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Presidency and Executive Staff

Av. Brigadeiro Luís Antônio, 3421 - Sala 903 -
Jardim Paulista, São Paulo, SP, Brazil
CEP: 01401-001
Phone.: (+55 11) 5573-4919
www.febrasgo.org.br
presidencia@febrasgo.org.br

RBGO Editorial Office

editorial.office@febrasgo.org.br

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Editorial

Sexual Wellness: A Movement Happening Worldwide

Lucia Alves da Silva Lara[1]

[1] Ribeirão Preto Medical School, São Paulo University, Ribeirão Preto, SP, Brazil

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Sexual Health (SH) is an area that requires special attention, as it is directly related to sexual behavior, which has the potential to impact sexual and reproductive health. In 1994, in Cairo, the Program of Action of the International Conference on Population and Development included SH in the definition of reproductive health,[1] indicating that SH extends beyond reproductive care and counseling for sexually transmitted diseases, but aims to promote sexual pleasure, which is fundamental to human health. Thus, Sexual and Reproductive Health (SRH) became the area responsible for promoting SH from the perspective of pleasure, which is essential for physical and mental health, as well as for people's quality of life. A pleasurable sexual life promotes assertiveness in social and marital relationships, contributing to the longevity of interpersonal relationships.[2] Conversely, sexual dysfunctions negatively interfere with people's quality of life and mental health, being associated with conditions such as anxiety, emotional stress, depression, cardiovascular disease, chronic pelvic pain, among other.[3]

Historically, issues related to sexual experience have been permeated by taboo, and in the clinical context, most Sexual and Reproductive Health (SRH) programs focus on contraception, teenage pregnancy, and sexually transmitted infections, which represent the tip of an iceberg supported by a broad base of biological, psychological, and environmental factors associated with risky sexual behaviors that lead to these pathologies. Each individual has their own motivations for seeking sexual pleasure; however, physiological sexual drive is a common motivation for most individuals. During puberty, the increase in androgens promotes an exacerbation of spontaneous sexual thoughts and an increase in interest in emotional relationships and sexual activity.[4] Sexual drive is the result of a complex motivation and reward mechanism elaborated in the limbic system (LS) and certain areas of the prefrontal córtex.[5] This mechanism is linked to certain neurotransmitters that act in the LS, such as the nucleus accumbens and the anteromedial preoptic area, which are associated with dopamine, playing a central role in the experience of reward and pleasure. These areas are connected to certain cortical regions of the central nervous system, which play a role in either positively or negatively modulating sexual impulse.[6]

The motivation and reward mechanism plays a crucial role in habit formation and the repetition of motivating behaviors. Through this mechanism, the positive reinforcement of a pleasurable or rewarding experience, such as a pleasurable sexual activity, activates the memory of the pleasure sensation in specific areas of the LS, leading to the release of dopamine, creating a sense of reward and pleasure. This, in turn, activates intrinsic motivation to repeat the behavior that led to the reward. This mechanism is an essential part of what drives humans to seek pleasurable experiences and form habits, as it represents the connection between stimulus, behavior, and reward.[5] In experimental studies with animals, sex steroids testosterone, estrogen, and progesterone have a modulating effect on this sexual motivation mechanism.[6] Understanding the mechanism of motivation for seeking pleasure facilitates the comprehension that sexual pleasure is a natural and important aspect of the human experience.

Building upon older studies that have shown the benefits of sexual pleasure for human health, since 2008, the World Association for Sexual Health (WAS) has been urging both public and private academic institutions and society as a whole to recognize the connection between sexual pleasure, rights, and health. It emphasizes that sexual pleasure is a component of overall health and human well-being.[7] This has reinforced the importance of considering the experience of sexual pleasure in research, healthcare service delivery, and public policies. In Brazil, this movement was led by the Brazilian Federation of Gynecology and Obstetrics (FEBRASGO), which, through its Sexology Commission, developed a competency framework in sexual health for the training of Obstetrics and Gynecology resident physicians in the care of women with sexual complaints.[8] This document was validated by the Ministry of Education and Culture (MEC) in accordance with the Ministry of Health.

Address for correspondence Lucia Alves da Silva Lara, Av. Bandeirantes, 3900, Monte Alegre, 14049-900, Ribeirão Preto, SP, Brazil (e-mail: luciaalvess2010@gmail.com).

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More recently, the global Sexual Wellness movement has been gaining momentum in the direction of promoting a holistic and positive approach to sexuality and SH. This movement embraces the idea that sexuality is a fundamental part of human life and should be addressed comprehensively, taking into account physical, emotional, psychological, and social aspects related to the development and experience of sexuality. Key points of this movement include sexual education, which is considered crucial to its agenda, open and healthy communication about sexuality in society at large, as well as the recognition and respect for sexual and gender diversity. It also advocates raising awareness about issues related to sexual health, consent, disease prevention, contraception, and more.[9]

The discussion on the importance of sexual pleasure for the physical and mental health of individuals needs to be included on the public health agenda. Recognizing sexual pleasure as a vital component of human well-being is essential to address sexual health issues in a comprehensive and holistic manner. The Sexual Wellness movement calls for broader access to quality sexual health services for the population, ensuring that everyone has the opportunity to take care of their sexual health.

Conflicts to Interest
None to declare.

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Placenta Accreta Spectrum Disorders – The Impact of the Creation of a Multidisciplinary Team on Maternal Outcomes in Portugal

Patologia do espectro do acretismo placentário – O impacto da criação de uma equipa multidisciplinar nos desfechos maternos em Portugal

Beatriz Teixeira[1] Pedro Viana Pinto[1,2,3] Rodrigo Realista[1] Manuela Silva[1] Antónia Costa[1,3] Ana Paula Machado[1] Marina Moucho[1]

[1] Department of Obstetrics and Gynecology, Centro Hospitalar Universitário de São João, Porto, Portugal
[2] Department of Anatomy, Faculdade de Medicina, Universidade do Porto, Porto, Portugal
[3] Department of Obstetrics and Gynecology, Faculdade de Medicina, Universidade do Porto, Porto, Portugal

Address for correspondence Beatriz Teixeira, MD, Alameda Prof. Hernâni Monteiro, 4200-319, Porto, Portugal (e-mail: anabeatrizgteixeira@gmail.com; ana.beatriz.teixeira@chsj.min-saude.pt).

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Abstract

Objective To describe a cohort of placenta accreta spectrum (PAS) cases from a tertiary care institution and compare the maternal outcomes before and after the creation of a multidisciplinary team (MDT).

Methods Retrospective study using hospital databases. Identification of PAS cases with pathological confirmation between 2010 and 2021. Division in two groups: standard care (SC) group – 2010–2014; and MDT group – 2015–2021. Descriptive analysis of their characteristics and maternal outcomes.

Results During the study period, there were 53 cases of PAS (24 - SC group; 29 - MDT group). Standard care group: 1 placenta increta and 3 percreta; 12.5% (3/24) had antenatal suspicion; 4 cases had a peripartum hysterectomy – one planned due to antenatal suspicion of PAS; 3 due to postpartum hemorrhage. Mean estimated blood loss (EBL) was 2,469 mL; transfusion of packed red blood cells (PRBC) in 25% (6/24) - median 7.5 units. Multidisciplinary team group: 4 cases of placenta increta and 3 percreta. The rate of antenatal suspicion was 24.1% (7/29); 9 hysterectomies were performed, 7 planned due to antenatal suspicion of PAS, 1 after intrapartum diagnosis of PAS and 1 after uterine rupture following a second trimester termination of pregnancy. The mean EBL was 1,250 mL, with transfusion of PRBC in 37.9% (11/29) - median 2 units.

Keywords
- placenta accreta spectrum disorders
- multidisciplinary team
- maternal morbidity

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Conclusion After the creation of the MDT, there was a reduction in the mean EBL and in the median number of PRBC units transfused, despite the higher number of invasive PAS disorders.

Resumo

Objetivo Descrever uma coorte de casos do espectro do acretismo placentário (PAS) de uma instituição terciária e comparar os resultados maternos antes e depois da criação de uma equipa multidisciplinar (MDT).

Métodos Estudo retrospectivo utilizando bancos de dados hospitalares. Identificação de casos de PAS com confirmação patológica entre 2010 e 2021. Divisão em dois grupos: grupo *Standard Care* (SC) – 2010–2014; e grupo MDT – 2015–2021. Análise descritiva de suas características e desfechos maternos.

Resultados Durante o período do estudo, houve 53 casos de PAS (24 - grupo SC; 29 - grupo MDT). Grupo Standard Care: 1 placenta increta e 3 percretas; 12,5% (3/24) tiveram suspeita anteparto; 4 casos tiveram histerectomia periparto – uma eletiva devido à suspeita anteparto de PAS; 3 devido a hemorragia pós-parto. A média de perda hemática estimada (EBL) foi de 2.469 mL; transfusão de concentrado eritrocitário (PRBC) em 25% (6/24) - mediana 7,5 unidades. Equipa multidisciplinar: 4 casos de placenta increta e 3 percretas. A taxa de suspeita anteparto foi de 24,1% (7/29); foram realizadas 9 histerectomias, 7 eletivas por suspeita anteparto de PAS, 1 após diagnóstico intraparto de PAS e 1 após rotura uterina após interrupção da gravidez no segundo trimestre. A EBL média foi de 1.250 mL, com transfusão de PRBC em 37,9% (11/29) - mediana de 2 unidades.

Conclusão Após a criação da MDT, houve redução na média de EBL e na mediana do número de unidades de PRBC transfundidas, apesar do maior número de PAS invasivos.

Palavras-chave
- distúrbios do espectro da placenta acreta
- equipe multidisciplinar
- morbidade materna

Introduction

Placenta accreta spectrum (PAS) disorders are an abnormality in placentation in which the chorionic villi adhere or invade the myometrium to a variable extent.[1] This complex clinical entity is associated with important morbidity, not only due to heavy bleeding but also to invasion of the surrounding pelvic organs by placental tissue. In a recent meta-analysis revising the main outcomes of PAS, 46.9% of the cases were complicated by hemorrhage, requiring transfusion.[2]

Although, historically, PAS is a rare complication of pregnancy, its incidence has been rising in the last decades, with recent reported occurrences in 1:533 to 1:730 deliveries.[3,4] This is often attributed to the increasing cesarean rates, as there is an association between the number of prior cesarean deliveries and PAS, particularly in the presence of placenta previa.[5] Other risk factors include a previous pregnancy with PAS, in vitro fertilization, and antecedents of manual removal of the placenta or uterine surgeries, such as curettage, endometrial ablation, and myomectomy.[6–8]

The most widely accepted treatment for PAS is cesarean hysterectomy with the placenta left in situ.[9–11] There is also a consensus in the recommendation that PAS should be treated by a multidisciplinary team (MDT) with expertise in complex pelvic surgery.[9–11] The MDT approach has been associated with better maternal outcomes, such as a reduction in estimated blood loss with lower transfusion requirements and a lower likelihood of reoperation within a week of delivery.[12–18]

The aim of the present study was to describe the cohort of PAS cases from a tertiary care institution and to compare the maternal outcomes before and after the creation of a dedicated MDT.

Methods

This is a retrospective cohort study including all the cases of PAS confirmed histologically that were treated in a tertiary teaching hospital in Porto, between 2010 and 2021. The diagnosis of PAS was made by identification of myometrial tissue adjacent to the chorionic villi (with immunohistochemical confirmation) with focal interruption of the decidua basalis, according to the classification by Hecht et al.[19] The cases were grouped according to the FIGO classification for PAS in placenta accreta (grade 1), increta (grade 2), and percreta (grades 3a–3c),[1] based on intraoperative findings and the pathology report. The cases of PAS were identified by the search of various key terms (low-lying placenta; manual removal of the placenta; peripartum hysterectomy; postpartum hemorrhage) in hospital-based electronic databases Obscare – Virtual Care (Porto, Portugal) and S Clínico – SPMS (Lisbon, Portugal).

After review of the medical records, data was collected regarding baseline characteristics (age, body mass index,

parity and mode of conception); risk factors for PAS disorders (placenta previa, prior caesarean section, and number and history of other uterine surgeries); timing of diagnosis; antepartum and intrapartum management; gestational age at delivery; neonatal outcomes (birthweight, neonatal intensive care unit [NICU] admission, and Apgar scores); immediate maternal morbidity; reoperations (early or delayed, defined as urgent surgical procedures performed during the first 7 days after birth/spontaneous abortion or after 7 days, respectively). Early maternal morbidity was defined as the occurrence of one or more of the following: maternal admission to the intensive care unit (ICU), transfusion of packed red blood cells (PRBCs), coagulopathy, ureteral or bladder wall injury, or early reoperation (up to 7 days after the surgery). Late maternal morbidity was defined as the occurrence of one or more of the following: hospital readmission within 6 weeks or delayed reoperation (performed between 7 days to 6 weeks after the surgery).

The standard care (SC) group was composed by the cases occurring before 2015 (prior to the creation of the MDT) and were managed on a case-by-case basis, as defined by the medical team. This approach could include formal or informal consultations with other specialties. In this group, the management of patients with antenatal suspicion of PAS ranged from planned peripartum hysterectomy with the placenta left in situ (with the possibility of placement of balloon catheters on the common iliac arteries as decided by the surgeons) to the performance of cesarean sections with an attempt to remove the placenta.

The MDT group included the cases treated in our institution after the creation of the MDT in 2015. Our team includes members from the following areas: maternal-fetal medicine, gynecological oncology, obstetrical ultrasound, interventional radiology, and urology; consultations were also made with the blood bank, anesthesiology, intensive care unit and neonatal intensive care unit. In cases with suspicion of deeper invasion, ultrasound evaluation of the placenta by a dedicated sonographer and magnetic resonance imaging of the placenta are performed. Whenever possible, the delivery is planned to the late preterm period (35–37 weeks of gestation). The woman is electively admitted for preoperative optimization and planning: a cycle of corticosteroids for fetal lung maturity is performed according to the gestational age. Adequate blood products should be available at the time of delivery for scheduled PAS and are prepared in advance.

On the day of the surgery for cases of a suspicion of higher degree invasion, in the same operative room, the patient is submitted to a cystoscopy with evaluation of bladder involvement and placement of ureteric stents; catheters on the common iliac arteries are also placed (inflated intraoperatively, if necessary). When a cesarean hysterectomy is performed, it is initiated under locoregional anesthesia and converted to general anesthesia after fetal extraction. A vertical midline skin incision is performed, and the uterus is exteriorized. The hysterotomy is made above the superior border of the placenta, usually in a fundal location. After fetal extraction, the placenta is left in situ with no attempts to remove it and no uterotonics are used. After closure of the uterine incision, either a simple or a modified radical hysterectomy is performed (depending on the degree of placental invasion), with a bilateral opportunistic salpingectomy, in accordance with recent recommendations for reduction of the risk of ovarian cancer.[20]

For the study, 'elective' deliveries were classified as deliveries performed non-urgently in prearranged designated theaters at least 24 hours in advance. 'Emergency' deliveries were deliveries performed expeditiously due to concerns for either maternal or fetal wellbeing. Reasons for emergency deliveries are discussed further.

The two groups were characterized using descriptive statistics. Due to the design of the study and the number of cases, no other statistical tests were performed. Approval was obtained from the ethics committee of Centro Hospitalar e Universitário de São João.

Results

During the study period, there were 29,517 deliveries, with 53 cases of histologically confirmed PAS disorders identified, making an incidence of 1.8/1,000. Of these, 24 occurred before 2015 and were part of the SC group; 29 were in the MDT group (2015–2021). The maternal baseline characteristics are presented in ►**Table 1**. In the SC group, half of the patients (12/24) were primiparous, and 5 had previous cesarean deliveries (20.8%). Regarding other risk factors for PAS, one patient had been submitted to previous hysteroscopic procedures (three ressectoscopic myomectomies), and there were 6 cases of placenta previa (25%). As for the MDT group, the rate of primiparous patients was 24.1% (7/29) and in more than half of the cases (51.7% - 15/29), there was a history of previous cesarean deliveries. Most cases corresponded to placenta accreta (FIGO grade 1), as expected (SC group – 20/24 [83.3%]; MDT group – 22/29 [75.9%]).

