson Classification System, A Retrospective Analysis"* offering valuable insights. This retrospective analysis applies both the C-Model and Robson Classification to understand cesarean trends, underscoring the complexity of defining ideal cesarean rates. By analyzing factors contributing to cesarean rates, this study provides a nuanced view that could guide future policies and practices for optimal delivery outcomes.

In exploring surgical innovations, *"Nazik Neovagina Technique: A Case Series"* presents a novel approach to neovagina construction, offering a potential alternative for patients requiring vaginal reconstruction. The technique demonstrates promising outcomes, which may expand options for patients with congenital or acquired vaginal defects.

Lastly, *"A Case Report of Uterine Myometrial Defect Following Hayman and Square Sutures for Postpartum Hemorrhage"* addresses complications following life-saving postpartum hemorrhage interventions. Documenting such cases is vital, as it encourages ongoing evaluation of current practices and highlights the need for further refinement of surgical techniques.

In sum, this issue reflects the diversity of modern obstetrical and gynecological research, addressing reproductive health challenges, maternal outcomes, cancer screening, and surgical innovations. These studies not only contribute to the existing body of knowledge but also pave the way for future advancements in patient-centered obstetrical and gynecological care. By continuing to address these pressing topics, we move closer to achieving optimal health outcomes for women worldwide.

Cemil Oğlak, MD
Associate Professor

Review

DOI: 10.4274/anajog.galenos.2024.29392

Anat J Obstet Gynecol Res 2024;2:41-47

Thrombocyte-rich Plasma in Gynecology: A Review

Hilal Gözüyukarı[1], Sevtap Hamdemir Kılıç[2]

[1]Yüksek İhtisas Hospital, Clinic of Obstetrics and Gynecology, Kırıkkale, Turkey
[2]Atılım University Faculty of Medicine, Department of Obstetrics and Gynecology, Ankara, Turkey

ABSTRACT

Platelet-rich plasma (PRP) is a specialized plasma preparation containing extremely high concentrations of platelets. The medical use of PRP has been accepted for many years and has produced favorable and better outcomes for disease management and prognosis in various fields. PRP has been widely used in orthopedics, plastic surgery, and many other fields since the early 1970s, but in recent years, its use in gynecology has become increasingly common. In gynecology, regenerative medicine was among the first areas to adopt PRP therapy, with largely positive results, which led to more extensive research in other areas of gynecology. The results of these studies demonstrate the importance of PRP in the treatment of gynecologic disorders, including genitourinary syndrome, urinary retardation, vesical vaginal fistulas, thin endometrium, and lymphatic sclerosis. This review summarizes the various uses of PRP in gynecology.

Keywords: Gynecology, thrombocyte-rich plasma, PRP

INTRODUCTION

Platelet-rich plasma (PRP) is an autologous plasma preparation enriched by increased concentrations of thrombocytes compared to those in full blood.[1] PRP is obtained by the centrifugation of whole blood.[2] It maakes use of the endogenous growth factors present in the thrombocyte granules to support the regeneration and repair processes of damaged tissues, cells, and organs. It is one of the most commonly used preparations in regenerative medicine today.[3] Depending on the preparation and method of thrombocyte activation, the following types of preparation are available: standard PRP and platelet-rich fibrin (PRF).

In 2014, Dohan Ehrenfest et al.[4] proposed the division of PRP presented in Table 1. There are four classifications of PRP: pure platelet-rich plasma (P-PRP), leukocyte- and platelet-rich plasma (L-PRP), pure platelet-rich fibrin (P-PRF), and leukocyte- and platelet-rich fibrin (L-PRF).[5] In order to categorize platelet products, they are separated according to their leukocytes concentration and the presence or absence of solid fibrin architecture. Pure P-PRP products are preparations with a low-density fibrin network, existing in the form of a liquid solution or activated gel, devoid of leukocytes.[5] Similarly, L-PRP products also exist in liquid or activated gel form, but these products also contain higher concentrations of leukocytes.[5,6] In contrast to plasma products, fibrin products exist exclusively as strong fibrin matrices, which can be handled as solid materials rather than liquids or gels. Within this category exist two subsets of PRF products: pure P-PRF as well as L-PRF products.[5] Due to the polymerization technique of these PRF products, the stable matrix allows for an extended, continuous release of growth factors for up to 28 days, which was hypothesized to enhance healing.[7] Delong et al.[8] created a new PRP classification system called platelet, activation, and white blood cells (WBC), which is based on the following parameters: the absolute number of platelets; the form of activation adopted; and the presence or absence of white cells. In this classification, the authors defined four different levels of platelet concentration: P1 (≤ baseline); P2 (> baseline-750,000 cells/μL); P3 (>750,000-1,250,000 cells/μL); and P4 (>1,250,000 cells/μL). Other

Table 1. Dohan Ehrenfest et al.[4] PRP classification

Preparation	Acronym	Leukocytes	Fibrin density
Pure platelet-rich plasma	P-PRP	Poor	Low
Leukocyte and platelet-rich plasma	L-PRP	Rich	Low
Pure platelet-rich fibrin	P-PRF	Poor	High
Leukocyte and platelet-rich fibrin	L-PRF	Rich	High

P-PRP: Platelet-rich plasma, L-PRP: Leukocyte- and platelet-rich plasma

Address for Correspondence: Hilal Gözüyukarı, Yüksek İhtisas Hospital, Clinic of Obstetrics and Gynecology, Kırıkkale, Turkey
Phone: +90 552 704 55 48 **E-mail:** hilalulucan202@gmail.com **ORCID ID:** orcid.org/0000-0003-0282-2596
Received: 23.07.2024 **Accepted:** 23.08.2024

considerations relate to the use or not of platelet activators, the presence of WBC, and neutrophils (above or below the basal value present in whole blood). According to the authors, the precise identification of the cellular components and the use and type of activator adopted are important information when comparing studies with PRP.[8] Mautner et al.[9] identified some variables for which none of the previously published PRP classifications showed all the characteristics that could influence PRP activity and efficacy. In this context, the authors showed that it is important to define platelet count (absolute number/μL), leukocyte content (as positive or negative) and percentage of neutrophils when present, red blood cells (RBCs) content (as positive or negative), and activation (yes or no for exogenous activation) in the platelet, leukocyte, red blood cell and activation classification.

The main advantage of using PRP is that the preparation is autologous and thus there is no risk of immune response and infection of microorganisms from other donors.[10] Another important advantage is that its preparation is simple and fast (about 30 minutes from blood extraction to application), and its cost is low.[11]

It is known that growth factors play an important role in the healing process and tissue regeneration.[12,13] This resulted in research examining various growth factors and their role in tissue repair.[12,14] However, there are conflicting reports about potential benefits. While some authors reported improved tissue healing with PRP, other researchers were less successful.[12,15,16] Alpha granules are storage units in platelets containing pre-packaged growth factors in inactive form. The growth factors contained in these granules are transformative growth factor-beta (TGF-β), vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), and epithelial growth factor (EGF) (Table 2). These growth factors are essential for increasing cell recruitment, proliferation, and differentiation during tissue regeneration, vascular remodeling, angiogenesis, inflammatory processes, and coagulation.[17]

Table 2. Growth factor chart

Platelet-derived growth factor	Stimulates cell replication
	Promotes angiogenesis
	Promotes epithelialization
	Promotes granulation tissue formation
Transforming growth factor	Promotes formation of extracellular matrix
	Regulates bone cell metabolism
Vascular endothelial growth factor	Promotes angiogenesis
Epidermal growth factor	Promotes cell differentiation and stimulates re-epithelialisation, angiogenesis and collagenase activity
Fibroblast growth factor	Promotes proliferation of endothelial cells and fibroblasts
	Stimulates angiogenesis

PRP Preparation

Different blood separation devices have different preparation stages but basically similar purposes. The Biomet Biologics GPS III (Platelet Concentration System, the patient's own platelets can be separated into a highly concentrated formula) system is briefly described. Approximately 30-60 mL of venous blood is taken from an anti-cubital vein by aseptic technique. It is recommended to use a butterfly needle of 18 or 19 g to prevent irritation and trauma to resting platelets. The blood is then placed in the Food and Drug Administration (FDA)-approved device and centrifuged for 15 minutes at 3200 rpm. Subsequently, the blood platelets are divided into weak platelet-poor plasma (PPP), RBC, and PRP. PPP is then removed through a dedicated port and discarded from the device. While the PRP is in a vacuumed space, the device is shaken for 30 seconds to re-suspend the platelets. The PRP is then withdrawn. Approximately 3 cc or 6 cc of PRP is obtained.[18]

The properties of PRP preparation systems are given in Table 3.[19] There were no significant differences between PRP separation systems in average PRP thrombocytes, RBCs, active TGF-β1, or fibrinogen concentrations (Table 4). There was only a significant difference in the effectiveness of thrombocyte capture.

The highest thrombocyte capture efficiency was achieved with Cascade, which is comparable to Magellan but significantly higher than GPS III. Significant differences in concentrations of WBC, PDGF-αβ, PGDF-ββ, and VEGF were observed among all systems. The cascade system concentrated weak PRP compared to leukocyte-rich PRP from the GPS III and

Table 3. PRP preparation system properties

Manufacturer	Device	Whole blood volume (mL)	Anticoagulant	Procedure	Centrifuge	Centrifuge time	mL
Emcyte	Genesis CS	54	ACD-A, 6 mL	Single spin	3600 RPMx10 min	10 min	6.0±0.0
Harvest	Smart PRP	54	ACD-A, 6 mL	Double spin	2500±150 RPMx1-3 min 2300±140 RPMx6-9 min	14 min	7.0±0.0
Arteriocyte	Magellan	52	ACD-A, 8 mL	Double spin	2800 RPM 3800 RPM	17 min	5.3±1.6
Biomet	GPS III	54	ACD-A, 6 mL	Single spin	3400 RPMx15 min	15 min	6.1±0.2

PRP: Platelet-rich plasma

Table 4. Mean platelet-rich plasma growth factor concentrations, ng/mL°

		PDGF-αβ	PDGF-ββ	TGF-β1	VEGF
Separation system, company	Cascade, MTF	9.7±3.6	14.8±2.5	0.1±0.08	0.3±0.3
	GPS III, Biomet	18.7±12.8	23.1±10.1	0.1±0.08	2.4±1.1
	Magellan, Arteriocyte	34.4±10.7	33.0±8.2	0.2±0.1	1.2±0.8
Comparison (p-values)	Among all systems (analysis of variance)	0.006	0.009	0.37	0.005
	Cascade vs. GPS III	0.52	0.33	0.99	0.004
	Cascade vs. Magellan	0.006	0.008	0.97	0.28
	GPS III vs. Magellan	0.08	0.20	0.54	0.12

°PDGF-αβ: Platelet-derived growth factor alpha-beta, PDGF-ββ: Platelet-derived growth factor beta-beta, TGF-β1: Transforming growth factor-beta 1 VEGF: Vascular endothelial growth factor

Magellan systems. GPS III and Magellan leukocytes were compared from Cascade to leukocytes poor PRP to increase concentrations of rich PRP, WBCs, PDGF-αβ, and VEGF.[20]

PRP injection is a relatively recent treatment modality, and therefore, robust data on the dosing of treatment, the location, frequency, and duration of its administration are scarce. Nevertheless, the adverse events of PRP therapy, such as infection, bleeding, and nerve damage, appear to be minimal.[21] It can be prepared manually, or there are different FDA-approved commercial PRP preparation kits, such as the GPSIII, Cascade and Magellan. With these kits, PRP substrates with different concentrations, using different coagulation activators, and with different leukocyte contents are obtained. Choukroun and Ghanaati[22] investigated growth factor release and total leukocyte and platelet counts for the first time in relation to the systematic variation of relative centrifugal force (RCF) exposure. The data showed that reducing RCF from a high range to a low spectrum in autologous PRF-based matrices resulted in a significant increase in leukocyte and platelet count as well as growth factor concentration of VEGF and TGF-β1. Furthermore, PRF clots produced with glass tubes showed higher weight (average 1.9±0.4 g) compared to silica-coated plastic tubes (average 1.6±0.3 g), although this difference was not significant. Recently, the importance of centrifuge tubes in the final production of PRF matrices has been reported.[23] Yamaguchi et al.[24] reported different platelet distributions in the concentrated growth factor (CGF) matrix when prepared with silica-coated plastic tubes or glass tubes. Platelets were distributed mainly on the distal side of the CGF matrix prepared with glass but homogeneously in the CGF matrix prepared with plastic.

In 2014, Ghanaati et al.[25] proposed a new protocol increasing the time of centrifugation and decreasing speed (A-PRF, RCFclot, 193 g, RCFmax: 276 g for 14 minutes) using glass tubes for blood collection. Recently, the same group introduced another modification by reducing centrifugation speed and duration even further (A-PRF+, RCFclot 145 g, RCFmax 208 g for 8 minutes). Reducing RCF resulted in an increase in the release of growth factors and in the concentration of leucocytes and platelets.[22]

REVIEW

In a case report by Kim et al.,[26] a 67-year-old woman who had been complaining of vaginal itching, irritation, and the appearance of her external genitalia for five years initially tried estrogen therapy but failed to achieve symptomatic relief. A total of 36 cc of autologous fat was collected from the abdomen using a 10 cc Luer-Lok syringe and a blunt-tipped two-hole cannula. A total of 4 cc autologous PRP was prepared from 30 cc whole blood using SmartPreP® APC-30 kit. A total of 40 cc autologous fat mixed with PRP was transferred into 1 cc syringes and injected aseptically into the subcutaneous layer of the labia majora through four ports injected into the subcutaneous layer of the labia majora. Within a month, itching and irritation disappeared, and there was a noticeable increase in volume in the labia majora in the immediate postoperative period.[26]

A prospective phase II pilot study conducted by Hersant et al.[27] enrolled twenty breast cancer survivors affected by vulva vaginal atrophy. Patients with a Gloria Bachman vaginal health index (VHI) score <15 received an A-PRP+HA (Regenkit A-PRP) combination intramucosally. Clinical evaluations were performed using VHI and female sexual distress (FSD) scores at months 0, 1, 3, and 6. Improvement in vaginal dryness and dyspareunia symptoms was observed, with a significant increase in VHI scores at six months ($p<0.0001$) and a significant decrease in FSD scores during the study ($p<0.0001$).[27]

A pilot study conducted by Long et al.[28] investigated the efficacy of A-PRP injections for the treatment of women with stress urinary incontinence (SUI). Twenty women with SUI received A-PRP injections through the anterior vaginal wall, near the middle of the urethra. Symptom severity was assessed using self-reported questionnaires before and six months after treatment. The study found a significant improvement in incontinence symptoms at both time points, with no adverse reactions. The treatment did not show a significant effect on sexual function. These results suggest that A-PRP injections may be a mildly effective, safe treatment for mild to moderate SUI in women and may open avenues for further research in this area.[29] Prodromidou et al.[29] conducted a systematic review to evaluate the efficacy of PRP in the treatment of urogynecologic disorders. The review included studies with patients who had vaginal atrophy, pelvic organ prolapse,

urinary incontinence, vaginal fistulas, and mesh exposure. The results suggested that PRP was a viable alternative method, especially when hormone therapy was contraindicated.

However, the study also concluded that more extensive, randomized trials are needed to fully establish the efficacy of PRP in these treatments.

In a meta-analysis by Maged et al.[30] in 2023, intrauterine and subendometrial PRP injection was proven to improve in vitro fertilization (IVF) cycle outcomes, such as rates of implantation, clinical pregnancy, live birth, and endometrial thickness in previously implantation failure and refractory infertile women with thin endometrium.

In obstetrics and gynecology, several different studies with small sample sizes have been conducted to investigate the effects of PRP injection into the uterus and ovaries.[31] The first reviews of intraovarian injections of PRP were published by Sills et al.[31] They reported improvement in laboratory values after intraovarian PRP in four women with premature ovarian failure. Later, Sfakianoudis et al.[32] reported the first pregnancy in a menopausal woman after intraovarian PRP injection.

The other indication for PRP administration is Asherman syndrome. According to studies by Aghajanova et al.,[33,34] treatment with intrauterine PRP infusion has been shown to improve endometrial function, as demonstrated by successful conception and ongoing clinical pregnancies without short- or long-term side effects. Together with robust *in vitro* data on human endometrial cells, these pilot clinical results were very reassuring, but the primary results obtained after a pilot study of 30 patients were not very instructive compared to standard therapy. Shen et al.[35] recruited women with moderate to severe intrauterine adhesions and randomly assigned them to either the PRP group or the control group. The results showed that intrauterine infusions of PRP did not improve clinical pregnancy rates. In contrast, Wang et al.[36] reported a significant improvement in clinical pregnancy rates and menstrual duration in the PRF group compared to the control group. PRF is a second-generation platelet concentrate containing mainly fibrin, platelets, and leukocytes.[37] Unlike PRP, PRF does not use anticoagulant in the preparation process and has a weak, flowing gel structure.[37] In the study, no significant difference was found between the cytokine concentrations measured in PPP supernatant and those measured in the actual PRF clot. Moreover, PRF may prolong cytokine lifespan by promoting the slow release of cytokines.[38] However, further research is needed to assess whether the therapeutic effect of PRF is superior to PRP.

Molina et al.[39] followed 19 patients with resistant endometrium, aged between 33 and 45 years, who had undergone IVF and in whom PRP was infused into the uterine cavity via a catheter. PRP was used twice, after the 10th day of hormone replacement therapy and 72 hours after the first administration. The endometrial thickness was reported to be >7.0 mm after the first application, and in all cases, the endometrial thickness was >9.0 mm after the second application. The entire study group was qualified for embryo transfer at the blastocyst stage. Pregnancy tests were positive in 73.7% of cases, 26.3% of which resulted in live births; 26.3% had ongoing pregnancies; 10.5% had biochemical pregnancies; and 5.3% had fetal death by 16 weeks.[39] In another publication, Zadehmodarres et al.[40] reported that they enrolled ten patients with a history of insufficient endometrial thickness in frozen-thawed embryo transfer cycles. In each patient, PRP treatment increased endometrial thickness, and embryo transfer was performed. Five patients became pregnant after treatment, and in four cases, the pregnancy progressed normally.

PRP is emerging as a promising therapeutic modality that shows promise in the treatment of refractory conditions.[41,42] PRP is rich in growth factors that have been implicated in cellular growth, differentiation, angiogenesis, and tissue repair.[43] The administration of PRP into the ovaries is thought to stimulate the activation of potential ovarian stem cells, resulting in the secretion of factors that facilitate follicular growth and development.[44] Furthermore, PRP may augment ovarian blood flow through the promotion of angiogenesis, thereby improving the delivery of oxygen and nutrients to developing follicles.[43,45] In cases where ovarian dysfunction makes it difficult to conceive, PRP injection into both ovaries has been attempted. The effect of the administration was an increase in the number of ovarian oocytes.[45] Autologous intraovarian PRP treatment in women with poor ovarian reserve and early menopause also increased anti-Müllerian hormone levels and decreased follicle-stimulating hormone concentrations; clinical and live birth rates tended to increase.[47,48] In a related study, Farimani et al.[46] published a study involving 19 women. The mean number of oocytes before and after PRP injection was 0.64 and 2.1, respectively. A spontaneous pregnancy occurred in two patients.

In the third case, clinical pregnancy was achieved, and a healthy baby was born.

PRP infiltrations may play a role in symptom relief in selected cases of patients with severe lichen sclerosis (LS) who have not responded to first-line therapy or for whom other treatments are poorly tolerated or contraindicated. Medina Garrido et al.[49] administered three PRP infiltrations to 28 postmenopausal female patients with biopsy-proven LS and an inadequate response to steroid therapy. The change in score according to the Clinical Scoring System for Vulvar Lichen Sclerosus was measured six times over the course of one year and they reported a statistically significant improvement. In another study involving the largest number of patients to date (94 patients), both female and male patients showed a significant reduction in symptoms and improvement in sexual function and quality of life after six months of PRP treatment.[50]

Sukgen et al.[51] investigated the effects of PRP injection into the lower third of the anterior vaginal wall on sexual function, orgasm, and genital perception in women with sexual dysfunction. The study revealed that PRP administration to the distal part of the anterior vaginal wall as a minimally invasive method can improve female sexuality and provide higher satisfaction. In another study of 68 women aged between 32 and 97 years, O-shot injection, the application of PRP to the vulvovaginal area, was found to be a satisfactory method for women with problems, such as stress incontinence, overactive bladder, lack of lubrication, and sexual dysfunction, such as

lack of libido, arousal, and dyspareunia. The results also showed that 94% of these patients were satisfied but did not show improvement in 6% of all patients with an overactive bladder.[52]

Gorlero et al.[53] evaluated the effect of PRP in patients with recurrent pelvic organ prolapse surgery. PRF was prepared in 10 patients using the Vivostat PRF system developed by Vivostat A/S and applied over the dissected pubourethral fascia before vaginal skin closure. The authors observed an anatomical success rate of 80%, and patients reported a 100% improvement in symptoms. Despite these excellent results, the authors did not go on to study a larger group of women affected by vaginal prolapse.

CONCLUSION

PRP has been one of the most widely used preparations in reconstructive medicine for over 20 years. Its growth factors and proteins have proven to be effective in wound healing and regeneration processes. Its low cost, ease of preparation, and minimally invasive application make the clinical use of PRP increasingly widespread. The absence of any risk of side effects is another reason for preference. Autologous PRP is a new alternative approach for the treatment and management of some etiologies of infertility, especially in women resistant to standard therapy. PRP is known to be effective in demonstrating endometrial regeneration, restoring the menstrual cycle, improving folliculogenesis, enhancing endometrial receptivity, and increasing clinical pregnancy and live birth rates. PRP has a wide range of applications in reproductive medicine, such as Asherman's syndrome, cases of thin endometrium, urinary incontinence, and adjunctive treatment of recurrent genitourinary fistulas. Although this would require a randomized controlled trial with a larger sample size, the small amount of information from the few studies currently published shows promise that PRP therapy, with the appropriate preparation, may in the near future be able to solve many of the challenges currently faced in obstetrics and gynecology. There are many factors in PRP applications that are not yet agreed upon. One of these is the effectiveness of serial injections. There are physicians who administer PRP injections at intervals of 2-4 weeks, as well as physicians who wait at least two months for a new injection or longer in chronic cases. The consequences of these differences in practice on the efficacy of PRP are not yet known. Other issues that have not been standardized are the buffering of PRP with the addition of bicorbanate and the addition of platelet activating agents, such as calcium chloride and thrombin to PRP for optimal release of growth factors from platelets. Other limitations include the shortcomings of existing PRP classification systems, the lack of standard protocols and definitions for centrifugation and preparation, cellular components such as platelet concentration, red and WBC counts, and the platelet activation procedure.

PRP injections are a new prospective treatment modality for chronic refractory diseases that we frequently encounter in gynecology practice and the treatment of which has failed with existing conservative methods.

The increasing popularity of PRP should not ignore the fact that there is still insufficient data concerning its use. In studies, groups are small, randomization is insufficient, and the level of evidence is low.

Larger scale, well-designed randomized controlled trials are needed to determine the efficacy of PRP in gynecological conditions.

Footnote

Author Contributions

Surgical and Medical Practices: H.G., S.H.K., Concept: H.G., S.H.K., Design: H.G., S.H.K., Data Collection and Processing: H.G., S.H.K., Analysis and Interpretation: H.G., S.H.K., Literature Search: H.G., S.H.K., Writing: H.G., S.H.K.

Conflict of Interest: The authors have no conflict of interest to declare.

Financial Disclosure: The authors declared that this study has received no financial support.

REFERENCES

1. Wu PI, Diaz R, Borg-Stein J. Platelet-rich plasma. Phys Med Rehabil Clin N Am. 2016;27(4):825-853.
2. Paoloni J, De Vos RJ, Hamilton B, Murrell GA, Orchard J. Platelet-rich plasma treatment for ligament and tendon injuries. Clin J Sport Med. 2011;21(1):37-45.
3. Alves R, Grimalt R. A review of platelet-rich plasma: History, biology, mechanism of action, and classification. Skin Appendage Disord. 2018;4(1):18-24.
4. Dohan Ehrenfest DM, Rasmusson L, Albrektsson T. Classification of platelet concentrates: from pure platelet-rich plasma (P-PRP) to leucocyte- and platelet-rich fibrin (L-PRF). Trends Biotechnol. 2009;27(3):158-167.
5. Dohan Ehrenfest DM, Andia I, Zumstein MA, Zhang CQ, Pinto NR, Bielecki T. Classification of platelet concentrates (platelet-rich plasma-PRP, platelet-rich fibrin-PRF) for topical and infiltrative use in orthopedic and sports medicine: current consensus, clinical implications and perspectives. Muscles Ligaments Tendons J. 2014;4(1):3-9.
6. Everts PA, Hoffmann J, Weibrich G, et al. Differences in platelet growth factor release and leucocyte kinetics during autologous platelet gel formation. Transfus Med. 2006;16(5):363-368.
7. Zumstein MA, Berger S, Schober M, et al. Leukocyte- and platelet-rich fibrin (L-PRF) for long-term delivery of growth factor in rotator cuff repair: review, preliminary results and future directions. Curr Pharm Biotechnol. 2012;13(7):1196-1206.
8. DeLong JM, Russell RP, Mazzocca AD. Platelet-rich plasma: the PAW classification system. Arthroscopy. 2012;28(7):998-1009.
9. Mautner K, Malanga GA, Smith J, et al. A call for a standard classification system for future biologic research: the rationale for new PRP nomenclature. PM R. 2015;7(4 Suppl):S53-S59.
10. Mehta S, Watson JT. Platelet rich concentrate: basic science and current clinical applications. J Orthop Trauma. 2008;22(6):432-438.
11. Rutkowski JL, Thomas JM, Bering CL, et al. Analysis of a rapid, simple, and inexpensive technique used to obtain platelet-rich plasma for use in clinical practice. J Oral Implantol. 2008;34(1):25-33.
12. Anitua E, Sánchez M, Nurden AT, Nurden P, Orive G, Andía I. New insights into and novel applications for platelet-rich fibrin therapies. Trends Biotechnol. 2006;24(5):227-234.