The antenatal and intrapartum characteristics in both groups are summarized in ►**Table 2**. There was an antenatal suspicion of PAS in 3 cases of the SC group (1 case FIGO grade 1, 1 FIGO 2, and 1 FIGO 3) and 7 cases of the MDT group (corresponding to the 7 cases of abnormally invasive placenta). Of the SC cases with antenatal suspicion of PAS, in one of them a planned peripartum hysterectomy was performed with the placenta left in situ. In the other two, the delivery was made by a scheduled cesarean section, with attempts to remove the placenta that culminated in a hysterectomy to control the postpartum hemorrhage (PPH) in one instance. As for the 7 cases of the MDT group that were identified antenatally, all were submitted to a planned peripartum hysterectomy by the MDT, following the protocol described. Balloon catheters in the common iliac arteries were placed in 1 patient in the SC group (inflated in that case) and in 7 cases in the MDT group (inflated in 3 cases).

►**Table 3** summarizes the maternal and neonatal morbidity associated with PAS in this series. In the SC group, the mean estimated blood loss (EBL) was 2,469 mL, with a need for transfusion of PRBCs in 25% (6/24) and a median of 7.5 units of PRBCs transfused. In the MDT group, the EBL was

Table 1 Maternal characteristics

Characteristics	SC group ($n = 24$)	MDT group ($n = 29$)	Total ($n = 53$)
Maternal age in years, median (range)	33.5 (23–44)	37 (25–43)	36 (23–44)
Pregestational BMI (kg/m^2), median (range)	25.4 (18.5–47.5)	23.7 (17.3–30.4)	24.3 (17.3–47.5)
Assisted reproductive techniques – n	3	1	4
Primiparous – n (%)	12 (50)	7 (24.1)	19 (35.8)
Gestational age at delivery, median (range)	38 (32–41)	38 (27–41)	38 (27–41)
Previous cesarean delivery – n (%)	5 (20.8)	15 (51.7)	20 (37.7)
Number of previous cesarean deliveries – n (%)			
0	19 (79.2)	14 (48.3)	33 (62.3)
1	4 (16.7)	12 (41.3)	16 (30.2)
2 or more	1 (4.2)	3 (10.3)	4 (6.9)
Other uterine surgical procedures - n	1	1	2
Placenta previa – n (%)	6 (25)	7 (24.1)	13 (24.5)
Placenta previa and previous cesarean deliveries – n (%)	1 (4.2)	6 (20.7)	7 (13.2)
No identifiable risk factors for PAS	14 (58.3)	10 (34.5)	24 (45.3)

Abbreviations: BMI, body mass index; MDT, multidisciplinary team; n, number; SC, standard care.

Table 2 Antenatal and intrapartum characteristics

	SC group ($n = 24$)	MDT group ($n = 29$)	Total ($n = 53$)
Type of PAS – n (%)			
Accreta (FIGO Grade 1)	20 (83.3)	22 (75.9)	42 (79.2)
Increta (FIGO Grade 2)	1 (4.2)	4 (13.8)	5 (9.4)
Percreta (FIGO Grade 3)	3 (12.5)	3 (10.3)	6 (11.3)
Grade 3a	2 (8.4)	1 (3.4)	3 (5.7)
Grade 3b	1 (4.2)	1 (3.4)	2 (3.8)
Grade 3c	0 (0)	1 (3.4)	1 (1.9)
Antenatal suspicion of PAS – n (%)	3 (12.5)	7 (24.1)	10 (18.9)
Intrapartum suspicion of PAS – n (%)	13 (54.2)	15 (51.7)	28 (52.8)
Gestational age at delivery, median (range)	38 (32–41)	38 (27–41)	38 (27–41)
Mode of delivery – n (%)			
Cesarean section	13 (54.2)	18 (62.1)	31 (58.5)
Vaginal delivery	11 (45.8)	8 (27.6)	19 (36.5)
Birthweight in grams (mean)	2,926	2,732	2,772
Hysterectomy – n	4	9	13
Planned	1	7	8
Urgent (in the setting of PPH)	3	1[a]	4
After intrapartum diagnosis of PAS disorder	0	1	1
Placement of catheters on common iliac arteries – n	1	7	8
Inflated – n	1	3	4

Abbreviations: FIGO, International Federation of Gynecology and Obstetrics; MDT, multidisciplinary team; n, number; PAS, placenta accreta spectrum; PPH, postpartum hemorrhage; SC, standard care.
[a]Hysterectomy performed after uterine rupture in the context of a second-trimester termination of pregnancy.

Table 3 Maternal and neonatal morbidity

	SC group ($n = 24$)	MDT group ($n = 29$)	Total ($n = 53$)
Estimated blood loss in mL (mean, range)	2,469 (450–8,200)	1,250 (450–4,500)	1,807 (450–8,200)
Early maternal morbidity - n	6	11	17
ICU admission	4	2	6
Need for PRBC transfusion – n (%)	6 (25%)	11 (37.9%)	17 (32.1%)
Number of PRBC units (median, range)	7.5 (2–12)	2 (1–7)	5,5 (1–12)
Coagulopathy	2	0	2
Bladder injury	2	1	3
Ureteral injury	1	0	1
Early re-operation	2	0	2
Late maternal morbidity - n	2	1	3
Hospital re-admission	2	1	3
Delayed re-operation	1	1	2
Neonatal morbidity – n			
NICU admission	5	7	12
5 minute Apgar score < 7	2	0	2

Abbreviations: ICU, intensive care unit; MDT, multidisciplinary team; n, number; NICU, neonatal intensive care unit; PRBC, packed red blood cells; SC, standard care.

1,250 mL, and there was a need for transfusion of PRBCs in 11 cases (37.9% - median of 2 units). Prior to 2015, a total of 6 cases fulfilled the criteria previously defined for early maternal morbidity; after 2015, there were 11 cases of early maternal morbidity.

As for the late maternal morbidity, there were 2 cases of hospital readmission in the SC group – one of them motivated by a pyelonephritis in a patient with ureteric stents and the other by a late postpartum hemorrhage that required two suction curettages for complete removal of placental fragments. The MDT group had one readmission in a patient that had a second trimester termination for a fetus with trisomy 21 and presented with a pelvic abscess that motivated surgical treatment culminating in a hysterectomy.

Regarding the neonatal outcomes, there were 5 admissions to the NICU in the SC group and 7 in the MDT group. There was a fetal death in the first group, subsequent to the rupture of an undiagnosed vasa previa at 32 weeks of gestation. In the latter group, in addition to the termination of pregnancy described above, there was one uterine rupture at 20 weeks in a woman with a history of a previous cesarean delivery and a hysteroscopic septostomy.

Looking only at the cases of deeper placental invasion (FIGO grades 2 and 3), there were 4 cases in the SC group and 7 cases in the MDT; half of them were antenatally suspected in the SC group and all of them were suspected in the MDT group; the mean EBL was 5,300 mL in the SC group (in 3 hysterectomy was not planned antenatally), with a universal need of transfusion of PRBCs (median of 7.5 units). In the MDT group, all cases were managed with planned hysterectomy; the mean EBL was 1,614 mL, with transfusion of PRBCs required in 5 of the 7 cases (median of 1 unit).

Discussion

The present study describes a cohort of PAS of a tertiary hospital, looking at two different groups in different periods of time: a group of cases when there was no standardized protocol regarding the management of these situations and the most recent group, after the creation of a MDT. After 2015, there were more cases with deeper placental invasion (4 of placenta increta – FIGO 2 - and 3 of percreta – FIGO 3), a result of referral from other centers to the newly established team. This factor, combined with a greater percentage of women with a previous cesarean delivery and placenta previa, probably explains the higher rate of antenatal suspicion of PAS in the MDT group; in this group, all the cases with higher degrees of invasion were identified antenatally (7/7). Despite having more cases of PAS FIGO 2 and 3, the mean EBL and the median number of PRBC units transfused were lower (1,250 versus 2,469 mL; 2 versus 7.5 units). The difference is even more pronounced in cases of PAS FIGO 2 and 3: mean EBL of 5,300 mL and transfusion of a median of 7.5 units of PRBC units in the SC group versus 1,614 mL and 1 unit, respectively, in the MDT group. Our results are in line with the evidence of lower maternal morbidity in cases of PAS managed by a MDT.[12–18] Antepartum suspicion of PAS is paramount to the management of this condition by MDT, leading to the improvement of maternal outcomes.[21,22] The combination of anterior placenta previa and a prior cesarean

delivery should alert for the high risk of PAS, as highlighted by several guidelines on this subject.[23]

Placenta accreta (FIGO grade 1), the more benign of PAS disorders, accounts for most of the cases in our study. When compared with invasive PAS (FIGO grades 2 and 3), this clinical entity is associated not only with a lower rate of antenatal diagnosis but also with less severe maternal morbidity.[24] This is congruent with our findings, as most of these cases were not identified antenatally, and there was even a subset that had no intrapartum suspicion, with the diagnosis of placenta accreta made incidentally in the pathology exam. Most of these cases could be classified as basal plate myometrial fibers, according to Hecht et al.[19]

The absence of antenatal suspicion of PAS or placenta previa in most cases explains the relatively high gestational age at delivery (38 weeks), even in the MDT group. This, as well as the fact that an important subset of patients had no identifiable risk factors for PAS (i.e., previous cesarean sections, previous uterine surgeries, or even medically assisted pregnancies), highlights the importance of clinical awareness for this entity and the implementation of best practice protocols in the event of intrapartum diagnosed PAS. In the absence of the MDT, every obstetrician should be familiarized with the basic management of PAS to ensure the best possible maternal outcomes, remembering that the cases are not always antenatally suspected and that even FIGO 1 cases can be associated with important morbidity. In cases of intrapartum suspicion, it is important to be aware of different approaches, from leaving the placenta in situ (at least the attached fragments) to hysterectomy (immediate or delayed, according to the emergency of the situation and the availability of the MDT).

As the incidence of PAS rises, and with the growing experience of the MDTs, the management of this condition is constantly evolving.[25] An example of this is the recently described Soleymani-Alazzam-Collins (SAC) technique regarding the surgical treatment of severe PAS.[26] Our center has also adopted a similar approach over the years, getting closer to the SAC technique in some points, such as the transversal skin incision, the identification and slinging of the ureters without catheter placement, and the identification of the common and internal iliac arteries (with the objective of reducing the need for balloons in the common iliac arteries). There is conflicting evidence in the literature regarding the need for placement of vascular balloons.[27,28] While some authors regard them as very useful, others believe that with a surgically experienced team with there may be no need for them.[29]

Our study has some limitations. First, its retrospective nature and the small sample size did not allow us to perform statistical tests comparing both groups. Another potential weakness in our study is the subjectivity of EBL in our primary outcome, which may have biased the study and the poor data obtained for the SC group. In fact, the percentage of PRCB transfusion is higher in the MDT group despite a lower mean EBL, which may be explained by a more aggressive approach toward anemia in the contemporary practice as well as by the difficulty of obtaining good data in the SC group. Also, some of the less severe cases had no EBL recorded. With the introduction of our multidisciplinary approach, the rigor of blood loss estimation was significantly increased. If anything, patients in the MDT group were more likely to have their blood loss overestimated and to receive more blood than the patients managed without this approach. Lastly, although we consider the histopathological definition of PAS in our study to be a strength, allowing for a more objective and standardized classification,[1,19] it also raises some limitations. The pathological examination of delivered placentas is more challenging when compared with hysterectomy samples. There is still some uncertainty in the literature regarding the clinical significance of basal plate myometrial fibers, as this entity may include cases of placenta accreta, with increased maternal morbidity, but may also be an incidental finding.[19] There is also the possibility that our study design might miss cases of PAS that had no antenatal suspicion and no pathological examination of the placenta. However, considering the limitations mentioned above, we believe that these cases probably corresponded to situations with low morbidity, and, therefore, their clinical significance is dubious.

Conclusion

In conclusion, our study describes the maternal outcomes of PAS cases before and after the creation of a specialized MDT. Despite the higher number of invasive PAS disorders, there was a reduction in the mean EBL, highlighting the importance of a MDT for diagnosis and treatment of PAS cases.

Contributions

All of the authors contributed with the project and data interpretation, the writing of the article, the critical review of the intellectual content, and with the final approval of the version to be published.

Conflict of Interests

The authors have no conflict of interests to declare.

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Prediction of Preterm Delivery Using Serum Ischemia Modified Albumin, Biglycan, and Decorin Levels in Women with Threatened Preterm Labor

Previsão de parto prematuro usando albumina modificada por isquemia sérica, biglicano e níveis de decorina em mulheres com ameaça de trabalho de parto prematuro

Ismail Biyik[1] Cenk Soysal[1] Ozlem Ulas Onur Ince[1,2] Sinem Durmus[3] Efser Oztas[1] Nadi Keskin[1] Ozben Ozden Isiklar[4] Oğuz Han Karaagac[1] Remise Gelisgen[3] Hafize Uzun[5]

[1] Department of Obstetrics and Gynecology, School of Medicine, Kutahya Health Sciences University, Kutahya, Turkey
[2] Department of Statistics, Faculty of Arts and Sciences, Middle East Technical University, Ankara, Turkey
[3] Department of Medical Biochemistry, School of Medicine, Istanbul University-Cerrahpasa, Istanbul, Turkey
[4] Department of Medical Biochemistry, School of Medicine, Kutahya Health Sciences University, Kutahya, Turkey
[5] Department of Medical Biochemistry, Faculty of Medicine, Istanbul Atlas University, Istanbul, Turkey

Address for correspondence Ismail Biyik, Associate Professor, Kütahya Sağlık Bilimleri Üniversitesi Alipaşa Mahallesi Fatih Sultan Mehmet Bulvarı No:22 Merkez/Kütahya, Turkey (e-mail: dribiyik@hotmail.com).

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Abstract

Objective The serum ischemia modified albumin (IMA), biglycan, and decorin levels of pregnant women who were hospitalized for threatened preterm labor were measured.

Methods Fifty-one consecutive pregnant women with a single pregnancy between the 24th and 36th weeks with a diagnosis of threatened preterm labor were included in the present prospective cohort study.

Results As a result of multivariate logistic regression analysis for predicting preterm delivery within 24 hours, 48 hours, 7 days, 14 days, $\leq$ 35 gestational weeks, and $\leq$ 37 gestational weeks after admission, area under the curve (AUC) (95% confidence interval [CI[) values were 0.95 (0.89–1.00), 0.93 (0.86–0.99), 0.91 (0.83–0.98), 0.92 (0.85–0.99), 0.82 (0.69–0.96), and 0.89 (0.80–0.98), respectively. In the present study, IMA and biglycan levels were found to be higher and decorin levels lower in women admitted to the hospital with threatened preterm labor and who gave preterm birth within 48 hours compared with those who gave birth after 48 hours.

Conclusion In pregnant women admitted to the hospital with threatened preterm labor, the prediction preterm delivery of the combined model created by adding IMA, decorin, and biglycan in addition to the TVS CL measurement was higher than the TVS CL measurement alone.

Keywords
- ischemia modified albumin
- biglycan decorin
- preterm delivery prediction
- threatened preterm labor
- preterm delivery

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Clinical trial registration The present trial was registered at ClinicalTrials.gov, number NCT04451928.

Resumo

Objetivo Medir os níveis séricos de albumina modificada por isquemia (IMA), biglicano e decorina de gestantes hospitalizadas por ameaça de parto prematuro.

Métodos Cinquenta e uma mulheres grávidas consecutivas com uma única gravidez entre a 24ª e a 36ª semanas com diagnóstico de ameaça de trabalho de parto prematuro foram incluídas no presente estudo de corte prospectivo.

Resultados Como resultado da análise de regressão logística multivariada para prever parto prematuro dentro de 24 horas, 48 horas, 7 dias, 14 dias, $\leq$ 35 semanas gestacionais e $\leq$ 37 semanas gestacionais após a admissão, área sob a curva (AUC) (95% de confiança os valores de intervalo [CI[) foram 0,95 (0,89–1,00), 0,93 (0,86–0,99), 0,91 (0,83–0,98), 0,92 (0,85–0,99), 0,82 (0,69–0,96) e 0,89 (0,80–0,98), respectivamente. No presente estudo, os níveis de IMA e biglican foram maiores e os níveis de decorin menores em mulheres admitidas no hospital com ameaça de trabalho de parto prematuro e que tiveram parto prematuro em 48 horas em comparação com aquelas que deram à luz após 48 horas.

Conclusão Em gestantes admitidas no hospital com ameaça de trabalho de parto prematuro, a predição de parto prematuro do modelo combinado criado pela adição de IMA, decorin e biglican, além da medição do TVS CL, foi maior do que a medição do TVS CL isoladamente.

Registro do ensaio clínico O presente ensaio foi registrado em ClinicalTrials.gov, número NCT04451928.

Palavras-chave
- albumina modificada por isquemia
- biglicano decorina
- previsão de parto prematuro
- ameaça de parto prematuro
- parto prematuro

Introduction

Births occurring after the 20th week of pregnancy and before the 37th week are called preterm delivery. It has been reported by the World Health Organization (WHO) that 9.6% of all births are preterm deliveries.[1] Preterm labor is one of the most important causes of infant mortality and morbidity. Risk factors for preterm delivery include systemic and genital tract infections, periodontal disease, reduced cervical length, previous cervical surgeries, congenital abnormalities of the uterus, smoking and substance abuse, nutritional deficiency, black race, low socioeconomic level, low educational level, genetic predisposition to preterm delivery, having a premature birth, and multiple pregnancies.[2] Unfortunately, half of preterm deliveries occur in pregnant women without any risk factors. Numerous studies have been conducted in the literature to predict preterm birth in women in threatened preterm labor. However, there is no single or combined screening method for high-sensitivity preterm birth to clearly identify women at risk of preterm birth.[3–10] Current markers give low predictions of which pregnancies will have preterm delivery.[11,12] The unclear pathogenesis contributes to the unpredictability.[13] The most cited mechanisms include premature activation of the maternal or fetal hypothalamic-pituitary-adrenal axis, inflammation and infection, decidual hemorrhage, and pathological uterine distension. Forty to 45% of the PTB cases are idiopathic (spontaneous). Previous preterm birth, maternal nutritional status, presence of infection or inflammation, and various demographic factors such as age and race are important risk factors for spontaneous PTB. Infection and/or inflammation are thought to play a role in $\sim$ 30% of spontaneous PTB cases.[14] Despite an unproven link between vaginal microbiology and PTB, an abundant body of literature exists on the subject. Bacterial vaginosis, increased colonization of F. nucleatum, Mycoplasma hominis, Bacteriodes urealytocius and the loss of Lactobacillus species are some of the proposed mechanisms between the change in vaginal microbiome and PTB.[15,16] In a recent study, it was shown that BV-associated bacterium 1(BVAB1), *Prevotella* cluster 2, *S. amnii* and TM7-H1, and other taxa may have roles in the etiology of PTB.[17]

Cervical length measurement by transvaginal sonography (TVS CL) is one of the most common tests to predict preterm delivery. Knowledge of cervical length in women with threatened preterm labor may improve outcome but data are limited.[18]

Albumin is abundant in human plasma and acts as a buffer for toxic molecules. The N-terminus of albumin binds nucleic acids, lipids, other proteins, and metals. In ischemia, the structure of albumin changes. When ischemia develops, free oxygen radicals emerge in the environment and damage the N terminus of albumin. It becomes difficult for albumin affected by ischemia to bind divalent metals in the N-terminus,[19] and this new molecule whose structure has changed is called ischemia-modified albumin (IMA).[20]

Ischemia modified albumin is used in cardiac ischemic diseases to determine the early stages of ischemia in which necrosis has not yet occurred. It has been claimed that it increases in the early stages in response to ischemia and will prevent the progression of myocardial damage. It has been shown that IMA levels are higher in pregnant women compared with nonpregnant women.[21] Also, IMA increases in cases where placental perfusion is impaired during pregnancy and oxidative stress and inflammation increase.[22] In cases of increased oxidative stress where this balance cannot be achieved, it may cause pathologies such as pre-eclampsia, intrauterine growth restriction (IUGR), preterm labor, and spontaneous abortion.[23] Ischemia modified albumin increases in pregnancies complicated by early pregnancy loss,[22] recurrent pregnancy loss,[24] hyperemesis gravidarum,[25] gestational diabetes,[26,27] pre-eclampsia,[28] small for gestational age (SGA) fetuses[29] and IUGR.[30] However, there is no study investigating the significance of IMA in preterm labor. It is proposed that the oxidative stress and inflammation are related to the pathogenesis of preterm birth in various studies.[31] Increase of IMA in preterm birth seems to be related to the increase of oxidative stress and inflammation in preterm birth rather than having a role in the pathogenesis of preterm birth.

Biglycan and decorin are proteoglycans found in the intermediate and reticular layers of human fetal membranes.[32] These proteoglycans form the extracellular matrix. The extracellular matrix increases the tensile strength of connective tissue.[33,34] It stabilizes the architecture of tissues by binding to decorin collagen fibres.[33–36] Biglycan destabilizes the decorin-collagen relationship.[34,35] During the 3rd trimester of pregnancy and active labor, the ratio of biglycan to decorin increases in fetal membranes. This increased rate is thought to contribute to the mechanical weakening of the membranes.[37] Premature rupture of fetal membranes (PPROM) was observed in the 2nd trimester of pregnancy in asymptomatic pregnant women with increased serum biglycan levels in the following weeks of pregnancy. Also, it was found that while biglycan was high in these pregnant women, serum decorin levels decreased.[38] In mouse studies, in models with genetic mutations and lack of informative or decorin, when biglycan decreases, decorin was found to be higher.[39] It is thought that these two molecules are compensating for each other. However, this coordination could not be demonstrated in human fetal membranes.[24] The relationship between increased decorin levels and decorin to biglycan ratio and the increase of these in maternal serum has not been explained in the literature. Uzun Cilingir et al. found increased maternal serum and placental tissue levels of preeclamptic women in their study, which included women in the 3rd trimester. Although a correlation analysis has not been performed for the placental and maternal serum decorin levels in this study, it is shown that both increase concurrently.[40] However, this relationship has not been performed yet for biglycan and decorin to biglycan ratio in the literature. Probably, increased decorin levels in membranes, hence the increase in decorin to bigylcan ratio in membranes, are possibly reflected and to amniotic fluid and passage to the maternal serum during preterm birth.

In the present study, the levels of IMA, biglycan, and decorin in the serum of pregnant women hospitalized for threatened preterm labor (preterm delivery within 24 hours, 48 hours, 7 days, 14 days, $\leq$ 35 gestational weeks, and $\leq$ 37 gestational weeks after admission) were examined. Serum levels were compared between women having preterm and term delivery.

Methods

The present prospective cohort study was conducted between December 2019 and December 2020 at the Evliya Çelebi Training and Research Hospital of the Kütahya Health Sciences University. Ethics committee approval was obtained prior to the study (2019-01/1). Informed consent was obtained from every patient included in the study. The present trial was registered at ClinicalTrials.gov, number NCT04451928

Fifty-one consecutive pregnant women aged between 18 and 42 years old who were hospitalized with a diagnosis of threatened preterm labor and who had a singleton pregnancy between the 24th and 36th6 weeks were included in the present study. Women were also enrolled only if they had intact amniotic membrane, uterine contraction $\geq$ 3 times in 30 minutes, cervical dilatation $<$ 3 cm, and cervical effacement $<$ 80%.[9] Women were excluded if they had multiple pregnancies, PPROM, abnormal placentation (such as placenta previa), uterine anomaly, maternal heart disease, inflammatory or infectious disease, pre-eclampsia, fetal growth restriction, congenital fetal anomaly, polyhydramnios, acute chorioamnionitis, and medically-induced preterm delivery.

Patients admitted to the hospital due to threatened preterm labor primarily received bed rest and hydration. When cervical changes persisted or contractions continued after 2 hours after intravenous hydration, tocolytic treatment was started. Calcium channel blockers were used as a tocolytic drug when needed. Maternal corticosteroid (12 mg intramuscular betamethasone within 24 hours) was given when needed to accelerate fetal lung development. Forty-eight hours after the steroid administration, tocolysis was stopped. Demographic data of the patients were recorded. Patients were followed until delivery. The gestational week was determined according to the last menstrual date and confirmed by early ultrasonographic measurements. The gestational week at birth and the time between admission to the hospital and birth were recorded. Delivery time was divided into groups as preterm delivery within 24 hours, 48 hours, 7 days, 14 days, $\leq$ 35 gestational weeks, and $\leq$ 37 gestational weeks after admission.[41–45] Mode of delivery, birth weight and APGAR score were recorded.

Spontaneous preterm labor (sPTL) leading to PTB is a heterogeneous condition, with a multifactorial etiology. Various different mechanisms with different pathways, including increased contractility, membrane rupture, and cervical changes leads to preterm birth.[46] Due to its multifactorial nature, it has not been possible to predict sPTL and PTB with a single marker. So, combinations of various markers were

evaluated in similar prediction studies. That is why we also tried to use a combination of several different markers each concerning different etiopathogenetic pathways. Our proposed model and the serum markers used in our study are not in daily clinical use in predicting threatened preterm delivery. Additionally, we do not claim the clinical use of our results in until future stronger studies support our results.

Clinically available predictive methods for women with symptoms of preterm labor are sonographic transvaginal cervical length (CL) measurement and bedside biomarker tests in cervical/vaginal fluid, such as fetal fibronectin (fFN), phosphorylated insulin-like growth factor binding protein-1 (phIGFBP-1 or Actim Partus), or placental alpha microglobulin-1 (PAMG-1 or Partosure).[47] However, the utility of these tests has not been validated in either large or randomized clinical trials.

Similar to our study, studies in the literature using various combinations of serum or vaginal biomarkers with CL measurement for prediction of preterm delivery in threatened preterm labor also performed serum biomarker measurements as early as possible at the time of diagnosis of threatened preterm birth.[48]

Cervical length measurement by transvaginal sonography during evaluation for preterm labor symptoms was measured with a 4 to 10 MHz transvaginal probe (Toshiba Medical Systems Corporation, Japan) with an empty bladder. Research personnel performing transvaginal CL measurement were trained, and all images were reviewed for adequacy and accuracy using the protocol described by Iams et al. at the time of image ascertainment.[49] The shortest CL measurement was used for each patient.

Blood withdrawal for serum biomarkers in our study was performed as soon as the threatened labor diagnosis was confirmed when uterine contractions with cervical changes persisted after 2 hours of bed rest and hydration.

Venous blood samples were taken from the antecubital vein of the patients. Blood samples were transferred to non-heparinized tubes. The tubes were centrifuged at 1,500 xg for 10 minutes. Serum samples obtained afterwards were stored in a freezer at - 80°C until analysis.

Levels of serum IMA were assayed with an ELISA kit (Human [IMA] ischemia modified albumin, Cat. No: E-EL-H5422, Elabscience, Texas, USA). Results were expressed as ng per mL of serum (ng/mL). The sensitivity of this kit was 1.88 ng/mL. Intra- and inter-CV were 5.2 and 6.4%, respectively.

Levels of serum DCN were assayed with an ELISA kit (Human [DCN] decorin, Cat. No: E-EL-H2248, Elabscience, Texas, USA). Results were expressed as ng per mL of serum (ng/mL). The sensitivity of this kit was 0.75 ng/mL. Intra- and inter-CV were 5.4 and 6.7%, respectively.

Levels of serum BGN were assayed with an ELISA kit (Human [BGN] biglycan, Cat. No: E-EL-H6091, Elabscience, Texas, USA). Results were expressed as pg per mL of serum (pg/mL). The sensitivity of this kit was 18.75 pg/mL. Intra- and inter-CV were 5.3 and 6.2%, respectively.

For data analysis, the IBM SPSS Statistics for Windows version 21.0 (IBM Corp., Armonk, NY, USA) and R statistical computing software (version 3.6.1, https://www.r-project.org/) were used. Data are presented as mean ± standard deviation (SD) and median (25^{th} percentile; 75^{th} percentile). Conformity to normal distribution was evaluated with the Shapiro-Wilk or the Kolmogorov-Smirnov test. Quantitative data of the groups were compared with the Student t-test or the Mann-Whitney U test. Univariate logistic regression analyses were performed to determine associations between each individual marker and preterm delivery. Multivariate logistic regression analysis of candidate serum biomarkers along with CL was performed to determine a combined model for predicting preterm delivery within 24 hours, 48 hours, 7 days, 14 days, ≤ 35 gestational weeks, and ≤ 37 gestational weeks after admission. Receiver operating characteristic (ROC) curve analysis was performed to calculate the area under the curve (AUC) values for the different markers and the combined model. A value of $p < 0.05$ was considered statistically significant.

Results

Forty-nine percent (26/51) of the threatened preterm labor cohort group resulted in preterm delivery (< 37 weeks). Characteristics of the study population of threatened preterm labor are shown in ►**Table 1**. A total of 29.4% (15/51) of the newborns needed neonatal intensive care. A total of 39.2% (20/51) of the newborns were female. A total of 56.9% (29/51) of the deliveries were performed vaginally. There was a history of preterm delivery in 29.4% (15/51) of the cases.

In the present study, IMA and biglycan levels were found to be higher and decorin levels were lower in women admitted to the hospital with threatened preterm labor and who had a preterm delivery within 48 hours compared to preterm delivery after 48 hours (respectively, $p = 0.043$, $p = 0.029$, and $p = 0.014$). Diagnostic indices of three candidate protein biomarkers, CL, and the final combined model for predicting preterm delivery within 24 hours, 48 hours, 7 days, 14 days, ≤ 35 weeks of gestation, and ≤ 37 weeks of gestation women with threatened preterm labor in the total cohort are shown in ►**Table 2** and ►**Table 3**.

Serum IMA level was found to be significant in predicting preterm delivery within 24 hours, 48 hours, 7 days, and 14 days after admission as a result of multivariate logistic regression analysis (respectively, $p = 0.039$, $p = 0.040$, $p = 0.031$, and $p = 0.031$). Decorin level was significant in predicting preterm delivery within 24 hours, 48 hours, 7 days, 14 days, and ≤ 37 gestational weeks after admission (respectively, $p = 0.042$, $p = 0.022$, $p = 0.025$, $p = 0.025$, and $p = 0.047$). Biglycan level was insignificant in predicting preterm delivery within 24 hours, 48 hours, 7 days, 14 days, ≤ 35 gestational weeks, and ≤ 37 gestational weeks after admission ($p > 0.05$). Cervical length was significant in predicting preterm delivery within 24 hours, 48 hours, 7 days, 14 days, ≤ 35 gestational weeks, and ≤ 37 gestational weeks after admission ($p = 0.018$, $p = 0.016$, $p = 0.044$, $p = 0.044$, $p = 0.020$, and $p = 0.012$, respectively). Area under the curve values of the final combined model 1 (3 biochemical markers) for predicting preterm delivery

Table 1 Characteristics of the study population of threatened preterm labor ($n = 51$)

Age (years old)	29.13 ± 6.65
	29.00 [23.0–34.0]
BMI (kg/m2)	22.72 ± 3.66
	22.6 [20.3–25.0]
Gravidity	2.27 ± 1,11
	2 [1–3]
Parity	1.01 ± 0.88
	1 [0–2]
Gestational age (weeks)	34.23 ± 2.71
	34.86 [33.43–36.29]
IMA (ng/mL)	43.17 ± 14.08
	50.53 [34.34–49.07]
Decorin (ng/mL)	14.87 ± 6.27
	12.65 [9.53–18.73]
Biglycan (pg/mL)	465.07 ± 175.68
	435.0 [382.0–477.0]
Cervical length (mm)	30.81 ± 6.84
	32.0 [28.0–35.2]
Gestational age at delivery (weeks)	36.94 ± 2.70
	37.14 [36.29–39.0]
Newborn weight (g)	2951.09 ± 552.30
	3130.0 [2710.0–3280.0]
Steroid/tocolytic administration	50.98% (26/51)
Neonatal intensive care need	39.2% (20/51)

Abbreviations: BMI, body mass index; IMA, ischemia modified albumin. Data are presented as mean ± standard deviation, median [interquartile range] or number (percentage).

within 24 hours, 48 hours, 7 days, 14 days, ≤ 35 gestational weeks, and ≤ 37 gestational weeks after admission were 0.88 (0.78–0.98), 0.86 (0.76–0.96), 0.88 (0.78–0.97), 0.86 (0.76–0.96), 0.69 (0.50–0.88), and 0.89 0.81 (0.68–0.93), respectively. Area under the curve values of the final combined model 2 (CL plus other 3 biochemical markers) for predicting preterm delivery within 24 hours, 48 hours, 7 days, 14 days, ≤ 35 gestational weeks, and ≤ 37 gestational weeks after admission were 0.95 (0.89–1.00), 0.93 (0.86–0.99), 0.91 (0.83–0.98), 0.92 (0.85–0.99), 0.82 (0.69–0.96), and 0.89 (0.80–0.98), respectively.