13. Werner S, Grose R. Regulation of wound healing by growth factors and cytokines. Physiol Rev. 2003;83(3):835-870.
14. Kirker-Head CA. Potential applications and delivery strategies for bone morphogenetic proteins. Adv Drug Deliv Rev. 2000;43(1):65-92.
15. Froum SJ, Wallace SS, Tarnow DP, Cho SC. Effect of platelet-rich plasma on bone growth and osseointegration in human maxillary sinus grafts: three bilateral case reports. Int J Periodontics Restorative Dent. 2002;22(1):45-53.
16. Raghoebar GM, Schortinghuis J, Liem RS, Ruben JL, van der Wal JE, Vissink A. Does platelet-rich plasma promote remodeling of autologous bone grafts used for augmentation of the maxillary sinus floor? Clin Oral Implants Res. 2005;16(3):349-356.
17. Cecerska-Heryć E, Goszka M, Serwin N, et al. Applications of the regenerative capacity of platelets in modern medicine. Cytokine Growth Factor Rev. 2022;64:84-94.
18. Sampson S, Gerhardt M, Mandelbaum B. Platelet rich plasma injection grafts for musculoskeletal injuries: a review. Curr Rev Musculoskelet Med. 2008;1(3-4):165-174.
19. Degen RM, Bernard JA, Oliver KS, Dines JS. Commercial separation systems designed for preparation of platelet-rich plasma yield differences in cellular composition. HSS J. 2017;13(1):75-80.
20. Castillo TN, Pouliot MA, Kim HJ, Dragoo JL. Comparison of growth factor and platelet concentration from commercial platelet-rich plasma separation systems. Am J Sports Med. 2011;39(2):266-271.
21. Dawood AS, Salem HA. Current clinical applications of platelet-rich plasma in various gynecological disorders: An appraisal of theory and practice. Clin Exp Reprod Med. 2018;45(2):67-74.
22. Choukroun J, Ghanaati S. Reduction of relative centrifugation force within injectable platelet-rich-fibrin (PRF) concentrates advances patients' own inflammatory cells, platelets and growth factors: the first introduction to the low speed centrifugation concept. Eur J Trauma Emerg Surg. 2018;44(1):87-95.
23. Castro A, Andrade CX, Li X, Pinto NR, Teughels W, Quirynen M. Impact of g force and timing on the characteristics of platelet-rich fibrin matrices. Sci Rep. 2021;11:6038.
24. Yamaguchi S, Aizawa H, Sato A, et al. Concentrated growth factor matrices prepared using silica-coated plastic tubes are distinguishable from those prepared using glass tubes in platelet distribution: application of a novel near-infrared imaging-based, quantitative technique. Front Bioeng Biotechnol. 2020;8:600.
25. Ghanaati S, Booms P, Orlowska A, et al. Advanced platelet-rich fibrin: a new concept for cell-based tissue engineering by means of inflammatory cells. J Oral Implantol. 2014;40(6):679-689.
26. Kim SH, Park ES, Kim TH. Rejuvenation using platelet-rich plasma and lipofilling for vaginal atrophy and lichen sclerosus. J Menopausal Med. 2017;23(1):63-68.
27. Hersant B, SidAhmed-Mezi M, Belkacemi Y, et al. Efficacy of injecting platelet concentrate combined with hyaluronic acid for the treatment of vulvovaginal atrophy in postmenopausal women with history of breast cancer: a phase 2 pilot study. Menopause. 2018;25(10):1124-1130.
28. Long CY, Lin KL, Shen CR, et al. A pilot study: effectiveness of local injection of autologous platelet-rich plasma in treating women with stress urinary incontinence. Sci Rep. 2021;11(1):1584.
29. Prodromidou A, Zacharakis D, Athanasiou S, et al. The emerging role on the use of platelet-rich plasma products in the management of urogynaecological disorders. Surg Innov. 2022;29(1):80-87.
30. Maged AM, Mohsen RA, Salah N, Ragab WS. The value of intraovarian autologous platelet rich plasma in women with poor ovarian reserve or ovarian insufficiency: a systematic review and meta-analysis. BMC Pregnancy Childbirth. 2024;24(1):85.
31. Sills ES, Rickers NS, Li X, Palermo GD. First data on *in vitro* fertilization and blastocyst formation after intraovarian injection of calcium gluconate-activated autologous platelet rich plasma. Gynecol Endocrinol. 2018;34(9):756-760.
32. Sfakianoudis K, Simopoulou M, Nitsos N, et al. autologous platelet-rich plasma treatment enables pregnancy for a woman in premature menopause. J Clin Med. 2018;8(1):1.
33. Aghajanova L, Sundaram V, Kao CN, et al. Autologous platelet-rich plasma treatment for moderate-severe Asherman syndrome: the first experience. J Assist Reprod Genet. 2021;38(11):2955-2963.
34. Aghajanova L, Cedars MI, Huddleston HG. Platelet-rich plasma in the management of Asherman syndrome: case report. J Assist Reprod Genet. 2018;35(5):771-775.
35. Shen M, Duan H, Lv R, Lv C. Efficacy of autologous platelet-rich plasma in preventing adhesion reformation following hysteroscopic adhesiolysis: a randomized controlled trial. Reprod Biomed Online. 2022;45(6):1189-1196.
36. Wang Z, Yang M, Mao L, et al. Efficacy and safety of autologous platelet-rich fibrin for the treatment of infertility with intrauterine adhesions. J Obstet Gynaecol Res. 2021;47(11):3883-3894.
37. Dohan DM, Choukroun J, Diss A, et al. Platelet-rich fibrin (PRF): a second-generation platelet concentrate. Part I: technological concepts and evolution. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2006;101(3):e37-e44.
38. Dohan DM, Choukroun J, Diss A, et al. Platelet-rich fibrin (PRF): a second-generation platelet concentrate. Part II: platelet-related biologic features. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2006;101(3):e45-e50.
39. Molina A, Sánchez J, Sánchez W, Vielma V. Platelet-rich plasma as an adjuvant in the endometrial preparation of patients with refractory endometrium. JBRA Assist Reprod. 2018;22(1):42-48.
40. Zadehmodarres S, Salehpour S, Saharkhiz N, Nazari L. Treatment of thin endometrium with autologous platelet-rich plasma: a pilot study. JBRA Assist Reprod. 2017;21(1):54-56.
41. Everts P, Onishi K, Jayaram P, Lana JF, Mautner K. Platelet-rich plasma: New performance understandings and therapeutic considerations in 2020. Int J Mol Sci. 2020;21(20):7794.
42. Streit-Ciećkiewicz D, Kołodyńska A, Futyma-Gąbka K, Grzybowska ME, Gołacki J, Futyma K. Platelet rich plasma in gynecology-discovering undiscovered-review. Int J Environ Res Public Health. 2022;19(9):5284.
43. Sills ES, Wood SH. Autologous activated platelet-rich plasma injection into adult human ovary tissue: molecular mechanism, analysis, and discussion of reproductive response. Biosci Rep. 2019;39(6):BSR20190805.
44. Marchante M, Buigues A, Ramirez-Martin N, et al. Single intraovarian dose of stem cell- and platelet-secreted factors mitigates age-related ovarian infertility in a murine model. Am J Obstet Gynecol. 2023;228(5):561.e1-561.e17.
45. Seckin S, Ramadan H, Mouanness M, Kohansieh M, Merhi Z. Ovarian response to intraovarian platelet-rich plasma (PRP) administration: hypotheses and potential mechanisms of action. J Assist Reprod Genet. 2022;39(1):37-61.
46. Farimani M, Heshmati S, Poorolajal J, Bahmanzadeh M. A report on three live births in women with poor ovarian response following intra-ovarian injection of platelet-rich plasma (PRP). Mol Biol Rep. 2019;46(2):1611-1616.
47. Sharara FI, Lelea LL, Rahman S, Klebanoff JS, Moawad GN. A narrative review of platelet-rich plasma (PRP) in reproductive medicine. J Assist Reprod Genet. 2021;38(5):1003-1012.
48. Elnashar AM. Intraovarian platelet-rich plasma: current status. Middle East Fertil Soc J. 2021;26:30.
49. Medina Garrido C, Cano García A, de la Cruz Cea L, Oreja Cuesta AB. Mid-term symptomatic relief after platelet-rich plasma infiltration in vulvar lichen sclerosus. Arch Dermatol Res. 2023;315(6):1527-1532.
50. Tedesco M, Garelli V, Bellei B, et al. Platelet-rich plasma for genital lichen sclerosus: analysis and results of 94 patients. Are there

gender-related differences in symptoms and therapeutic response to PRP? J Dermatolog Treat. 2022;33(3):1558-1562.

51. Sukgen G, Ellibeş Kaya A, Karagün E, Çalışkan E. Platelet-rich plasma administration to the lower anterior vaginal wall to improve female sexuality satisfaction. Turk J Obstet Gynecol. 2019;16(4):228-234.
52. Neto JB. O-Shot: Platelets rich plasma in intimate female treatment. J Women's Health Care. 2017;6:395.
53. Gorlero F, Glorio M, Lorenzi P, Bruno-Franco M, Mazzei C. New approach in vaginal prolapse repair: mini-invasive surgery associated with application of platelet-rich fibrin. Int Urogynecol J. 2012;23(6):715-722.

Original Article

DOI: 10.4274/anajog.galenos.2024.65375

Anat J Obstet Gynecol Res 2024;2:48-53

The Role of Matrix Metalloproteinases in Copper IUD-Induced Dysmenorrhea and Prolonged Menstruation

Hayal Uzelli Şimşek[1], Ömer Doğukan Saraç[2], Ebru Coşkun[3], Bahar Müezzinoğlu[4], Aydın Çorakçı[1]

[1]Kocaeli University Faculty of Medicine, Department of Obstetrics and Gynecology, Kocaeli, Turkey
[2]Kocaeli City Hospital, Clinic of Obstetrics and Gynecology, Kocaeli, Turkey
[3]Private Asya Hospital, Clinic of Gynecology and Obstetrics, İstanbul, Turkey
[4]Medipol University Faculty of Medicine, Department of Obstetrics and Gynecology, İstanbul, Turkey

ABSTRACT

Purpose: This study was conducted to determine the role of matrix metalloproteinase (MMP) and tissue inhibitors of metalloproteinase (TIMP) expression in copper intrauterine device (IUD)-induced dysmenorrhea and prolonged menstruation.

Methods: This prospective clinical study included 30 women who were willing to use copper IUD contraception. Endometrial biopsies were performed before and 3 months after the insertion of the IUD. Correlations between menstrual bleeding abnormalities, visual analog scale (VAS) dysmenorrhea scores related to copper-IUD usage and MMP-1, MMP-2, MMP-9, TIMP-1, and TIMP-2 endometrial expression were investigated.

Results: The mean ± standard deviation VAS score at 3 months after IUD insertion increased compared with before IUD use (3.5±1.8 vs. 2.8±0.9; p=0.003). The mean duration of menstrual bleeding was significantly prolonged three months after IUD use compared to before use (4.5±1.1 days vs. 4.06±0.8; p<0.001). MMP-1 expression in the luminal epithelium was present in 1.4±2.98 (0-8) cells per 100 cells before IUD use and significantly increased to 14.7±20.7 (0-76) cells three months after IUD insertion (p<0.001). MMP-1 expression was positively correlated with the presence of dysmenorrhea VAS score (p<0.001) and duration of menstruation (p=0.04) three months after IUD insertion. MMP-9 expression was increased three months after IUD use (47.9±40.2, 4-90 cells) compared to before IUD use (26.8±23.5, 4-80 cells, p=0.01) in luminal epithelium. MMP-9 expression grade in luminal epithelium (p=0.003) and stroma glandular epithelium (p=0.001) increased significantly as the menstrual pattern progressed from light to heavy. As MMP-9 expression grade in luminal epithelium and stroma glandular epithelium increased the mean duration of menstrual bleeding became longer (p=0.01, p=0.01, respectively).

Conclusion: With the use of copper-IUDs, VAS score increased and duration of menstruation was prolonged. MMP-1 expression increased in cases with menstrual pain and duration of menstruation while MMP-9 expression increased as the duration and/or the severity of menstrual bleeding increased.

Keywords: Intrauterine device, menstrual bleeding, dysmenorrhea, matrix metalloproteinases, tissue inhibitors of matrix metalloproteinase

INTRODUCTION

Matrix metalloproteinases (MMPs), are a group of enzymes involved in matrix degradation.[1,2] The family comprises interstitial collagenases, gelatinases, stromelysins, and membrane-type MMPs. MMPs are inhibited either by specific tissue inhibitors of metalloproteinases (TIMPs), or less specifically, by alfa 2-macroglobulin.[3,4]

MMPs are involved in several key reproductive events, such as ovulation, embryo implantation, menstruation and postpartum uterine involution.[5-8] Progesterone withdrawal increases production of pro-MMP-2 by decidualized stromal cells and activates endometrial proteases MMP-1, -2, -3, and -9.[9] MMP-9 is found in the epithelium only during the early secretory phase, while during menstruation it is predominantly present in a variety of leukocytes.[10]

TIMPs are the major endogenous regulators of MMPs and consist of four homologous members (TIMP 1-4). TIMP-1 binds to and inhibits the active form of MMPs. TIMP-2 is differentially regulated from TIMP-1 and has been proposed to act selectively on different MMPs. TIMP-3 has a high affinity

Address for Correspondence: Hayal Uzelli Şimşek, Kocaeli University Faculty of Medicine, Department of Obstetrics and Gynecology, Kocaeli, Turkey
Phone: +90 505 539 48 28 **E-mail:** jinekolog.dr@hotmail.com **ORCID ID:** orcid.org/0000-0002-1197-1326
Received: 17.04.2024 **Accepted:** 28.04.2024

for MMP-9 and has the ability to inhibit membrane type-1 MMP (MT1-MMP). However, unlike TIMP-1 or TIMP-2, TIMP-3 is secreted and then bound to the extracellular matrix (ECM). TIMP-4 is a good inhibitor for all classes of MMPs without remarkable preference for specific MMPs.[11]

Copper-intrauterine devices (IUDs) cause a sterile inflammatory reaction in the endometrium.[12] The most common side effects of an IUD are increased menstrual bleeding and pain and the removal rate for these complaints within the first year of IUD use is 5-15%.[13] Labied et al.[14] investigated MMP expressions in the endometrium in levonorgestrel-releasing intrauterine system users. However, the effect of copper-IUD on these inflammatory systems remains unclear and how the copper-IUD causes side effect, such as abnormal menstrual bleeding, is unknown.

In this study, we aimed to determine the role of copper-IUD on the expression of MMP-1, MMP-2, MMP-9, TIMP-1 and TIMP-2 in luteal phase endometrium. We assessed the relationship between tissue expression of these MMPs and TIMPs in menstrual bleeding abnormalities and dysmenorrhea associated with copper-IUD usage.

METHODS

The study population consisted of women whose midluteal phase progesterone level was 10 ng/mL (31.8 nmol/L) and who were willing to use a copper-IUD for contraception. The women were menstruating regularly (cycle varying between 28-35 days). The exclusion criteria were pregnancy, acute or chronic pelvic inflammatory disease, metrorrhagia for unknown reason, cervicitis, dysplasia in the cervix, genital tumor, copper allergy, usage of contraceptive pills within the previous three months, lactating women, hypothyroidism, hyperthyroidism, diabetes mellitus, hyperprolactinemia, abnormalities in blood clotting and severe dysmenorrhea. Menstrual day-3 serum thyroid stimulating hormone levels were 1.7±1.5 μIU/mL, follicule stimulating hormone levels were 7.2±2.4 mU/mL, luteinizing hormone levels were 5.1±2.1 mU/mL, estradiol levels were 42.7±24.6 pg/mL, international normalized ratios were 1.02±0.07, fasting blood glucose levels were 92±4.3 mg/dL, and prolactin levels were 14.9±6.7 ng/mL. Informed consent was taken from the women and the study was approved by the the Ethics Committee of the Kocaeli University Faculty of Medicine (approval number: KOÜ 2008/124, date: 2008).

All patients underwent gynecological examination and had a Papanicolaou smear taken during the previous 12 months. Menstruation period, bleeding quantity and dysmenorrhea was recorded before and three months after the IUD insertion. A menstrual calendar was used to evaluate the amount and the duration of menstrual bleeding. The amount of menstrual bleeding was defined as mild (<1/4 of tampon or pad surface stained with blood), moderate (1/4-3/4 of tampon or pad surface stained with blood) or heavy (>3/4 of tampon or pad surface stained with blood). Dysmenorrhea were estimated using the visual analogue scale (VAS) at the initial examination and after IUD insertion. The VAS score (0-10) of each patient was calculated as an arithmetic mean of the first three days of the menstrual cycle. Prolonged menstruation was defined as a menstrual period lasting more than eight days according to the Federation of Gynecology and Obstetrics ovulatory disorders classification.[15]

The biopsy specimens were taken with a Pipelle curette without dilatation of cervix and without anesthesia. Endometrial biopsies were taken from the mid-uterine cavity before and three months after the insertion of the IUD, on day 20-24 of the first and third cycle. The biopsy specimens were fixed in 10% neutral buffered formalin for at least six hours. After overnight tissue processing they were embedded in parafin. Tissue sections for histological examination were 5 μm thick and stained with hematoxylene and eosin. Sections for immunohistochemical examination were also 5 μm thick and mounted onto adhesive-coated slides (Superfrost® Plus, Menzel-Gloser, Germany). For immunohistochemical staining, sections were kept at 56 °C overnight and then soaked in xylene for 30 mins. After washing with a decreasing series of ethanol, sections were washed with distilled water and phosphate-buffered saline (PBS) for 15 minutes. Then, coated slides were dried in an incubator at 56 °C for two hours. Antigen unmasking was performed for MMP-1 antibody, MMP-2 antibody, MMP-9 antibody, TIMP-1 antibody, and TIMP-2 antibody in a citrated buffer solution in the commercially available pressure cooker at 1 atmosphere pressure for 90 seconds.

After antigen unmasking, step slides were washed with PBS (pH 7.4) and then in order to block endogenous peroxidase activity, the slides were incubated in 3% hydrogen peroxide for 20 minutes. Slides were washed again with PBS for five minutes. Sections were then blocked with Super Block (REF:AAA 125 LOT:12232, CP Lab Safety, California, USA) at room temperature for 15 minutes and afterwards washed with PBS. Slides were immunostained with rabbit polyclonal antibody against human MMP-1 (Collagenase-1) (Neo-Markers, Fremont, CA, USA, Cat No: RB-1536-P) at 1:50 dilution for one hour in the microwave, with rabbit monoclonal antibody against MMP-2 (72kDa Collagenase IV) Ab-4 (Neo-Markers Fremont, CA, USA, Cat No: MS-806-P at 1:100 dilution for one hour in the pressure cooker, with rabbit polyclonal antibody against MMP-9 (92 kDa Collagenase type IV) Ab-9 (Neo-Markers Fremont, CA, USA, Cat No:RB-1539-P) at 1:50 dilution for two hours, with mouse monoclonal antibody against TIMP-1 (NCL-TIMP1-485, Novacastra, Newcastle, UK) at 1:150 dilution for one hour and with mouse monoclonal antibody against TIMP-2 (Clone 3A4) (Neo-Markers Fremont, CA, USA, Cat No:MS-1485-P) at 1:25 dilution for two hours at room temperature (20-250C).

Afterwards, slides were washed with PBS and slides were incubated with UltraTek Anti-Polyvalent Biotinylated Antibody (REF:ABN 125, LOT:11461, ScyTek Laboratories, Utah, USA) at room temperature for 25 minutes. Slides were washed with PBS again and incubated with UltraTek horse radish peroxidase (REF:ABL125, LOT:11460, ScyTek Laboratories, Utah, USA) at room temperature for 25 minutes. Slides were washed with PBS and incubated with Ultravision Detection System Large Volume AEC Substrate System (REF:TA-125-HA, LOT:AHA60718, LabVision, Fremont, CA, USA) at room temperature for 15 minutes. The sections were finally

counterstained using Mayer's hematoxylin and mounted in an aqueous medium.

Slides were analyzed with a BX50 conventional light microscope (Olympus, Tokyo, Japan) by BM at 100 and 200 magnification twice. Staining intensity was graded as; "0= no staining", "+1 or 1-10% cells stained = weak staining", "+2 or 10-49% cells stained = mild staining" and "+3 or 50-100% cells stained = strong staining". Immunohistochemical staining in luminal and stromal glandular epithelium cytoplasm were graded in 60 sections counting 100 cells separately at 400x magnification.

Statistical Analysis

The statistical analysis of the study data was performed using SPSS, version 11.5, for Windows (IBM Inc., Armonk, NY, USA). All values reported are mean (± SD) or percentage. The McNemar Bowker test was used for immunostaining grade and intensity before and three months after IUD insertion. Analysis of classified data was performed using chi-square test and/or Fisher's exact test. Comparison of classified data was performed using McNemar-Bowker test before and after IUD use. Correlation of the data was determined using Pearson correlation test. Comparison of continuous variables between different grades of staining was performed using Kruskall-Wallis analysis. Probability (p) values <0.05 were considered statistically significant.

RESULTS

The study group numbered 30 women with a mean ± SD age of 32.8±5.3 years, ranging from 25 to 40 years. The first biopsy was made at cycle day 22.7±1.3 before IUD insertion and the second biopsy was made at cycle day 22.1±1.5 three months after IUD insertion (p=0.06). Before IUD insertion, menstrual bleeding was mild in 3 (10%) patients and moderate in 27 (90%) patients while it was mild in 2 (6.7%) patients, moderate in 20 (66.7%) patients, and heavy in 8 (26.7%) patients three months after IUD (p=0.1). The mean ± SD VAS score at the cycle three months after IUD insertion increased significantly compared to before IUD use (3.5±1.8 vs. 2.8±0.9, p=0.003). The mean ± SD duration of menstrual bleeding was longer three months after IUD use compared to before use (4.5±1.1 days vs. 4.06±0.8, p<0.001).

MMP-1 expression of the luminal epithelium was present in 1.4±2.98 (0-8) cells per hundred cells before IUD use and significantly increased to 14.7±20.7 (0-76) cells three months after IUD insertion (p<0.001). MMP-1 expression was positively correlated with the VAS score (p<0.001) three months after IUD insertion. Three months after IUD insertion, women with dysmenorrhea (n=9) had significantly higher mean luminal epithelium cytoplasm MMP-1 expression of 21.6±24.2% compared to women without dysmenorrhea (n=21) in whom the expression was 1.9±2.4% (p=0.04). Grade of expression of MMP-1 in the luminal epithelium with respect to menstrual bleeding amount and duration is presented in Table 1. As MMP-1 expression increased, the mean duration of menstruation became longer (p=0.04), but the severity of menstrual bleeding was similar.

MMP-2 expression of the luminal epithelium was similar before compared to three months after IUD insertion (36±26.7, 5-80 cells versus 41.2±22.6, 6-80 cells, respectively; p=0.41). No correlation was found between MMP-2 expression in the luminal epithelium and the mean duration of menstruation, the severity of menstrual bleeding or VAS score (Table 2).

MMP-9 expression increased three months after IUD use compared to before IUD use (47.9±40.2, 4-90 cells versus 26.8±23.5, 4-80 cells, respectively; p=0.01) in luminal epithelium. MMP-9 expression in the stroma was similar before compared to three months after IUD use (11.3±20.7, 0-70 cells versus 13.7±17.4, 0-40 cells, respectively; p=0.6). No correlation was found between increase of the MMP-9 expression of the luminal epithelium or the endometrial

Table 1. Menstrual bleeding volume and duration with respect to the expression grade of the MMP-1 antibody in the luminal epithelium

Grade of MMP-1 immunostaining in luminal epithelia	0	+1	+2	+3	p-value
Menstrual bleeding (n) Light	1	1	0	0	
Moderate	8	10	2	0	0.7
Heavy	3	4	0	1	
Duration of menstrual Bleeding (days)	4.3±1.1	4.4±1.1	5.5±0.7	7	0.04*

MMP: Matrix metalloproteinases, *p<0.05, statistically significant

Table 2. Menstrual bleeding volume and duration with respect to MMP-2 expression grade in luminal epithelium

Grade of MMP-2 immunostaining in luminal epithelia	0	+1	+2	+3	p-value
Menstrual bleeding (n) Light	0	1	1	0	
Moderate	0	3	10	1	0.08
Heavy	0	0	11	53	
Duration of menstrual bleeding (days)	0	4.5±1.2	4.3±1.1	5.1±1.4	0.4

MMP: Matrix metalloproteinases

stroma glandular epithelium and VAS score. Menstrual bleeding amount and duration of menstrual bleeding with respect to expression grade of the MMP-9 antibody in the luminal epithelium and in the endometrial stroma glandular epithelium is presented in Table 3. MMP-9 expression grade in luminal epithelium (p=0.003) and stroma glandular epithelium (p=0.001) increased significantly as the menstrual pattern progressed from light to heavy. As MMP-9 expression grade in luminal epithelium and stroma glandular epithelium increased the mean duration of menstrual bleeding become longer (p=0.01, p=0.01, respectively).

Expression grade of TIMP-1 in the luminal epithelium and endometrial stroma glandular epithelium is presented in Table 4. TIMP-1 expression in luminal epithelium and stroma glandular epithelium was similar after three months compared to before IUD insertion (p=0.1, p=0.06 respectively). No correlation was found between TIMP-1 expression in the luminal epithelium and endometrial stroma glandular epithelium and the VAS score (p=0.7 and p=0.36, respectively).

Expression grade of TIMP-2 in the luminal epithelium and endometrial stroma is presented in Table 4. TIMP-2 expression in luminal epithelium and stroma glandular epithelium was similar after three months compared to before IUD insertion (p=0.1 and p=0.06, respectively). No correlation was found between TIMP-2 expression in the luminal epithelium and endometrial stroma glandular epithelium and the VAS score (p=0.6 and p=0.43, respectively).

DISCUSSION

The World Health Organisation advises that copper-IUD is one of the most effective contraceptive methods used to date.[16] Menstrual abnormalities, including spotting and/or mild bleeding or heavy and/or prolonged bleeding were reported to be common in the first 3-6 months of IUD use, and persisted thereafter in a minority of women.[17] Studies have shown that menstrual bleeding alone and bleeding with pain were the most common reasons for requesting IUD removal.[18,19]

Table 3. Menstrual bleeding volume and duration with respect to MMP-9 expression grade in luminal epithelium and in endometrial stroma glandular epithelium

Immunostaining of MMP-9 in luminal epithelium	**0**	**+1**	**+2**	**+3**	**p-value**
Menstrual bleeding (n) Light	0	2	0	0	
Moderate	0	9	11	0	0.003*
Heavy	0	1	4	3	
Duration of menstrual bleeding (days)	0	3.9±0.8	4.6±1.1	6.3±0.5	0.01*
Immunostaining for MMP-9 in the endometrial stromal glandular epithelium	0	+1	+2	+3	p-value
Menstrual bleeding (n) Light	0	2	0	0	
Moderate	0	3	17	0	0.001*
Heavy	0	1	4	3	
Duration of menstrual bleeding (days)	0	3.6±0.5	4.5±1.1	6.6±0.5	0.01*

MMP: Matrix metalloproteinases, *p<0.05, statistically significant

Table 4. Expression grades of TIMP-1 and TIMP-2 in luminal and endometrial stromal glandular epithelium

Antibody	**Expression**	**Before IUD (n=30) n (%)**	**After IUD (n=30) n (%)**	**p-value**
Grade of TIMP-1 immunostaining in luminal epithelia	0 +1 +2 +3	18 (60) 9 (30) 2 (6.6) 1 (3.3)	26 (86.7) 4 (13.3)	0.1
Grade of TIMP-1 immunostaining in the endometrial stromal glandular epithelium	0 +1 +2 +3	24 (80) 4 (10) 2 (10)	27 (90) 3 (10)	0.06
Grade of TIMP-2 immunostaining in luminal epithelia	0 +1 +2 +3	21 (70) 5 (16.6) 3 (10) 1 (3.3)	25 (83) 5 (16.6)	0.1
Grade of TIMP-2 immunostaining in the endometrial stromal glandular epithelium	0 +1 +2 +3	25 (83) 3 (10) 2 (6.6)	28 (93) 2 (6.6)	0.07

p<0.05, statistically significant, TIMP: Tissue inhibitors of metalloproteinase, IUD: Intrauterine device

It has been reported that endometrial explants from metrorrhagic women released considerably more MMP-1, -2, -3, and -9, and lower amounts of TIMP-1.[20,21] In the literature review, we found only one report investigating the relationship between Copper-IUD and MMP-1. It suggested that the Copper-IUD may enhance the activity of MMPs in human endometrium and that MMPs may participate in the development of IUD-induced menorrhagia.[22] In a limited number of studies, it has been reported that the increase in MMP expressions due to IUD use is correlated with bleeding disorders.[14,23] In the present study, after three months of IUD usage, MMP-1 expression in endometrial luminal epithelium cytoplasm increased significantly. While the increase was not correlated with heavier menstruation, it was found to be positively correlated with the mean duration of menstruation as it became longer and the *de novo* dysmenorrhea VAS score related to IUD use.

MMP-2 and MMP-9 are synthesized by various stromal cells, including macrophages, fibroblasts, and endothelial cells.[24] One of the aims of this study was to investigate the effect of copper-IUD insertion on MMP-2 expression in luteal phase endometrium. Menstruation is characterized by the lysis of collagen-rich argyrophilic fibers in the endometrial stroma, followed by tissue collapse and fragmentation, collectively described as "stromal breakdown".[25,26] Thus, MMPs are prime candidates for triggering endometrial bleeding because they are able to collectively degrade most proteins of the ECM at neutral pH.[27] In the present study, while MMP-2 expression in endometrial luminal epithelial cytoplasm did not change significantly after three months of IUD usage, MMP-9 expression increased in luminal epithelium significantly after three months of IUD usage. No correlations were found between MMP-2 expressions in the luminal epithelium and prolonged and/or heavier menstruation. Furthermore, MMP-2 and MMP-9 expressions after IUD insertion were not correlated with the VAS score.

In normal endometrium, epithelial expression of MMP-2, MMP-9 and TIMP-2 were reported to increase during the proliferative phase of the menstrual cycle, and MMP-2 expression was negatively correlated with TIMP-2 expression. MMP-9 and TIMP-2 expression had been found not to vary with the phase of the menstrual cycle.[28] Studies reported that more MMP-1, -2, -3, and -9 and lower amounts of TIMP-1 were released when the endometrium was sampled during bleeding episodes.[20,21] At least two studies have indicated that TIMP-1 and TIMP-2 were expressed in small arteriolar and capillary vascular tissues in the secretory endometrium, suggesting that TIMPs might be involved in stabilization of uterine vasculature during the reproductive cycle and pregnancy.[29,30] Although the role of TIMP expression during endometrial vascularization remains to be fully elucidated, TIMP-1 and TIMP-2 have each been recently reported to have anti-angiogenic activity that may be related to an inhibition of vascular endothelial growth factor expression.[31] We found that TIMP-1 and TIMP-2 expression in endometrial luminal epithelial cytoplasm and endometrial stroma did not change significantly after three months of IUD use. Furthermore, luminal epithelial and stromal TIMP-1 and TIMP-2 expressions were not found to be correlated with prolonged and/or heavier menstruation and VAS score.

CONCLUSION

In conclusion, the pathogenesis of menstrual bleeding disturbances and dysmenorrhea associated with copper-IUD use is multifactorial and not dependent on a single variable. Our results show a positive correlation of MMP-1 expression with increased VAS score and prolonged mean menstrual duration. IUD-related prolonged menstruation and severity of menstrual bleeding appear to be associated with changes only in MMP-9 expression in luminal epitelium, while no associations were found with MMP-2, TIMP-1, TIMP-2 expressions in the endometrium. There is a need to conduct further studies with MMPs and develop new treatment modalities for IUD-related menstrual bleeding disorders and dysmenorrhea, which are the main reasons for the removal of copper IUDs.

Footnote

Acknowledgement

The authors would like to thank Mr. Jeremy Jones for critical appraisal and English language editing of the manuscript.

Ethics Committee Approval: This study was approved by the Ethics Committee of the Kocaeli University Faculty of Medicine (approval number: KOÜ 2008/124, date: 2008).

Informed Consent: Informed consent was obtained.

Authorship Contributions

Surgical and Medical Practices: H.U.Ş., B.M., Concept: H.U.Ş., Ö.D.S., B.M., A.Ç., Design: H.U.Ş., Ö.D.S., B.M., A.Ç., Data Collection or Processing: H.U.Ş., E.C., Analysis or Interpretation: H.U.Ş., B.M., A.Ç., Literature Search: H.U.Ş., Ö.D.S., E.C., Writing: H.U.Ş., Ö.D.S., E.C., A.Ç.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study received no financial support.