Discussion

In the present study, IMA and biglycan levels were higher and decorin levels were lower in women admitted to the hospital with threatened preterm labor and who had a preterm delivery within 48 hours compared with those who gave birth after 48 hours (respectively, $p = 0.043$, $p = 0.029$, and $p = 0.014$). In predicting the diagnostic indices of the final combined model (3 candidate protein biomarkers plus CL) for predicting preterm delivery within 24 hours, 48 hours, 7 days, 14 days, ≤ 35 gestational weeks, and ≤ 37 gestational weeks after admission, prediction AUC (95% confidence interval [CI]) values were 0.95 (0.89–1.00), 0.93 (0.86–0.99), 0.91 (0.83–0.98), 0.92 (0.85–0.99), 0.82 (0.69–0.96), and 0.89 (0.80–0.98), respectively.

Numerous studies have been conducted in the literature to predict preterm birth in women in threatened preterm labor. However, there is no single or combined screening method for high-sensitivity preterm birth to clearly identify women at risk of preterm birth.[3–10] In the previously conducted studies, it was shown that while the levels of biglycan increase in fetal membranes after labor, decorin levels decrease.[37] Atalay et al. found that serum decorin has a limited effect in the prediction of preterm delivery within 1 week or before 34 weeks. But a combination of serum decorin with CL measurements predicted preterm delivery before 37 weeks.[43] In the study by Underhill and et al., patients with PPROM had high serum biglycan levels and low decorin levels.[38] In the present study, in the univariate analysis, similar to Underhill et al., decorin and biglycan were found to be significant in predicting preterm delivery. However, in the multivariate analysis, biglycan was not significant in predicting preterm delivery. In the study by Atalay et al., pregnant women with 24 to 32 weeks of gestation are included, similarly to our study.[43] However, their study was a case control study, unlike ours, which we designed as a cohort study which makes it not right to make comparison between studies.

In the study by Underhill et al. (which is a retrospective case control study), PPROM risk was tried to be predicted by the serum biglycan and decorin levels in 15 to 20 weeks of pregnancy.[38] They found an AUC value of 0.659 for biglycan and 0.563 for decorin. However, in our study, AUC values range between 0.69 and 0.73 for biglycan and between 0.61 and 0.87 for decorin for 5 different primary outcomes, as can be seen in ►**Table 2**. Thus, the study design of Underhill et al. and ours differ considerably, which makes it not right to make comparison between studies. The reason that our AUC values are higher than the values in the study of Underhill et al. is that our cohort consists of women with threatened preterm labor.

Cervical length measurement by transvaginal sonography is one of the most common tests to predict preterm delivery. Knowledge of cervical length in women with threatened preterm labor may improve outcome but data are limited.[18] Ness et al. stated in their study that > 50% of pregnant women who were admitted to the hospital with threatened preterm labor and who had TVS CL ≥ 30 mm were discharged and the probability of delivery within 7 days after admission was < 2%.[50] In the literature, it was aimed to increase the prediction rates of preterm delivery by adding markers to the TVS CL measurement to determine the risk of preterm delivery in pregnant women presenting with threatened preterm labor. However, in routine clinical practice, there is so far no solid marker in addition to TVS CL measurement to determine preterm delivery risk in symptomatic women with threatened labour.[43] In the present study, the AUC values of the TVS CL measurement in preterm delivery

Table 2 Diagnostic indices of three candidate protein biomarkers, cervical length and the final combined model for predicting spontaneous preterm birth within 24 hours, 48 hours, and 7 days with preterm labor in the total cohort

	24 hours			48 hours			7 days		
Preterm birth ratio	**25.5% (13/51)**			**29.4% (15/51)**			**37.3% (19/51)**		
	OR (95%CI)	***p-value***	**AUC (95%CI)**	**OR (%95CI)**	***p-value***	**AUC (95%CI)**	**OR (%95CI)**	***p-value***	**AUC (95%CI)**
IMA	1.07 (1.02–1.15)	**0.014***	0.69 (0.51–0.872)	1.07 (1.02–1.14)	**0.014***	0.68 (0.50–0.86)	1.07 (1.02–1.15)	**0.013***	0.68 (0.52–0.84)
Decorin	0.88 (0.75–1.00)	0.080	0.67 (0.51–0.83)	0.85 (0.72–0.97)	**0.032***	0.72 (0.57–0.8679)	0.85 (0.73–0.95)	**0.014***	0.73 (0.59–0.87)
Biglycan	1.01 (1.00–1.02)	**0.019***	0.74 (0.56–0.91)	1.01 (1.00–1.02)	**0.024***	0.70 (0.52–0.87)	1.01 (1.00–1.02)	**0.019***	0.71 (0.56–0.87)
Cervical Length	0.80 (0.67–0.90)	**0.002***	0.89 (0.80–0.98)	0.82 (0.70–0.92)	**0.003***	0.85 (0.75–0.96)	0.85 (0.74–0.94)	**0.007***	0.80 (0.67–0.93)
Combined model 1									
IMA	1.11 (1.01–1.25)	**0.048***	0.88 (0.78–0.98)	1.09 (1.01–1.21)	0.059	0.86 (0.76–0.96)	1.10 (1.01–1.22)	**0.043***	0.88 (0.78–0.97)
Decorin	0.84 (0.63–1.03)	0.157		0.81 (0.61–0.98)	0.069		0.82 (0.65–0.97)	**0.043***	
Biglycan	1.02 (1.01–1.04)	**0.028***		1.01 (1.00–1.03)	**0.033***		1.02 (1.01–1.03)	**0.015***	
Combined model 2									
IMA	1.17 (1.03–1.42)	**0.039***	0.95 (0.89– 1)	1.13 (1.02–1.29)	**0.040***	0.93 (0.86–0.99)	1.12 (1.02–1.25)	**0.031***	0.91 (0.83–0.98)
Decorin	0.71 (0.47–0.93)	**0.042***		0.74 (0.55–0.93)	**0.022***		0.79 (0.61–0.95)	**0.025***	
Biglycan	1.02 (1.00–1.04)	0.100		1.01 (1.00–1.03)	0.161		1.01 (1.00–1.03)	0.061	
Cervical Length	0.65 (0.41–0.86)	**0.018***		0.74 (0.55–0.91)	**0.016***		0.83 (0.67–0.98)	**0.044***	

Abbreviations: AUC, area under the curve; CI, confidence interval; IMA, ischemia modified albumin; OR, odds ratio.
Statistically significant comparisons were marked with *.

Table 3 Diagnostic indices of three candidate protein biomarkers, cervical length, and the final combined model for predicting spontaneous preterm birth within 14 days of sampling of and before 35 weeks of gestation, before 37 weeks of gestation women with preterm labor in the total cohort

	14 days			< 35 weeks			< 37 weeks		
Preterm birth ratio	**45.1% (23/51)**			**23.5% (12/51)**			**51.0% (26/51)**		
	OR (95%CI)	***p-value***	**AUC (95%CI)**	**OR (95%CI)**	***p-value***	**AUC (95%CI)**	**OR (95%CI)**	***p-value***	**AUC (95%CI)**
IMA	1.07 (1.02–1.15)	**0.013***	0.67 (0.52–0.82)	1.01 (0.96–1.05)	0.827	0.51 (0.31–0.71)	1.04 (1.00–1.10)	0.085	0.60 (0.44–0.76)
Decorin	0.85 (0.73–0.95)	**0.014***	0.72 (0.57– 0.86)	0.93 (0.81–1.04)	0.245	0.61 (0.43–0.79)	0.87 (0.77–0.96)	**0.012***	0.71 (0.56–0.86)
Biglycan	1.01 (1.00–1.02)	**0.019***	0.73 (0.59–0.88)	1.00 (1.00–1.01)	0.271	0.70 (0.54–0.86)	1.01 (1.00–1.02)	**0.017***	0.71 (0.57–0.85)
Cervical Length	0.85 (0.74–0.94)	**0.007**	0.83 (0.72–0.95)	0.88 (0.78–0.97)	**0.014***	0.82 (0.70–0.93)	0.77 (0.63–0.89)	**0.002***	0.81 (0.68–0.93)
Combined model 1									
IMA	1.08 (1.01–1.18)	0.060	0.86 (0.76–0.96)	0.98 (0.92–1.04)	0.503	0.69 (0.50–0.88)	1.03 (0.97–1.10)	0.386	0.82 (0.70–0.94)
Decorin	0.85 (0.71–0.99)	0.051		0.92 (0.80–1.04)	0.215		0.88 (0.76–0.99)	**0.046***	
Biglycan	1.02 (1.01–1.03)	**0.008***		1.00 (1.00–1.01)	0.213		1.01 (1.00–1.02)	**0.020***	
Combined model 2									
IMA	1.12 (1.02–1.25)	**0.031***	0.92 (0.85–0.99)	0.99 (0.93–1.05)	0.719	0.82 (0.69–0.96)	1.04 (0.96–1.14)	0.319	0.89 (0.80–0.98)
Decorin	0.79 (0.61–0.95)	**0.025***		0.91 (0.78–1.04)	0.193		0.85 (0.71–0.99)	**0.047***	
Biglycan	1.01 (1.00–1.03)	0.061		1.00 (0.99–1.00)	0.966		1.01 (1.00–1.02)	0.192	
Cervical Length	0.83 (0.67–0.98)	**0.044***		0.87 (0.76–0.97)	**0.020***		0.81 (0.67–0.94)	**0.012***	

Abbreviations: AUC, area under the curve; CI, confidence interval; IMA, ischemia modified albumin; OR, odds ratio.
Statistically significant comparisons were marked with *.

prediction were > 0.8. In addition to the TVS CL measurement, the preterm delivery prediction of the combined model, which was created by adding IMA, decorin, and biglycan, was higher than the TVS CL measurement alone.

In addition to TVS CL measurement, the most investigated measurement in the literature is fetal fibronectin. Although many studies implicated the role of fetal fibronectin in vaginal secretions in prediction of preterm delivery in symptomatic women, routine clinical use has not gained widespread use. Fetal fibronectin testing in singleton gestations with threatened preterm labor is not associated with the prevention of preterm birth or improvement in perinatal outcome but is associated with higher costs.[51] In addition to the cost and questionable effectiveness, fetal fibronectin results may be affected by coitus within 48 hours preceding testing.[52]

An AUC value of 0.78 was determined in predicting preterm delivery before 34 weeks using quantitative fetal fibronectin for symptomatic high-risk women in a large prospective study.[52] In another prospective study, the AUC was 0.95 using a model combining TVS CL measurement with fetal fibronectin in symptomatic cases.[51] Our combined model using three serum biochemical markers in addition to TVS CL had nearly the same AUC value.

In a recent meta-analysis, the AUC for predicting preterm delivery at ≤ 7 days for placental alpha microglobulin-1 (PAMG-1), fetal fibronectin (fFN) and insulin-like growth factor-binding protein-1 (phIGFBP-1) were 0.961, 0.874, and 0.801, respectively, in symptomatic women.[53] In a recent study, using an application (QUiPP App prototype) that uses fetal fibronectin and TVS CL measurement for the prediction of preterm delivery, AUC values were 0.96, 0.85, 0.77, 0.91, and 0.92 for preterm delivery < 30 weeks, < 34 weeks, < 37 weeks, < 1 week, and < 2 weeks, respectively.[54] Although we obtained lower AUC values for each single marker, our combined model reached an AUC of 0.95, which is compatible with the highest values in the relevant literature. However, we are aware that our findings need to be substantiated given the small number of subjects.

As a result, IMA and biglycan levels were found to be higher and decorin levels lower in women admitted to the hospital with threatened preterm labor and who had preterm birth within 48 hours compared with those who gave birth after 48 hours. Preterm delivery prediction of the combined model created by adding IMA, decorin, and biglycan in addition to the TVS CL measurement in pregnant women presenting with threatened preterm labor was higher than the TVS CL measurement alone for all women in the present study. The results show that serum IMA, decorin, and biglycan concentrations and the TVS CL measurement may be a useful marker for monitoring preterm delivery in symptomatic women.

The smaller case number is the major limitation of our study. Additionally, it needs to be noted that the predictive performance and utility of the test would be different if the concept of our study was to predict preterm birth by measuring these serum biochemical markers and CL in the 2nd trimester before the threatened preterm labor has taken place. We believe that an important contribution to the literature for predicting preterm labor can be made if our parameters could be studied in a low-risk population during the 2nd trimester.

Contributions

Surgical and medical practices: Biyik I., Soysal C., OU, OI, Karaagac O. H.; Concept and design: Biyik I., Oztas E., Keskin N., Gelisgen R., Uzun H.; Data collection or processing: Biyik I., Soysal C., OU, Karaagac O. H., Durmus S., Isiklar O. O.; Analysis or interpretation: Biyik I., OI, Oztas E., Isiklar O. O.; Literature search: Biyik I., Durmus S., Gelisgen R., Uzun H.; Writing: Biyik I., Oztas E., Keskin N..

Conflict of Interests

The authors have no conflict of interests to declare.

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Arabin-pessary or McDonald Cerclage in Cervical Shortening?

Pessário de Arabin ou cerclagem McDonald no encurtamento cervical?

Aytaj Jafarzade[1] Sveta Aghayeva[1] Tamer Mungan[1] Aydan Biri[1] Osman Ufuk Ekiz[2]

[1] Obstetrics and Gynecology Department, Koru Hospital Ankara, Ankara, Turkey.
[2] Statistic Department, Gazi University, Yenimahalle, Ankara, Turkey.

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Address for correspondence Aytaj Jafarzade, MD, Koru Hospital Ankara, Obstetrics and Gynaecology Department, Kızılırmak, 1450. Sk. No:13, 06510 Çankaya/Ankara, Turkey (e-mail: jafarzade_aytac@yahoo.com).

Abstract

Objective The aim of the present study is to compare the effectiveness of Arabin pessary and McDonald cervical cerclage on preterm delivery.

Methods We conducted a retrospective analysis of data from patients who underwent either Arabin pessary or McDonald cerclage between January 1, 2019, and January 1, 2023. A total of 174 patients were included in the study, with 31 undergoing Arabin pessary and 143 receiving cervical cerclage using the McDonald technique in singleton pregnant women with cervical insufficiency, which applied between 14 and 22 gestational weeks. We included singleton pregnant women with normal morphology, and with normal combined test. The primary outcome was the impact of each method on preterm delivery (< 34 gestational weeks).

Results The weeks of cervical cerclage or pessary application were compatible with each other ($p < 0.680$). The pessary group had a statistically significant longer time to delivery compared with the Cerclage group (cerclage group mean 30.8 c 7.1 standard deviation [SD] versus pessary group mean 35.1 ± 4.4 SD; $p < 0.002$). A statistically significant difference was found between the pessary and cerclage groups in terms of delivery at < 34 weeks ($p = 0.002$). In patients with cervical length between 25 and 15mm and < 15mm, no significant difference was found between the pessary and cerclage groups in terms of delivery week ($p < 0.212$; $p < 0.149$). Regardless of the technique applied, no statistically significant difference was observed between cervical length and birth < 34 weeks.

Conclusion Our study found that pessary use for cervical insufficiency is statistically more effective than cervical cerclage surgery in preventing preterm births < 34 weeks in singleton pregnancy.