REFERENCES

1. Xie J, Zhou X, Wang R, et al. Identification of potential diagnostic biomarkers in MMPs for pancreatic carcinoma. Medicine (Baltimore). 2021;100:e26135.
2. Wu XM, Li YX, Zheng HS, et al. The effect and mechanism of low-molecular-weight heparin on the decidualization of stromal cells in early pregnancy. J Matern Fetal Neonatal Med. 2024;37:2294701.
3. Ahmadighadykolaei H, Radisky ES, Raeeszadeh-Sarmazdeh M. Engineering Selective TIMPs Using a Counter-Selective Screening Strategy. Methods Mol Biol. 2024;2747:257-278.
4. Müller D, Roessner A, Röcken C. Distribution pattern of matrix metalloproteinases 1, 2, 3, and 9, tissue inhibitors of matrix metalloproteinases 1 and 2, and alpha 2-macroglobulin in cases of generalized AA- and AL amyloidosis. Virchows Arch. 2000;437:521-527.

5. Nguyen TT, Shynlova O, Lye SJ. Matrix metalloproteinase expression in the rat myometrium during pregnancy, term labor, and postpartum. Biol Reprod. 2016;95:24.
6. Irwin JC, Kirk D, Ralph B, et al. Human endometrial matrix metalloproteinase-2, a putative menstrual proteinase. Hormonal regulation in cultured stromal cells and messenger RNA expression during the menstrual cycle. J Clin Invest 1996;97:438-447.
7. Endo T, Kiya T, Goto T, et al. Significance of matrix metalloproteinases in the pathophysiology of the ovary and uterus. Reprod Med Biol. 2006;5:235-243.
8. Salamonsen LA, Woolley DE. Matrix metalloproteinases and menstruation. Hum Reprod. 1996;11:124-133.
9. Marbaix E, Kokorine I, Henriet P, et al. The expression of interstitial collagenase in human endometrium is controlled by progesterone and by oestradiol and is related to menstruation. Biochem J. 1995;305:1027-1230.
10. Jeziorska M, Nagase H, Salamonsen LA, et al. Immunolocalization of the matrix metalloproteinases, gelatinase B and stromelysin-1 in human endometrium throughout the menstrual cycle. J Reprod Fertil. 1996;107:43-51.
11. Endo T, Kiya T, Goto T, et al. Significance of matrix metalloproteinases in the pathophysiology of the ovary and uterus. Reprod Med Biol. 2006;5:235-243.
12. Patai K, Szilagyi G, Noszal B, et al. Local tissue effects of copper-containing intrauterine devices. Fertil Steril. 2003;80:1281-1283.
13. Speroff L, Glass HG, Kase NG. The intrauterine device (IUD). In: Clinical Gynecoloic Endocrinology, and Infertility, editors. Williams and Wilkins, Baltimore, 1994;777-93.
14. Labied S, Galant C, Nisolle M, et al. Differential elevation of matrix metalloproteinase expression in women exposed to levonorgestrel-releasing intrauterine system for a short or prolonged period of time. Hum Reprod. 2009;24:113-121.
15. Munro MG, Balen AH, Cho SH, et al. The FIGO ovulatory disorders classification system. Int J Gynecol Obstet. 2022;159:1-20.
16. World Health Organization (WHO). Medical Eligibility Criteria for Contraceptive Use. Geneva, Switzerland: WHO, 2000.
17. World Health Organization (WHO). Selected Practice Recommendations for Contraceptive Use. Geneva, Switzerland: WHO, 2002.
18. Cox M, Tripp J, Blacksell S. Clinical performance of the Nova T380 intrauterine device in routine use by the UK family planning and reproductive health research network: 5-year report. J Fam Plan Reprod Health Care. 2002;28:69-72.
19. Wildemeersch D, Andrade A, Goldstuck ND, et al. Intrauterine levonorgestrel delivery with frameless fibrous delivery system: review of clinical experience. Int J Womens Health. 2017;9:49-58.
20. Marbaix E, Vekemans M, Galant C, et al. Circulating sex hormones and endometrial stromelysin-1 (matrix metalloproteinase-3) at the start of bleeding episodes in levonorgestrel-implant users. Hum Reprod. 2000;15:120-34.
21. Galant C, Vekemans M, Lemoine P, et al. Temporal and spatial association of matrix metalloproteinases with focal endometrial breakdown and bleeding upon progestin-only contraception. J Clin Endocrinol Metab. 2000;85:4827-4834.
22. Li W, Cao L, Chen Z, et al. Study on the effects of FCu-IUD and FICu-IUD on matrix metalloproteinases in human uterine flushing and endometrium. J Huazhong Univ Sci Technolog Med Sci. 2002;22:9-11,16.
23. Maruo T, Ohara N, Yoshida S, et al. Translational research with progesterone receptor modulator motivated by the use of levonorgestrel-releasing intrauterine system. Contraception. 2010;82:435-441.
24. Fujimura T, Ohuchi K, Ikawa T, et al. Possible effects of plasminogen activator inhibitor-1 on promoting angiogenesis through matrix metalloproteinase 9 in advanced mycosis fungoides. Hematol Oncol. 2024;42:e3244.
25. Ferenczy A. Pathophysiology of endometrial bleeding. Maturitas. 2003;45:1-14.
26. Mazur MT, Kurman, RJ. Dysfunctional uterine bleeding. In: Mazur MT, Kurman RJ, editors. Diagnosis of Endometrial Biopsies and Curettings. New York: Springer-Verlag, 1995;89-108.
27. Eeckhout Y, Henriet P. Matrix metalloproteinases. In: Creighton TEE, editor. Wiley Encyclopedia of Molecular Medicine. New York: John Wiley & Sons, 2002.
28. Graesslin O, Cortez A, Fauvet R, et al. Metalloproteinase-2, -7, and -9 and tissue inhibitor of metalloproteinase-1 and -2 expression in normal, hyperplastic and neoplastic endometrium: a clinical-pathological correlation study. Ann Oncol. 2006;17:637-645.
29. Freitas S, Meduri G, Le Nestour E, et al. Expression of metalloproteinases and their inhibitors in blood vessels in human endometrium. Biol Reprod. 1999;61:1070-1082.
30. Guedez L, McMarlin AJ, Kingma DW, et al. Tissue inhibitor of metalloproteinase-1 alters the tumorigenicity of Burkitt's lymphoma via divergent effects on tumor growth angiogenesis. Am J Pathol. 2001;158:1207-1215.
31. Hajitou A, Sounni NE, Devy L, et al. Down-regulation of vascular endothelial growth factor by tissue inhibitor of metalloproteinase-2: effect on in vivo mammary tumor growth and angiogenesis. Cancer Res. 2001;61:3450-3457.

Original Article

DOI: 10.4274/anajog.galenos.2024.03511

Anat J Obstet Gynecol Res 2024;2:54-60

Decreasing and Maintaining Low Maternal Mortality Rate and Near Miss in Kocaeli District

Yasin Ceylan[1], Yasemin Doğan[2], Engin Yurtçu[3], Şule Yıldırım Köpük[4], Mahmut Sabri Medişoğlu[5]

[1]Fethiye State Hospital, Clinic of Obstetrics and Gynecology, Muğla, Turkey
[2]Kocaeli University Faculty of Medicine, Department of Obstetrics and Gynecology, Kocaeli, Turkey
[3]Düzce University Faculty of Medicine, Department of Obstetrics and Gynecology, Düzce, Turkey
[4]Private Pendik Medicalpark Hospital, Clinic of Obstetrics and Gynecology, İstanbul, Turkey
[5]Kocaeli Health and Technology University Faculty of Dentistry, Department of Anatomy, Kocaeli, Turkey

ABSTRACT

Purpose: Every dying woman goes through a phase where her life could have been saved with effective and timely intervention. In this study, we aimed to report how the maternal mortality ratio (MMR) and maternal near-miss ratio (MNMR) were impressively reduced in Kocaeli between 2009 and 2014.

Methods: Patient files who delivered in Kocaeli between 2009-2017 were retrospectively reviewed. These data were obtained from Kocaeli Health Directorate, maternal deaths were determined by the Maternal Mortality Research Commission between 2009-2017, and the data were sorted according to three delay models.

Results: There were 192,815 deliveries assessed, 312 near miss cases and 32 death reported in 2009-2015 at Kocaeli. MNMR was 3.75 in 2010, it decreased significantly to 0.61 in 2015 (p=0.02). The MMR had decreased significantly from 32.9 in 2009 to 3.3 per 100,000 live births in 2014 in Kocaeli (p=0.007) whereas it was 15.2 in 2014 in Turkey.

Conclusion: A citywide organization and cooperation betwen all levels of health facilities, health care providers, together with education of practitioners, midwifes, obstetrics and gynecology specialists, weekly evaluation of citywide high risk pregnancies and providing 7/24 online consultation of high risk deliveries resulted in a significant decrease in MMR and MNMR.

Keywords: Maternal near-miss, maternal mortality, pregnancy

INTRODUCTION

Maternal mortality is a globally accepted indicator of the quality and accessibility of maternal healthcare services. Maternal death, though rare, represents the most critical complication associated with pregnancy. Despite global efforts, progress in reducing maternal mortality has been slow, particularly in Turkey. Each day, over 1,000 women die from pregnancy-related causes worldwide, with the majority of these deaths occurring in developing nations.[1] Maternal mortality remains a significant public health issue in low- and middle-income countries. Strengthened health systems and effective health services for women experiencing acute pregnancy-related complications are considered key factors in reducing maternal mortality.[2]

Every maternal death could potentially be prevented with effective and timely intervention. This situation was defined by the World Health Organization (WHO) in 2009 as a maternal near-miss: "A woman who nearly died but survived a complication that occurred during pregnancy, birth, or within 42 days of pregnancy termination".[3] Near-miss cases are more common than maternal deaths, offering important insights into the challenges and barriers within the healthcare system. Consequently, near-miss cases have emerged as a vital metric for assessing and enhancing maternal health services, especially in developing countries.

The objective of this study was to assess the impact on maternal mortality reduction of the emergency obstetric hemorrhage team, which was established for the first time in Kocaeli province in Turkey. This investigation focused on the influence of the team's establishment on the maternal mortality rate (MMR) and maternal near-miss rate (MNMR), highlighting the substantial reductions observed over time.

Address for Correspondence: Yasin Ceylan, Fethiye State Hospital, Clinic of Obstetrics and Gynecology, Muğla, Turkey
Phone: +90 535 748 97 29 **E-mail:** md.yasinceylan@yahoo.com **ORCID ID:** orcid.org/0000-0001-5517-8461
Received: 09.04.2024 **Accepted:** 31.07.2024

METHODS

We conducted a retrospective study encompassing all women who met the WHO criteria for maternal near-miss or maternal death between 2009 and 2017 in Kocaeli. This retrospective study was initiated following the decision of the Ethics Committee of Kocaeli University. The WHO criteria were employed to identify cases of maternal near-miss (Table 1). Women exhibiting potentially life-threatening conditions were identified according to the WHO criteria (see Table 2).[4]

Data were obtained from the Kocaeli Health Directorate, with maternal deaths determined by the Maternal Mortality Research Commission between 2009-2017. The data were categorized according to the Three Delays Model. Phase 1 delay involves delays in deciding to seek appropriate medical care due to familial and community factors. Phase 2 delay occurs when timely access to an obstetric facility is hindered by multiple referrals or transfers between health facilities. Phase 3 delay involves delays in receiving appropriate and timely care within the healthcare facility.

According to the Kocaeli District Health Commission Circular, all members of the emergency obstetric team were authorized to conduct examinations, consultations, visits, and surgical operations across all district hospitals. Family practitioners, nurses, and midwives stationed at Family Health Care units, Mother and Child Health centers, and Obstetrics and Gynecology clinics underwent categorization into two groups. They were subjected to mandatory education sessions covering various aspects, including triage of high-risk pregnancies, obligatory notification of high-risk mothers, utilization of emergency call centers such as the "112 Emergency Call Center" protocols for patient transfer, predefined patient data and laboratory work-up requirements before or during patient transfer, management of delivery rooms including the use of partograms, fetal monitoring techniques employing ultrasound and hand doppler ultrasound, and cardiotocography, management of postpartum hemorrhage, timely maintenance of blood and blood products, and team management of obstetric emergencies. This educational program was implemented within the initial three months of 2009.

The Health Directorate formed a High-Risk Pregnancy Evaluation Commission that met weekly, a Maternal Mortality Commission that met monthly, and an Emergency Obstetric Coordination Team under the "112 Emergency" call centers. In addition, the Kocaeli University and Red Crescent blood banks, along with hospital transfusion units, provided daily updates to the health directorate regarding the availability of blood products, while intensive care units (ICUs) reported their bed capacities daily. Furthermore, various decrees were enacted to streamline patient transfers, mandating their coordination via the 112 ambulance system, ensuring patient stabilization prior to transfer, and organizing the allocation of blood products through the 112 emergency system.

All data presented here are provided by the authors: the director perinatologist of the Emergency Obstetric Team and the provincial health director. MMR is defined as the number of maternal deaths per 100,000 live births, and MNMR is defined

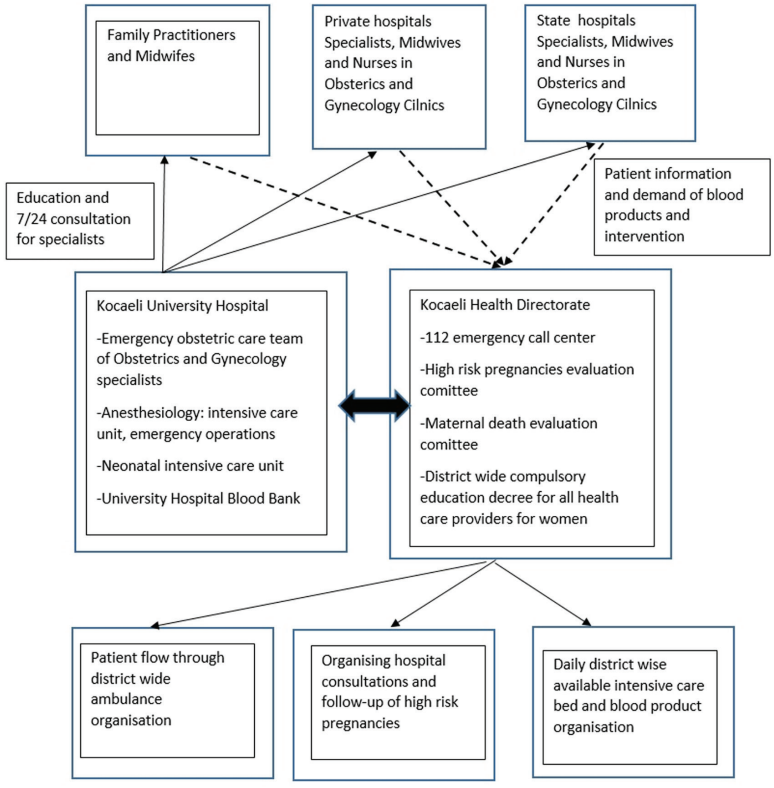

Figure 1. Organisation and work flow of maternal care in Kocaeli District

Table 1. The World Healt Organization maternal near miss criteria

Clinical criteria	Laboratory-based criteria	Management-based criteria
Shock	Severe hypoperfusion (lactate >5 mmol/L or >45 mg/dL)	Use of continuous vasoactive drugs
Cardiovascular arrest	Severe acidosis (pH <7.1)	Cardio-pulmonary resuscitation
Acute cyanosis	Severe hypoxemia (O_2 saturation <90% for ≥60 minutes or PAO_2/FiO_2<200)	Intubation and ventilation notrelated to anesthesia
Gasping	Severe acute azotemia (creatinine ≥300 µmol/mL or ≥3.5 mg/dL)	Dialysis for acute renal failure
Severe tachypnea (respiratory rate >40 breaths perminute) or severe bradypnea (respiratoryrate <6 breaths per minute)	Severe acute thrombocytopenia <50 000 platelets/mL)	Massive transfusion of blood or red cells (≥5 units)
Oliguria non responsive to fluids or diuretics	Severe acute hyperbilirubinemia (bilirubin >100 µmol/L or >6.0 mg/dL)	Uterine hemorrhage or infection leading tohysterectomy
Failure to form clots		
Jaundice in the presence of preeclampsia		
Any loss of consciousness not medicallyinduced, lasting >12 hours		
Stroke		
Uncontrollable fit/status epilepticus		
Total paralysis		

Table 2. Potentially life-threatening conditions

Women with severe complications	Women undergoing critical interventions
Severe postpartum haemorrhage Severe pre-eclampsia Eclampsia Sepsis or severe systemic infection Ruptured uterus Other complications associated with severe maternal outcome	Use of blood products Interventional radiology Laparotomy Admission to intensive care unit

as the number of maternal near-miss cases per 1,000 live births.

Statistical Analysis

The data collected through the questionnaires were analyzed using IBM SPSS Statistics (version 25; IBM Corporation, Armonk, NY). Statistical significance was defined as $p<0.05$.

RESULTS

A total of 255,653 deliveries were assessed between 2009 and 2017 in Kocaeli, resulting in 312 near-miss cases and 41 deaths. The mean age of women was 28 (18-42) years, with a mean gestational week of 28 (9-39) weeks. In terms of maternal mortality, most (75%) occurred in the postpartum period, with 31% occurring between one week and 42 days postpartum, 29% within the first 48 hours, and 15% between 48 hours and one week. Maternal deaths were predominantly observed in the early postpartum period, with an average duration of 2.9 days (1-8), and those during the puerperal period averaged 18 (1-39) days.

In 2009, Turkey's MMR stood at 18.4 maternal deaths per 100,000 live births, while Kocaeli's MMR was significantly higher at 34.7 ($p=0.01$). By 2014, Kocaeli's MMR had significantly ($p=0.004$) decreased to 3.3 per 100,000 live births, compared to the average rate in Turkey of 15.2 (Table 3).[5]

The measures implemented, including emergency obstetric intervention workflows and healthcare provider training, resulted in a significant decrease in delay models 2 and 3 within two years (Table 4). However, maternal deaths attributed to delay model 1 remained relatively unchanged throughout the study period.

From 2009 to 2017, 31.7% of maternal deaths were due to direct obstetric causes, and 43.9% were due to indirect causes in Kocaeli (Table 5). The causes of death of the 41 patients are detailed in Table 5.

The majority (95%) of pregnant women accessed prenatal care services, with 81% utilizing primary health clinics, 55% attending state hospitals, 34% visiting private hospitals, and 19% receiving care at tertiary hospitals. Furthermore, 77% of women underwent prenatal care visits more than four times, 13% received care two to three times, and 5% sought prenatal care only once.

Between 2010 and 2015, among the cohort of 312 near-miss cases, 134 patients received ICU treatment, while 32 patients underwent surgical interventions at the primary healthcare facility. These surgical procedures encompassed hypogastric artery ligation for 25 patients, hysterectomy for 6 patients, and uterine re-transplantation for 1 patient. Following this, 24 patients were referred to tertiary care centers, as outlined in Table 6.

The MNMR exhibited a significant ($p=0.02$) decline from 3.75 per 1000 live births in 2010 to 0.61 per 1000 live births in 2015. The correlation between the MMR and MNMR is delineated in Table 7.

Table 3. Maternal mortality rate of Kocaeli and Turkey

Years	Number of live birth rate	Maternal deaths (n=41) n (%)	Direct causes	Maternal mortality rate per 100,000 live birth in Kocaeli	Maternal mortality rate per 100,000 live birth in Turkey
2009	25,876	9 (23.6)	6	34.7	18.4
2010	25,864	5 (13.1)	1	19.3	16.3
2011	25,770	3 (7.8)	1	11.6	15.9
2012	27,334	5 (13.1)	1	18.2	15.2
2013	27,521	5 (13.1)	0	18.1	15.8
2014	29,879	1 (2.6)	0	3.3	15.2
2015	30,901	4 (10.5)	1	12.9	14.7
2016	31,188	6 (15.7)	2	19.2	14.7
2017	31,320	3 (9)	1	9.5	17

Table 4. Analysis of maternal mortality according to delay models in Kocaeli

Year	Model 1 delay	Model 2 delay	Model 3 delay	Total
2009	2	4	3	9
2010	2	2	1	5
2011	2	1	0	3
2012	4	0	1	5
2013	4	1	0	5
2014	1	0	0	1
2015	3	0	1	4
2016	4	1	1	6
2017	2	0	1	3
Total	24	9	8	41

DISCUSSION

The determinants underlying maternal mortality necessitate meticulous documentation and analysis. Utilizing maternal death reviews, establishing local committees dedicated to enhancing the quality of care, and implementing targeted interventions to augment healthcare providers' competencies are strategies proven to mitigate MMRs.[6]

Direct obstetric deaths stem from complications directly linked to pregnancy or its management, encompassing instances of incorrect treatments, omissions, and specific pregnancy-related complications such as hemorrhage and uterine rupture.[7] In contrast, indirect obstetric deaths arise from preexisting conditions, like diabetes or cardiac disease, or from new conditions that develop during pregnancy and are not directly related to it but are aggravated by the physiological effects of pregnancy. It is estimated that approximately 25% of maternal deaths are attributable to indirect obstetric causes.[8]

In developed nations, prevalent causes of maternal mortality comprise hemorrhage, thromboembolism, cardiac disease, sepsis, hypertensive disorders, and amniotic fluid embolism.[9] In Africa and Asia, hemorrhage accounts for 30.8% to 33.9% of maternal deaths, with hypertensive disorders following at 9.7%. Conversely, in high-income countries, hypertensive disorders are the foremost cause of maternal death (16.1%), followed by pulmonary embolism (14.9%).[10]

On a global scale, spanning from 2003 to 2009, hemorrhage constituted 27.1% of maternal fatalities, hypertensive disorders accounted for 14.0%, and sepsis contributed to 10.7% of the total maternal mortality.[11-13] Conversely, within the Kocaeli region during the period from 2009 to 2017, direct obstetric causes attributed to 31.7% of maternal deaths, while indirect obstetric causes were responsible for 43.9% (Table 5).

Measuring maternal near-miss cases can serve as a valuable indicator of the quality of care provided to pregnant women. The assessment of near-miss incidents yields more analyzable data than maternal deaths due to their higher frequency.[14] Souza et al.[15] reported that the overall maternal near-miss ratio was 8.3 per 1000 live births in a WHO study conducted in 29 developing countries. In a retrospective cohort study by Ozimek et al.,[16] out of 16,323 deliveries, 386 (2%) were screened positive for severe maternal morbidity. Upon detailed review, true severe maternal morbidity was identified in 150 (0.9%) deliveries. A multidisciplinary committee found that there was an opportunity for improvement in care in 66 (44%) of these cases.[16]

Several critical factors contribute to maternal survival during obstetric emergencies. These include the regularity of follow-up and registration of pregnant women, proximity to health facilities, presence of trained and qualified health workers during childbirth, availability of emergency obstetric care services, blood transfusion capabilities, and access to quality intensive care services. The Three-Delays model has been widely applied to identify and address preventable causes of maternal mortality and morbidity.

Between 2012 and 2015, prenatal care services were accessed by 95% of pregnant women. Among these, 81% sought care at primary health clinics, 55% at state hospitals, 34% at private hospitals, and 19% at tertiary hospitals. Throughout this timeframe, 77% of women attended prenatal care visits more than four times, 13% attended two to three times, and 5% attended only once.

Numerous studies have demonstrated that near-misses and maternal deaths are significantly higher among patients without registration and follow-up compared to those who receive consistent prenatal care. Regular prenatal follow-

Table 5. Causes of maternal death in 2009-2017 in Kocaeli

Year	Cause of death	Direct or Indirect causes
2017	Systemic lupus erythamatosis	Indirect
2017	Mucopolysaccharidosis	Indirect
2017	Postpartum bleeding and disseminated intravascular coagulation	Direct
2016	Pulmonary embolism	Direct
2016	Acute pancreatitis	Indirect
2016	Gunshot wounds	Accidental
2016	Hemoperitoneum	Indirect
2016	Postpartum hemorrhage	Direct
2016	Brain tumour	Indirect
2015	Gastric cancer	Indirect
2015	Traffic accident	Accidental
2015	Congestive hearth failure	Indirect
2015	Massive pulmonary embolism	Direct
2014	Acute myeloid leukemia	Indirect
2013	Traffic accident	Accidental
2013	Traffic accident	Accidental
2013	Circulatory system disease	Indirect
2013	Aortic aneurysm and dissection	Indirect
2013	Acute myocard infarction	Indirect
2012	Postpartum hemorrhage	Direct
2012	Subarachnoid hemorrhage	Accidental
2012	Gunshot wounds	Accidental
2012	Chronic obstructive pulmonary disease	Indirect
2012	Jumping from high place	Accidental
2011	Preeclampsia	Direct
2011	Takayasu	Indirect
2011	Gunshot wounds	Accidental
2010	Cerebral venous thrombosis	Indirect
2010	Preeclampsia	Direct
2010	Pulmonary hypertension, CHF	Indirect
2010	Traffic accident	Accidental
2010	Mental illness and nervous system disease	Indirect
2009	Preeclampsia	Direct
2009	Venous thromboembolism	Direct
2009	Suicide	Indirect
2009	Postpartum bleeding	Direct
2009	Postpartum sepsis	Direct
2009	Peripartum bleeding	Direct
2009	Disseminated intravascular coagulation	Direct
2009	Pschizophrenia, house fire	Indirect
2009	Traffic accident	Accidental

Direct (31.7%), Indirect (43.9%), Accidental (24.4%). CHF: Congestive heart failure

Table 6. Maternal near miss cases evaluated and managed by the emergency obstetric care team 2010-2015

Year	Intensive care support	Surgical operation	Onsite consultation	Total
2010	33	17	47	97
2011	32	13	36	81
2012	27	8	19	54
2013	19	8	9	36
2014	12	7	6	25
2015	11	3	5	19
Total	134	56	122	312

Table 7. Change in MMR and MNMR over the years of Kocaeli

Years	Maternal deaths (n=41) n (%)	MMR per 100,000 live birth	Maternal near-miss cases per 1000 live birth
2009	9 (21.9)	34.7	
2010	5 (12.1)	19.3	3.75
2011	3 (7.3)	11.6	3.14
2012	5 (12.1)	18.2	1.97
2013	5 (12.1)	18.1	1.30
2014	1 (2.4)	3.3	0.83
2015	4 (9.7)	12.9	0.61
2016	6 (14.6)	19.2	
2017	3 (7.3)	9.5	

MMR: Maternal mortality ratio, MNMR: Maternal near miss ratio

up is crucial for differentiating between high- and low-risk patients, predicting complications, and planning interventions, all of which contribute to the reduction of severe maternal morbidities. The absence of regular prenatal follow-up remains a significant challenge in developing countries.[11,17,18]

Data from the United States Pregnancy Mortality Surveillance indicate that higher levels of education serve as protective factors against maternal mortality. Moreover, women who receive prenatal care have lower maternal death risks than those who do not receive any care.[19-21] Tunçalp et al.[22] reported that the prevalence rates for maternal near-miss varied between 0.6% and 14.98% based on disease-specific criteria. In Rwanda, the MMR decreased from 487 in 2010 to 210 in 2015, and facility-based deliveries increased from 69% in 2010 to 91% in 2015, likely due to the prohibition of traditional birth attendants and the availability of health insurance.[23]

In Turkey, the health system involves family practitioners recording new pregnancies in the Ministry of Health database, with monthly examinations and follow-up by family physicians. Between 2006 and 2015, primary health clinics actively reached out to newly pregnant women through home visits or telephone calls, providing immunizations. During this period, 26 social workers and 278 guidance specialists educated the public on prenatal and antenatal care, encouraging hospital deliveries among women aged 15-49 years, thereby improving the quality and quantity of follow-up care.

The number of pregnant women transported to the hospital via emergency ambulance and healthcare clinic vehicles increased markedly. High-risk patients were referred to obstetricians specializing in their care, with access to a comprehensive range of specialized services. A case-control study of 77 maternal deaths at Birmingham University found that longer distances to the hospital were a significant predictor of maternal death.[9] Kocaeli, the largest city in Turkey's northwestern region, with a population of 1.75 million based on the 2015 census, has an extensive transportation network and qualified healthcare providers. The city is served by one university hospital, one training and research hospital, four state hospitals, and fifteen private hospitals.

In the United States, the mean MMR increased from 14.3 in 2005 to 17.2 in 2014, a rise attributed to increased immigration rates, cesarean delivery rates, and the prevalence of medical conditions.[24] Following the onset of the Syrian civil war, Turkey experienced a substantial influx of uncontrolled immigration, with Kocaeli, as an industrial city, having the highest migration rate. Consequently, the MMR in Kocaeli increased from 3.3 in 2015 to 13, and further to 19.2 in 2016.

The third delay in addressing maternal mortality is attributed to untrained healthcare professionals, diagnostic inadequacies, urgent blood transfusion requirements, and overburdened ICUs in tertiary institutions. Many of these limitations can be mitigated through comprehensive training for healthcare workers and the implementation of surveillance and control systems to enhance care quality. In Malaysia, the national MMR decreased by 94%, from 530 per 100,000 live births in 1950 to 28 per 100,000 live births in 2009. This significant reduction was largely due to the introduction of competency-based training and the deployment of midwives to rural areas, as well as improvements in the healthcare delivery system.[25]

In 2013, the Intensive Care National Audit and Research Center Case Mix Program reported the development of the first validated early warning scoring system for pregnant women. This system has proven to be a highly effective tool for the early identification of women at increased risk of mortality. Obstetric early warning systems, which are based on the identification of predetermined abnormal values in vital signs or laboratory parameters, have been proposed as a potential strategy to reduce maternal morbidity and mortality by enabling a rapid and effective medical response.[26] A prospective observational study of over 1000 deliveries reported that the early warning charting system had a positive predictive value of 53.8% and a negative predictive value of 96.9% for the identification of maternal morbidity.[27,28]

The Turkish Gynecology and Obstetrics Society (TGOS) has played a pivotal role in educating obstetricians, gynecologists, and midwives on the management of obstetric emergencies and the additional risks posed by underlying medical conditions. Furthermore, TGOS has provided training for clinical staff in surgical procedures and the management of postpartum hemorrhage.