Keywords
- cerclage
- pessary
- cervical insufficiency
- McDonald cerclage

Introduction

Preterm birth is a serious condition that results in high rates of mortality and morbidity[1] and affects ~ 10% of all pregnancies.[2] Preterm labor requires high health expenditures and to prevent cervical insufficiency, which is one of the causes of preterm labor, cervical length is measured by transvaginal ultrasonography at the end of the 1st trimester and the beginning of the 2nd trimester.[3] Women with

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asymptomatic cervical shortening detected by this low-cost method have a high risk of preterm delivery.[4] There is a meta-analysis that advocates its application during routine controls[5] and argues that routine screening is more appropriate only for high-risk patients, since it does not significantly reduce preterm birth rates.[6] Cervical insufficiency is when dilation and slackening of the cervix (≤ 25 mm) occur before 24 weeks gestation without contraction, bleeding, infection, rupture of the membranes, or labor and history one or more second trimester losses, or prior spontaneous preterm birth at < 34 gestational weeks.[7]

Treatment options for patients with cervical shortening include cervical cerclage (abdominal and vaginal), pessary, and observation/medical management. Vaginal cerclage can be performed using either the Shirodkar or the McDonald technique.[8] The pessary applied in cases of cervical insufficiency prevents the cervical shortening and dilation by changing the angle between the uterine lower segment and the cervix.[9] It is minimally invasive, easily accessible, can be applied in an ambulatory setting, and is relatively low-cost, although it may have some negative effects on pregnancy.[2] Studies have shown that the pessary changes the uterocervical angle and reduces cervical dilation and effacement.[10]

The main aim of our study is to compare the rate of preterm birth (< 34 weeks of gestation) between pessary and McDonald cerclage methods in patients diagnosed with cervical insufficiency.

Methods

Our study was conducted retrospectively at the Koru Ankara Hospital between January 1, 2019, and January 1, 2023. We included singleton pregnant women with cervical insufficiency (diagnosed via transvaginal sagittal B-mode ultrasound), normal morphology, and who had undergone the combined test. Patients with uterine malformation, stillbirths, fetal anomalies, preeclampsia, intrauterine grow restriction, placenta previa, and placenta accreta spectrum were excluded from the study.

Diagnostic Methods for cervical insufficiency (CI)

(a) **Ultrasound-based diagnosis:** Used when there have been ≥ 1 pregnancy losses or preterm births of 14 to 36 weeks in the past and a cervical length (CL) < 25 mm is measured by transvaginal ultrasound (TVU) before 24 weeks of gestation.

(b) **Physical examination-based diagnosis:** Used when painless cervical dilatation or prolapsed fetal membranes have been detected on manual or speculum examination before 24 weeks of gestation regardless of whether a history of midtrimester pregnancy loss or preterm birth exists.

(c) **History-based diagnosis:** Used when there was painless cervical dilatation, leading to recurrent miscarriages in the second trimester and preterm births without other reasons.

Ultrasonography and physical examination were performed simultaneously for those with cervical insufficiency diagnosis. We included patients who underwent pessary or McDonald cerclage due to cervical insufficiency in the study and compared their results. We defined births < 34 weeks as births primary results. Secondary outcomes were the effect of progesterone use on the week of birth, delivery week (< 24, < 28, and < 37), and birthweight.

In patients without vaginal infection, the pessary was placed in the examination room in accordance to the position of the cervical neck, without the need for anesthesia. Following the procedure, the patient was observed for several hours to ensure that there was no discomfort, bleeding, or uterine activity. A speculum inspection was performed to ensure that the pessary was fitted correctly. The pessary was removed in cases of vaginal bleeding, pain and soreness, or rupture of membranes.

All patients underwent cervical cerclage using the McDonald technique while under analgesia. The cervical neck was held with ovarian forceps, carefully pushed in, and sutured at 12, 9, 6, and 3 o'clock with a 5-mm Mersilene tape. Patients who experienced rupture of the amniotic membrane within 48 hours following the procedure were excluded from the study.

We performed statistical analysis using IBM SPSS Statistics for Windows, version 22.0 (IBM Corp., Armonk, NY, USA). Continuous variables were expressed as mean, median, and standard deviation (SD) and were compared between groups using the Mann-Whitney U test. Categorical variables were expressed as the number of patients and percentage and were compared between groups using the Pearson chi-squared test for independent attributes or the Fisher exact test if appropriate. A p-value < 0.05 was considered statistically significant.

The present study was conducted in accordance with the principles of the Helsinki Declaration. All subjects participated voluntarily. The participants provided their written informed consent to participate in the present study. The Declaration of Helsinki was adequately addressed, and the study was approved by the Ethics committee of Gazi University.

Results

A total of 174 patients who underwent cerclage with 31 pessary and 143 McDonald technique due to cervical insufficiency were included in the study. In the study, pessary and cerclage application was performed according to the history in 37 patients (21.3%) and based on physical examination plus ultrasound in 137 patients (78.7%). The diagnosis of cervical insufficiency was made according to the history in 15 patients (48.3%) in the pessary group and as a result of physical examination (physical examination and ultrasound were performed simultaneously) in 16 patients (42.7%). In the cerclage group, 22 patients (15.3%) were diagnosed with cervical insufficiency based on their history and 121 patients (84.7%) on physical examination. No statistically significant difference was observed between the two groups in terms of patient

Table 1 Comparison of pessary and cerclage

	Cerclage	Pessary	
	mean	mean	*p-value*
Number of patients (*n*)	143	31	—
Age, years old (mean ± SD)	30.5 ± 4.4	29.9 ± 3.9	0.526
BMI (mean ± SD)	23.8 ± 3.5	23.5 ± 2.2	0.680
Parity (mean ± SD)	0.45 ± 0.8	0.38 ± 0.5	0.651
Number of abortions (mean ± SD)	0.53 ± 1.0	0.64 ± 1.1	0.611

Abbreviation: BMI, body mass index.

age, body mass index (BMI), parity number, and number of abortions (►**Table 1**).

While the cervical effacement was significantly lower in the cerclage group ($p = 0.000$), there was no significant difference in cervical dilatation between the 2 groups ($p = 0.823$). The application weeks of cerclage or pessary were compatible with each other ($p < 0.680$). However, there was a statistically significant difference between the birth weeks of the 2 groups ($p < 0.002$). The birth week of the pessary group was advanced. The use of progesterone was also statistically significant in the pessary group ($p < 0.000$). Nevertheless, there was no significant difference in prenatal mortality rates between the 2 groups ($p < 0.030$) (►**Table 2**).

Birthweights of the babies in the pessary group were statistically significantly higher than those in the cerclage group ($p = 0.002$). In the subgroup analysis, the rate of infants <1,500 g was 16% ($n = 5$) in the pessary group and 42.6% ($n = 61$) in the cerclage group. The birth rate of 1,500 to 2,500 gr infants was 9. 6% ($n = 3$) in the pessary group and 15.3% ($n = 22$) in the cerclage group. Babies with a birthweight > 2,500 gr comprised 74.3% ($n = 23$) in the pessary group and

Table 2 Comparison of pessary and cerclage

	Cerclage	Pessary	*p-value*
	Mean	Mean	
Cervical effacement (cm)	12.9 ± 3.7	18.6 ± 5.2	0.000
Cervical dilatation (cm)	26.4 ± 6.4	26.7 ± 5	0.823
Processing week	18 ± 3.1	17.8 ± 2.4	0.680
Birth week	30.8 ± 7.1	35.1 ± 4.4	0.002
Progesterone user	0.8 ± 0.4	1 ± 0	0.000
Prenatal death	0.24 ± 0.4	0.06 ± 0.2	0.030
Birth weight, gr(mean)	1855.85 ± 98.668	2566.48 ± 156.581	0.002

Table 3 Comparison of the Pessary and Serklyaj groups according to the birth weight of the patients

	Cerclage (*n*)	Pessary (*n*)	*p-value*
< 1,500 gr	61	5	0.005[c]
1,501 to 2,500gr	22	3	0.411[c]
> 2,501gr	60	23	0.001[c]

[c]Chi-squared Test.

41% ($n = 61$) in the cerclage group. The number of patients with a birthweight < 1,500 gr and > 2,500 gr in the cerclage group was statistically significantly higher than in the pessary group (►**Table 3**).

When we compared the results of patients using progesterone and not using progesterone between the groups in which cervical cerclage and pessary were applied, and compared the pessary and cerclage groups using progesterone, the gestational week of the pessary group was significantly advanced. However, no significant difference was observed between the cerclage group using and not using progesterone (►**Table 4**).

When we examined the cervical length of the patients who underwent the procedure as 25–15 mm and < 15mm, the delivery week of the patients with a cervical length of < 15 mm and who had pessary applied on was significantly advanced compared with the other groups (►**Table 5**). There was no statistically significant difference between the week of birth of patients with a cervical length of 25 to 15 mm based on cervical length and who underwent cerclage or pessary ($p < 0.212$). The week of delivery was not statistically significant in patients with cervical length <15 mm and pessary applied ($p < 0.149$). There was no statistically significant difference between cervical length and babies born < 34 weeks, regardless of the technique applied (►**Table 5**).

When we compared the two groups based on the week of birth, rate of birth < 24 weeks of gestation was statistically higher in the cerclage group, and the rate of births > 37 weeks of gestation was statistically higher in the pessary group. There was no statistically significant difference between the other subgroups (►**Table 6**).

However, Kaplan-Meier analysis of survival for the pessary and cerclage groups showed less preterm births before 34 weeks in a singleton pregnancy in the pessary group. The Breslow-Wilcoxon test was used to determine the significant difference between the groups ($p = 0.002$) (►**Fig. 1**).

Table 4 Birth weeks of progesterone user and non-user patients among patients who underwent cerclage and pessary

	Cerclage	Pessary	
	Mean	Mean	*p-value*
Progesterone user (Birth week)	30.8 SD ± 7.1	35.1 ± 4.5	0.0021
Progesterone nonuser (Birth week)	30.9 ± 7.3	0	—

Table 5 Birth week by cervical length

	CERCLAGE			PESSARY			
Cervical length (mm)	*n*	Birth week (mean)	Rate birth < 34 w (%)	*n*	Birth week (mean)	Rate birth < 34 w (%)	*p-value*
I 25-15 mm	32	30.5225	(*n* = 12) 37.5%	23	34.3750	(*n* = 5) 21.7%	0.212[c]
II < 15 mm	111	32.0000	(*n* = 57) 51.4%	8	35.3913	(*n* = 2) 25%	0.149[c]
Total	143			31			

[c]Chi-squared Test.

Table 6 Comparison of groups based on birth weeks in both groups

	CERCLAGE		PESSARY		
Birth week	*n*	Rate (%)	*n*	Rate (%)	*p-value*
< 24	34	23.8	2	6.5	0.031[c]
24 to 27 + 6	16	11.2	1	3.2	0.175
28 to 33 + 6	19	13.3	4	12.9	0.954
34 to 36 + 6	24	16.7	3	9.7	0.321
> 37	50	35	21	67.7	0.007
Total	143	100	31	100	

[c]Chi-squared Test.

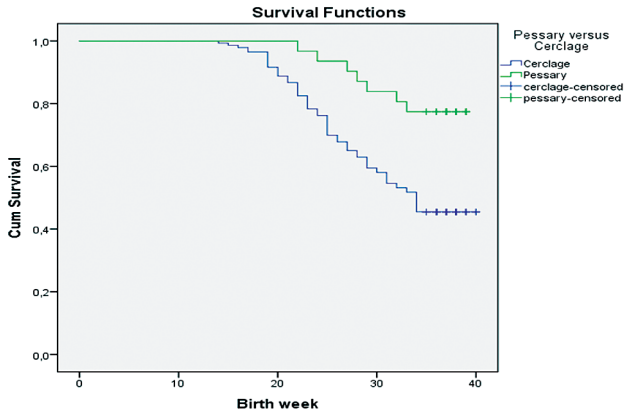

Fig. 1 Comparison of Kaplan-Meier survival analysis for pessary and cerclage groups to evaluate the occurrence of preterm birth before 34 weeks in singleton gestation (Breslow-Wilcoxon $p = 0.02$).

Discussion

In a study conducted by Saccone et al, pregnant women with a cervical length < 25 mm who underwent pessary treatment were compared with those who received observational treatment. The study found that cervical pessary did not reduce the rate of preterm delivery or improve perinatal outcomes.[11]

In our study, we compared the results of pessary and cervical cerclage and found that the delivery week of patients who received pessary treatment was significantly more advanced than those who received cervical cerclage ($p = 0.002$). However, we should consider that the cervical length in the cerclage group was shorter (mean 12.99 ± 3.76 SD for the cerclage group versus mean 18.69 ± 5.23 SD for the pessary group) at the time of pessary and cervical cerclage application. It is important to note that cervical length is known to be the most important factor affecting the week of delivery, when interpreting these results.[12]

In their prospective studies, Archarya et al. demonstrated the effectiveness of pessary in both primiparous and multiparous patients with cervical insufficiency.[13]

In our study, we found no significant difference in the effectiveness of pessary and cervical cerclage between primiparous and multiparous patients. Specifically, we observed no difference in delivery week between the two groups and found that both groups could benefit from pessary and cervical cerclage application.

While the first randomized controlled trial (PECEP)[14] investigating the efficacy of pessary showed that pessary reduces preterm birth rate and perinatal mortality, studies that did not support these results were also published.[15] Later, the effectiveness of pessary in the prevention of preterm labor was demonstrated in randomized controlled and nonrandomized controlled studies.[16]

In our study, some of the patients who underwent cervical cerclage used progesterone while others did not. No statistically significant difference was observed in terms of the week of birth between these two groups, and there was no evidence of progesterone use being beneficial in terms of week of preterm birth. However, as all the patients in the pessary group were using progesterone, a comparison could not be made with the group in which the pessary was applied but progesterone was not used. Nevertheless, when we compared the group that received pessary without progesterone and the group that underwent cervical cerclage without progesterone, we found that the week of delivery was significantly higher in the pessary group.

Melcer et al. conducted a study comparing the results of patients using pessary and progesterone to those using only progesterone due to cervical shortness and found that patients using pessary plus progesterone had a longer gestational week.[17] Although subsequent studies have supported these findings,[18] there is also a study indicating that pessary and progesterone are not more effective than progesterone use alone.[19]

The analysis of cervical length in the two groups (Group I: 25 to 15 mm, Group II: < 15 mm) did not reveal any statistically significant difference between the pessary and cerclage groups. Therefore, our findings did not support the hypothesis that cerclage would be more effective in patients with cervical shortness < 15 mm. This is in contrast to the findings

of Owen et al., who suggested that cervical cerclage would be more effective when the cervical length was < 15 mm.[20] Nicolaides et al. compared pessary and progesterone treatment in cases with cervical shortness and found that pessary was not superior to progesterone.[21] However, in our study, since progesterone was preferred in patients with cervical length < 15 mm, it is possible that this could potentially reduce any benefit of cervical pessary in this group. Combining methods is not recommended due to lack of benefit according to reviews.[22]

In our study, we compared the efficacy of pessary and cerclage in terms of weeks of birth and found birth < 24 weeks of gestation was statistically higher in the Cerclage group, and the rate of births > 37 weeks of gestation was statistically higher in the pessary group. As a primary result, we found a significant difference between the pessary and cerclage groups in terms of delivery at < 34 weeks ($p = 0.002$). Alfirevic et al. compared vaginal progesterone, pessary, and cerclage outcomes in patients with a history of preterm birth and cervical shortness.[23] The study found that all three methods were not superior to each other in terms of preterm birth and perinatal loss. There was no significant difference in the number of preterm births before 37 weeks between the cerclage, vaginal progesterone, and pessary groups.

Mouzakiti et al. conducted a retrospective study comparing the effectiveness of pessary and McDonald cerclage methods for cervical shortening (< 25 mm) with and without funneling and found that pessary had no superiority over cervical cerclage, but the rate of neonatal intensive care unit (NICU) hospitalization was lower in the pessary group.[24]

The limitations of our study are that it is retrospective, with a limited number of cases and does not account for factors that may contribute to cervical insufficiency, such as smoking or ethnicity.

After considering all of this information, we conclude that pessary application appears to be at least as effective as cervical cerclage in preventing preterm labor. Cervical cerclage is a costly procedure that requires general anesthesia and hospitalization, while pessary application is a less expensive procedure which does not require anesthesia or sedation in outpatient conditions and does not require hospitalization afterwards. Therefore, the use of Arabin pessary in appropriate patients appears to be a suitable choice.

Conclusion

The use of pessary application for cervical insufficiency appears to be equally effective as the surgical procedure of cervical cerclage in preventing preterm delivery. Pessary application may be a preferred option in terms of healthcare expenditures due to its noninvasive, easily accessible, and low-cost nature. Nevertheless, randomized controlled studies are necessary to further investigate the efficacy of pessary application compared with cervical cerclage.

Contributions
Jafarzade A.: Conception, design, writer, supervision, data collection, literature review. Aghayeva S.: Data collection and/or processing. Munga T.: Critical review. Biri A.: Critical review. Ekiz O. U.: Analysis and/or interpretation.

Conflict of Interests
The authors have no conflict of interests to declare.

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Correlation between Anatomopathological Aspects and Pelvic Pain in Women with Deep Infiltrating Endometriosis

Correlação entre aspectos anatomopatológicos e dor pélvica em mulheres com endometriose profunda

Daniela Angerame Yela[1] Mariana Sousa Sguerra Silva[1] Larissa Eloy[1] Cristina Laguna Benetti-Pinto[1]

[1] School of Medicine, University of Campinas, Campinas – SP, Brazil.

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Address for correspondence Daniela Angerame Yela, MD, PhD, 101 Alexander Fleming Street, 13083-881, Campinas, SP, Brazil (e-mail: yela@unicamp.br).

Abstract

Objective To correlate the morphological aspects with pelvic pain in women with deep infiltrating endometriosis.

Methods A retrospective study with 67 women with deep endometriosis who underwent surgical treatment in a tertiary hospital from 2007 to 2017. The following variables were considered: age, parity, body mass index, site of involvement, hormonal treatment before surgery, pelvic pain, and morphometric analysis. The histological slides of the surgical specimens were revised and, using the ImageJ software for morphometric study, the percentages of stromal/glandular tissues were calculated in the histological sections.

Results The mean age of the women was 38.9 ± 6.5 years. The mean pain score was 8.8 ± 1.9 and the mean time of symptomatology was 4.7 ± 3.5 years, with 87% of the patients undergoing hormone treatment prior to surgery. The average expression of CD10, CK7, and S100 markers was $19.5 \pm 11.8\%$, $9.4 \pm 5.9\%$, and $7.9 \pm 5.8\%$ respectively. It was found that the greater the expression of CD10, the greater the level of pain ($p = 0.02$). No correlation was observed between the expression of CD10, CK7, and S100 markers and age and duration of symptoms.

Conclusion Women with deep infiltrating endometriosis have a positive association between the level of pain and the fibrosis component in the endometrial tissue's histological composition.

Keywords
- Deep infiltrating endometriosis
- Histology
- Pain

Resumo

Objetivo Correlacionar os aspectos morfológicos com a dor pélvica em mulheres com endometriose profunda.

Métodos Estudo retrospectivo com 67 mulheres com endometriose profunda submetidas a tratamento cirúrgico em hospital terciário de 2007 a 2017. As seguintes variáveis foram consideradas: idade, paridade, índice de massa corporal, local do acometimento, tratamento hormonal antes da cirurgia, dor pélvica e análise

Palavras-chave
- Endometriose profunda
- Histologia
- Dor

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morfométrica. As lâminas histológicas das peças cirúrgicas foram revisadas e, por meio do software ImageJ para estudo morfométrico, foram calculadas as porcentagens de tecidos estromais/glandulares nos cortes histológicos.

Resultados A média etária das mulheres foi de $38,9 \pm 6,5$ anos. O escore de dor médio foi de $8,8 \pm 1,9$ e o tempo médio de sintomatologia foi de $4,7 \pm 3,5$ anos, sendo que 87% das pacientes realizavam tratamento hormonal antes da cirurgia. A expressão média dos marcadores CD10, CK7 e S100 foi de $19,5 \pm 11,8\%$, $9,4 \pm 5,9\%$ e $7,9 \pm 5,8\%$, respectivamente. Verificou-se que quanto maior a expressão de CD10, maior o nível de dor ($p = 0,02$). Não foi observada correlação entre a expressão dos marcadores CD10, CK7 e S100 com a idade e duração dos sintomas.

Conclusão Mulheres com endometriose profunda apresentam associação positiva entre o nível de dor e o componente de fibrose na composição histológica do tecido endometrial.

Introduction

Endometriosis can be conceptualized as the presence of ectopic endometrial tissue, gland and/or stroma outside the uterine cavity inducing a chronic inflammatory reaction.[1] It is a common gynecological pathology, affecting one in every ten women in the general population.[2] Endometriosis can be didactically classified as peritoneal, ovarian, and deep, the latter being defined as the presence of invasion by more than 5 mm of the peritoneal tissue.[3,4]

The clinical picture of endometriosis is mainly determined by pain (dysmenorrhea, dyspareunia, chronic pelvic pain, dyschezia, and dysuria) and infertility. Endometriosis can cause pain through different mechanisms such as inflammation, pressure, adherence, neural involvement, psychological factors, and peritoneal prostaglandins. The pain can be continuous/intermittent, cyclical (related to the menstrual period), or acyclical.[5–7]

The treatment of endometriosis can be clinical, surgical, or combined. The treatment indication must be individualized, and its choice depends on the woman's age, desire for pregnancy, severity of the symptoms, type and location of lesions, and the disease's stage.[3,6] Currently, one of the main surgical indications in the treatment of deep endometriosis is poor response to clinical treatment.[8]

This broad spectrum of response to hormone therapy may be due to different cell populations found in endometriosis foci. It is believed that women who have foci with a greater glandular population in relation to the stromal and/or fibrotic may have a better response to hormone therapy in terms of pain, as well as women whose endometriosis foci have a greater number of estrogen receptors / progesterone.[9]

There is also the possibility that different neural densities and inflammatory cytokines in endometriotic tissue are closely related to pain intensity. The ectopic endometriotic tissue would favor the production of cytokines, generating local inflammation and a greater proliferation of local neural fibers, which would intensify the pain symptoms.[9]

Although the painful manifestation of endometriosis is a frequent complaint, compromising the lives of affected women, its relationship with the degree of endometriosis and the different types of injury has not yet been fully elucidated. Studies correlating the different types of injury, percentage of different types of cells present, and their relationship with drug response may aid the choice of therapeutic options available. Thus, this study aims to correlate morphological aspects with pelvic pain in women with deep infiltrating endometriosis undergoing surgical treatment.

Methods

This is a retrospective study of 67 women with deep infiltrating endometriosis who underwent surgical treatment at a tertiary hospital from 2007 to 2017. The women were identified through the electronic medical record system. Of the 876 women followed up during this period, only 70 were referred for surgical treatment, and 67 were included in the study. The surgical blocks were then located in the Department of Pathological Anatomy. The block was selected by reviewing the slides stained with hematoxylin and eosin from the surgical specimens, and those with highest percentages of endometriotic cells were chosen.

The areas corresponding to the stromal and glandular components of the endometriosis lesions were measured, and the respective percentages were calculated for each one. For better identification of these components, markers were used. The markers used were CD10 and S100 to identify stromal cells and cytokeratin 7 (CK7) to identify the glandular component.

The results of the histological analysis with the percentages of stromal/glandular tissues were related to the following clinical data: age, parity, body mass index (BMI, calculated based on weight in kilograms divided by the square of height in meters), site of endometriosis involvement, use of medication before surgery (combined oral contraceptives or progestins), pelvic pain with intensity being graded from 0 to 10 according to the visual analogue scale (VAS), and time of pain symptom. Data were collected

by the researchers responsible through the information contained in medical records.

This study was approved by the Ethics and Research Committee of the University of Campinas, Brazil, under the number CAAE 34929120.4.0000.5404.

Immunohistochemical reactions for CD10, S100, and CK7 were performed at the Laboratory of Immunohistochemistry of the hospital.

The materials, previously fixed in 10% formalin and embedded in paraffin, were submitted to histological sections of 4 micrometers of thickness, which were placed on signed slides, after which they were deparaffinized with 3 xylol baths at room temperature. Afterwards, the slides were bathed in absolute alcohols three times, once in 80% alcohol and once in 50% alcohol for progressive hydration. They were then washed in running water for 3 minutes and, finally, rinsed in distilled water. In order to inhibit endogenous peroxidase, the slides were bathed for 5 minutes each in a hydrogen peroxide solution at room temperature, then washed again in running water for 3 minutes and then rinsed in distilled water.

Antigenic retrieval was performed by immersing the slides in 0.05M Tris-EDTA pH 8.9 buffer for 30 minutes at approximately 95°C in a steam pan, with subsequent cooling for 15 minutes for anti-CK7 monoclonal mouse antibodies (clone OV-TL12/30, DAKO, Glostrup, Denmark), monoclonal mouse anti-CD10 (clone 56C6, DAKO) and polyclonal rabbit anti-S100 (DAKO), all with dilution 1:100. Afterwards, they were washed in running water and distilled water.

The primary antibodies were dripped onto the respective histological preparations at the aforementioned dilutions, and the slides were incubated in an oven at 37°C for 30 minutes. Afterwards, they were incubated for 16 to 20 hours (overnight) at 4°C in a humid chamber.

Subsequently, they were washed three times in phosphate buffer saline (PBS) pH 7.4 solution at room temperature. The slides were then incubated for one hour at 37°C with a cocktail of polymers labeled with peroxidase, using the advanced detection system (DAKO), and then immersed again in PBS.

Staining was performed with the diamino-benzidine (DAB) brown chromogen kit (DAKO, REF – 3468) for 5 minutes at 37°C. The material was washed in running water, counterstained with the Mayer hematoxylin, dehydrated (three baths in ethyl alcohol and three baths in xylene) and the coverslips were glued with the Entellan resin (ProSciTech Pty. Ltd. Kirwan, QLD, Australia).

In addition to the cases in the study group, a case of liver in the cirrhosis phase was selected to control the immunohistochemical reactions for the three markers, for which the positivity of the reaction is considered: CD10–membrane expression in the bile canaliculi of the hepatocytes; CK7–cytoplasmic expression in bile duct epithelium; and S100–cytoplasmic expression in neural plexuses.

After this process, the slides were photographed in a Zeiss Axiophot 2 microscope (Carl Zeiss Meditec, Jena, Thuringia, Germany), with an Olympus DP72 camera (Evident Corp., Shinjuku-ku, Tokyo, Japan) using the cellSens Standard (Evident Corp.) software that processes the images for the computer. Each slide was photographed in three different regions, where markers were expressed in greater proportions. The histological analysis was performed with the help of a pathologist. After being photographed, each image was inserted into the ImageJ (LOCI, University of Wisconsin, WI, USA) software for morphometric study, which measures the percentage of expression of the analyzed markers.[10]

For morphological evaluation, a histological classification was applied comprising four different forms of endometriosis, based on the degree of differentiation.[11–13] The first is a well-differentiated glandular form, with the presence of surface epithelium or epithelium with glandular to cystic formations; the cells are indistinguishable from a normal endometrium during different phases of the menstrual cycle (proliferative/follicular, secretory/luteal, menstrual, and regenerative). The second is a pure stromal form of endometriosis, without any surface or glandular epithelium; the stroma also closely resembles that of the normal endometrium during different phases of the menstrual cycle. The third is a glandular pattern of mixed differentiation, with the epithelium being composed of cylindrical to columnar endometrial cells, cuboidal to low flattened cells, undifferentiated cells, and, sometimes, cells with other Mullerian histological patterns (serous or mucinous cells). The fourth is a poorly differentiated form that is characterized by a uniquely undifferentiated glandular pattern in which the surface epithelium or glandular/cystic formations are lined exclusively by low to flat cuboidal, mesothelial cells, or appear as small epithelial nests or islands.

The variables were described as frequency, mean, and standard deviation (SD). The Chi-square and Fisher exact tests were used to detect associations between categorical variables. The Kruskal-Wallis and Mann-Whitney tests were used to detect the association between continuous variables. The Spearman correlation coefficient was used to analyze the relationships between numerical variables. A probability value (p-value) of <0.05 was considered statistically significant. The SAS software (SAS Inc., Cary, NC, USA) version 9.04 was used for all statistical analyses.

Results

The mean age of the women was 38.9 ± 6.5 years and the mean BMI was 25.8 ± 4.5 kg/m2. The average pain level was 8.8 ± 1.9, and the average time of symptom onset was 4.7 ± 3.5 years. Among the 67 women, 55.2% were nulliparous and 87% underwent hormone treatment prior to surgery. The sites of disease involvement were: rectosigmoid (65.6%), ovary (43.2%), peritoneum (16.4%), ureter (0.5%), and uterosacral (0.1%) (►**Table 1**).

The average expression of anatomopathological markers CD10, CK7, and S100 was $19.5 \pm 11.8\%$, $9.4 \pm 5.9\%$, and $7.9 \pm 5.8\%$, respectively. Regarding the histological classification, 80% of the slides showed an undifferentiated glandular tissue pattern. There was no significant difference between the percentage of CD10, CK7, and S100 markers and the site of involvement of endometriosis in women ($p = 0.37$, 0.12, and 0.09, respectively). No difference was observed between

Table 1 Clinical characteristics of women with deep infiltrating endometriosis undergoing surgical treatment (n = 67)

Variables	Mean ± SD / n(%)
Age (years)	38.9 ± 6.5
BMI (kg/m^2)	25.8 ± 4.5
Nulliparous	37(55.2)
Pelvic pain	8.8 ± 1.9
VAS (7–10)	60(89.5)
VAS (4–6)	7(10.5)
Hormonal treatment	58(86.5)
Pain time (years)	4.7 ± 3.5

Abbreviations: SD, standard deviation; BMI, body mass index; VAS, visual analog pain scale.

Table 3 Correlations between age, pain level, duration of symptoms and body mass index with expression of markers CD10, CK7 and S100 (n = 67)

		Age	Pain score	Pain time	BMI
CD10	r	0.069	0.271	0.108	0.054
	p	0.57	0.02	0.38	0.66
CK7	r	0.021	−0.022	−0.106	−0.100
	p	0.85	0.85	0.39	0.41
S100	r	−0.008	0.080	0.166	0.359
	p	0.94	0.51	0.17	0.002

Abbreviation: BMI, body mass index. **Note:** r = Spearman correlation coefficient.

expression of CD10, CK7, and S100 markers and hormone treatment ($p = 0.79$, 0.83, and 0.74, respectively). Women who did not undergo hormone treatment had a mean CD10 expression of 20.2 ± 10.6%; of 8.4 ± 5.0% for CK7; and 8.0 ± 7.5% for S100, while those who underwent hormonal treatment had an average of CD10 of 19.4 ± 12.0; 9.5 ± 6.1 for CK7; and 7.9 ± 5.6 for S100 (►**Table 2**).

It was found that a greater the expression of CD10 correlated with greater levels of pain ($p = 0.02$). A positive correlation was also found between the expression of S100 and the BMI ($p = 0.002$). No correlation was observed between the expression of CD10, CK7, and S100 markers and age or duration of symptoms (►**Table 3**).

Discussion

This study observed a positive correlation between the level of pain and the percentage of CD10 (stromal marker). There was no significant difference between marker expression and the site of endometriosis involvement and treatment performance. There was no correlation between marker expression and age or duration of symptoms.

Most of the women in our study had a histological pattern classified as undifferentiated glandular. Studies in the literature indicate that women with deep infiltrating endometriosis present this undifferentiated pattern.[11,12] Additionally, studies point out that women who present the undifferentiated glandular pattern have the worse response to hormonal treatment.[11,12] In our study, most women underwent surgical treatment due to poor response to hormone treatment—the mean pain score was nine, which can be explained by the undifferentiated histological pattern of the lesions.

Histological analysis of deep infiltrating endometriosis often shows undifferentiated glandular and/or stromal cells surrounded by a significant amount of fibrotic tissue.[13,14] Endometriosis-related fibrosis represents a complex phenomenon, with underlying mechanisms that are still unclear. Fibrosis is consistently present in all forms of this disease and contributes to the classic symptoms of pain and infertility related to endometriosis.[9] The main component of nodular lesions is not endometrial tissue, but fibromuscular tissue with sparse finger-like extensions of glandular tissue and stroma.[15]

Our results showed higher levels of fibrosis in the evaluated biopsies (higher expression of CD10). Therefore, this finding suggests that hormone treatment would not be effective for the fibrotic portion of the disease. Pain mechanisms, especially in women who did not respond to hormone treatment, are complex and could also be related to other multifactorial elements such as inflammation, oxidative stress, and genetics.[16]

There are several mechanisms that contribute to chronic pain in endometriosis. Among them are neurogenic inflammation, neuroangiogenesis, peripheral sensitization, and central sensitization. As women with endometriosis may also have other comorbid conditions such as irritable bowel syndrome and overactive bladder syndrome, the study by Maddern et al. highlights how the common nerve pathways that innervate the colon, bladder, and female reproductive tract may contribute to pain through crossorgan sensitization.[17]

Table 2 Evaluation of the percentage of anatomopathological markers CD10, CK7 and S100 according to the site of involvement of endometriosis and hormonal treatment in women (n = 67)

	Ovary	Peritoneum	Rectosigmoid	*p**	Treatment	Without treatment	*p***
CD10 (%)	17.6 ± 13.8	15.6 ± 12.7	17.5 ± 11.0	0.37	19.4 ± 12	20.2 ± 10.6	0.79
CK7 (%)	10.7 ± 8.6	8.3 ± 4.4	8.7 ± 5.9	0.12	9.5 ± 6.1	8.4 ± 5.0	0.83
S100 (%)	9.3 ± 8.3	6.4 ± 2.9	6.1 ± 4.2	0.09	7.9 ± 5.6	8.0 ± 7.5	0.74

Notes: *Kruskal-Wallis test. **Mann-Whitney test.