In Turkey, the Emergency Obstetric Intervention Programme (EOI), implemented in 2009, has been the most effective organization for reducing maternal mortality. The EOI Programme aims to decrease the MMR by providing qualified prenatal and postnatal care. Similarly, the Mother Care Country Projects, conducted between 1989 and 1993 in Bolivia, Guatemala, Indonesia, and Nigeria, emphasized two crucial aspects of reducing maternal and perinatal mortality: ensuring referral facilities and training midwifery and obstetrics staff; and involving women and their families in recognizing danger signs and mobilizing for referral.[29] These projects demonstrated improvements in referral systems and a reduction in perinatal mortality.

A 2014 study published by the WHO highlighted significant advancements made by the Acute Emergency Obstetrics Team in providing pregnant women with accessible obstetric care facilities, managed by qualified healthcare personnel.[8] The TGOS conducted a comprehensive analysis of the causes of maternal and fetal mortality and morbidity in Turkey, formulating new strategies aimed at reducing mortality, morbidity, and cesarean rates.

In 2009, the establishment of a postpartum hemorrhage team in Kocaeli marked a pioneering effort. Comprising three academicians equipped to handle various scenarios, this team commenced operations to effectively address postpartum hemorrhage. As a result, the MNMR decreased substantially from 3.75 in 2010 to 0.61 in 2015. The model, initially implemented in Kocaeli, was subsequently adopted in all cities of Turkey.

The TGOS conducted educational sessions to enhance the management of obstetric emergencies, particularly focusing on postpartum hemorrhage. These sessions provided training to 308 obstetricians, covering blood transfusion criteria and surgical procedures. Furthermore, 620 midwives received education on various topics including postpartum hemorrhage management, external fetal monitoring techniques, partogram use, nutritional guidelines, exercise during pregnancy, and methods for inducing labor.

CONCLUSION

The initiatives implemented since 2009 have resulted in the recognition of near-miss cases and have enhanced the skills of healthcare professionals in managing these cases. In addition, the establishment of postpartum bleeding teams in every province has ensured prompt, appropriate, and precise interventions for all near-miss cases, which has significantly contributed to a notable reduction in both the MMR and the MNMR.

Footnote

Ethics Committee Approval: This retrospective study was initiated following the decision of the Ethics Committee of Kocaeli University.

Informed Consent: Retrospective study.

Authorship Contributions

Surgical and Medical Practices: Y.C., Y.D., Concept: Y.C., Y.D., Design: Y.C., E.Y., Data Collection or Processing: E.Y., Ş.Y.K.,

Analysis or Interpretation: E.Y., M.S.M., Literature Search: M.S.M., Writing: Y.C., Ş.Y.K.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study received no financial support.

REFERENCES

1. World Health Organization (WHO), UNICEF, UNFPA, The WB. Trends in Maternal Mortality: 1990-2008. Geneva: WHO, 2010.
2. Maine D. Detours and shortcuts on the road to maternal mortality reduction. Lancet. 2007;370(9595):1380-1382.
3. Pattinson R, Say L, Souza JP, Broek Nv, Rooney C. WHO Working Group on Maternal Mortality and Morbidity Classifications. WHO maternal death and near-miss classifications. Bull World Health Organ. 2009;87(10):734.
4. World Health Organization (WHO) (2011). Evaluating the qualityof care for severe pregnancy complications: The WHO near-miss approach for maternal health. Geneva: WHO.
5. Turkish Statistical Institute, Population Statistics Portal. (tuik.gov.tr)
6. Pattinson RC, Buchmann E, Mantel G, Schoon M, Rees H. Can enquiries into severe acute maternal morbidity act as a surrogate for maternal death enquiries? BJOG. 2003;110(10):889-893.
7. Louie JK, Acosta M, Jamieson DJ, Honein MA; California Pandemic (H1N1) Working Group. Severe 2009 H1N1 influenza in pregnant and postpartum women in California. N Engl J Med. 2010;362(1):27-35.
8. Say L, Chou D, Gemmill A, et al. Global causes of maternal death: a WHO systematic analysis. Lancet Glob Health. 2014;2(6):e323-e333.
9. Frölich MA, Banks C, Brooks A, Sellers A, Swain R, Cooper L. Why do pregnant women die? A review of maternal deaths from 1990 to 2010 at the University of Alabama at Birmingham. Anesth Analg. 2014;119(5):1135-1139.
10. Cantwell R, Clutton-Brock T, Cooper G, et al. Saving mothers' lives: Reviewing maternal deaths to make motherhood safer: 2006-2008. The eighth report of the confidential enquiries into maternal deaths in the United Kingdom. BJOG. 2011;118 (Suppl 1)1-203.
11. Trends in maternal mortality: 1990 to 2008 Estimates developed by WHO, UNICEF, UNFPA and the World Bank. https://www.unfpa.org/publications/trends-maternal-mortality-1990-2008
12. Say L, Souza JP, Pattinson RC; WHO working group on maternal mortality and morbidity classifications. Maternal near miss-towards a standard tool for monitoring quality of maternal health care. Best Pract Res Clin Obstet Gynaecol. 2009;23(3):287-296.
13. Pattinson RC, Hall M. Near misses: a useful adjunct to maternal death enquiries. Br Med Bull. 2003;67:231-243.
14. Geller SE, Cox SM, Kilpatrick SJ. A descriptive model of preventability in maternal morbidity and mortality. J Perinatol. 2006;26(2):79-84.
15. Souza JP, Gülmezoglu AM, Vogel J, et al. Moving beyond essential interventions for reduction of maternal mortality (the WHO multicountry survey on maternal and newborn health): a cross-sectional study. Lancet. 2013;381(9879):1747-1755.
16. Ozimek JA, Eddins RM, Greene N, et al. Opportunities for improvement in care among women with severe maternal morbidity. Am J Obstet Gynecol. 2016;215(4):509.
17. Chang J, Elam-Evans LD, Berg CJ, et al. Pregnancy-related mortality surveillance-United States, 1991-1999. MMWR Surveill Summ. 2003;52(2):1-8.
18. Louis JM, Menard MK, Gee RE. Racial and ethnic disparities in maternal morbidity and mortality. Obstet Gynecol. 2015;125(3):690-694.
19. Centers for disease control and prevention pregnancy mortality surveillance system. Reviewed January 31, 2017. https://www.cdc.gov/maternal-mortality/php/pregnancy-mortality-surveillance/index.html
20. Centers for Disease Control and Prevention (CDC). State-specific maternal mortality among black and white women-United States, 1987-1996. MMWR Morb Mortal Wkly Rep. 1999;48(23):492-496.
21. Tucker MJ, Berg CJ, Callaghan WM, Hsia J. The Black-White disparity in pregnancy-related mortality from 5 conditions: differences in prevalence and case-fatality rates. Am J Public Health. 2007;97(2):247-251.
22. Tunçalp O, Hindin MJ, Souza JP, Chou D, Say L. The prevalence of maternal near miss: a systematic review. BJOG. 2012;119(6):653-661.
23. Nathan LM, Shi Q, Plewniak K, et al. Decentralizing maternity services to increase skilled attendance at birth and antenatal care utilization in rural rwanda: a prospective cohort study. Matern Child Health J. 2015;19(9):1949-1955.
24. Moaddab A, Dildy GA, Brown HL, et al. Health care disparity and pregnancy-related mortality in the United States, 2005-2014. Obstet Gynecol. 2018;131(4):707-712.
25. Kaur J, Singh H. Maternal health in Malaysia: a review. Webmed Central public health. 2011;2(12):WMC002599.
26. Paternina-Caicedo A, Miranda J, Bourjeily G, et al. Performance of the Obstetric Early Warning Score in critically ill patients for the prediction of maternal death. Am J Obstet Gynecol. 2017;216(1):58.e1-58.e8.
27. Singh A, Guleria K, Vaid NB, Jain S. Evaluation of maternal early obstetric warning system (MEOWS chart) as a predictor of obstetric morbidity: a prospective observational study. Eur J Obstet Gynecol Reprod Biol. 2016;207:11-17.
28. Lewis G, (ed) 2007. The Confidential Enquiry into Maternal and Child Health (CEMACH). Saving Mothers' Lives: reviewing maternal deaths to make motherhood safer-2003-2005. The Seventh Report on Confidential Enquiries into Maternal Deaths in the United Kingdom. London: CEMACH. http://www.publichealth.hscni.net
29. Kwast BE. Reduction of maternal and perinatal mortality in rural and peri-urban settings: what works? Eur J Obstet Gynecol Reprod Biol. 1996;69(1):47-53.

Original Article

DOI: 10.4274/anajog.galenos.2024.03522

Anat J Obstet Gynecol Res 2024;2:61-65

Could Clinically Suspicious Cervix Predict Cervical Premalignant and Malignant Lesions in Postmenopausal Women?

Tuğba Özcan Aydın[1], Yasin Ceylan[2], Doğan Ege Aydın[1], Ömer Doğukan Saraç[3], Hakan Güraslan[1]

[1]University of Health Sciences Turkey, Bağcılar Training and Research Hospital, Clinic of Obstetrics and Gynecology, İstanbul, Turkey
[2]Fethiye State Hospital, Clinic of Obstetrics and Gynecology, Muğla, Turkey
[3]University of Health Sciences Turkey, Kocaeli City Hospital, Clinic of Obstetrics and Gynecology, Kocaeli, Turkey

ABSTRACT

Purpose: Assessing the role of clinically suspicious cervix in detecting premalignant and malignant lesions in postmenopausal women, ındependent of cervical cytology and human papilloma virus (HPV)-deoxyribonucleic acid (DNA) testing.

Methods: This study retrospectively analyzed 392 postmenopausal women aged 45-86 who underwent colposcopic biopsy at our clinic between 2017 and 2021. Data collected included patient age, parity, cervical cytology results, HPV-DNA test outcomes, and colposcopic biopsy findings. Patients were categorized based on the indication for colposcopy into three groups: clinically suspicious cervix, high-risk group HPV-DNA positivity, and abnormal cytology. The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) for detecting premalignant and malignant cervical lesions were subsequently calculated.

Results: Among the 174 patients referred for colposcopic biopsy due to a clinically suspicious cervix, 50% were found to have cervical premalignant lesions classified as cervical intraepithelial neoplasia-1 or higher, and 10% were diagnosed with malignancy. In the subset of 30 patients aged 65 and older with a clinically suspicious cervix, 17 were diagnosed with either premalignant or malignant cervical lesions. The sensitivities of clinically suspicious cervix, HPV-DNA positivity, and abnormal cytology for detecting premalignant or malignant lesions were 43%, 80%, and 37%, respectively. The specificities were 53%, 49%, and 73%; accuracies were 47%, 67%, and 51%; PPV were 60%, 68%, and 69%; and NPV were 36%, 64%, and 42%, respectively. Additionally, among the 392 patients, 11 were diagnosed with premalignant or malignant lesions solely through endocervical curettage.

Conclusion: The presence of a clinically suspicious cervix serves as a significant indication for colposcopy, comparable to traditional screening tests, in the detection of cervical premalignant and malignant lesions. The findings from our study indicate that cervical cancer screening should be maintained in women over the age of 65.

Keywords: Clinically suspicious cervix, postmenopause, pap smear, colposcopy, HPV-DNA test

INTRODUCTION

Cervical cancer ranks as the fourth most prevalent malignancy among women globally. Despite a reduction in incidence and mortality rates in developed countries, attributed to the systematic implementation of screening programs, cervical cancer continues to pose a significant public health challenge in developing and underdeveloped regions.[1] Screening methods for cervical cancer include pelvic examination, cervical cytology, human papilloma virus (HPV)-deoxyribonucleic acid (DNA) testing, and co-testing (a combination of cytology and HPV-DNA testing). A key characteristic of cervical cancer is that its premalignant lesions can be detected through screening tests, and these lesions may progress to malignancy over a long period.[2]

Over the past 50 years, the widespread use of cytology in cervical cancer screening has led to a reduction in disease-related mortality by approximately 70%. However, the broad range of false-negative rates, between 5% and 40%, is a major disadvantage of cytological assessment.[2]

HPV 16 is responsible for 50% of cervical cancer cases, while HPV 18 is associated with 20% of cases.[3] HPV-DNA-based

Address for Correspondence: Yasin Ceylan
Phone: +90 535 748 97 29 **E-mail:** md.yasinceylan@yahoo.com **ORCID ID:** orcid.org/0000-0001-5517-8461
Received: 31.07.2024 **Accepted:** 25.08.2024

testing is widely regarded as the primary screening method in many countries because of its high sensitivity in detecting cervical cancer. However, the use of these tests is restricted in underdeveloped and developing countries, primarily due to their cost.[4]

Patients referred for colposcopy based on symptoms and lesions suggestive of malignancy, as identified through anamnesis and pelvic examination, are categorized as having a "clinically suspicious cervix".[5] In addition to cytology and HPV-DNA testing, a clinically suspicious cervix is an indication for colposcopy that covers all age groups and does not require additional costs. The existing literature on the colposcopic findings associated with a clinically suspicious cervix is limited, with studies involving relatively small patient cohorts.[5-7]

In this study, we aim to demonstrate the role of a clinically suspicious cervix in detecting premalignant and malignant lesions, particularly in cases where cytology and/or HPV-DNA testing results are negative.

Additionally, we seek to emphasize the importance of cervical examination in guiding the decision for colposcopic biopsy. Given the age range of our study population, we also aim to contribute to the literature on the necessity of continuing cervical cancer screening for women aged 65 and older.

METHODS

In this retrospective observational study, we reviewed the medical records of 2,040 patients who presented to our clinic and were referred for colposcopy between 2017 and 2021. We identified 486 postmenopausal women among these patients. 94 patients were excluded from the study for the following reasons: having a history of premalignant or malignant cervical lesions and currently undergoing follow-up or treatment; receiving exogenous hormone therapy; having undergone hysterectomy; presenting with lesions indicative of obvious invasive cervical cancer who had undergone cervical biopsy without prior colposcopic examination; and possessing incomplete medical records. The remaining 392 postmenopausal women, aged 45-86, were analyzed for age, parity, cervical cytology, HPV-DNA test results, colposcopy indications, and pathology reports. Patients were categorized based on colposcopy indications into clinically suspicious cervix, high-risk group HPV (hr- HPV) positivity, and abnormal cytology. Ethics committee approval for our retrospective observational study was received from the Prof. Dr. Cemil Taşçıoğlu Training and Research Hospital Clinical Research Impact Committee (decision numbered: 248/2021-06-21).

"Clinical suspicion" was defined as postmenopausal bleeding, postcoital bleeding, and treatment-resistant malodorous vaginal discharge. The following criteria were used to diagnose abnormal cervical appearance in postmenopausal patients:

- Presence of ectropion/erosion, markedly hyperemic lesions with contact bleeding,
- Increased abnormal vascularization,
- Presence of leukoplakia or condyloma-like lesions,
- Presence of exophytic millimeter-sized masses.

Patients with lesions indicative of stage 1a[1] or greater cervical cancer, who underwent direct biopsy, were excluded from the study.

Colposcopy Technique in Postmenopausal Patients

The cervix was washed with physiological saline and treated first with a 3-5% acetic acid solution and then with Lugol's solution under colposcopic illumination. The cervix was evaluated for abnormal colposcopic findings, including acetowhite epithelium, mosaic pattern, punctation, and atypical vascularization, and biopsies were taken from areas with detected abnormalities. All postmenopausal patients also underwent endocervical curettage (ECC).

Statictical Analysis

Data analysis was performed using SPSS version 20.0 (SPSS Inc., Chicago, IL, USA). Normality and homogeneity were assessed using the Kolmogorov-Smirnov and Levene's tests, respectively, and the chi-square test was used to examine the distribution of categorical data between groups. Descriptive statistics were conducted to determine mean and standard deviation values for age and parity. The colposcopic biopsy results for clinically suspicious cervix indications in postmenopausal patients were compared with the results of colposcopy performed due to abnormal cervical cytology and positive high-risk group HPV indications.

Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) for detecting premalignant and malignant lesions were calculated. A 95% confidence interval and a significance level of $p=0.05$ were applied for all data evaluations.

RESULTS

Among these patients, 174 were referred for colposcopic evaluation due to clinical suspicion and abnormal cervical appearance, 146 due to HPV-DNA positivity, and 72 due to abnormal cervical cytology.

Age and parity distributions were similar among the three groups. Table 1 provides the descriptive statistics for patients in each group.

As shown in Table 2, 60% of the 174 patients with a clinically suspicious cervix were found to have cervical premalignant or malignant lesions of cervical intraepithelial neoplasia (CIN)-1 or higher, based on pathology results. This percentage was similar to those referred for colposcopy due to positive HPV-DNA testing (63%) and abnormal cytology (58%). Among the

Table 1. Common descriptive characteristics of patients

Patient groups and their numbers	Age Mean ± SD (min.-max.)	Parity Mean
Cervix with clinical suspicion (n=174)	57±9 (45-86)	4
HPV positive (n=146)	54±5 (46-66)	3
Abnormal cytology (n=72)	57±7 (45-70)	3
p-value	0.064	0.091

HPV: Human papilloma virus, min.-max.: Minimum-maximum

patients referred for colposcopy due to clinical suspicion, 10% (n=18) had detected malignancies, while only one patient each in the HPV-DNA positive and abnormal cytology groups exhibited malignancies. Among 43 patients aged 65-86, 27 (62.7%) had pathological results indicating pre-malignant/malignant cervical lesions. Specifically, 11 patients had CIN1, 2 patients had CIN2, 3 patients had CIN3 and 11 (25.5%) patients had malignancy. In contrast, among 349 patients aged 45-65, malignancy was found in 2% (n=7). Malignacy detection in cases of clinically suspicious cervix was significantly more frequent in elderly postmenopausal patients aged 65 and greater compared to postmenopausal patients younger than age 65 (p=0.0001).

Among patients with abnormal cytology (n=72), the distribution was as follows: 70% (n=50) had an atypical squamous cells of undetermined significance, 14% (n=10) had a low-grade squamous intraepithelial lesion or, 8% (n=6) had an atypical squamous cells, cannot exclude high-grade squamous intraepithelial lesion, and 7% (n=5) had a high-grade squamous intraepithelial lesion. One patient had an atypical glandular cells.

In the classification of HPV-DNA positive cases (n=146) based on oncogenic risk potential, 125 cases were identified as high-risk types, while 21 cases were categorized as having unknown types.

The primary indications for referral to colposcopy due to clinically suspicious cervix were exophytic mass (28%), postcoital bleeding (17%), postmenopausal bleeding (20%), and increased cervical vascularity (11%) (Table 3). We observed a higher detection rate of CIN1 or more severe lesions in cases presenting with postcoital and postmenopausal bleeding which are among the most significant symptoms of cervical cancer. In instances where malignancy was identified, postmenopausal and postcoital bleeding, along with exophytic mass, were the predominant clinical suspicions.

Table 4 shows that the PPV among the three groups were nearly equivalent. HPV positivity had the highest sensitivity, while clinically suspicious cervix had higher sensitivity compared to abnormal cytology, but lower specificity.

Among the 392 patients, 11 who had negative colposcopic biopsies were diagnosed solely through ECC. Pathology results for these patients included CIN3 in 5 patients, CIN2 in 1 patient, CIN1 in 3 patients, and invasive cancer in 2 patients (Table 5).

DISCUSSION

Contemporary indications for colposcopy predominantly include high-risk HPV positivity and abnormal cytology.[8] The American Society for Colposcopy and Cervical Pathology risk-

Table 2. Pathology results distribution based on colposcopy indications

Biopsy result	Benign	CIN1	CIN2	CIN3	Malign
Cervix with clinical suspicion (n=174) n (%)	69 (40%)	66 (38%)	9 (5%)	12 (7%)	18 (10%)
HPV positive (n=146) n (%)	54 (37%)	64 (44%)	12 (8%)	15 (10%)	1 (1%)
Abnormal cytology (n=72) n (%)	30 (42%)	34 (47%)	5 (7%)	2 (3%)	1 (1%)
Total	**153**	**164**	**26**	**29**	**20**

HPV: Human papilloma virus, CIN: Cervical intraepithelial neoplasia

Table 3. Distribution of pathology results by clinical suspicious cervix diagnostic criteria

Colposcopic biopsy result	Normal	CIN1	CIN2	CIN3	Malignite	Total
Exophytic mass	20 (40.8%)	20 (40.8%)	0	3 (6%)	6 (12%)	49 (28%)
Erosion/Ectropion	11 (45.8%)	7 (29%)	4 (16.6%)	0	2 (8.3%)	24 (13.7%)
Postmenopausal bleeding	12 (36%)	9 (27%)	1 (3%)	5 (15%)	6 (18%)	33 (20%)
Increased vascularity	9 (45%)	9 (45%)	1 (5%)	0	1 (5%)	20 (11.4%)
Postcoital bleeding	10 (33%)	13 (43%)	1 (3%)	3 (10%)	3 (10%)	30 (17%)
Abnormal vaginal discharge	4 (36.3%)	5 (45.4%)	1 (9%)	1 (9%)	0	11 (6%)
Leukoplakia	3 (43%)	4 (57%)	0	0	0	7 (4%)
Total	**69 (40%)**	**66 (38%)**	**9 (5%)**	**12 (7%)**	**18 (10%)**	**174 (100%)**

CIN: Cervical intraepithelial neoplasia

Table 4. Predictive value of clinically suspicious cervix, HPV positivity and abnormal cytology in cervical premalignant and malignant lesions

	Sensitivity	Specificity	Confirmation rate	PPV	NPV
Cervix with clinical suspicion	43%	53%	47%	60%	36%
HPV positive	80%	49%	67%	68%	64%
Abnormal cytology	37%	73%	51%	69%	42%

HPV: Human papilloma virus, PPV: Positive predictive value, NPV: Negative predictive value

Table 5. Comparison of colposcopic biopsy and ECC results

Colposcopic biopsy + ECC			
Colposcopic biopsy	**Negative**	**Positive**	**Total**
Negative	141	11	152
Positive	0	240	240
Total	141	251	392

ECC: Endocervical curettage

based management algorithm also utilizes these two screening methods to calculate and manage the risk of developing cervical pre-invasive lesions.[8] Other indications for colposcopy include clinical suspicion and abnormal cervical appearance. However, the boundaries and objective criteria for defining clinically suspicious cervixes for identifying pre-malignant and malignant lesions are unclear. Cervical examination, whether performed visually or via colposcopy, remains a subjective assessment.

There is limited information on clinical suspicion in evaluating the cervix and the definition of abnormal cervical appearance. In a guide published by Casey et al.,[9] abnormal-looking cervical lesions were categorized as cervical polyps and fibroids, cervical ectropion, cervical endometriosis, cervicitis, lesions associated with postcoital bleeding, and in utero dietilstilbestrol exposure. In another study, cervical ectopy/ectropion, suspicious masses, ulcers, hypertrophy, leukoplakia, and cervical warts were additionally included among these lesions.[10] In our study, under the heading of the clinically suspicious cervix, we aggregated cases of exophytic masses, erosion/ectropion, increased vascularization, contact bleeding lesions, abnormal chronic vaginal discharge, leukoplakia, condyloma, and postmenopausal/postcoital bleeding.

In our study, among the 174 patients referred for clinically suspicious cervix, colposcopic biopsy results revealed CIN1 or higher cervical pre-malignant or malignant lesions in 60% of cases. Reviewing similar studies in the literature, a 60% positive result rate for cervical dysplasia in cases of clinically suspicious cervix was reported.[5] However, other studies have shown this rate to be between 20% and 30%.[6,7] We hypothesize that these difference in the literature may be attributed to limited number of patients, subjective definitions of clinically suspicious cervix, and differences in background risk of the populations the studies were conducted.

During the postmenopausal period, the squamocolumnar junction typically shifts towards the endocervical canal. Cervical ectropion and erosion are not expected to be seen during this period. Since it is not possible to definitively differentiate cervical ectropion from CIN and cervical cancer using macroscopic imaging in postmenopausal patients, it is necessary to distinguish between cervical pre-malignant and malignant lesions using cytology and/or colposcopy in suspicious cases.[11] In our study, half of the 24 postmenopausal patients who were evaluated colposcopically for cervical erosion or ectropion had abnormal pathology findings.

In a study examining 314 women presenting with postcoital bleeding, colposcopy results revealed invasive cancer in 4% of cases and CIN in 17%.[12] Our study identified postcoital bleeding as a significant symptom indicative of cervical lesions. Among the 14 postmenopausal patients who underwent colposcopic biopsy due to this symptom, CIN1 or higher pre-malignant/malignant lesions were detected in 10 cases. A study examining 148 patients with cervical cancer found that 70% of the cases were symptomatic, with postmenopausal bleeding being the most common symptom at a rate of 33%.[13] In our study, among the 21 patients with postmenopausal bleeding, 2/3 had pre-malignant or malignant pathology on colposcopy. Specifically, 6 patients had CIN1, 4 patients had CIN3 and 4 patients had invasive cancer. These data suggest that postmenopausal bleeding is a significant indicator of cervical cancer.

Currently, cervical cancer screening programs enable the detection of cervical lesions in the pre-invasive stage, allowing for monitoring and treatment to prevent progression to invasive lesions. Guidelines for cervical screening methods and their management are continuously updated with emerging studies. According to international guidelines, cervical screening programs are recommended to be terminated at age 65.[14-16] In our study, malignacy detection in cases of clinically suspicious cervix was significantly more frequent in elderly postmenopausal patients aged 65 and greater compared to postmenopausal patients younger than age 65.

Based on the pathological results of the 43 patients aged 65 and over in our study, the cumulative risk for cervical cancer continues, continuing screening beyond age 65 appears reasonable as the life expectancy in the world is increasing. Additionally, these data indicate that in developing countries like ours, the risk of cervical malignancy persists in older age, and continued screening could significantly prevent malignancy. Supporting studies in the literature include Rustagi et al.,[17] who reported that HPV infection risk persists in older women, and cervical cancer screening programs should include women aged 65 and over. Another study involving 2,753 women with invasive cervical cancer found that approximately 20% of cervical cancer cases were in the 55-69 age group, and 19% were in those aged 70 and over. This suggests that the risk of cervical cancer continues with age, most cancer cases still occur in women over 65, and screening should continue at older ages.[18]

In a study by Gage et al.,[19] among 13,115 colposcopic biopsies detecting CIN2 and above, approximately 1% of cases had a positive ECC only, and they recommended the addition of ECC to colposcopic procedures, especially in older women. In our study, among 392 patients, 11 had normal colposcopic cervical biopsy results but had pathological diagnoses identified only through ECC. ECC alone detected CIN1 in 3 patients, CIN2 in 1 patient, CIN3 in 5 patients, and invasive cancer in 2 patients. Our results support that ECC should be performed routinely with colposcopic biopsy, particularly in postmenopausal patients.

In our study, HPV positivity exhibited the highest sensitivity at 80%, whereas colposcopic biopsy conducted in the presence of a clinically suspicious cervix demonstrated greater sensitivity (43%) compared to cytology results (37%). The positive PPV for all three groups we examined were similar.

This indicates that a clinically suspicious cervix is at least as effective as screening tests in identifying actual cases of cervical pathology.

Study Limitations

The limitations of our study include the restricted sample size and the fact that it was conducted on a cross-sectional subset of the population. This does not address whether similar results would be obtained in premenopausal patients. Additionally, the subjective nature of defining a clinically suspicious cervix represents another limitation of our study. On the other hand, our study could contribute to establishing standard criteria for the definition of a clinically suspicious cervix. Given that it includes data from the postmenopausal patient group outside of screening programs, it highlights the importance of gynecological examinations and the necessity for continued screening in this group of patients.

CONCLUSION

The gold standard method for the diagnosis of cervical lesions is colposcopy-guided cervical biopsy and histopathologic examination. Currently, the majority of patients referred for colposcopy are those with positive cervical cytology and/or high-risk group HPV-DNA test results. Nevertheless, our study identified a clinically suspicious cervix as a significant indicator for detecting pre-malignant and malignant lesions, comparable to screening tests. The importance of clinical suspicion increases particularly when considering the age group and menopausal status examined in our study. In the postmenopausal patient group, we concluded that directing patients to immediate colposcopic biopsy after evaluating cervical examination and symptoms by a clinician is at least as effective as screening tests in detecting pre-malignant and malignant lesions. This suggests that cytological screening should be continued in the postmenopausal patient group.

Footnote

Ethics Committee Approval: Prof. Dr. Cemil Taşçıoğlu Training and Research Hospital Clinical Research Impact Committee (decision numbered: 248/2021-06-21).

Informed Consent: Retrospective study.

Authorship Contributions

Surgical and Medical Practices: T.Ö.A., Y.C., Concept: T.Ö.A., H.G., Design: T.Ö.A., H.G., Data Collection or Processing: D.E.A., Ö.D.S., Analysis or Interpretation: T.Ö.A., Y.C., H.G., Literature Search: D.E.A., Ö.D.S., Writing: T.Ö.A., Y.C.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study received no financial support.