Endometriosis is a complex disease characterized by a relevant component of fibrosis and adhesions. The identification of specific histopathological characteristics remains extremely important for the diagnosis of endometriosis.[18] This study establishes a relationship between the level of pain and the highest expression of the CD10 marker, which stains stromal cells. Thus, it corroborates the prerogative that women with more pain would have more fibrosis in relation to glandular cells and, therefore, would not respond to hormonal treatment.

Current therapeutic options provide pain relief for over 6 months in only 40 to 70% of women.[19,20] As such, a greater understanding of the mechanisms underlying endometriosis-induced pain is needed to achieve better clinical outcomes in the future.[17]

This study has limitations, such as a small sample size and the absence of a control group. Further studies and the evaluation of a larger number of cases are needed to establish the relationship between fibrosis and the degree of clinical response. In this way, we intend a greater understanding of the chronic pain associated with endometriosis and the identification of possible targets for pain control that can help improve the quality of life of people who suffer from this disease.

Conclusion

Women with deep infiltrating endometriosis have a positive association between the morphometric aspects of endometriotic lesions (CD10 marker) and pain.

Contributions

All the authors participated actively in the study, as follows: DA Yela conceptualized and designed the study, she also wrote and reviewed the manuscript. She was also involved in the interpretation of results. MSS Silva conceptualized and designed the study and helped in the acquisition of data, interpretation of results, and writing the manuscript. L Eloy was responsible for the anatomopathological analyzes. CL Benetti-Pinto reviewed the manuscript.

Conflict of Interests

The authors have no conflict of interests to declare.

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Mortality from Breast Cancer in Women under 50 Years of Age in Colombia

Mortalidade por câncer de mama em mulheres com menos de 50 anos de idade na Colômbia

Mario Arturo González Mariño[1]

[1] Department of Obstetrics and Gynecology, Faculty of Medicine, Universidad Nacional de Colombia, Bogotá, Colombia

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Address for correspondence Mario Arturo González Mariño, Department of Obstetrics and Gynecology, Faculty of Medicine, Universidad Nacional de Colombia, Bogotá, Colombia (e-mail: marioar90@hotmail.com).

Abstract

Objective To calculate and analyze the mortality rates from breast cancer in women under 50 years of age in Colombia and to compare them with those of other countries in the region.

Methods Based on data from the registry of deaths in 2018 and the results of the National Population and Housing Census of Colombia for the same year, specific mortality rates in women with breast cancer, specific mortality according to age group, standardized by age, proportional mortality, potential years of life lost, and years of life expectancy lost in women under 50 years of age who died from breast cancer were calculated. The mortality rate of regional countries was consulted on the Global Cancer Observatory webpage.

Results In the group from 20 to 49 years, the specific mortality rate was higher in the age range from 45 to 49 years, with a rate of $23.42 \times 100{,}000$, a value that was above the specific mortality rate due to breast cancer in women in Colombia, 15.17×100.000. In the age range of 45 to 49 years, the potential years of life lost were 42.16. Of the 0.275 years of life expectancy lost by the population due to this neoplasia, women under 50 years of age represented 0.091 (33%). Colombia is the fifth in the rank of mortality in Latin American countries in this age group.

Conclusion Breast cancer in patients from 30 to 59 years is the number one cause for the decrease in life expectancy of women in Colombia. Women under 50 years of age represent one third of this decrease. This neoplasm is also the leading cause of mortality in women younger than 50 years in South America.

Keywords

- neoplasms
- breast
- mortality
- women
- demography
- Colombia
- South America

Introduction

The most diagnosed cancer in the world is breast cancer (with an estimated 2.3 million new cases annually), and it is the leading cause of cancer death in women.[1] In women under 50 years of age, this position is maintained, with an estimated of 665,508 new cases and a mortality of 131,322.[2] In low- and middle-income countries, breast cancer patients tend to be younger than in high-income countries.[3] Breast cancer, particularly in women under 40 years of age, has a worse prognosis than in older women,[4] especially luminal A-type tumor (regardless of the state at the time of diagnosis).[5]

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In these women, the tumors are characterized by a higher proportion of histological grade 3 tumors, triple negative or HER2 overexpression, lymphovascular invasion, and lymphocytic infiltration. Intrinsic basal-like subtypes and HER2-enriched tumors are more frequent.[6] Their detection is usually reported in a more advanced stage[7] although in other studies, young age is not an independent factor of delay in diagnosis.[8,9]

Breast cancer at a young age is associated with higher costs in medical treatment, loss of productivity,[10–12] labor, and financial problems due to reduced working hours, and in the long term, a higher unemployment rate.[13] Increased risk of psychosocial morbidity after diagnosis has been documented, particularly in those receiving chemotherapy and/or undergoing the menopausal transition with treatment.[3,14,15]

Compared with North America and Europe, Latin American women are also diagnosed at a younger age.[16] The rising predicted trend in the 20 to 49 age group is possibly due to changes in menstrual (early menarche) and reproductive factors as well as in lifestyle habits.[16] However, specific risk factors for breast cancer trends in Latin America remain largely unknown.[17]

In Colombia, the highest proportion of new cancer cases in the female population corresponds to breast cancer (4,506 new cases),[18] which also has the highest number of cancer deaths,[18] with 3,428 deaths, of which 763 (22.25%) were of women under 50 years of age in 2018, 3 of them between 20 and 24 years as the lower limit.[19] According to the census, the population of women between 20 and 49 years old was 10,007,332 (44.29% of the total number of women).[20]

Mortality rates can serve as a measure of disease severity and help determine whether the treatment has become more effective over time.[21] They can be classified in general, as a summary form of the risk of dying in the general population, but since the risk of dying is strongly related to age, to perform a more precise analysis of the risks of dying, specific mortality rates are used.[22] To compare mortality rates considering differences in age distribution between populations or the same population in different periods, standardized or adjusted mortality rates are used. The goal is to eliminate the influence of different age structures on the mortality rates being compared.[22,23] However, care must be taken in the comparison since the data used in its calculation may come from different sources, be estimates, or differ in the selected standard population.

Proportional mortality is a useful measure to describe the relative weight of the various causes of total deaths. It is a frequently used indicator despite its limitations. A change in the proportional mortality of a certain disease over time may be due not to changes in the mortality of that disease, but to changes in the mortality of some other disease.[24]

Years of potential life lost (YPLL) refer to the losses suffered by society because of the deaths of young people or premature deaths. It has been increasingly used to set health priorities. This indicator in years is higher the younger the person who died. Its limits vary; the most recommended by developing countries are 0 to 65 years, while developed countries suggest 1 to 70 years.[25]

The years of life lost index is derived based on life table functions and is related to the decomposition of changes in life expectancy.[26]

The GLOBOCAN, used in this study, is an online database that provides global cancer statistics and estimates of incidence and mortality in 185 countries for 36 types of cancer and all cancer sites combined.[27] The data are part of International Association of Cancer Registries (IARC)'s Global Cancer Observatory, a web-based platform presenting global cancer statistics to inform cancer control and cancer research on cancer.[2]

Methods

This is a descriptive study that collects data on the registry of deaths in 2018, results of the National Population and Housing Census of Colombia in the same year, both reported by the National Administrative Department of Statistics (DANE, in the Spanish acronym) and information from the references cited in the text with which the indicators are constructed and developed. The specific mortality rate due to breast cancer in women, the specific mortality rate due to breast cancer according to age group in women, the standardized rate by age (in groups by age and gender), proportional mortality, potential years of life lost, and years of life expectancy lost (corresponding to the years 2017 to 2019) in women under 50 years of age who died from breast cancer were calculated.

The data for comparison of breast mortality in women under 50 years of age among South American countries were obtained from GLOBOCAN by the Global Cancer Observatory.

Of the 4,506 new cases of breast cancer in Colombia in 2018, there were 1,362 (30.22%) under 50 years of age. The intrinsic subtypes of these tumors have not been well characterized in the country, but in an institutional study,[28] it was found that of 468 patients with breast cancer, 131 cancers were in patients under 50 years of age, with luminal A being the most frequent (30.53%), followed by luminal B Her2 negative (27.48%). Triple-negative cancer was found in 16.79% of women under 50 years of age with breast cancer, but its proportion to the total of its intrinsic subtype was the highest (36.06 of the total triple negatives). In cancers in women 50 years of age or older, the same order is maintained. Overall survival according to age was reported to be 88.4% (95%CI: 85.5–90.8) in women younger than 50 years with a median follow-up of 41 months, and 88.3% (95%CI: 86.3–90.0) with a median follow-up of 40 months in women aged 50 and over.[29]

Results

Colombia's specific mortality rate for breast cancer in women was $15.17 \times 100{,}000$. The proportional mortality in women with breast cancer under 50 years of age was 0.71. The specific mortality rates for breast cancer according to the age group, age-standardized rates, and potential years of life lost are presented in ►**Table 1**. Women under 50 years of age with breast cancer contributed 0.091 years of life

Table 1 Number of deaths from breast cancer, population of women by age group, specific mortality rates for this pathology by age group, age-standardized rates, and years of potential life lost in Colombia

Age	Population	Deaths	SMR	Weighing*	ASR	YPLL
20–24	1 956 735	3	0.15	0.0867	0.01	0.65
25–29	1 857 016	28	1.50	0.0837	0.12	5.72
30–34	1 700 746	72	4.23	0.0803	0.33	13.97
35–39	1 656 227	143	8.63	0.0755	0.65	24.17
40–44	1 436 336	189	13.15	0.0695	0.91	30.26
45–49	1 400 272	328	23.42	0.0637	1.49	42.16

Abbreviations: ASR, age-standardized rates; SMR, specific mortality rates; YPLL, years of potential life lost.
Weighting* = weighting in the standard population (WHO world standard population).
Source: Pan American Health Organization (PAHO) 2009. Age-standardized death rates per population of 100,000. https://www.paho.org/hq/dmdocuments/2010/Tasas-de-mortalidad-por-edad-estandarizadas-hoja-de-resumen.pdf. Accessed 2023 January 15. (Spanish).[23]

expectancy lost to the population. The mortality rate from breast cancer standardized by age per 100,000 from 0 to 49 years in the region shows the Bolivarian Republic of Venezuela and Uruguay (5) as the countries with the highest rates followed in descending order by Brazil (4.6), Argentina (4.5), Colombia (3.9), Ecuador (3.3), and Peru (3.1).

Discussion

Mortality is one of the most important indicators for monitoring the health of patients with breast cancer.[30] This neoplasm is the most frequent type of cancer in Latin American countries and the leading cause of cancer mortality among women. The mean age of diagnosis and death from this neoplasia in the region is $\sim$ 10 years younger than that reported in developed countries (except Argentina and Uruguay).[16]

The age-standardized mortality rate in women under 50 years of age in 2008 was reported in Uruguay and Argentina at 7, in Brazil and Ecuador at 14, and $15 \times 100{,}000$ in Colombia.[16] For the year 2020, Colombia reported lower mortality rates than Uruguay and Argentina.[2]

The mortality from cancer in Latin American countries is approximately 2-fold higher than it is in more developed countries,[31] and the incidence and mortality are likely to continuously increase in the coming decades.[32] It must be considered that developing countries like those in Latin America are subjected to serious problems in access to health services, including those to obtain diagnosis and modern treatments,[33] which contributes to the increased mortality rates from breast cancer. The Organization for Economic Co-operation and Development (OECD) and the World Bank, within the indicators to measure the quality control of medical care, describe the survival rates for breast cancer as a reflection of the quality of preventive and curative care. Among the countries of Latin America and the Caribbean with data for this indicator, women with an early diagnosis of breast cancer had an average 78% probability of surviving at least 5 years. Below this percentage were Ecuador (76%), Brazil (75%), and Colombia (72%), while Argentina (84%) and Peru (82%) exceeded this threshold.[34]

The number one cause for the decrease in life expectancy of women in Colombia from the age of 30 to 59 is malignant breast tumors. Of the 0.275 years of life expectancy lost by the population due to this neoplasm in the country,[21] women under 50 years of age contributed one third.

The data reported in this study showed that the age-standardized breast cancer mortality rate in women under 50 years of age in Colombia was lower than that reported in other studies.[2,17] In the case of the publication by Carioli et al.,[17] the mortality from breast cancer standardized by age (20–49 years) expected for 2019 was 5.65 (5.13–6.16), while in this study, in 2018, it was 3.52. However, the census data sources used in this study do not allow direct comparison with other reports made with population estimation.

The mortality indicators calculated in this article become important when studies are performed with the same methodology over time. Brazil has mortality trend studies that show that breast cancer mortality in young women has increased in the last 2 decades,[35] with an average increase of 0.18 per year; $p < 0.001$, with regional differences, particularly in the 20 to 49 years age group (0.07 per year; $p < 0.001$).[30] Some studies suggest that this increasing breast cancer incidence in young women occurs in the absence of family clustering, reflecting a change in the distribution patterns for this neoplasm.[36]

The limitations of this study in its report have to do with the quality of the information source. Death registries can present flaws in their processing, although this aspect has been improving, with figures that report 92.8% of well-certified cancer mortality.[37] Comparison with other countries is difficult due to different sources of data, estimate-based studies, differing in the standard population selected, reference ages, and different calculations of death indicators in women.

Conclusion

Mortality is an index of the severity of a disease from a clinical and public health viewpoint. Knowing its indicators

is important in planning public policies. Breast cancer is the leading cause of cancer mortality among women in Latin America. The growing mortality in the population of women under 50 years of age should attract the interest of health offices to generate clinical and population awareness of breast cancer in women in this age group.

Conflict of Interests

The authors have no conflict of interests to declare.

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Systemic Inflammatory Patterns in Ovarian Cancer Patients: Analysis of Cytokines, Chemokines, and Microparticles

Padrões inflamatórios sistêmicos em pacientes com câncer de ovário: Análise de citocinas, quimiocinas e micropartículas

Aline Evangelista Santiago[1] Sálua Oliveira Calil de Paula[1] Andréa Teixeira de Carvalho[2] Eduardo Batista Cândido[3] Rafaela de Souza Furtado[3] Agnaldo Lopes da Silva Filho[1]

[1] Department of Gynecology and Obstetrics, Faculty of Medicine, Universidade Estadual Paulista "Júlio de Mesquita Filho", Botucatu, SP, Brasil
[2] Instituto René Rachou – Fiocruz Minas, Belo Horizonte, MG, Brasil
[3] Department of Gynecology and Obstetrics, Faculty of Medicine, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brasil,

Address for correspondence Aline Evangelista Santiago, PhD, Universidade Estadual Paulista "Júlio de Mesquita Filho", Botucatu, SP, Brazil (e-mail: alinevajf@gmail.com).

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Abstract

Objective To compare the patterns of systemic inflammatory response in women with epithelial ovarian cancer (EOC) or no evidence of malignant disease, as well as to evaluate the profile of systemic inflammatory responses in type-1 and type-2 tumors. This is a non-invasive and indirect way to assess both tumor activity and the role of the inflammatory pattern during pro- and antitumor responses.

Materials and Methods We performed a prospective evaluation of 56 patients: 30 women without evidence of malignant disease and 26 women with EOC. The plasma quantification of cytokines, chemokines, and microparticles (MPs) was performed using flow cytometry.