REFERENCES

1. Sung H, Ferlay J, Siegel RL, et al. Global Cancer Statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2021;71(3):209-249.
2. Breitenecker G. Zervixkarzinom-screening: Gestern-heute-morgen [Cervical cancer screening: past-present-future]. Pathologe. 2009 ;30(Suppl 2):128-135.
3. de Sanjose S, Quint WG, Alemany L, et al. Human papillomavirus genotype attribution in invasive cervical cancer: a retrospective cross-sectional worldwide study. Lancet Oncol. 2010;11(11):1048-1056.
4. Catarino R, Petignat P, Dongui G, Vassilakos P. Cervical cancer screening in developing countries at a crossroad: Emerging technologies and policy choices. World J Clin Oncol. 2015;6(6):281-290.
5. Goyal R, Mohi KM, Bal Singh M. AgNOR Count and its Correlation with colposcopy in suspicious cervix. J Obstet Gynaecol India. 2012;62(4):437-441.
6. Milingos DS, Harry VN, Cruickshank ME. The clinically suspicious cervix-how often is it cancer? J Low Genit Tract Dis. 2010;14(3):196-199.
7. KAUR A, Singh G. Colposcopy in postmenopausal women: a challenging procedure. International Journal of Reproduction Contraception Obstetrics and Gynecology. 2020;9(8):3341.
8. Perkins RB, Guido RS, Castle PE, et al. 2019 ASCCP risk-based management consensus guidelines for abnormal cervical cancer screening tests and cancer precursors. J Low Genit Tract Dis. 2020;24(2):102-131.
9. Casey PM, Long ME, Marnach ML. Abnormal cervical appearance: what to do, when to worry? Mayo Clin Proc. 2011;86(2):147-150.
10. Darwish AM, Kamel MA, Zahran K, Aboulela M. Office cervicoscopy versus stationary colposcopy in suspicious cervix: a randomized controlled trial. J Midlife Health. 2019;10(3):115-122.
11. Aggarwal P, Ben Amor A. Cervical ectropion. 2020 Nov 17. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2021.
12. Rosenthal AN, Panoskaltsis T, Smith T, Soutter WP. The frequency of significant pathology in women attending a general gynaecological service for postcoital bleeding. BJOG. 2001;108(1):103-106.
13. Myriokefalitaki E, Potdar N, Barnfield L, Davies Q, Moss EL. Cervical cancer still presents symptomatically 20 years after the introduction of a structured national screening programme. Cytopathology. 2016;27(4):229-236.
14. US Preventive Services Task Force; Curry SJ, Krist AH, et al. Screening for cervical cancer: US preventive services task force recommendation statement. JAMA. 2018;320(7):674-686.
15. Fontham ETH, Wolf AMD, Church TR, et al. Cervical cancer screening for individuals at average risk: 2020 guideline update from the American Cancer Society. CA Cancer J Clin. 2020;70(5):321-346.
16. Practice Bulletin No. 157: Cervical cancer screening and prevention. Obstet Gynecol. 2016;127(1):e1-e20.
17. Rustagi AS, Kamineni A, Weiss NS. Point: cervical cancer screening guidelines should consider observational data on screening efficacy in older women. Am J Epidemiol. 2013;178(7):1020-1022.
18. Sasieni P, Adams J, Cuzick J. Benefit of cervical screening at different ages: evidence from the UK audit of screening histories. Br J Cancer. 2003;89(1):88-93.
19. Gage JC, Duggan MA, Nation JG, Gao S, Castle PE. Detection of cervical cancer and its precursors by endocervical curettage in 13,115 colposcopically guided biopsy examinations. Am J Obstet Gynecol. 2010;203(5):481.

Original Article

DOI: 10.4274/anajog.galenos.2024.85057

Anat J Obstet Gynecol Res 2024;2:66-70

MMP-1, MMP-2, MMP-9, TIMP-1, TIMP-2, MUC1 and CD29 Expression in Endometrial Polyps at Implantation Window Compared to Neighbouring Normal Endometrium

Emre Köle[1], Ömer Doğukan Saraç[2], Nihan Erdoğan Atalay[3], Bahar Müezzinoğlu[4]

[1]Alanya Alaaddin Keykubat University Faculty of Medicine, Department of Obstetrics and Gynecology, Antalya, Turkey
[2]Kocaeli City Hospital, Clinic of Obstetrics and Gynecology, Kocaeli, Turkey
[3]Bolu İzzet Baysal State Hospital, Clinic of Obstetrics and Gynecology, Zonguldak, Turkey
[4]Medipol Mega University Hospital, Clinic of Pathology, İstanbul, Turkey

ABSTRACT

Purpose: The aim of this study was to evaluate the expression of potential mediators of embryo implantation and invasion on polyps such as Matrix metalloproteinases (MMPs), Tissue inhibitors of Metalloproteinases (TIMPs), Mucin-1 and CD29 in women with uterine polyps compared to healthy adjacent endometrium to elucidate causal relationship between infertility and endometrial polyps (EPs).

Methods: The study was conducted on 15 women presented with unexplained infertility and EPs. Office hysteroscopy was performed, and the polyps were removed via scissors and these polyps formed the Study group (n=15). The endometrium adjacent to the polyps was punch-biopsied and formed the Control group (n=15). Sections were cut, antigens unmasked, and immunohistochemical staining was performed. Immunohistochemical staining in luminal and glandular epithelium cytoplasm was graded.

Results: MMP-1 immunostaining in luminal epithelium cytoplasm did not differ between the groups, whereas luminal epithelium cytoplasm had statistically significant ($p<0.01$) staining for MMP-2 and 9 among the study and control groups. TIMP-1 and TIMP-2 expression in polyps were statistically significantly prominent compared to the control group. While MUC1 expression did not differ between the groups, CD29 integrin expression was higher in the control group ($p<0.01$).

Conclusion: The presence of EPs in infertile women may adversely affect the delicate balance of mediators unfavorably. As it is well studied, the hyperreactivity of MMPs in chronic inflammatory states may decrease the chance of successful implantation. Consequently, we believe that removing even asymptomatic EPs may increase implantation success in infertile women.

Keywords: Endometrial polyps, MMP, TIMP, MUC1, CD29

INTRODUCTION

Endometrial polyps (EPs) were reported to be present in 3 to 10% of women of reproductive age.[1] The prevalence is reported to be even higher among infertile patients which is 8 to 34%.[2,3] Although routine use of transvaginal ultrasonography during gynecologic examination may lead to the diagnosis of EPs in asymptomatic patients, sonohysterography in premenopausal women with and without abnormal bleeding is believed to be the best non-invasive method to diagnose EPs accurately. However, the gold standard for more accurate diagnosis is accepted to be hysteroscopy-guided biopsy.[4,5]

A healthy uterine environment plays a pivotal role in facilitating the successful implantation of a viable embryo, underscoring the importance of exploring factors that may influence uterine receptivity. Uterine polyps are benign growths within the uterine cavity often associated with abnormal uterine bleeding, recurrent miscarriage, and impaired fertility. Despite their prevalence among infertile patients, the mechanisms linking uterine polyps to infertility are not thoroughly elucidated.[6,7]

Furthermore, uterine polyps have been speculated to exert detrimental effects on fertility through various mechanisms, including hindrance of sperm transport, obstruction of embryo implantation due to their space-occupying nature, and induction of local inflammatory changes.[8-10] These inflammatory responses, characterized by an increased presence of mast cells and upregulation of Matrix metalloproteinase 2 (MMP2) and MMP9 activity, suggest a potential association between

Address for Correspondence: Emre Köle, Alanya Alaaddin Keykubat University Faculty of Medicine, Department of Obstetrics and Gynecology, Antalya, Turkey
Phone: +90 530 922 13 37 **E-mail:** emre.kole.41@gmail.com **ORCID ID:** orcid.org/0000-0002-4940-8862
Received: 31.07.2024 **Accepted:** 25.08.2024

uterine polyps and altered molecular pathways implicated in infertility.[6,7]

MMPs are a family of calcium-dependent homologous enzymes containing zinc in the active site and capable of degrading extracellular matrix (ECM) and basement membrane components.[11] They are essential for physiological processes such as implantation, placentation, and the dynamic remodeling of uterine structures during pregnancy. MMP-1 belongs to the collagenase group and is up-regulated by trophoblast invasion while MMP-2 and MMP-9 belong to the gelatinase group. MMP-1 as a collagenase, breaks down collagen fibers in the ECM, while MMP-2 and MMP-9 are gelatinases that primarily degrade gelatin substrates in the ECM.[12]

Recent studies suggest a potential association between altered MMP activity and infertility, with elevated intrauterine levels observed in infertile women compared to fertile ones.[13] MMP enzymes are regulated transcriptionally, activated by proenzyme conversion, and regulated by tissue inhibitors [Tissue inhibitors of Metalloproteinases (TIMPs)]. There are genetically four different TIMPS in humans. TIMPS are expressed coordinately with MMPs. The hyperactivity of MMP-2 and MMP-9 is associated with an unfavorable uterine environment, as seen in endometrial inflammation. The simultaneous expression and inhibition of MMP-9 during the luteal phase are regulated by TIMP.[14,15]

Mucin-1 (MUC1) is a member-associated protein that is highly expressed in the luminal and glandular epithelium during the implantation window.[16,17] MUC1 is crucial in aiding embryo implantation and the establishment of pregnancy. It accomplishes this by safeguarding the uterine lining, regulating immune reactions, and fostering communication and attachment between the embryo and the mother. Fertile women showed a higher level of endometrium MUC1 expression than infertile patients.[18] The reduction of anti-adhesive factors such as MUC1 in infertility cases may contribute to the increased adhesiveness of pinopods.[19] CD29 assists in the initial bonding of the embryo to the uterine lining and promotes the invasive actions of trophoblast cells during implantation, thus playing a vital part in establishing pregnancy.[20]

This study aimed to evaluate the expression of potential mediators of embryo implantation and invasion on polyps such as MMPs, TIMPs, MUC1, and CD29 in women with uterine polyps compared to healthy adjacent endometrium to elucidate the causal relationship between infertility and EPs.

METHODS

The study was conducted on 15 women presented with unexplained infertility complaints and ultrasound-diagnosed EPs which were consecutively removed via hysteroscopy for pathological examination. Office hysteroscopy was performed, and the polyps were removed via scissors and these polyps formed the Study group (n=15). The endometrium adjacent to the polyps was punch-biopsied and formed the Control group (n=15). The biopsy specimens were taken without dilatation of the cervix and without anesthesia.

EPs and endometrial biopsies of normal endometrial neighbouring the polyps were taken from the mid-uterine cavity on day 20-24 of the menstrual cycle, at the time of embryo implantation window in regularly menstruating women. Participation in the study was voluntary and all participants read and approved the informed consent form.

The biopsy specimens were fixed in 10% neutral buffered formalin for at least 6 hours. After overnight tissue processing, they were embedded in paraffin. The histologic sections were in 5 µm thickness and stained with Hematoxylin and Eosin. Sections for immunohistochemical examination were cut in 5 µm thickness and mounted onto adhesive-coated slides (Menzel-Gloser, Superfrost® Plus, Germany). For immunohistochemical staining, sections were kept at 56 °C overnight and then soaked in xylene for 30 minutes. After washing with a decreasing series of ethanol, sections were washed with distilled water and phosphate-buffered saline (PBS) for 15 minutes. After that, coated slides were dried in an incubator at 56 °C for 2 hours. Antigen unmasking was performed for the MMP-1 antibody, MMP-2 antibody, MMP-9 antibody, TIMP-1 antibody, and TIMP-2 antibody in a citrated buffer solution in the commercially available pressure cooker at 1 atmosphere pressure for 1.5 min.

After antigen unmasking, step slides were washed with PBS (pH: 7,4) and thereafter to block endogenous peroxidase activity, the slides were incubated in 3% hydrogen peroxide for 20 mins. Slides were washed with PBS for 5 mins. Sections were then blocked with Super Block (REF: AAA 125 LOT:12232) at room temperature for 15 mins and afterward washed with PBS. Slides were immunostained with commercially available rabbit polyclonal antibody MMP-1 (Collagenase-1) (Neo-Markers, Fremont, CA, USA, Cat No: RB-1536-P) at 1:50 dilution for 1 hour in the microwave, with commercially available rabbit monoclonal antibody MMP-2 (72kDa

Collagenase IV) Ab-4 (Neo-Markers Fremont, CA, USA, Cat No: MS-806-P) at 1:100 dilution for 1 hour in the pressure cooker, with commercially available rabbit polyclonal antibody MMP-9 (92 kDa Collagenase type IV) Ab-9 (Neo-Markers Fremont, CA, USA, Cat No: RB-1539-P) at 1:50 dilution for 2 hours, with commercially available mouse monoclonal antibody TIMP-1 (Novocastra™, NCL-TIMP1-485, Newcastle, UK) at 1:150 dilution for 1 hour, and with commercially available mouse monoclonal antibody TIMP-2 (Clone 3A4) (Neo-Markers Fremont, CA, USA, Cat No: MS-1485-P) at 1:25 dilution for 2 h at room temperature (20-25 °C).

Afterward, slides were washed with PBS and slides were incubated with UltraTek Anti-Polyvalent Biotinylated Antibody (REF: ABN 125, LOT:11461, ScyTek Laboratories, Utah, USA) at room temperature for 25 mins. Slides were washed with PBS again and incubated with UltraTek HRP (REF: ABL125, LOT:11460, ScyTek Laboratories, Utah, USA) at room temperature for 25 mins. Slides were washed with PBS and incubated with Ultravision Detection System Large Volume AEC Substrate System (RTU) (REF: TA-125-HA, LOT: AHA60718, LabVision, Fremont, CA, USA) at room temperature for 15 mins. The sections were finally counterstained using Mayer's hematoxylin and mounted in an aqueous medium.

Slides were analyzed with a BX50 conventional light microscope (Olympus, Tokyo, Japan) by BM at 100 and 200 magnifications two times. Staining intensity was graded as; "0 = no staining", "+1 or 1-10% staining = weak staining", "+2 or 10-49% staining = mild staining" and "+3 or 50-100% staining = strong staining". Immunohistochemical staining in luminal and glandular epithelium cytoplasm were graded in 60 sections counting 100 cells separately at 400X magnification.

Statistical Analysis

The statistical analysis of the study data was performed using SPSS 23.0 for the Windows packet program. The age of the patients were normally distributed according to Shapiro-Wilk test and presented as mean and standard deviation (SD) of the mean. Classified data was presented as number and percentages. Analysis of classified data was performed using the chi-square test, Fisher's Exact test. Probability (p) value smaller than 0.05 was considered significant. All values reported are mean (± SD) or percentage.

RESULTS

The mean age of the patients was 32.4±4.8 years. The mean menstrual cycle timing of the hysteroscopic polypectomy and concomitant endometrial biopsy was 22.2±1 days. According to common wisdom, any hysteroscopic intervention is advised to be done in the early proliferative phase of the cycle. However, the so-called- implantation window, is believed to be around the 21st-23rd day of the cycle. So, we decided to do hysteroscopic interventions around these days to elucidate the problem in implantation and avoid other confounders. MMP-1 immunostaining in luminal epithelium cytoplasm did not differ between the groups, whereas luminal epithelium cytoplasm had statistically significant ($p<0.01$) staining for MMP-2 and 9 in the study group (Table 1). Not surprisingly the TIMP-1 and TIMP-2 expression in polyps were statistically significantly prominent compared to the control group (Table 2). While MUC1 expression did not differ between the groups, CD29 integrin expression was higher in the control group ($p<0.01$) (Table 3).

DISCUSSION

EPs are frequently encountered in asymptomatic women during routine gynecologic examinations. However, the prevalence of EPs in infertile women seems to be somewhat higher than in the normal population.[2,3] The causal relation between EPs and implantation failure has not been extensively studied in the literature. Despite huge advances in this field implantation is still believed to be the least understood phase of human reproduction. There are multiple delicate regulatory mechanisms with interactions ultimately leading to the hosting of a genetically diverse group of cells within the cells of the mother. Among these, cytokines, cell adhesion molecules, prostaglandins, and growth factors, MMPs are believed to play an integral role in human embryo implantation and are the main rate-limiting enzymes in ECM remodeling during implantation. Successful implantation is believed to depend on

Table 1. MMP-1, MMP-2, and MMP-9 expression in polyps and adjacent endometrium

Variable	Study group (n=15)	Control group (n=15)	p-value
MMP-1 immunostaining in luminal epithelium cytoplasm			
No stain	7 (46.7)	9 (60)	0.5
+1	3 (20)	4 (26.7)	
+2	4 (26.7)	2 (13.3)	
+3	1 (6.7)	0	
MMP-2 immunostaining in luminal epithelium cytoplasm			
No stain	13 (86.7)	0	<0.0*
+1	2 (13.3)	5 (33.3)	
+2	0	8 (53.3)	
+3	0	2 (13.3)	
MMP-9 immunostaining in luminal epithelium cytoplasm			
No stain	13 (86.7)	5 (33.3)	<0.01*
+1	1 (6.7)	4 (26.7)	
+2	1 (6.7)	4 (26.7)	
+3	0	2 (13.3)	
MMP-9 immunostaining in luminal epithelium stroma			
No stain	11 (73.3)	6 (0.4)	0.8
+1	2 (13.3)	6 (0.4)	
+2	1 (6.7)	2 (13.3)	
+3	0	1 (6.7)	

*Likelihood ratio, statistically significant, $p<0.05$. MMP: Matrix metalloproteinase

Table 2. TIMP-1 and TIMP-2 expression in polyps and adjacent endometrium			
Variable	**Study group (n=15)**	**Control group (n=15)**	**p-value**
TIMP-1 immunostaining in luminal epithelium cytoplasm			
No stain	11 (73.3)	2 (13.3)	<0.01*
+1	3 (20)	6 (40)	
+2	1 (6.7)	3 (20)	
+3	0	4 (26.7)	
TIMP-1 immunostaining in endometrial stroma			
No stain	10 (66.7)	0	<0.01*
+1	1 (6.7)	2 (13.3)	
+2	4 (26.7)	12 (80)	
+3	0	1 (6.7)	
TIMP-2 immunostaining in luminal epithelium cytoplasm			
No stain	15 (100)	11 (73.3)	0.04*
+1	0	3 (20)	
+2	0	1 (6.7)	
TIMP-2 immunostaining in endometrial stroma			
No stain	15 (100)	13 (86.6)	0.1
+1	0	2 (13.3)	
*Likelihood ratio, statistically significant, p<0.05. TIMP: Tissue inhibitors of Metalloproteinase			

Table 3. MUC1 and CD29 integrin expression on polyps and adjacent endometrium			
Variable	**Study group (n=15)**	**Control group (n=15)**	**p-value**
MUC-1			
+2	2 (13.3)	4 (26.7)	0.36
+3	13 (86.7)	11 (73.3)	
CD29 integrin			
No stain	10 (66.7)	1 (6.7)	<0.01*
+1	5 (33.3)	6 (40)	
+2	0	8 (53.3)	
*Likelihood ratio, statistically significant, p<0.05. MUC1: Mucin-1			

a strict balance between activation and inhibition of MMPs.[14,15] Hyperactivity of MMP2 and 9 are reported to be found in the endometrial tissues in chronic or mild inflammatory states.

In our study, we aimed to highlight the hyperexpression of MMP 2 and 9 in the tissues extracted from the EPs and compared them with normal neighboring endometrial tissue. The reason for not choosing a control group from the endometrium of fertile women is that there might be innate differences between the fertile and infertile women. To overcome other confounding factors, we preferred to sample the same uterus for comparison. It was not surprising to find the overexpression of TIMPs, since they usually reflect the increased levels of MMPs in response.

CD29 integrin assists in the initial bonding of the embryo to the uterine lining and promotes the invasive actions of trophoblast cells during implantation, thus playing a vital part in establishing pregnancy. The immunohistochemical staining for CD29 antigen in the study group was significantly less than the control group. This finding may also constitute another explanation for implantation failure in the infertile patients diagnosed with EPs.

In our study, we did not include EPs dimensions. Hence, we could not compare and quantify whether the magnitude of the volume of the polyps has an effect on the staining levels of the markers that we studied. This might be regarded as a weakness of our study.

CONCLUSION

The presence of EPs in infertile women may adversely affect the delicate balance of mediators unfavorably. As it is well studied, the hyperreactivity of MMPs as seen in chronic inflammatory states may lead to a decrease in the chance of a successful implantation. Consequently, we believe that removing even asymptomatic EPs may increase implantation success in infertile women.

Footnote

Acknowledgments

The authors acknowledge their gratitude to all of the participants in this work.

Ethics Committee Approval: The study received the approval of Kocaeli University Clinical Research Ethics Committee (approval number: 17/18, dated: 2008/124).

Informed Consent: Participation in the study was voluntary and all participants read and approved the informed consent form.

Authorship Contributions

Surgical and Medical Practices: E.K., B.M., Concept: B.M., Design: E.K., B.M., Data Collection or Processing: Ö.D.S., N.E.A., Analysis or Interpretation: Ö.D.S., N.E.A., Literature Search: E.K., Ö.D.S., Writing: E.K.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study received no financial support.

REFERENCES

1. Clevenger-Hoeft M, Syrop CH, Stovall DW, Van Voorhis BJ. Sonohysterography in premenopausal women with and without abnormal bleeding. Obstet Gynecol. 1999;94(4):516-520.
2. Hinckley MD, Milki AA. 1000 office-based hysteroscopies prior to in vitro fertilization: feasibility and findings. JSLS. 2004;8(2):103-107.
3. Lieng M, Istre O, Qvigstad E. Treatment of endometrial polyps: a systematic review. Acta Obstet Gynecol Scand. 2010;89(8):992-1002.
4. American Association of Gynecologic Laparoscopists. AAGL practice report: practice guidelines for the diagnosis and management of endometrial polyps. J Minim Invasive Gynecol. 2012;19(1):3-10.
5. Jansen FW, de Kroon CD, van Dongen H, Grooters C, Louwé L, Trimbos-Kemper T. Diagnostic hysteroscopy and saline infusion sonography: prediction of intrauterine polyps and myomas. J Minim Invasive Gynecol. 2006;13(4):320-324.
6. Spiewankiewicz B, Stelmachów J, Sawicki W, Cendrowski K, Wypych P, Swiderska K. The effectiveness of hysteroscopic polypectomy in cases of female infertility. Clin Exp Obstet Gynecol. 2003;30(1):23-25.
7. Al-Jefout M, Black K, Schulke L, et al. Novel finding of high density of activated mast cells in endometrial polyps. Fertil Steril. 2009;92(3):1104-1106.
8. Richlin SS, Ramachandran S, Shanti A, Murphy AA, Parthasarathy S. Glycodelin levels in uterine flushings and in plasma of patients with leiomyomas and polyps: implications for implantation. Hum Reprod. 2002;17(10):2742-2747.
9. Maia H Jr, Pimentel K, Silva TM, et al. Aromatase and cyclooxygenase-2 expression in endometrial polyps during the menstrual cycle. Gynecol Endocrinol. 2006;22(4):219-224.
10. Ben-Nagi J, Miell J, Yazbek J, Holland T, Jurkovic D. The effect of hysteroscopic polypectomy on the concentrations of endometrial implantation factors in uterine flushings. Reprod Biomed Online. 2009;19(5):737-744.
11. Al-Jefout M, Black K, Schulke L, et al. Novel finding of high density of activated mast cells in endometrial polyps. Fertil Steril. 2009;92(3):1104-1106.
12. Renaud SJ, Kubota K, Rumi MA, Soares MJ. The FOS transcription factor family differentially controls trophoblast migration and invasion. J Biol Chem. 2014;289(8):5025-5039.
13. Inagaki N, Stern C, McBain J, Lopata A, Kornman L, Wilkinson D. Analysis of intra-uterine cytokine concentration and matrix-metalloproteinase activity in women with recurrent failed embryo transfer. Hum Reprod. 2003;18(3):608-615.
14. Liu G, Zhang X, Lin H, et al. Effects of E-cadherin on mouse embryo implantation and expression of matrix metalloproteinase-2 and -9. Biochem Biophys Res Commun. 2006;343:832-838.
15. Mulayim N, Savlu A, Guzeloglu-Kayisli O, Kayisli UA, Arici A. Regulation of endometrial stromal cell matrix metalloproteinase activity and invasiveness by interleukin-8. Fertil Steril. 2004;81 Suppl 1:904-911.
16. Hey NA, Li TC, Devine PL, Graham RA, Saravelos H, Aplin JD. MUC1 in secretory phase endometrium: expression in precisely dated biopsies and flushings from normal and recurrent miscarriage patients. Hum Reprod. 1995;10(10):2655-2662.
17. Aplin JD, Hey NA, Graham RA. Human endometrial MUC1 carries keratan sulfate: characteristic glycoforms in the luminal epithelium at receptivity. Glycobiology. 1998;8(3):269-276.
18. Margarit L, Taylor A, Roberts MH, et al. MUC1 as a discriminator between endometrium from fertile and infertile patients with PCOS and endometriosis. J Clin Endocrinol Metab. 2010;95(12):5320-5329.
19. Horne AW, White JO, Lalani el-N. Adhesion molecules and the normal endometrium. BJOG. 2002;109(6):610-617.
20. Lu H, Jin LP, Huang HL, et al. Trophoblast-derived CXCL12 promotes CD56bright CD82- CD29+ NK cell enrichment in the decidua. Am J Reprod Immunol. 2020;83(2).

Original Article

DOI: 10.4274/anajog.galenos.2024.41861

Anat J Obstet Gynecol Res 2024;2:71-78

What is an Ideal Cesarean Birth Rate? The Use of the C-Model and the Further Interpretation with the Robson Classification System, A Retrospective Analysis

Esra Ayanoğlu[1], Arzu Bilge Tekin[1], Mahmut Yassa[2], Kemal Sandal[1], Niyazi Tuğ[1]

[1]University of Health Sciences Turkey, Sancaktepe Şehit Prof. Dr. İlhan Varank Training and Research Hospital, Clinic of Obstetrics and Gynecology, İstanbul, Turkey
[2]University of Health Sciences Turkey, Başakşehir Çam and Sakura City Hospital, Clinic of Obstetrics and Gynecology, İstanbul, Turkey

ABSTRACT

Purpose: Our aim was to evaluate the clinical implementation of the World Health Organization (WHO) recommended an optimal cesarean sections (CS) rate of 10-15%. However, this recommendation has been increasingly challeng WHO C-Model in combination with the Ten-Group Classification System (TGCS).

Methods: The data of women who gave birth between December 2019 and February 2020 was retrospectively analyzed. The over- or under-use of CS was assessed by comparing the observed CS rate and the mean estimated CS rate obtained by C-Model for each TGCS group and for the study cohort. The standardized CS ratio was calculated by the ratios between the observed CS rate and the mean estimated CS rate and between the observed CS rate and the estimated CS rate using the determined cut-off.

Results: One thousand two hundred thirty-two women were included in the study. The observed CS rate was 37.42% (n=461). The area under curve for the C- Model to predict CS was 0.952 [95% confidence interval (CI)=0.940-0.965]. The diagnostic odds ratio of the C-Model (determined based on a cut-off point of 19.6%) was 75.9 (95% CI=52.1 to 110.5, Z=22.56, p<0.0001). The standardized CS ratios between the observed CS rate (37.42%) and the estimated CS rate using the determined cut-off value (38.9%) and the mean estimated CS rate (30.5%) were 0.96 and 1.23, respectively.

Conclusion: The C-Model is a promising tool for initially assessing the balance between observed and estimated CS rates pragmatically. Combining the use of the C-Model with detailed TGCS interpretation can be helpful in dedicated clinical settings to achieve the desired CS rates that might eventually improve neonatal and maternal morbidity and mortality.

Keywords: Cesarean section rate, C-Model, Robson classification, ten-group

INTRODUCTION

Cesarean section (CS) is a life-saving operation for the mother and the baby when performed within medical indications.[1] However, its overuse can increase maternal-neonatal morbidity and mortality[2] or at least does not reduce the adverse outcomes beyond certain limits.[3] CS rates have long been a subject of global debate due to the varying outcomes associated with their overuse and underuse. In 1985, the World Health Organization (WHO) recommended an optimal CS rate of 10-15%.[4] However, this recommendation has been increasingly challenged due to differing socioeconomic contexts and evolving obstetric practices.[5,6]

The worldwide CS target rate's generalizability has already been questioned.[7,8] The optimal CS rate in large, global studies can vary based on the time frame and the socioeconomic features of included countries.[9] Institutional optimal CS rate can directly be influenced by the maternity unit's infrastructure, which includes qualifications (such as a reference center for complex cases), adequate resources, obstetric protocols, and availability of services (such as external cephalic version, vaginal birth after CS). In addition, the common obstetric profile of the targeted population including maternal age, parity, obstetric characteristics, and accompanying morbidities can affect the desired population-based CS rate. Patient safety should be the paramount goal when aiming to reduce the

Address for Correspondence: Esra Ayanoğlu, University of Health Sciences Turkey, Sancaktepe Şehit Prof. Dr. İlhan Varank Training and Research Hospital, Clinic of Obstetrics and Gynecology, İstanbul, Turkey
Phone: +90 544 544 62 42 **E-mail:** dresraayanoglu@gmail.com **ORCID ID:** orcid.org/0000-0001-7525-1822
Received: 13.08.2024 **Accepted:** 25.08.2024

overuse of CS at an appropriate level.[7,8] Therefore, it is wise to keep the CS rate at a certain level with an optimal balance of maternal and perinatal outcomes.[10]

The C-Model was developed in a multi-country cross-sectional study by the WHO to provide more tailored CS rate benchmarks.[11] It utilizes the Ten-Group Classification System (TGCS) as its basis to incorporate specific maternal and fetal characteristics, as well as healthcare settings.[11,12] This approach addresses the variability in global CS rates and focuses on optimizing outcomes based on institutional and demographic factors. The use of C-Model was previously tested in a limited number of studies and was found as a valid tool in obtaining optimal CS rates for their settings.[13]

The aim of the present study was to evaluate the C-Model predictivity and clinical implementation of C-Model in combining with TGCS in a tertiary hospital.

METHODS

This retrospective observational study was conducted in a tertiary training and research hospital. The data of women who were admitted for delivery to the inpatient clinic of the maternity ward and who gave birth during three months-period between December 2019 and February 2020 were analyzed. Those who gave birth at less than 22 weeks of gestational age and those whose newborns weighed less than 500 grams at birth were excluded. Data on maternal and obstetric characteristics and outcomes were extracted from electronic medical files. The University of Health Sciences Turkey, Sancaktepe Şehit Prof. Dr. İlhan Varank Training and Research Hospital, Scientific Research Ethics Committee has approved the study (approval number: 2020/57, date: 16.12.2020). This study was conducted in accordance with the principles of the Declaration of Helsinki.

The perinatal events and outcomes were analyzed from the extracted data and the CS rates were analyzed using TGCS. The TGCS group details were reproduced with the utilization of the Robson Classification Implementation Manual[14] and therefore, the previously recorded TGCS data was not used for this study to increase the data quality and lower the missing information. The probability of delivery by CS was calculated for each woman using the WHO's C-Model online calculator (https://cmodel.fmrp.usp.br/). The over-or under-use of CS rate was assessed by comparing the observed CS rate and the mean estimated CS rate obtained by C-Model for each TGCS group.

The C-Model calculates the CS probability using some specific maternal conditions including obstetric characteristics, demographics and severity, and maternal complications domains. The relevant data for obstetric characteristics include parity, previous CS, multiple pregnancy, provider-initiated childbirth (spontaneous labor, induced or CS before labor), fetal head presentation and week of gestation. The data for demographics include maternal age, while data for severity encompass conditions requiring intensive care unit (ICU) admission or those involving significant organ dysfunction. The data for maternal complications include placenta previa, placental abruption, chronic hypertension, preeclampsia, renal disease, and human immunodeficiency virus.

The sample size was calculated using OpenEpi version 3 (www.openepi.com/SampleSize/SSPropor.htm), on the basis of the following formula: n=(DEFFxNp [1-p])/(d2/Z2 1-α/2x[N-1]+px[1-p]). The total number of deliveries (N) per year at the study hospital was 4,500, and the cesarean delivery (CD) rate (estimated proportion, p) was 38%. With a level of precision (d) of 5%, a Z-score (Z) of 1.96 (value from the standard normal distribution corresponding to the desired confidence level of 95%), and a design effect (DEFF) of 1 (assuming simple random sampling), the sample size needed was 336.

Statistical Analysis

SPSS for Windows version 22.0 (SPSS, Chicago, IL, USA) was used for statistical analysis. The proportion of variables examined by descriptive statistical tests and was specified as numbers and percentages. Quantitative data were expressed as mean ± standard deviation and median [interquartile range (IQR)] according to distribution characteristics. The observed CS rate, group size, the contribution of the group, and the estimated CS rate based on mean values of C-Model results were calculated for each TGCS group. In addition, the Receiver Operator

Characteristic (ROC) curve analysis was used to obtain the optimal cut-off value of C-Model predicting the probability of CS regarding the entire study population. Overall estimation of CS rate was also calculated using the determined cut-off value. The standardized CS ratio was calculated twice for good measure: once as the ratio between the observed CS rate and the mean estimated CS rate, and again as the ratio between the observed CS rate and the estimated CS rate using the specified cut-off.

RESULTS

One thousand two hundred thirty-two women gave birth and data of them were analyzed in the present study. Maternal and obstetric characteristics are presented in Table 1. The observed rate of cesarean birth was 37.42% (461/1232).

The 10-group Robson classification details and the estimated CS rates were presented in Table 2. The overall mean estimated CS rate of the study cohort was 30.5±1.06% (ranging between 0.8% and 99.9%).

The ROC curve analysis of the comparison between the estimated and observed CS rates was conducted (Figure 1). The area under the curve (AUC) of the C-Model to predict CS was 0.952 [95% confidence interval (CI)=0.940-0.965]. The optimal cut-off value of C-Model to predict the probability of CS was found as 19.6% with a sensitivity, specificity, positive and negative predictive value of 89%, 91%, 87% and 94%, respectively (Youden index: 0.79). The diagnostic odds ratio of the C-Model (determined based on cut-off point of 19.6%) was 75.9 (95% CI: 52.1 to 110.5, Z: 22.56, p<0.0001).

The overall estimated CS rate using the determined cut-off value (19.6%) of CS probability was 38.9%. The standardized CS ratios between the observed CS rate (37.42%) and the estimated CS rate using the determined cut-off value (38.9%) and mean estimated CS rate (30.5%) were 0.96 and 1.23, respectively.

Table 1. Demographics and pregnancy related clinical characteristics

Variables		Values
Age, mean (SD), y		27.45 (5.74)
BMI, mean (SD), kg/m^2		26.36 (4.41)
Parity, median (IQR)		1 (2)
Gestational age, mean (SD), week		38.67 (1.98)
		n (%)
Singleton pregnancy		1219 (98.9%)
Twin pregnancy		13 (1.1%)
Previous CS		312 (25.3%)
Fetal presentation (singleton)		
	Cephalic	1165 (94.6%)
	Breech	50 (4.1%)
	Transvers or obliqe lie	4 (1.1%)
Fetal status		
	Alive	1236 (99.28%)
	Stillbirth	9 (0.72%)
Complications of pregnancy		
	Oligohydramnios[a]	55 (4.5%)
	Pre-eclampsia	29 (2.4%)
	Gestational diabetes mellitus	29 (2.4%)
	Gestational hypertension	21 (1.7%)
	Polyhydramnios	19 (1.5%)
	Fetal growth restriction	16 (1.2%)
	Prelabour rupture of membrane	14 (1.1%)
	Chronic hypertension	7 (0.6%)
	Placental abruption	7 (0.6%)
	Cholestasis	6 (0.5%)
	Postterm	5 (0.4%)
	Placenta previa	5 (0.4%)
	Fetal macrosomia	5 (0.4%)
	Cardiac/renal dysfunction	4 (0.3%)
	Third-trimester bleeding	3 (0.3%)
	Eclampsia	2 (0.2%)
	Severe hyperemesis	1 (0.1%)
Maternal morbidities		
	Hypothyroidism	22 (1.8%)
	Hyperthyroidism	4 (0.3%)
	Other[b]	8 (0.6%)

[a]The common pregnancy complication, 26 of them were in group 2 (25.4% of group 2) and 14 of them were in group 4 (15.2% of group 4), [b]HIV, second degree burn on the abdomen, thalassemia minor, type 2 diabetes, hypophysis tumor, asthma, uterine prolapse, glaucoma for one of each 1(0.1%), SD: Standard deviation, IQR: Interquartile range, BMI: Body mass index, CS: Cesarean section, HIV: Human immunodeficiency virus

Analysis of the observed CS rates and mean estimated CS rates with regard to 10-group classification showed an overuse of CS in all Robson groups except group 4 (Table 2). The major contributor to CS rate was group 5 with 21.5% and this was followed by groups 2 and 10 (3.7% and 3.4%, respectively).

Birth characteristics and obstetric outcomes of the present cohort are presented in Table 3. The congenital fetal malformation rate was found as 4.1% (n=51). Those were as follows; testicular hydrocele (0.6%), hypospadias (0.5%), patent foramen ovale (0.5%), atrial septal defect (0.6%), umbilical hernia (0.2%), pes equinovarus (0.2%), Down Syndrome (0.2%), ventricular septal defect (0.2%), patent ductus arteriosus (0.2%) and others 12 (1%; including spina bifida aperta, cleft palate, tricuspid atresia, undescended testis, inguinal hernia, aortopulmonary collateral artery, Fallot tetralogy, meningomyelocele, amelia, trigonocephaly, tethered cord, polycystic kidney).

The CS indications of the most clinically relevant Robson groups are given in Table 4 (Groups 1-4, 7 and 10). The CS indications of groups 5, 6 and 8 were previous uterine scar, breech presentation and multiple pregnancy, respectively.

Seventy-seven (14.4%) women underwent labor induction. The indications for labor induction were oligohydramnios (25.7%), premature rupture of membrane (17.9%), post-term pregnancy (16.2%), polyhydramnios (7.3%), non-reassuring fetal heart rate trace (6.7%), gestational hypertension (5.6%), preeclampsia (5.6%), intrauterine fetal death (4%), cholestasis (4%), gestational diabetes (2.8%), fetal growth restriction (1.1%), uterovaginal bleeding in third trimester (0.6%) and reduced fetal movement (0.6%).

There was no maternal death in this cohort. Three women were admitted to the ICU and 12 women received blood transfusions (due to prepartum/postpartum hemorrhage or maternal anemia). There was one neonatal mortality and admission to the neonatal ICU rate was 10.8% (n=134).

The total rate of complications directly related to either vaginal or CD was 2.1% (n=26). These included uterine atony (0.5%, n=6), grade 3 or higher obstetrical anal sphincter tear (0.3%, n=4), wound infection (0.2%, n=3), vaginal hematoma (0.2%, n=2), and retained placenta (0.2%, n=2). Other complications specific to delivery were manual removal of the placenta, vulvar hematoma, subcutaneous hematoma, uterine rupture, difficulty in extraction of the fetal head in CS (T-incision), intraabdominal bleeding, re-laparotomy (0.7%, one case each). Additionally, there was one case of posterior reversible encephalopathy syndrome, which, while associated with preeclampsia rather than directly with delivery, was observed during the postpartum period.

DISCUSSION

In this study, the WHO's C-Model was used to determine a CS rate in a tertiary health care setting. The optimization of the estimated reference CS rate was ensured with further interpretation of the observed TGCS reports. The estimation ability of the C-Model in predicting the expected CS rate was high at the institutional level with an AUC rate of 0.952 (95% CI 0.940-0.965).

Table 2. The ten group classification system for the study population with estimated CS rate of each group

Group	Description	Number of CS/ group number (461/1232)	Group size 1 (%)	Group CS rate 2 (%)	Contribution of each group 3 (37.4%)	Estimated CS rate (mean percentage of C-Model for each group) (%)
1	Nulliparous women with a singleton cephalic pregnancy at ≥37 wk in spontaneous labor	15/170	13.8	8.8	1.2	3.30
2	Nulliparous women with a singleton cephalic pregnancy at ≥37 wk who either had labor induced or were delivered by CS before labor (provider initiated childbirth)	45/99	8	45.4	3.7	35.45
2[a]	Labor induced	23/77	6.2	29.9	1.9	33.61
2[b]	Pre-labor CS	22/22	1.8	100	1.8	41.87
3	Multiparous women without a previous uterine scar, with a singleton cephalic pregnancy at ≥37 wk in spontaneous labor	14/449	36.4	3.1	1.1	1.69
4	Multiparous women without a previous uterine scar, with a singleton cephalic pregnancy at ≥37 wk, who either had labor induced or were delivered by CS before labor (provider-initiated childbirth)	18/92	7.5	19.6	1.5	20.59
4[a]	Labor induced	9/83	6.7	10.8	0.73	20.57
4[b]	Pre-labour CS	9/9	0.8	100	0.73	20.78
5	All multiparous women with at least one previous CS, with a single cephalic pregnancy, ≥37 weeks gestation	265/267	21.7	99.3	21.5	84.09
5.1	With one previous CS	165/167	13.6	98.8	13.4	76.39
5.2	With two or more previous CSs	100/100	8.1	100	8.1	96.94
6	All nulliparous women with a singleton breech pregnancy	17/18	1.5	94.4	1.4	73.73
7	All multiparous women with a singleton breech pregnancy, including women with previous uterine scars	31/32	2.6	96.9	2.5	73.24
8	All women with multiple pregnancies, including women with previous uterine scars	10/13	1.1	76.9	0.8	47.27
9	All women with a singleton pregnancy with a transverse or oblique lie, including women with previous uterine scars	4/4	0.3	100	0.3	93.25
10	All women with a singleton cephalic pregnancy at ≤36 wk, including women with previous scars	42/88	7.1	47.7	3.4	42.29

1 group size (%) = n of women in the group/total n women delivered in the hospital x100, 2 group CS rate (%) = n of CS in the group/total n of women in the group x100, 3 contribution of each group (%) = n of CS in the group/total n of women delivered in the hospital x100. CS: Cesarean section, wk: Week

C-Model was produced in an international multi-country cohort with low CS rates (<30%) and with low intrapartum-related perinatal deaths (<6.8 deaths per 1000 births) as a tool to generate reference CS rates.[11] The absolute deviation of the estimated rate from observed CS rate was reported as minimal for countries with low CS rate and good perinatal outcomes, and the ratio between observed and predicted CS (standardized CS ratio) was found near 1 in those countries (e.g., Finland, Sri Lanka, France).[11] In the current study, the observed CS rate, and estimated CS rate based on the calculated cut-off value were 37.4% and 38.9%, respectively. The standardized CS ratio was 0.96 with an absolute deviation of 1.5%. Our results at a referral tertiary institutional level were found similar to Argentina's data in the original study (ratio of 0.95 with a deviation of 1.8% in the original study).[11] The relatively good balance between the observed and estimated CS rates of the current study can be explained by the interpretation of the Robson Classification.

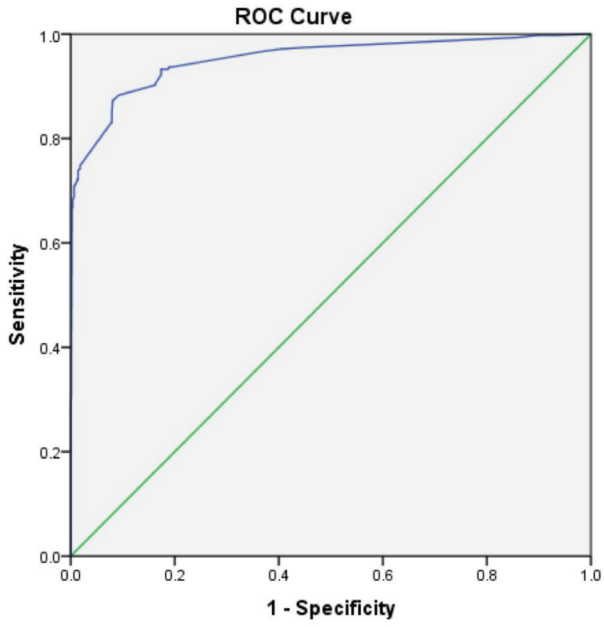

Figure 1. Comparison of the observed and the estimated CS rates of C-Model by ROC

ROC: Receiver operating curve, CS: Cesarean section

Our center fits the profile of a tertiary maternity hospital that manages high-risk pregnant women without a common practice of trial of labor after cesarean (TOLAC) and external cephalic version. The size of Group 5 was 21.5% and its relative contribution to the overall CS rate was 21.5%. The estimated CS rate by the C-Model and the observed CS rate in Group 5 were 84.1% and 99.3%, respectively, likely due to the absence of TOLAC practices at the study center. Previous CD was also the major CS indication (28.4%) in Group 10. These high CS rates were probably related to high overall cesarean rates mainly in Group 1 and 2 in past years.[14,15] The size of Group 10 was 7.1% which accounts for a higher rate of preterm births.[14,15] The ratio of the size of Group 1 versus Group 2 should usually be 2:1 or higher.[14,15] The ratio of 1.7 in the current study confirms the care of a high-risk population in nulliparous women with a high induction/prolabor CS rate of 14.4%. Similarly, almost one out of every five women had a high-risk pregnancy in this cohort (18.6%).

Both the observed and estimated CS rates of 37.5% and 38.9% were not consistent with the WHO's target of a 10-15% CS rate.[4] While the WHO's threshold aims to minimize unnecessary surgical interventions and associated risks, several factors make these rates challenging to achieve in certain populations. Firstly, changes in maternal demographics, such as increased maternal age and higher rates of obesity, have contributed to a rise in CS rates worldwide. Older mothers and those with higher body mass index are more likely to experience complications that necessitate a CD, such as hypertensive disorders, gestational diabetes, and obstructed labor.[16] Additionally, the growing prevalence of elective CS for non-medical reasons, driven by patient preference or cultural practices, has also contributed to higher rates.[17] Secondly, the management of high-risk pregnancies, particularly in tertiary care settings that handle a disproportionate number of complex cases, often justifies higher CS rates. For example, in settings with a high incidence of preterm births, multiple

Table 3. Birth characteristics and obstetric outcomes

		Values
Fetal birth weight, mean (SD), gr		3268.46 (517.2)
Labor initiation		n (%)
	Spontaneous	704 (57.1%)
	Induced labor	177 (14.4%)
	Cesarean before labor	351 (28.5%)
Mode of birth		
	Spontaneous vaginal	771 (62.6%)
	Elective cesarean	237 (19.2%)
	Emergency cesarean	159 (12.9%)
	Intrapartum cesarean	65 (5.3%)
5th minute Apgar		
	<7	17 (1.4%)
	≥7	1215 (98.6%)
Neonatal ICU admission		
	Yes	134 (10.8%)
	No	1111 (89.2%)
Blood transfusion		
	Yes	12 (1%)
	No	1220 (99%)
Maternal ICU admission		
	Yes	3 (0.2%)
	No	1229 (99.8%)
Fetal complications		
	Hyperbilirubinemia[b]	38 (2.8%)
	Transient tachypnea of newborn[b]	30 (2.4%)
	Respiratory distress[b]	15 (1.2%)
	Sepsis[b]	13 (1.1%)
	Prematurity[b]	12 (1%)
	Asphyxia[b]	6 (0.5%)
	Meconium aspiration[b]	4 (0.3%)
	Clavicle fracture	3 (0.2%)
	Othera	6 (0.5%)
Congenital malformation		
	Yes	54 (4.3%)
	No	1191 (95.7%)

[a]Acute myocarditis[b], cephalohematoma, fetal scalp incision, perinatal death, pulmonary hypertension[b], neonatal pneumonia[b] 1 each. [b]The fetal complications which were causes of ICU admission. ICU: Intensive care unit, SD: Standard deviation

Table 4. Robson groups 1-4, 7 and 10 CS indications

CS indications	1	2[a]	2[b]	3	4[a]	4[b]	7	10
Fetal distress	6 (3.5%)	17 (22.1%)	4 (18.2%)	6 (1.3%)	3 (4.8%)	1 (11.1%)		7 (8%)
Suspected fetal macrosomia	3 (1.8%)		10 (45.5%)	2 (0.4%)		5 (55.6%)		
Maternal medical reason[a]	1 (0.6%)		2 (9.1%)			2 (22.2%)		1 (1.1%)
Labor arrest	3 (1.8%)	2 (2.6%)		2 (0.4%)	4 (6.3%)			1 (1.1%)
CPD	2 (1.2%)							
Failed induction		3 (3.9%)						
Placental abruption			1 (4.5%)	1 (0.2%)	1 (1.6%)		1 (3.1%)	4 (4.5%)
Pre-eclampsia/eclampsia			1 (4.5%)			1 (11.1%)		1 (1.1%)
Maternal request			2 (9.1%)		1 (1.6%)			
Fetal growth restriction			1 (4.5%)					1 (1.1%)
Placenta previa			1 (4.5%)					1 (1.1%)
Previous CD							13 (40.6%)	25 (28.4%)
Cord presentation								1 (1.1%)
Occiput posterior				1 (0.2%)				
Cord prolapsus				1 (0.2%)				
Arm prolapsus				1 (0.2%)				
Breech presentation							17 (53.1%)	
Twin pregnancy								
Total	15 (8.8%)	22 (28.6%)	22 (100%)	14 (3.1%)	9 (14.3%)	9 (100%)	31 (96.9%)	42 (47.5%)

[a]Lumbar hernia, previous brain operation, otosclerosis, orthopedic problem in the mother. CPD: Cephalopelvic disproportion, CS: Cesarean section, CD: Cesarean delivery

gestations, or previous CS, the clinical imperative to minimize risks to both mother and baby may necessitate more frequent use of CD. This is consistent with findings from studies such as Silver et al.,[18] which highlight increased maternal morbidity associated with multiple repeat CS. Given these factors, setting targeted optimal CS rates for specific populations or maternal facilities and evaluating the rates using TGCS may be more realistic than adhering to a universal threshold. The use of tools like the C-Model, which provides customized reference CS rates based on specific population characteristics, is one way to establish more appropriate benchmarks that reflect local realities.

The study observed an overuse of CS across most Robson groups, except for group 4. This overuse is particularly pronounced in high-risk groups such as Groups 5 and 10, which consist of multiparous women with previous CS and all women with a singleton pregnancy with a transverse or oblique lie, respectively. Several factors contribute to the overuse of CS in high-risk groups, such as Groups 5 and 10. First, clinical decision-making is influenced by patient safety concerns, particularly with women who have had previous CS or have complex obstetric histories. The risk of uterine rupture in women attempting a vaginal birth after delivery (VBAC) often leads to opting for a repeat CS, despite guidelines suggesting VBAC as a safe option in appropriate circumstances.[19,20] Second, institutional policies favoring CD in specific clinical scenarios, especially where resources to manage potential complications are limited, can drive these decisions. Practitioner experience and comfort levels also play a role.[18]

The implications for clinical practice are substantial. Overuse of CS increases healthcare costs and resource utilization and exposes women to surgical risks, such as infections, hemorrhage, and prolonged recovery. It is crucial to promote evidence-based practices and shared decision-making between clinicians and patients. Implementing clinical audits and feedback mechanisms can help monitor CS rates and ensure that CS are medically justified.[21]

The C-Model, when used in conjunction with the TGCS, provides a valuable framework for institutional audits and policymaking. By identifying specific Robson groups where CS rates exceed expected benchmarks, institutions can target areas for improvement, such as promoting vaginal births when safe and appropriate, enhancing training for healthcare providers, and revising clinical guidelines to reduce unnecessary CSs. Additionally, regular audits using these tools can help track the impact of changes over time, ensuring continuous quality improvement and optimal maternal and neonatal outcomes.

This study's strengths include the large cohort size and the novel application of the C-Model in combination with the TGCS for assessing CS rates. Our findings contribute to the current literature by offering a detailed analysis of CS rates in a tertiary setting, identifying specific areas of overuse, and providing evidence-based recommendations for clinical practice and policy. This approach helps to optimize resource allocation and improve maternal and neonatal outcomes by setting realistic and tailored cesarean rate targets.

Study Limitations

The outcomes of TGCS are affected differently by the data quality, characteristics of the obstetric population, and practice variation.[11] Although the data were regarded as reliable, the present study was limited by the fact that it was collected from medical records retrospectively.

The data collection for this study was limited to a three-month period between December 2019 and February 2020. While this time frame was chosen to ensure data quality and completeness, we acknowledge that it could introduce seasonal or temporal biases, potentially affecting the generalizability of the findings. Different periods of the year may have varying CS rates due to seasonal trends in obstetric complications, staff availability, or policy changes. To mitigate this limitation, we ensured that the sample size was sufficiently large to provide robust statistical power, as detailed in our sample size calculation. Future studies could extend the data collection period to cover an entire year to explore seasonal variations more comprehensively.

CONCLUSION

As the original authors have emphasized, the C-Model should not be used prospectively in clinical decision-making within labor wards. However, it can serve as a valuable tool for initially assessing the balance between observed and estimated CS rates, guiding further interpretation of TGCS results. The C-Model offers a pragmatic approach to evaluate whether the standardized CS ratio is close to 1, helping to develop health policies based on realistic CS rates tailored to the obstetric profile of the population.

To maximize its utility, the C-Model should be combined with TGCS for comprehensive clinical audits, which are crucial for monitoring data quality and changes in obstetric practices over time. This combination enables healthcare institutions to identify specific Robson groups where CS rates exceed expected benchmarks, allowing for targeted interventions to optimize delivery outcomes.

Integrating the C-Model and TGCS into routine practice involves training healthcare providers on their interpretation, focusing on promoting vaginal births when appropriate. Establishing clinical guidelines that incorporate these tools can standardize decision-making and ensure medically justified CS. Regular audits can monitor CS rates, identify trends, and continuously refine practices.

By adopting these strategies, healthcare institutions can effectively use the combined insights of the C-Model and TGCS to optimize CS rates, improve resource allocation, and enhance maternal and neonatal outcomes. Future research should continue to refine these tools and explore their application across diverse settings to further improve global health outcomes.

Footnote

Ethics Committee Approval: University of Health Sciences Turkey, Sancaktepe Şehit Prof. Dr. İlhan Varank Training and Research Hospital Scientific Research Ethics Committee has approved the study (approval number: 2020/57, date: 16.12.2020).

Informed Consent: Retrospective study.

Authorship Contributions

Surgical and Medical Practices: E.A., A.B.T., K.S., N.T., Concept: A.B.T., Design: A.B.T., K.S., Data Collection or Processing: E.A., K.S., Analysis or Interpretation: M.Y., N.T., Literature Search: E.A., K.S., Writing: A.B.T., M.Y., N.T.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study received no financial support.

REFERENCES

1. World Health Organization (WHO). WHO Recommendations: intrapartum care for a positive childbirth experience. 2018.
2. Villar J, Valladares E, Wojdyla D, et al. Caesarean delivery rates and pregnancy outcomes: the 2005 WHO global survey on maternal and perinatal health in Latin America. Lancet. 2006 Jun 3;367(9525):1819-1829.
3. O'Dwyer V, Hogan JL, Farah N, Kennelly MM, Fitzpatrick C, Turner MJ. Maternal mortality and the rising cesarean rate. Int J Gynaecol Obstet. 2012;116(2):162-4.
4. World Health Organization (WHO). Appropriate technology for birth. Lancet. 1985;2:436-437.
5. Boerma T, Ronsmans C, Melesse DY, et al. Global epidemiology of use of and disparities in caesarean sections. Lancet. 2018;392(10155):1341-1348.
6. Gibbons L, Belizán JM, Lauer JA, Betrán AP, Merialdi M, Althabe F. The global numbers and costs of additionally needed and unnecessary caesarean sections performed per year: overuse as a barrier to universal coverage. World Health Report. 2010;30(1):1-31.
7. D'Alton ME, Hehir MP. Cesarean delivery rates: revisiting a 3-decades-old dogma. JAMA. 2015;314(21):2238-2240.
8. Robson SJ, De Costa CM. Thirty years of the World Health Organization's target caesarean section rate: time to move on. Med J Aust. 2017;206(4):181-185.
9. Molina G, Weiser TG, Lipsitz SR, et al. Relationship between cesarean delivery rate and maternal and neonatal mortality. JAMA. 2015;314(21):2263-2270.
10. Betrán AP, Merialdi M, Lauer JA, et al. Rates of caesarean section: analysis of global, regional and national estimates. Paediatr Perinat Epidemiol. 2007;21(2):98-113.
11. Souza JP, Betran AP, Dumont A, et al. A global reference for caesarean section rates (C-Model): a multicountry cross-sectional study. BJOG. 2016;123(3):427-436.
12. Robson M. A global reference for CS at health facilities? Yes, but there is work to do. BJOG. 2016;123(3):437.
13. Abdel-Aleem H, Darwish A, Abdelaleem AA, Mansur M. Usefulness of the WHO C-model to optimize the cesarean delivery rate in a tertiary hospital setting. Int J Gynecol Obstet. 2017;137(1):40-44.
14. World Health Organization (WHO). Robson classification: Implementation manual. Geneva: World Health Organization; 2019.
15. Robson M, Murphy M, Byrne F. Quality assurance: the 10-group classification system (Robson classification), induction of labor, and cesarean delivery. Int J Gynecol Obstet. 2015;131
16. Lean SC, Derricott H, Jones RL, Heazell AEP. Advanced maternal age and adverse pregnancy outcomes: A systematic review and meta-analysis. PLoS One. 2017;12(10):e0186287.

17. Mazzoni A, Althabe F, Gutierrez L, et al. Women's preferences and mode of delivery in public and private hospitals: a prospective cohort study. BMC Pregnancy Childbirth. 2016;16:34.
18. Silver RM, Landon MB, Rouse DJ, et al. Maternal morbidity associated with multiple repeat cesarean deliveries. Obstet Gynecol. 2006;107(6):1226-1232.
19. Landon MB, Hauth JC, Leveno KJ, et al. Maternal and perinatal outcomes associated with a trial of labor after prior cesarean delivery. N Engl J Med. 2004;351(25):2581-2589.
20. Practice bulletin no. 184: Vaginal birth after cesarean delivery. Obstet Gynecol. 2017;130(5):e217-e233.
21. World Health Organization (WHO) Human Reproduction Programme, 10 April 2015. WHO Statement on caesarean section rates. Reprod Health Matters. 2015;23(45):149-150.

Case Report

DOI: 10.4274/anajog.galenos.2024.68077

Anat J Obstet Gynecol Res 2024;2:79-86

Nazik Neovagina Technique: A Case Series

Hakan Nazik

Adana Private Ortadoğu Hospital, Clinic of Obstetrics and Gynaecology, Adana, Turkey

ABSTRACT

Mayer-Rokitansky-Küster-Hauser (MRKH) syndrome is the congenital absence of the vagina and uterus. The aim of this case series is to evaluate the anatomical and functional outcomes of a new vaginoplasty technique called the Nazik Neovagina Technique. Nine women with MRKH syndrome between the years of 2018-2024 were included in the study. The women underwent laparoscopic surgery using the Nazik Neovaginal Technique. The mean vaginal length was 9.78±1.39 cm at the first month after surgery and 8.56±1.13 cm at the sixth month. None of the patients developed complications. Vaginal epithelialization was complete in all patients. Six of the nine patients were sexually active at six months after surgery. The other three patients were not active because they did not yet have a partner. Two women who were sexually active developed vaginal infections. The Nazik Neovagina Technique is considered an easy-to-learn and easy-to-use surgical treatment option with less vaginal stricture.

Keywords: Mayer-Rokitansky-Küster Hauser syndrome, laparoscopic vaginoplasty, neovagina

INTRODUCTION

Vaginal agenesis, also known as Mayer-Rokitansky-Küster-Hauser (MRKH) syndrome, is indeed a complex condition that requires a multidisciplinary approach to diagnosis and treatment. It affects the development of the vagina and uterus, creating unique challenges for individuals, particularly in terms of sexual health and reproductive options.[1] The incidence of this condition is quite low, varying from 1/4000 to 10000.[2] These patients have secondary sexual characteristics like other females during puberty.

Patients usually present with primary amenorrhea and may not be aware of their condition until adolescence when they realize that they are not menstruating.[3] Diagnostic imaging is essential to evaluate potential urinary anomalies, particularly because Müllerian agenesis can be associated with other congenital malformations.[4]

There are several treatment options available, vaginal dilators are usually the first choice for non-surgical treatment. They help in gradually stretching the vaginal canal, promoting a functional vaginal length, which is vital for sexual activity. Each surgical method has its pros and cons, and the choice of technique often depends on the individual's specific circumstances, including their anatomy and any associated abnormalities. However, there is no consensus among gynecologists, pediatric surgeons, pediatric urologists, and plastic surgeons on the ideal method for creating a functional vagina.[5] Functional sexual success has been defined as vaginal acceptance of the largest dilator without discomfort or a vaginal length of 6 or 7 cm.[6,7]

The Nazik Neovagina Technique is an innovative approach that builds on previous models to provide better structural support and reduce the risk of vaginal stenosis by mimicking the cervix and ligaments. In the commonly used McIndoe technique, uses a skin graft to create the vaginal canal but involves significant morbidity due to extensive skin removal.[8] The Intestinal Graft Method can be performed laparoscopically, but concerns include the potential for foul-smelling discharge and complications associated with bowel surgery.[9] The Vechietti procedure allows vaginal stretching using a neovaginal set but requires continuous tension and can be cumbersome.[10] The original neovaginal technique described by Davydoy used parietal peritoneum for the vaginal wall. However, it has the disadvantage of placing a circular suture over the bladder, sigmoid colon, and ureter to create the vaginal dome. An important addition to this technique in the Uncu modification is the use of Müllerian remnants and parietal peritoneum to create the vaginal dome.[11]

The Nazik neovaginal technique described in this article was developed based on the Dayvdoy neovaginal technique and the Uncu modification. In this technique, the vaginal dome was redesigned using Müllerian remnants to mimic the cervix and ligaments to prevent vaginal stricture, which is the main problem of neovaginal surgical techniques. The Mold has been

Address for Correspondence: Hakan Nazik, Adana Private Ortadoğu Hospital, Clinic of Obstetrics and Gynaecology, Adana, Turkey
Phone: +90 542 686 11 20 **E-mail:** drhakannazik@gmail.com **ORCID ID:** orcid.org/0000-0001-6495-3511
Received: 10.08.2024 **Accepted:** 25.08.2024

modified to be more flexible and softer to increase functional sexual success.

METHODS

In this case series, laparoscopic-assisted neovaginal surgery, which we defined as Nazik Neovagina Technique, was performed in 9 patients between 2018-2024 years. It was planned to determine the duration of surgery, hospital stay, complications to evaluate the surgical technique, and anatomical and functional vaginal length, vaginal width, vaginal epithelialization, and postoperative sexual activity status to evaluate the functional outcomes throughout the study. The inclusion criteria of the patients were to have Müllerian agenesis, vaginal and uterine agenesis, no previous neovagina surgery, both ovaries and Müllerian remnants detected. Patients with rudimentary uterus, transwomen patients, and those who received previous dilatation treatment were excluded from the study.

Preoperative Preparation

Patients were preoperatively evaluated with a complete clinical examination, sonography, and magnetic resonance imaging for genitourinary and pelvic renal abnormalities. Detailed informed consent forms were obtained from the patients.

Surgical Technique

The stages have been numbered for a better understanding of the surgical technique.

The first stage was to prepare the patient on the operating table. All patients were operated in the dorsolithotomy position and under general anesthesia. Both vaginal and laparoscopic approaches were used in combination. After positioning the patient, the laparoscopic approach was performed first. The aim was to facilitate the opening of the vaginal tunnel from below under laparoscopic observation (Figure 1). For this purpose, a 10-mm trocar was inserted through the umbilicus and two 5-mm trocars were inserted into the abdomen from the left side. In patients with MRKH syndrome, the ovaries can be observed in both fossa ovarica or outside the pelvis. Remnants of Müllerian ducts were observed in and around both ovaries in all patients. Pelvic kidneys were seen in two patients. In cases where the pelvic kidney is located at the base, it becomes very difficult to create a vaginal dome. In one of these patients, the kidney had to be suspended from the abdominal wall during surgery (Figure 2).

Second stage, after laparoscopic observation, vaginal approach was performed. Before opening the rectovesical space, a ureteral catheter was placed in all patients to reduce urinary injury. Then, the dome of the vaginal mucosa was opened transversely with a sharp dissection using scissors, monopolar cautery or bipolar instrument with a diameter of 3 cm. After cutting the mucosa, blunt dissection was performed with a finger or dilator to reach the rectovesical space. Once this space is reached, it is found to be easily dissected with blunt dissection.

At this point, sharp dissection should be avoided as much as possible to reduce the possibility of rectal or urinary injury. Therefore, when a depth of approximately 6-7 cm was reached, a 70 Shore a silicone mold was placed. The mold was held in a pressurized position by the assistant and the laparoscopic procedure was repeated (Figure 3).

In the third stage, the silicone mold was pressed with the help of an assistant to expose the surgical area from the inside. To avoid injury to the bladder and rectum, a vertical incision was made using a harmonic scalpel under the guidance of this mold. As shown in Figure 4-1, the Müllerian remnants on the sides of both ovaries were connected transversely with a thin fibrous tissue in the middle. The bladder margin begins

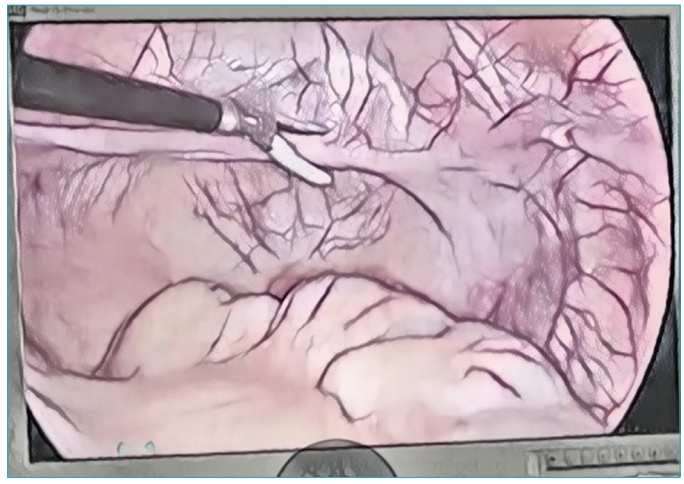

Figure 1. Laparoscopic observation of the pelvis

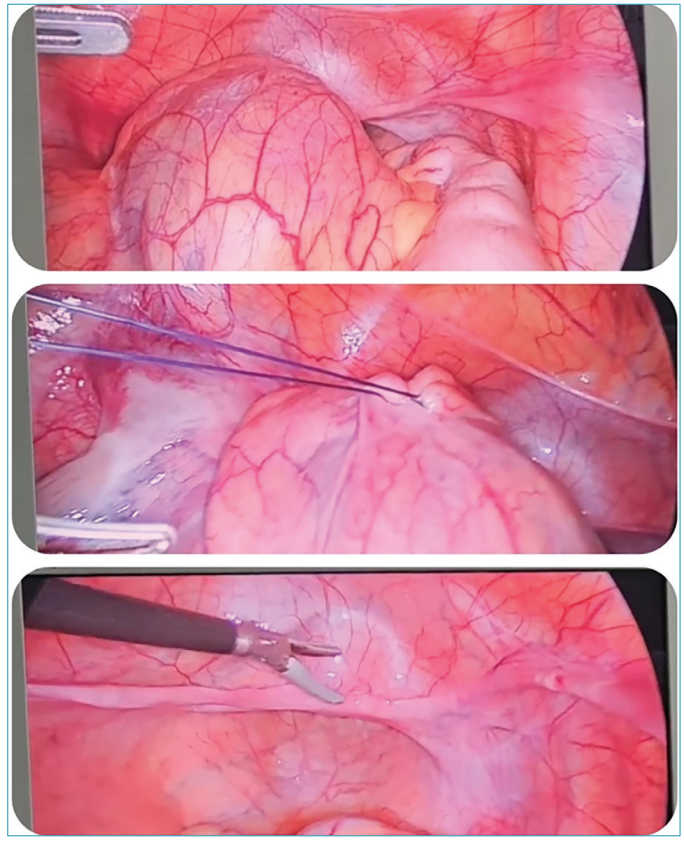

Figure 2. Pelvic kidney suspension

just above this fibrous tissue. This Müllerian fibrous tissue is of great help in determining the bladder boundary. If this fibrous tissue is exceeded by more than 1 cm, the likelihood of bladder injury may increase. The tunnel was completed with controlled surgery under the guidance of the mold. At this point, a tunnel was created in the rectovesical space. To prevent future stenosis of this tunnel, fibrous tissue consisting of Müllerian remnants was excised approximately 1 cm around the mold (Figure 4).

The fourth stage, the goal of this stage is to pull the peritoneum into the tunnel and fix it to the vaginal entrance. This requires dissection and release of the peritoneum. After completion of the tunnel, three sutures are placed from the intra-abdominal end of the tunnel at 2, 6, and 10 o'clock with a 40 mm needle and 0 Vicryl. A peritoneal suture is not placed at the 12 o'clock position because this is the exit point of the urethra. If this is done, the urethra and urinary axis will be altered. These sutures were removed vaginally with a clamp in a U shape without knotting the ends and without cutting the needle. If the peritoneum is knotted, it will cause difficulty in entering the vagina when using a mold after surgery. These sutures should not be sutured immediately to the vaginal entrance. At this stage, they should only be fixed to surgical drapes at the vaginal entrance, again symmetrically to the points inside. After the peritoneum around the tunnel is released intra-abdominally, the sutures should be fixed to the vaginal entrance. In this way, the peritoneum can be easily pulled to the vaginal entrance (Figure 5).

Fifth stage, peritoneal dissection is not performed immediately at this point. First, the Müllerian remnants on the lateral wall were brought closer to the midline to form the vaginal dome. For this purpose, they were detached from the lateral walls with blunt and sharp dissection and joined in the midline with a number 1 permanent suture. In this way, the Müllerian ducts were positioned in the midline similar to the cervix. To avoid peritoneal contact, the Müllerian remnants should be centralized by suturing them from the lateral points in the midline as much as possible. This will create a soft tissue support in the dome approximately the size of the cervix. The dome should not end in a funnel shape. This will increase the possibility of stricture due to peritoneal contact. To prevent this stricture, a vagina should be constructed with a cylindrical structure and a dome similar to the cervix (Figure 6).

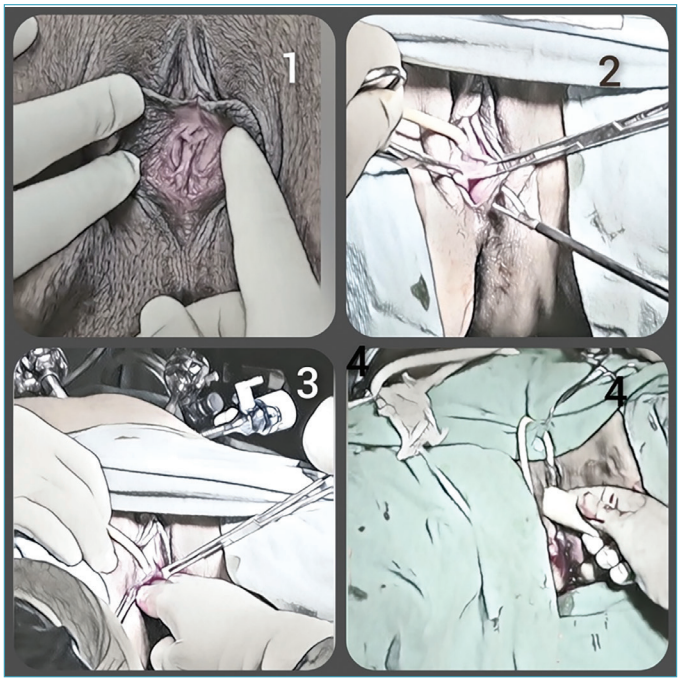

Figure 3. Vaginal tunnel opening

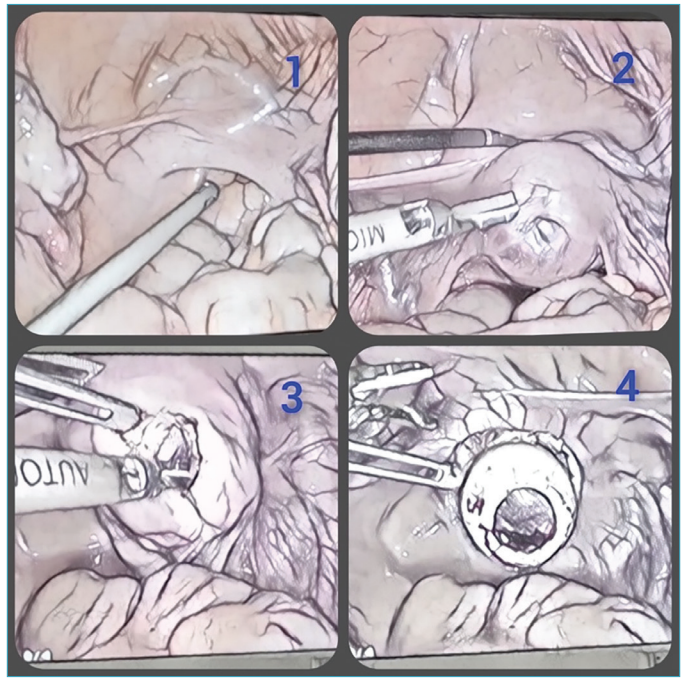

Figure 4. Completing the vaginal tunnel with mold

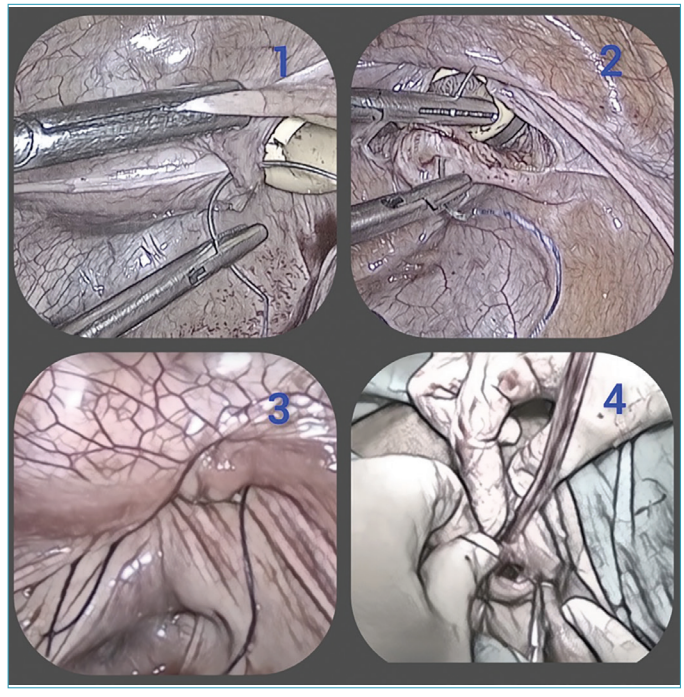

Figure 5. Retraction of the peritoneum into the vaginal tunnel

In the sixth stage, the peritoneal surface required to cover the anterior and posterior vaginal walls is identified. The peritoneum to be used for the anterior and posterior vaginal walls will be less because the Müllerian remnants meet in the midline. Peritoneal dissection was not performed circularly over the rectum. Dissection was performed parallel to the ureters from the medial aspect of both ureters to form the posterior wall of the dome. Inverted V peritoneal dissection should be performed up to the tunnel. Then, both peritoneal leaves were joined in the midline with the visceral part facing the inside of the tunnel. The created peritoneal posterior wall was sutured to the posterior part of the dome (Figure 7).

In the seventh stage, the anterior vaginal wall peritoneum was dissected over the bladder. Excessive dissection should be avoided at this point. The peritoneum was dissected to a size that would cover the anterior wall of the dome and released. After dissection, the peritoneal anterior vaginal wall was sutured to the dome. The anterior vaginal wall was then sutured to the created dome with 2/0 Vicryl. At this point the tunnel was completed (Figure 8).

When connecting the Müllerian remnant to the midline, the round ligament remnant should be dissected as little as possible. In this way, both the infundibulopelvic ligament and the round ligament provide support for the midline dome, similar to the uterus. At the end of the operation, after the anterior and posterior peritoneum of the dome have been released, the sutures taken out vaginally should be fixed at the

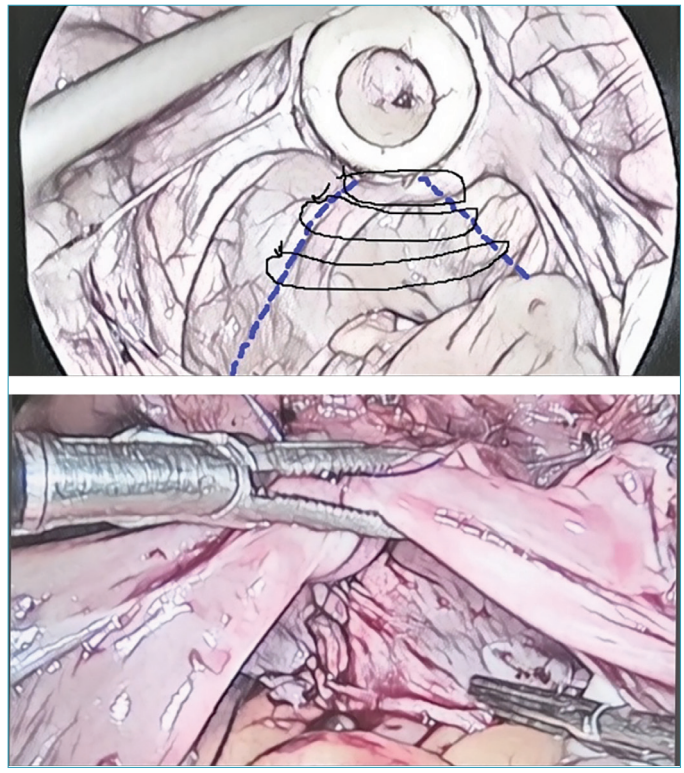

Figure 7. Creation of the posterior vaginal wall

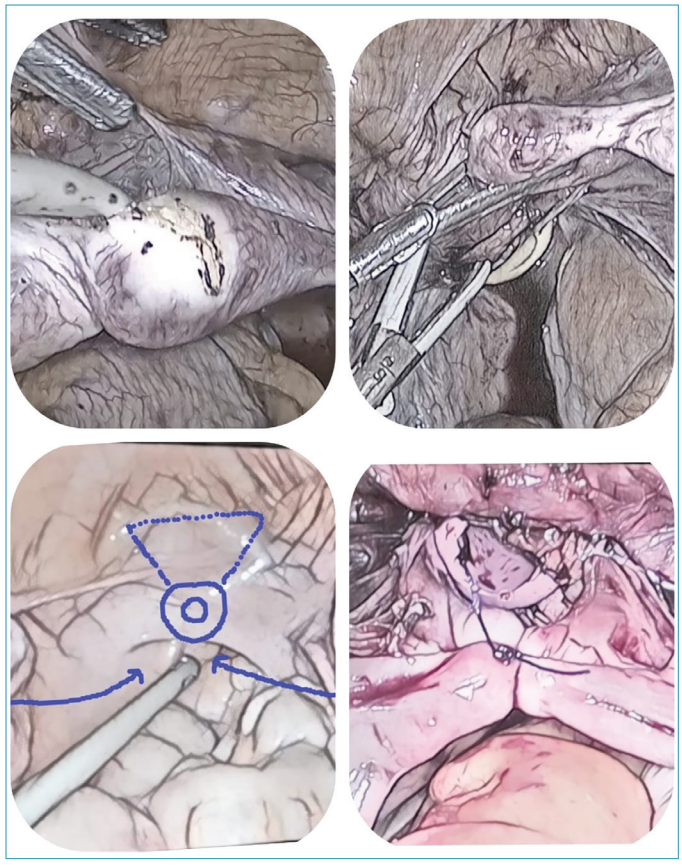

Figure 6. Creation of a cervix-like vaginal vault with the help of Müllerian rests

Figure 8. Formation of the anterior vaginal wall

2, 6, and 10 o'clock positions by pulling the peritoneum well toward the vaginal opening (Figure 9).

Vaginal Mold

The mold placed vaginally during surgery is 3 cm in diameter and 10-12 cm long. In most cases, a length of 12 cm is sufficient. A 10 cm long mold was used in one patient because of a pelvic kidney and in another patient because the patient was very short. A 70 Shore silicone mold was used during surgery, while a softer and more flexible 30 Shore mold was used on the third postoperative day. Harder molds should not be preferred because they make it difficult for the patient to sit and move. There is a 1.5 cm diameter hole in the center. This hole allows blood and fluids to drain from the inside and speeds up the healing process. In addition, this hole is wide enough to allow the patient's index finger to easily insert and remove the mold from the vagina. Stainless steel hooks are located on the back of the mold at 3 and 9 o'clock. These hooks help to secure the mold to the vaginal entrance with no. 1 Vicryl after surgery. The fixed mold was kept for 48 hours in the postoperative period. During this time, a urinary catheter was placed to allow the patient to urinate comfortably (Figure 10).

Postoperative Follow-up

Fourty-eight hours after surgery, patients were transferred to the gynecology table. First, the urinary catheter was removed. Then the fixation sutures at the 3 and 9 o'clock positions were removed and the mold was slowly removed. A cream containing 10% lidocaine was applied to the vagina so that the patient would not feel any pain. After waiting approximately two minutes, a soft hookless mold was placed. The patient was then instructed on how to insert and remove the mold at the correct angle. Depending on the patient's compliance and learning process, the patient was followed in the hospital for another day.

During this time, the patient was advised to remove the mold only when going to the bathroom and then reapply the mold in bed. The patient was advised to wear two tight underwear on top of each other. It was advised to place a thick pad between the mold and the underwear. Adequate pressure is important to prevent vaginal shortening. The patient was discharged with the advice to remove the mold only when going to the toilet for 40 days after surgery (Figure 11). Patients were advised to visit for follow-up on day 7, month 1, and month 6. After day 40, patients were allowed to have sexual intercourse.

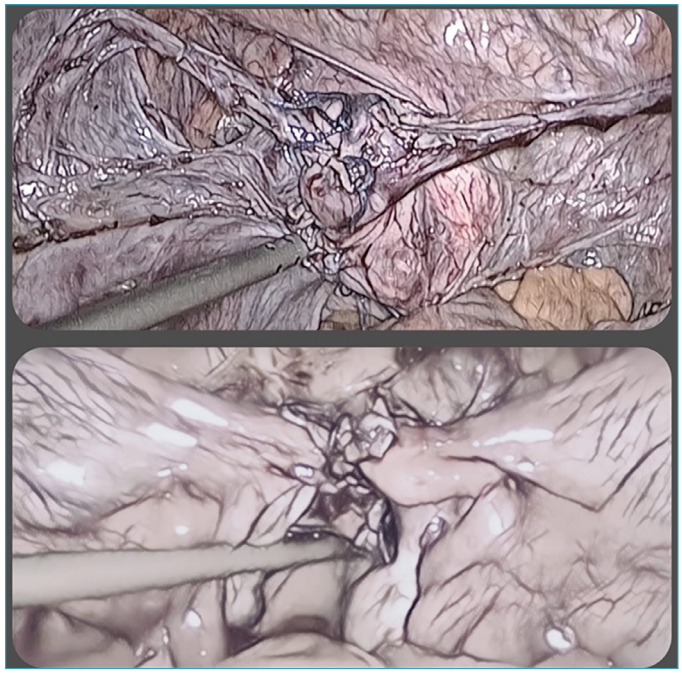

Figure 9. Final dome appearance supported by round and infundibulopelvic ligament

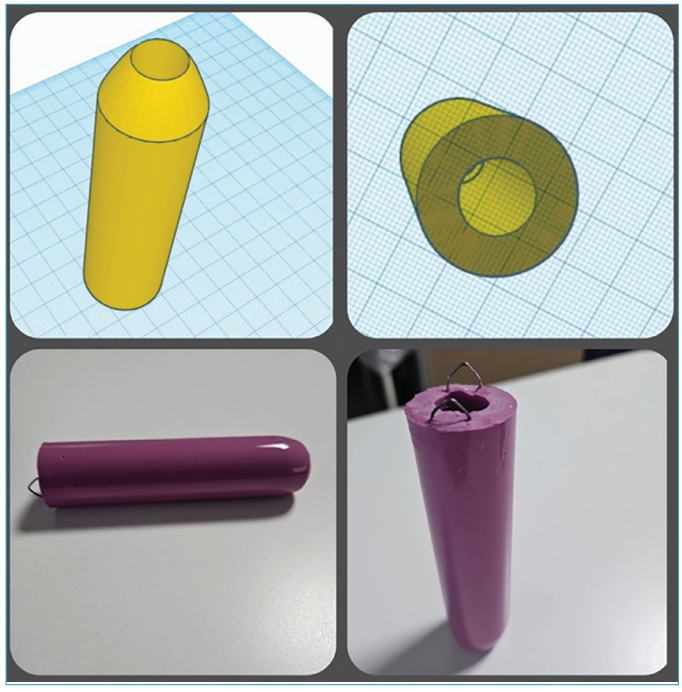

Figure 10. Silicone mold with a hole in the center

Figure 11. Soft silicone mold

Statistical Analysis

The SPSS 20 package program (SPSS Inc, Chicago, Illinois, USA) was used for statistical analysis of the data obtained in the study. Patient demographics and categorical variables such as descriptive characteristics were summarized as numbers (n) and percentages (%). Descriptive statistics related to continuous variables are summarized as mean ± standard deviation.

RESULTS

A total of 9 women with MRKH syndrome were operated with the Nazik Neovagina Technique. The mean age of the women was 23.33±4.09 years and the mean body mass index was 21.78±2.44 kg/m^2. Three of the women were married and six were single. None of the women had any previous treatment or surgery. Two patients had pelvic kidney as a congenital malformation. All patients had normal external genitalia and complete vaginal agenesis. All women had bilateral Müllerian ducts and ovaries.

The mean duration of surgery was 128.89±29.97 minutes. There were no surgical complications in any patient. A urinary catheter was placed in all patients. When the rigid mold was removed at 48 hours postoperatively, the urinary catheter was removed. All patients were discharged on postoperative day 3.

The mean vaginal length at discharge was 11.55±0.88 cm (10-12). In addition, the vaginal diameter of all patients was three centimeters wide as compatible with the mold. At the first postoperative month, the mean vaginal length was 9.78±1.39 cm and at the sixth postoperative month, the mean vaginal length was 8.56±1.13 cm. At the first month evaluation, vaginal epithelialization was complete in all patients. All patients had vaginal discharge that changed color from red to transparent for approximately 6 weeks. At the sixth month after surgery, 6 of 9 patients were sexually active. The other three patients were actively using their molds. Two women who were sexually active developed vaginal infections. These patients were treated for vaginitis.

DISCUSSION

Patients with MRKH syndrome have combined agenesis of the uterus, cervix, and upper vagina. On the other hand, the phenotypes, endocrinologic status, and external genitalia of these patients are normal. The main goal of MRKH syndrome vaginoplasty is to create a new anatomically adequate and functional vagina. The ideal vaginoplasty should have sufficient width, length, axis, and lubrication function. The best surgical technique is still controversial.[12]

Reconstruction of the vagina using dilators of increasing diameter and length was described by Frank in 1938. The time required to reconstruct the vagina varies from four months to several years, depending on patient compliance. Ingram's modification of the Frank procedure involves the use of a bicycle seat mounted on a stool to create pressure for vaginal dilation.[13] Although successful results have been reported with this technique, patient compliance, anxiety, and the fact that patients find the treatment extremely uncomfortable have been reported as disadvantages.[14]

Surgery is an option for women who have failed dilators or who choose surgery after counseling. It is extremely important for the patient to know that she will need to use a vaginal dilator postoperatively to prevent stricture or stenosis with surgical procedures. Each surgical procedure has its advantages and disadvantages, and there is no "perfect" option.

The Mcindoe technique is one of the most commonly used surgical procedures. It is a neovagina technique in which a mold wrapped with a skin graft taken from the patient's body is placed after blunt dissection of the space between the rectum and bladder. The mold is left in the vagina for 7 days postoperatively. While no abdominal access and low morbidity are considered advantages, poor cosmetic results at the graft site, the need to use a dilator after surgery, stenosis in the newly created vagina, and the need to use a lubricant during sexual intercourse are considered disadvantages.[15]

Intestinal vaginoplasty is a technique to create a new vagina using a segment of the rectum, sigmoid colon, or ileum. To create a neovagina, one end of the resected segment is pulled toward the introitus and the other end is closed to create a blind pouch. An end-to-end reanastomosis is performed to reconstruct the GI tract. According to McIndoe, the advantage of this procedure is that no dilators are required. The disadvantages are that women complain of chronic vaginal discharge and malodor. There is also a risk of adenocarcinoma developing in these grafts.[16]

In the modified Vecchietti procedure, a neovagina is created by traction using an acrylic "olive" placed in the vaginal dimple. This olive is attached to the abdominal device by laparoscopically placed subperitoneal sutures. Sufficient traction is applied to the olive to produce vaginal elongation of approximately 1.0 cm per day, creating a neovagina in approximately seven days. Once the neovagina is created, active dilation is required until regular sexual activity can be resumed.[16] An advantage of this technique over the Frank technique is that continuous traction is applied. In addition, prolonged hospitalization is not required. A study of 52 women reported 100 percent anatomic success and 98.1 percent functional success.[17]

Another laparoscopic approach is an adaptation of the Davydov procedure. The Davydov technique is a three-stage procedure that involves dissection of the rectovesical space, abdominal mobilization of the peritoneum to create vaginal fornices, and ligation of the peritoneum to the introitus.[18] In the Davydoy Neovagen technique, the vaginal vault is created by attaching a purse-string peritoneal suture to the pelvic floor. The use of peritoneum eliminates the disadvantages of other graft materials such as skin or intestine and allows epithelialization of the new vagina without excessive secretions and hair growth. Another advantage of this technique is the absence of scarring and granulation tissue formation. The Dovydoy neovagina technique was modified by Uncu et al.[11] In this technique, a paramesonephric remnant supported laparoscopic double layer peritoneal pull-down vaginoplasty was performed. In this technique, the pelvic peritoneum is first circularly dissected

and then connected to the midline with a purse-string suture. The Müllerian remnants are then brought closer to the midline and provide support for the peritoneal dome. It is claimed that this modification reduces the possibility of vaginal stricture and herniation. In our technique, to reduce vaginal stricture, the Müllerian remnants are first brought closer to the midline in a manner that mimics the cervix, then the anterior and posterior peritoneal walls are dissected and connected to the midline. This prevents contact between the anterior and posterior peritoneal walls. The reduction of peritoneal contact reduces vaginal stricture.

The Uncu modification uses a 360 degree peritoneal incision to create a vaginal dome. This extensive dissection prolongs the surgery and also creates a large area of dissection on the rectum and bladder. In our technique, the Müllerian structures are first dissected from the abdominal sidewall and attached to the midline. Thus, less peritoneum is dissected for the anterior and posterior vaginal walls. This technique shortens the duration of surgery and reduces the possibility of complications.

In the Nazik Neovagina Technique, the uterus is simulated by leaving the round and infundibulopelvic ligaments as support to prevent prolapse of the dome. In this way, the dome is supported and the vagina can remain cylindrical. In the Uncu modification, a rigid and imperforate acrylic mold was used. The mold design was changed in our method. The most important way to prevent vaginal stricture is to use the vaginal mold for a long time. For this purpose, we used a hard mold for the first 48 hours and then a soft silicone mold. The silicone mold was manufactured with a longitudinal hole in the middle. This facilitates the drainage of serohemorrhagic fluid and accelerates wound healing. There was no hook for suture fixation in the mold used in the Uncu modification. The mold we designed has stainless steel hooks at the end. In addition, a 30 Shore soft silicone mold is used on the third postoperative day to increase patient compliance. One of the limitations of the study is that the sample size was limited to 9 patients. Our results should be supported by future multicenter studies with a larger sample size. In addition, vaginal length measurements presented at 6 months should be reported at 1 year.

While the Uncu modification achieved a vaginal length of 7.5 cm at the end of one year, our technique achieved a vaginal length of 8.6 cm at the end of 6 months. Most authors consider 6 cm to be an adequate length for satisfactory intercourse.[19]

CONCLUSION

In the case series described in this article, the Dayvdoy Neovagina Technique and Uncu modification was developed. In this technique, Müllerian remnants were used to redesign the vaginal dome to mimic the uterine cervix and ligaments to prevent vaginal stricture, which is the main problem of neovaginal surgical techniques. This prevents peritoneal contact and reduces vaginal stenosis. With this technique, the operating time is shortened because less peritoneal dissection is required. The mold has been modified to be more flexible and softer to increase functional sexual success. In particular, although the number of patients in our report is limited to 9, the anatomical and functional vaginal length ratios are sufficient, six of the patients are satisfied with their sexual experience, and the operation is easy to apply.

Footnote

Informed Consent: Detailed informed consent forms were obtained from the patients.

Financial Disclosure: The authors declared that this study received no financial support.

REFERENCES

1. Williams LS, Demir Eksi D, Shen Y, et al. Genetic analysis of Mayer-Rokitansky-Kuster-Hauser syndrome in a large cohort of families. Fertil Steril. 2017;108(1):145-151.
2. Herlin M, Bjørn AM, Rasmussen M, Trolle B, Petersen MB. Prevalence and patient characteristics of Mayer-Rokitansky-Küster-Hauser syndrome: a nationwide registry-based study. Hum Reprod. 2016;31(10):2384-2390.
3. Fedele L, Bianchi S, Frontino G, Ciappina N, Fontana E, Borruto F. Laparoscopic findings and pelvic anatomy in Mayer-Rokitansky-Küster-Hauser syndrome. Obstet Gynecol. 2007;109(5):1111-1115.
4. Kapczuk K, Iwaniec K, Friebe Z, Kędzia W. Congenital malformations and other comorbidities in 125 women with Mayer-Rokitansky-Küster-Hauser syndrome. Eur J Obstet Gynecol Reprod Biol. 2016;207:45-49.
5. Laufer MR. Congenital absence of the vagina: in search of the perfect solution. When, and by what technique, should a vagina be created? Curr Opin Obstet Gynecol. 2002;14(5):441-444.
6. Edmonds DK, Rose GL, Lipton MG, Quek J. Mayer-Rokitansky-Küster-Hauser syndrome: a review of 245 consecutive cases managed by a multidisciplinary approach with vaginal dilators. Fertil Steril. 2012;97(3):686-690.
7. Gargollo PC, Cannon GM Jr, Diamond DA, Thomas P, Burke V, Laufer MR. Should progressive perineal dilation be considered first line therapy for vaginal agenesis? J Urol. 2009;182(Suppl 4):1882-1889.
8. Edmonds DK. Congenital malformations of the genital tract and their management. Best Pract Res Clin Obstet Gynaecol. 2003;17(1):19-40.
9. Louis-Sylvestre C, Haddad B, Paniel BJ. Creation of a sigmoid neovagina: technique and results in 16 cases. Eur J Obstet Gynecol Reprod Biol. 1997;75(2):225-229.
10. Csermely T, Halvax L, Sárkány A, et al. Sexual function after modified laparoscopic Vecchietti's vaginoplasty. J Pediatr Adolesc Gynecol. 2011;24(3):147-152.
11. Uncu G, Özerkan K, Ata B, et al. Anatomic and functional outcomes of paramesonephric remnant-supported laparoscopic double-layer peritoneal pull-down vaginoplasty technique in patients with Mayer-Rokitansky-Küster-Hauser syndrome: Uncu modification. J Minim Invasive Gynecol. 2018;25(3):498-506.
12. Bombard DS 2nd, Mousa SA. Mayer-Rokitansky-Kuster-Hauser syndrome: complications, diagnosis and possible treatment options: a review. Gynecol Endocrinol. 2014;30(9):618-623.
13. Ingram JM. The bicycle seat stool in the treatment of vaginal agenesis and stenosis: a preliminary report. Am J Obstet Gynecol. 1981;140(8):867-873.
14. Liao L, Doyle J, Crouch NS, Creighton SM. Dilation as treatment for vaginal agenesis and hypoplasia: a pilot exploration of benefits and barriers as perceived by patients. J Obstet Gynaecol. 2006;26(2):144-148.
15. Kapoor R, Sharma DK, Singh KJ, et al. Sigmoid vaginoplasty: long-term results. Urology. 2006;67(6):1212-1215.

16. Fedele L, Bianchi S, Berlanda N, et al. Neovaginal mucosa after Vecchietti's laparoscopic operation for Rokitansky syndrome: structural and ultrastructural study. Am J Obstet Gynecol. 2006;195(1):56-61.
17. Fedele L, Bianchi S, Zanconato G, Raffaelli R. Laparoscopic creation of a neovagina in patients with Rokitansky syndrome: analysis of 52 cases. Fertil Steril. 2000;74(2):384-389.
18. Davydov SN. Operatsiia kol'popeza iz briushiny matochno-priamokishechnogo prostranstva [Colpopoeisis from the peritoneum of the uterorectal space]. Akush Ginekol (Mosk). 1969;45(12):55-57.
19. Lloyd J, Crouch NS, Minto CL, Liao LM, Creighton SM. Female genital appearance: "normality" unfolds. BJOG. 2005;112(5):643-646.

Case Report

DOI: 10.4274/anajog.galenos.2024.14633

Anat J Obstet Gynecol Res 2024;2:87-90

A Case Report of Uterine Myometrial Defect Following Hayman and Square Sutures for Postpartum Hemorrhage

Yasemin Doğan[1], Lale Aksoy[2], Ömer Doğukan Saraç[3], Mehmet Zeki Türe[4], Ercan Koçkaya[5]

[1]Kocaeli University Hospital, Department of Obstetrics and Gynecology, Clinic of Perinatology, Kocaeli, Turkey
[2]Alanya Alaaddin Keykubat University Training and Research Hospital, Department of Obstetrics and Gynecology, Antalya, Turkey
[3]Kocaeli City Hospital, Clinic of Obstetrics and Gynecology, Kocaeli, Turkey
[4]Private Clinic, Clinic of Obstetrics and Gynecology, Mardin, Turkey
[5]Şırnak State Hospital, Clinic of Obstetrics and Gynecology, Şırnak, Turkey

ABSTRACT

Uterine atony due to postpartum hemorrhage (PPH) is an important factor in maternal morbidity and mortality. Various compression suture techniques have been described in order to manage PPH. Here, we describe a case complicated by myometrial necrosis following Hayman and square sutures. Here, we describe a case complicated with myometrial necrosis after Hayman and square sutures to control hemorrhage due to uterine atony after cesarean delivery (C-section). A 32-year-old female patient with a history of chronic hypertension and in vitro fertilization pregnancy after primary infertility was admitted to our clinic with vaginal bleeding on her 33rd week of gestation. Fetal bradycardia was observed, and emergency C-section was performed. PPH occurred due to uterine atony and hemorrhage was managed with Hayman and square sutures. Postpartum 1st year ultrasound revealed myometrial defect in uterine fundus. Patients should be informed about possible complications of compression sutures and postoperative follow-up is necessary to confirm uterine wall integrity. We suggest a national database in order to document the efficiency and long-term complications of the procedure.

Keywords: Postpartum hemorrhage, uterine atony, Hayman suture, square suture, uterine necrosis

INTRODUCTION

Postpartum hemorrhage (PPH) is the leading cause of maternal mortality worldwide.[1,2] Primary PPH (24 hours postpartum) occurs approximately in 4-6% of pregnancies and 80% of cases are caused by uterine atony.[3]

Various medical and surgical techniques are used to manage PPH. These techniques can be identified as bimanual uterine compression, use of uterotonic agents (oxytocine, carboprost tromethamine, methylergonovine, misoprostol, carbetocin), tranexamic acid, uterine tamponade and uterine balloon tamponade, ligation of uterine artery and hypogastric artery, uterine artery embolization and hysterectomy. In 1997, B-Lynch et al.[4] developed an alternative technique to cesarean hysterectomy and successfully performed this technique on 5 patients. In 2000, Cho et al.[5] reported a case series of 23 patients with primary PPH who were surgically treated with multiple square sutures to contract anterior and posterior uterine walls. In 2002, Hayman described a new technique in which 2 vertical sutures were placed on both sides of the uterus.[6]

Following a few reports of case series, B-Lynch and other compression suture techniques have been widely used in PPH due to uterine atony.[4-12] Here, we present a uterine atony followed by PPH and treated with square suture in which myometrial defect was observed 1 year after the cesarean delivery (C-section).

CASE REPORT

A 32-year-old female patient with a history of chronic hypertension and in vitro fertilization pregnancy after primary infertility was admitted to our clinic with vaginal bleeding on her 33rd week of gestation. It was seen that the patient's bleeding extended beyond her pad and fetal heart rate was 50/min on ultrasonographic (USG) examination. An emergency C-section was decided due to a probable placental abruption. 1480 gr fetus with no cardiac activity was delivered by a lower uterine segment incision and was handed over to pediatricians.

Address for Correspondence: Yasemin Doğan, Kocaeli University Hospital, Department of Obstetrics and Gynecology, Clinic of Perinatology, Kocaeli, Turkey
Phone: +90 262 303 65 37 **E-mail:** perinatolojidr@gmail.com **ORCID ID:** orcid.org/0000-0002-2614-4411
Received: 29.07.2024 **Accepted:** 13.08.2024

Approximately 40% of the placenta was observed to be abrupted. Twenty unites of intravenous (IV) oxytocin and 0.2 milligrams of methylergonovine maleate intramuscular was administered and uterine incision was sutured. Uterine tonus was consistent with uterine atony, uterine massage was performed and an addition of 10 units of IV oxytocin and 800 mcg rectal misoprostol tablets administered. Despite the perioperative medical treatment, uterine contractions and uterine tonus could not be obtained, therefore Hayman technique was applied, and C-section was completed. The patient was taken to recovery area in the operating room (OR). During the 1st hour observation in the OR, vaginal bleeding and uterine atony persisted and relaparotomy decision was given. Under general anesthesia, the Pfannenstiel incision, subcutaneous and peritoneal sutures were cut. Bleeding was seen due to atonic uterus; therefore, uterine artery was ligated bilaterally. Since the uterus was still atonic, multiple uterine square sutures were made with Vicryl®. Five units of erythrocyte suspension, 3 units of fresh frozen plasma, 2 units of pooled thrombocyte and 4 flacons of fibrinogen concentrate (Haemocomplettan P) was administered.

After bleeding control, the abdominal layers were sutured properly, and the operation was completed. The patient was transferred to the intensive care unit (ICU). Active vaginal bleeding was not observed during postoperative follow-up in the ICU and the patient was extubated on postoperative day 1.3 days after admission to the ICU, she was transferred to obstetrics and gynecology clinic where she was discharged on her postoperative 20th day.

One year after the C-section, the patient applied to our clinic for an embryo transfer procedure. Endometrial irregularity was seen during transvaginal USG therefore, diagnostic diagnostic hysteroscopy and diagnostic laparoscopy (L/S) was scheduled. During the hysteroscopy endometrium was atrophic, right tubal ostium was normal, but the left tubal ostium could not be visualized. Synechia on lateral uterine walls and fundus was dissected with hysteroscopic scissors. Laparoscopic visualization of the uterus revealed loss in integrity of the myometrium and large bulging appearances were observed at the fundus. Hydrosalpinx was seen in the left fallopian and chromopertubation with methylene blue dye showed no passage thorough the left fallopian tube. With the patient's consent, laparotomy for the uterine defect was scheduled for repair at a further date.

The patient was reoperated 6 months after the L/S operation with the plan of myometrial defect repair and salpingectomy. Under general anesthesia, a laparotomy was performed through Pfannenstiel incision in the low lithotomy position. Hydrosalpinx in the left tuba, left ovary and tuba adhered to each other, myometrial defects of 5 cm and 3 cm in diameter were observed on the uterine surface (Figures 1, 2), and endometrial tissues could be seen between the defects. Methylene blue was injected into the endometrial cavity with a Foley catheter. Then, the defects observed on the anterior posterior and fundal surfaces of the uterus were partially repaired with 1 vicrly. Left salpingectomy was performed with the use of advanced bipolar tissue sealer device and the procedure was terminated. The patient was discharged on postoperative day 3. Informed consent was obtained for this case report at the post-discharge outpatient clinic controls.

DISCUSSION

Nulliparous women, patients who have uterine overdistension (fetal macrosomia, multiple pregnancy, polyhydramnios), prolonged or augmented labor, obesity, operative delivery, chorioamnionitis and history of PPH in previous pregnancy are known to be in risk for uterine atony resulting in PPH.[1] Even though these risk factors are well defined to anticipate patients at risk, it is not always possible for clinicians to predict primary PPH. Recognition of bleeding in early stages, taking prompt action and multidisciplinary approach are crucial to manage PPH. It's estimated that one in every three women with uterine atony will undergo hysterectomy due to failure to respond to uterotonic agents, and nulliparous women account for one-quarter of these.[13]

Good results have been obtained with compression suturing techniques in terms of reducing maternal morbidity and mortality in uterine atony cases as well as preserving fertility.

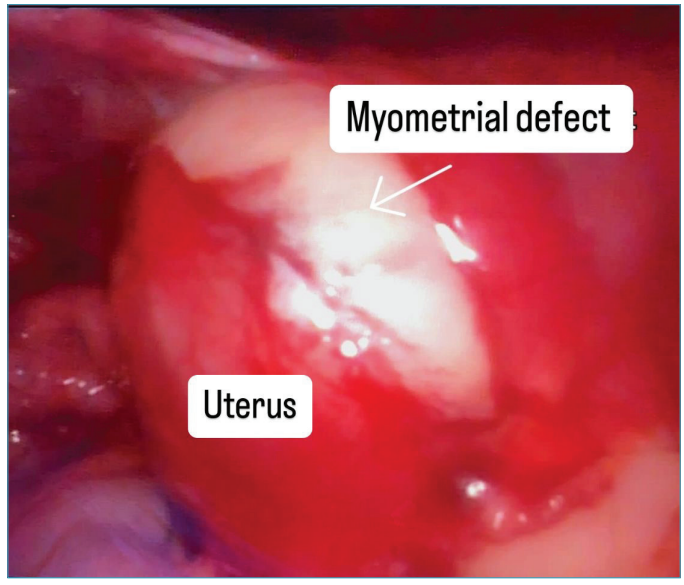

Figure 1. Five cm myometrial defect on the uterine surface

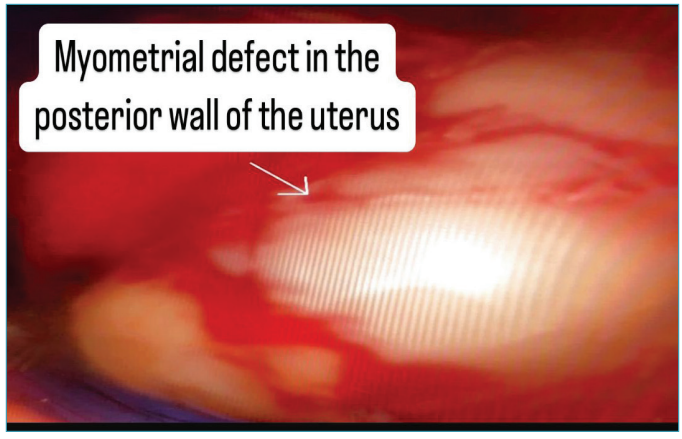

Figure 2. Myometrial defect in the posterior wall of the uterus

B-Lynch and square sutures are favorable since these techniques can be performed with ease (less hypogastric or uterine artery ligation is required, and operation time is reduced) and they are associated with fewer serious complications than devascularization procedures.[14] The square suture technique is superior to B-Lynch in controlling bleeding in cases of placenta previa. Thus, in PPH due to atony when medical treatment is insufficient to manage the hemorrhage alone, use of compression sutures have been widely accepted.

There are not enough studies on the superiority of compression sutures in terms of maternal morbidity in long-term follow-up. In their review, Doumouchtsis et al.[15] have demonstrated that compression sutures had a success rate of 92%. It is difficult to determine the exact efficacy of compression sutures in bleeding due to uterine atony due to the low number of reported cases and the possibility of underreporting of failed procedures. In a case series of 11 patients, Allahdin et al. reported that B-Lynch suture was performed with a 72% success rate in controlling secondary hemorrhage, with three cases proceeding to hysterectomy.[16] In a study of 35 patients who underwent B-Lynch after atony by Grotegut et al.,[17] 3 patients experienced failure and the success rate of the technique was 91.4%. Ferguson, on the other hand, suggested that B-Lynch technique may be associated with the risk of damage to uterus caused by over-compression of the uterus with uterine compression sutures.[7]

Another suturing technique, Hayman suture was investigated by Nanda and Singhal[18] and was found to be successful in 93.75% of the patients. The technique was rapid and easy to perform, requiring less experience.[18] In a study by Alouini et al.[19] the Cho square suture technique was reported to be efficient in 93% of the patients. However, it was demonstrated that intrauterine adhesions varying between thin to severe occurred in 60% of the patients.

In a review by García-Guerra et al.,[20] 66% of the uterine necrosis cases occurred after B-Lynch suturing technique and 25% of the cases occurred after the Cho suture.

After our literature search, we see that cases of partial myometrial necrosis after compression suture are rare. El-Hamamy[21] and Joshi and Shrivastava[22] have reported cases where partial necrosis of the uterus occurred subsequent to uterine brace compression suture. In 2009, Reyftmann et al.[23] reported a case of partial uterine necrosis which was treated with Cho sutures for PPH. Gottlieb et al.[24] reported fundal uterin necrosis 8 days after C-section and implementation of compression sutures. Although partial necrosis of the uterus is rare, combining compression sutures with the uterine artery ligation may increase the risk.

Postpartum pyometritis cases after square suture technique have also been reported.[25]

Sentilhes et al.[26] have estimated that around 5% of the cases to who were treated with compression sutures, are complicated with uterine synechia subsequently. In a study by Poujade et al.,[27] 15 women who underwent uterine compression sutures for PPH were investigated for uterine synechiae. Hysteroscopy or hysterosalpingogram revealed that 26.7% of the patients had developed uterine synechiae.[27]

In order to avoid postoperative complications like uterine necrosis or synechia, the removal of compression sutures 24-48 hours after the first surgery has been suggested and implemented by Hashida et al.[28]

Our patient in the present case did not show any unusual symptoms in the early or late postpartum period after Hayman and square suture and the mechanism of necrosis in the fundal zone has not been clarified. In the square suture technique, equal compression is applied to the anterior and posterior wall of the uterus at the site of the knot. However, due to the caliber and location of the blood vessels, necrosis due to ischemia may develop in selected areas compressed with sutures.

In another study, uterine erosion was found in an asymptomatic patient who underwent compression suturing due to uterine atony at the 6th week postpartum follow-up.[17] The authors recommend the use of rapid absorbable sutures to prevent this complication. We do not agree that the suture material used plays an active role. In the first postpartum days, the myometrium is released from compression as the uterus involution occurs and contracts rapidly. Therefore, damage due to pressure occurs at an early stage, depending on the duration and degree of stress. On the other hand, late absorbing type of suture material may cause synechiae due to compression of the endometrium and myometrium. Postpartum atonic uterus may trigger necrosis by impairing the function of intrinsic cells as a result of impaired uterine blood supply after compression suture.

CONCLUSION

The use of compression sutures is a simple method to effectively control PPH due to atony and to preserve fertility. However, patients who are treated with compression sutures such as B-Lynch, Hayman, Cho etc. should be informed about possible complications of these lifesaving procedures. We also recommend pelvic USG and sonohysterography for these women postoperatively, to identify uterine wall defects and uterine cavity adhesions. Three-dimensional ultrasound and magnetic resonance imaging are valuable to examine uterine wall integrity. Patients with uterine tenderness and persistent vaginal bleeding after compression suturing should be investigated for uterine wall necrosis.

As clinicians gain experience with compression suture techniques, the efficacy and related complications will be better understood and managed. Creation of a national registry of patients undergoing compression suturing and further research is needed to document the effectiveness and long-term complications of the procedures.

Footnote

Informed Consent: Informed consent was obtained for this case report at the post-discharge outpatient clinic controls.

Authorship Contributions

Surgical and Medical Practices: Y.D., Ö.D.S., M.Z.T., Concept: Y.D., L.A., Design: Y.D., Ö.D.S., E.K., Data Collection or Processing: Y.D., L.A., M.Z.T., Analysis or Interpretation: Y.D., Ö.D.S., Literature Search: Y.D., Ö.D.S., Writing: Y.D.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study received no financial support.

REFERENCES

1. American College of Obstetricians and Gynecologists. Postpartum hemorrhage. ACOG Educational Bulletin N0.243. Washington DC: American College of Obstetricians and Gynecologists; 1998.
2. Drife J. Management of primary postpartum hemorrhage. Br J Obstet Gynaecol. 1997;104(3)275-277.
3. Combs CA, Murphy EL, Laros RK Jr. Factors associated with postpartum hemorrhage with vaginal birth. Obstet Gynecol. 1991;77(1):69-76.
4. B-Lynch C, Coker A, Lawal Ah, Abu J, Cowen MJ. The B-Lynch surgical technique for the control of massive postpartum haemorrhage: an alternative to hysterectomy? Five cases reported. Br J Obstet Gynaecol. 1997;104(3):372-375.
5. Cho JH, Jun HS, Lee CN. Hemostatic suturing technique for uterine bleeding during caesarean delivery. Obstet Gynecol. 2000;96(1):129-131.
6. Hayman RG, Arulkumaran S, Steer PJ. Uterine compression sutures: surgical management of postpartum hemorrhage. Obstet Gynecol. 2002;99(3):502-506.
7. Ferguson JE. Bourgeois FJ, Underwood PB. B-Lynch for postpartum hemorrhage. Obstet Gynecol. 2000;95:1020-1022.
8. Smith KL, Baskett TF. Uterine compression sutures as an alternative to hysterectomy for severe postpartum haemorrhage. J Obstet Gynaecol Can. 2003;25(3):197-200.
9. Dacus JV, Busowski MT, Busowski JD, Smithson D, Masters K, Sibai BM. Surgical treatment of uterine atony employing the B-Lynch technique. J Matern Fetal Med. 2000;9(3):194-196.
10. Mazhar SB, Yasmin S, Gulzar S. Management of massive postpartum hemorrhage by "B-Lynch" brace suture. J Coll Physicians Surg Pak. 2003;13(1):51-52.
11. Roman AS, Rebarber A. Seven ways to control postpartum hemorrhage. Contemporary Ob/Gyn. 2003;3:34-35.
12. Danso D, Reginald P. Combined B-Lynch suture with intrauterine balloon catheter triumph over massive postpartum haemorrhage. BJOG. 2002;109(8):963.
13. El-Hamamy E, B-Lynch C. A worldwide review of the uses of the uterine compression suture techniques as alternative to hysterectomy in the management of severe post-partum haemorrhage. J Obstet Gynaecol. 2005;25(2):143-149.
14. AbdRabbo SA. Stepwise uterine devascularization: A novel technique for management of uncontrolled postpartum hemorrhage with preservation of the uterus. Am J Obstet Gynecol. 1994;171(3):694-700.
15. Doumouchtsis SK, Papageorghiou AT, Arulkumaran S. Systematic review of conservative management of postpartum hemorrhage: what to do when medical treatment fails. Obstet Gynecol Surv. 2007;62(8):540-547.
16. Allahdin S, Aird C, Danielian P. B-Lynch suture for major primary postpartum haemorrhage at caesarean section. J Obstet Gynaecol. 2006;26(7):639-64.
17. Grotegut C, Larsen F, Jones M, Livingston E. Erosion of a B-Lynch suture through the uterine wall. J Reprod Med. 2004;49(10):849-852.
18. Nanda S, Singhal SR. Hayman uterine compression stitch for arresting atonic postpartum hemorrhage: 5 years experience. Taiwan J Obstet Gynecol. 2011;50(2):179-181.
19. Alouini S, Coly S, Mégier P, Lemaire B, Mesnard L, Desroches A. Multiple square sutures for postpartum hemorrhage: results and hysteroscopic assessment. Am J Obstet Gynecol. 2011;205(4):335.e1-6.
20. García-Guerra R, Assaf-Balut M, El-Bakkali S, Pérez de Ávila-Benavides I, Huertas-Fernández MÁ. Uterine necrosis following hemostatic compression suture: case report and review of the literature. Rev Colomb Obstet Ginecol. 2022;73(4):378-387.
21. El-Hamamy E. Partial ischemic necrosis of the uterus following a uterine brace compression suture. BJOG. 2005;112(1):126.
22. Joshi VM, Shrivastava M. Partial ischemic necrosis of the uterus following a uterine brace compression suture. BJOG. 2004;111(3):279-280.
23. Reyftmann L, Nguyen A, Ristic V, Rouleau C, Mazet N, Dechaud H. Partial uterine wall necrosis following Cho hemostatic sutures for the treatment of postpartum hemorrhage. Gynecol Obstet Fertil. 2009;37(6):579-582.
24. Gottlieb AG, Pandipati S, Davis KM, Gibbs RS. Uterine necrosis: a complication of uterine compression sutures. Obstet Gynecol. 2008;112(Pt 2):429-431.
25. Ochoa M, Allaire AD, Stitely ML. Pyometra after haemostatic square suture technique. Obstet Gynecol. 2002;99(3):506-509.
26. Sentilhes L, Gromez A, Marpeau L. Fertility after pelvic arterial embolization, stepwise uterine devascularization, hypogastric artery ligation, and B-Lynch suture to control postpartum hemorrhage. Int J Gynaecol Obstet. 2010;108(3):249.
27. Poujade O, Grossetti A, Mougel L, Ceccaldi PF, Ducarme G, Luton D. Risk of synechiae following uterine compression sutures in the management of major postpartum haemorrhage. BJOG. 2011;118(4):433-499.
28. Hashida O, Hayashi M, Furuno A, Saito K, Takayasu Y, Ishikawa M. Laparoscopic removal of the threads for uterine compression suture: possible prophylaxis of uterine synechiae. Kanto J Obstet Gynecol. 2013;50:167-170.