Results Plasma levels of proinflammatory cytokines interleukin-12 (IL12), interleukin-6 (IL-6), tumor necrosis factor alpha (TNF-α) interleukin-1 beta (IL-1β), and interleukin-10 (IL-10), and C-X-C motif chemokine ligand 9 (CXCL-9) and C-X-C motif chemokine ligand 10 (CXCL-10) were significantly higher in patients with EOC than in those in the control group. Plasma levels of cytokine interleukin-17A (IL-17A) and MPs derived from endothelial cells were lower in patients with EOC than in the control group. The frequency of leukocytes and MPs derived from endothelial cells was higher in type-2 tumors than in those without malignancy. We observed an expressive number of inflammatory/regulatory cytokines and chemokines in the cases of EOC, as well as negative and positive correlations involving them, which leads to a higher complexity of these networks.

Keywords
- cytokines
- chemokines
- microparticles
- ovarian cancer
- inflammation

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Conclusion The present study showed that, through the development of networks consisting of cytokines, chemokines, and MPs, there is a greater systemic inflammatory response in patients with EOC and a more complex correlation of these biomarkers in type-2 tumors.

Resumo

Objetivo Comparar os padrões de resposta inflamatória sistêmica em mulheres com câncer epitelial de ovário (CEO) ou sem evidência de doença maligna, bem como avaliar o perfil de respostas inflamatórias sistêmicas em tumores dos tipos 1 e 2. Esta é uma forma não invasiva e indireta de avaliar tanto a atividade tumoral quanto o papel do padrão inflamatório durante as respostas pró- e antitumorais.

Métodos Ao todo, 56 pacientes foram avaliados prospectivamente: 30 mulheres sem evidência de doença maligna e 26 mulheres com CEO. A quantificação plasmática de citocinas, quimiocinas e micropartículas (MPs) foi realizada por citometria de fluxo.

Resultados Os níveis plasmáticos das citocinas pró-inflamatórias interleucina-12 (IL12), interleucina-6 (IL-6), fator de necrose tumoral alfa (*tumor necrosis factor alpha*, TNF-α, em inglês), interleucina-1 beta (IL-1β), e interleucina-10 (IL-10), e da quimiocina de motivo C-X-C 9 (CXCL-9) e da quimiocina de motivo C-X-C 10 (CXCL-10) foram significativamente maiores em pacientes com EOC do que nos controles. Os níveis plasmáticos da citocina interleucina-17A (IL17A) e MPs derivados de células endoteliais foram menores em pacientes com CEO do que no grupo de controle. A frequência de leucócitos e de MPs derivadas de células endoteliais foi maior nos tumores de tipo 2 do que naqueles sem malignidade. Observou-se um número expressivo de citocinas e quimiocinas inflamatórias/regulatórias nos casos de CEO, além de correlações negativas e positivas entre elas, o que leva a uma maior complexidade dessas redes.

Conclusão Este estudo mostrou que, por meio da construção de redes compostas por citocinas, quimiocinas e MPs, há maior resposta inflamatória sistêmica em pacientes com CEO e correlação mais complexa desses biomarcadores em tumores de tipo 2.

Palavras-chave
- citocinas
- quimiocinas
- micropartículas
- câncer do ovário
- inflamação

Introduction

Epithelial ovarian cancer (EOC) is the fifth most frequent cause of cancer-related death in women, with an approximate yearly mortality rate of 7.0 per 100 thousand women. Most diagnoses are made when the disease is in an advanced stage (III or IV), which implies a five-year survival rate lower than 30%.[1] Epithelial ovarian cancer comprises a heterogeneous group of tumors, subdivided according to histological differences, by the degree of proliferation, and considering epithelial invasion. A dualistic model has been proposed, and EOC has been divided into types 1 and 2, depending on the histological, immunohistochemical, and molecular characteristics of the tumor.[2] Type-1 tumors are considered low-grade and usually originate from mutations on the *KRAS*, *BRAF*, *ERBB2*, *CTNNB1*, *PTEN*, and *PIK3CA* genes. Since they are genetically stable, they are less aggressive, which leads to a more favorable prognosis. In contrast, type-2 tumors are high-grade and have more uncontrolled cell differentiation, which culminates in an aggressive behavior. This is why they are usually diagnosed at an advanced stage and have a less favorable prognosis. They show TP53 mutations in more than 80% of the cases and repair DNA damage. A recent study[1] showed better disease-specific survival in type-1 than that in type-2 tumors, as well as the importance of the stage of the disease at the time of diagnosis in determining the survival rate.

Understanding the carcinogenesis of EOC is very important to determine the mechanisms involved in the origin and pathogenesis of these tumors.[3] The molecular biology of oncogenesis in ovarian cancer consists of multiple complex pathways, and previous studies[4] on the identification of prognostic markers for EOC have not yielded definitive results. There is growing evidence that an inflammatory process contributes to the growth of ovarian tumors and metastases to the peritoneum.[5] Therefore, the present study focused on ovarian cancer carcinogenesis and the role of inflammatory infiltrates in tumor progression.

Inflammatory mediators and various cytokines produced by the activated innate immune cells, such as tumor necrosis factor alpha (TNF-α), interleukin-1 beta (IL-1β) and proinflammatory cytokine interleukin-6 (IL-6), have been shown to promote the genesis, growth, and progression of EOC, with IL-6 being considered a central immunoregulatory cytokine.[6] This cytokine activates signaling pathways that lead to tumor-cell proliferation, and it appears to be involved in the process of tumor metastasis.[7–9] There is also evidence that cytokines and their regulators participate both in the process of ovarian cancer progression and in the

chemoresistance of neoplastic cells, with IL-6 being one of the main immunoregulatory cytokines involved in this process.[7,8] Although the factors that regulate the activity of these cytokines in ovarian cancer are being studied, they are still unknown.[10] Regarding chemokines, C-C motif chemokine ligand 2 (CCL2) and C-C motif chemokine ligand 5 (CCL5), for example, are well recognized due to their activities in the immune context, stimulating the migration mainly of monocytes and T-cells to damaged or infected sites.[11]

Microparticles (MPs) are a group of heterogeneous membranous vesicles with different shapes and sizes (ranging from 0.1 µm to 1 µm) called microvesicles (MVs). They are released from the cell membrane by the budding process of the external membrane, and determine similarities between MPs and their source cells, including the contents of substances of the mother cell, such as chemokines and cytokines, as well as genetic information to carry messenger RNA (mRNA), microRNA (miRNA), and genomic DNA.[11,12] This ability to incorporate components of the original cell and bring them to the recipient cells characterizes the importance of MPs in the process of intercellular communication, causing them to participate in several stages of cancer progression and resistance, such as metastasis, tumor angiogenesis, development of drug resistance, and evasion of immune surveillance. This, along with the fact that their molecules are promising biomarkers for the diagnosis, prognosis, and follow-up of the disease, makes MPs a great research subject.[11,13–16] Measuring the plasma concentrations of cytokines, chemokines, and MPs in women with cancer is a noninvasive and indirect way to assess tumor activity and the associated inflammatory/regulatory systemic response during the pro- and antitumor responses of the host. In addition, these molecules may also serve as biomarkers of disease activity and be used to monitor the treatment. In that regard, the aim of the present study was to compare the patterns of systemic inflammatory response in women with EOC and with no evidence of a malignant disease, as well as to evaluate the profile of the systemic inflammatory responses for tumor types 1 and 2.

Materials and Methods

In the present study, we performed a prospective evaluation of 56 patients: 30 women with no evidence of malignant disease and 26 women with EOC. The study was approved by the Ethics Committee of Universidade Estadual Paulista "Júlio de Mesquita Filho" and by Hospital Vera Cruz Hospital, and all participants provided signed informed consent. The patients answered a questionnaire that encompassed many clinical and epidemiological variables, while the remaining clinical data were obtained from their medical records. The study included a control group composed of women with no evidence of malignancy or gynecological diseases and a second group of patients with EOC who underwent debulking surgery. The exclusion criteria for both groups were as follows: previous chemotherapy and/or radiotherapy; diagnosis of diseases of the immune system and/or use of corticosteroids or immunosuppressive drugs within the past six months, presence of any acute infectious processes in a laparotomy, and identification of a distinct EOC-related malignancy in the histopathological examination of the surgical specimen. In the EOC group, histological grading and disease staging were based on the International Federation of Gynecology and Obstetrics (Fédération Internationale de Gynécologie et d'Obstétrique, FIGO, in French) classification. In this study, ovarian cancer in FIGO stages I and II and FIGO stages III and IV were considered early and advanced diseases respectively. Only malignant epithelial tumors were included in the study, and borderline tumors were excluded. To distinguish between type-1 and type-2 tumors, the histological classification was not considered as the only parameter; we used clinical, histological, and immunohistochemical parameters. Type-1 tumors are diagnosed at early stages (I and II) with p53-negative immunohistochemical staining and classified as low-grade. Type-2 tumors are diagnosed in advanced stages (III and IV) with p53-positive immunohistochemical staining and classified as high-grade tumors. The histological subtypes included were endometrioid, clear-cell, mucinous, low- and high-grade serous, low- and high-grade adenocarcinoma/not otherwise specified, undifferentiated, carcinosarcoma, and granulosa-cell tumors. All cases were reviewed by a pathologist experienced in gynecological oncology.

Purification of Plasma MPs

Flow cytometry was used to quantify the MPs in the plasma. Centrifugation of the citrated (0.5 mL) blood was performed at 1,500 × g for 15 minutes. Afterwards, the plasma was cooled to -20°C prior to storage at -80°C. The samples were then subjected to centrifugation at 13,000 × g for 3 minutes to obtain platelet-free plasma. This plasma was diluted (1:3) in phosphate-buffered saline (PBS) with citrate containing heparin and centrifuged at 14,000 × g for 90 minutes at 15°C. The resulting MP pellet was then resuspended in 1X annexin V (BD Biosciences, San Jose, CA, Unite States).

Detection of Plasma MPs

Unless otherwise stated, all reagents and monoclonal antibodies (mAbs) used in the flow cytometry assays were obtained from BD Biosciences. The MPs isolated from plasma were gated (R1) based on their forward scatter (FSC) and side scatter (SSC) distribution in a density plot compared with the distribution of synthetic 0.7 µm to 0.9 µm SPHERO Amino Fluorescent Particles (Spherotech Inc., Libertyville, IL, United States). Considering the presence of the phosphatidylserine (PS) residues in the MP surface, the events present in R1 were assessed for positive annexin V staining (BD Biosciences), a classic microparticle marker, using mAbs conjugated with phycoerythrin (PE). Mouse immunoglobulin G (IgG) PE-conjugated isotype control mAbs were used to properly place the gates. Annexin V+ events gated in the R2 region were further assessed for immunolabeling with mAbs conjugated with fluorescein isothiocyanate (FITC) against cell markers CD66 (neutrophils), CD41a (platelets), CD51 (endothelial cells), CD235a (erythrocytes), CD45 (leukocytes), CD3 (lymphocytes), and CD14 (monocytes), or the corresponding

mouse IgG FITC-conjugated isotype control mAbs. The samples were analyzed using a FACSCalibur flow cytometer (BD Biosciences). More than 100 thousand events were acquired for each sample, with at least 2 thousand events within the MP gate.

Assessing Plasma Cytokine/Chemokine Levels using a Cytometric Bead Array Immunoassay

Analyzing secreted cytokine/chemokine with flow cytometry enables the simultaneous measurement of multiple biomarkers in a single sample.[12,17,18] To measure plasma biomarkers, whole blood samples were collected using ethylenediaminetetraacetic acid (EDTA) as the anticoagulant. Plasma was maintained at -80°C in aliquots and thawed just before use. The Cytometric Bead Array (CBA) immunoassay kit (BD Biosciences) was used for the quantitative analysis of the plasma biomarker levels. The CBA kit uses 7.5-µm polystyrene microbeads, distinct populations of beads that are unique due to their type-3 fluorescence intensity (fluorescence channel 3, FL- 3). Each bead is coupled to a biomarker-specific mAb, such as IL-1, IL-2, IL-6 IL-10, IL-12, IL-17a, TNF, interferon-gamma (IFN-γ), C-X-C motif chemokines ligands 8 , 9 and 10 (CXCL-8, CXCL-9, and CXCL-10), CCL- 2, and CCL-5, to capture the amount of protein detected in a direct immunoassay using a cocktail of different mAbs coupled to PE (fluorescence channel 2, FL-2). Briefly, 25 µL of plasma or standard (previously diluted in diluent G, as recommended by the manufacturer) were added to 15 µL of a bead cocktail and incubated for 90 minutes at room temperature in the dark. A biomarker standard calibrator mixture was used for each assay. After incubation, both the samples and standards were washed with 500 µL of wash buffer (supplied with the CBA kit) and centrifuged at $600 \times g$ for 7 minutes at room temperature. Subsequently, 20 µL of detection cocktail – consisting of six PE-conjugated mAbs – were added to each tube, and the mixture was reincubated for 90 minutes at room temperature in the dark. Following incubation, the samples and standards were washed again with 500µL of wash buffer and centrifuged at $600 \times g$ for 7 minutes at room temperature to remove the unbound detector reagent. After washing the samples, 250 µL of wash buffer were added to each tube prior to data acquisition using a fluorescence-activated cell sorter (FACS) Calibur flow cytometer (BD Biosciences). Although the fluorescently-labeled particles in the BD CBA immunoassay are designed to be excited by the 488-nm laser that is commonly found on all BD flow cytometers, they can also be excited by the red diode laser on dual-laser BD FACS Calibur instruments. The detection of particle emission on fluorescence channel 4 (FL-4) simplifies instrument setup and requires less fluorescence compensation. Thus, a total of 1,800 events/gate were acquired once the flow cytometer was properly setup to measure the FSC and SSC. Dual-color (FL-4 and FL-2) flow cytometric acquisition using a dual-laser BD CBA template was also conducted. Data analysis was performed using the BD Biosciences CBA software. The results were expressed in pg/mL.

Statistical Analyses

The Mann–Whitney test was used in the comparison between the groups and variables of interest.[18] The software used in the analyses was R (R Foundation for Statistical Computing, Vienna, Austria), version 3.5.2. Non-normal variables were expressed as median and interquartile range (IQR, 25^{th}–75^{th} percentiles) values. Correlations were analyzed using the Spearman two-sided test and the GraphPad Prism (GraphPad Software, Inc., San Diego, CA, United States) software, version 5.00. Values of $p < 0.05$ were considered statistically significant.

Network Analyses

The Spearman correlation test was used to assess the correlations involving biomarker levels. The correlations were classified as negative or positive, and the correlation index (r) was used to categorize the correlation as weak ($r < 0.35$), moderate (r ranging from 0.36 to 0.67), or strong ($r > 0.68$). Then, networks of biomolecular interactions were developed to evaluate the correlations among cytokines, chemokines, and MPs for each clinical group using the Cytoscape (open source) software, version 3.0.2.[19] Tables involving the characteristics of the correlations (type and strength) and the different parameters to be correlated (cytokines, chemokines, and MPs) were created. These tables were then imported to the Cytoscape software and built into the networks, in which the nodes represented the source and target interactions (cytokines, chemokines, and MPs) determined in the imported table. Dotted lines represented negative correlations and solid lines represented positive ones. The strength of the correlation was represented by the thickness of the lines: the thicker the line, the stronger the correlation. The positive and negative correlations were significant when $p < 0.005$.

Results

The mean ages of the control group and EOC patients were of 55.8 ± 6.8 years and 62.3 ± 14.1 years respectively ($p = 0.497$). In total, 10 (38.5%) of the EOC patients had stage-I/II ovarian cancer, and 16 (61.5%) had stage-III/IV ovarian cancer. Among them, 8 (30.8%) had type-1 tumors (7 in stage III and 1 in stage III/IV), and 18 (69.2%) had type-2 tumors (3 in stage III and 15 in stage III/IV), and patients with type-2 EOC were in more advanced stages compared with those with type-1 tumors ($p = 0.001$). ►**Figs. 1** and **2** present the description of the variables of interest for the groups. ►**Fig. 1** showed plasma levels of proinflammatory cytokines IL12 ($p = 0.028$), IL-6 ($p < 0.001$), TNF-α ($= 0.008$), IL-1β ($p = 0.04$), and IL-10 ($p < 0.001$), and chemokines CXCL-9 ($p < 0.001$) and CXCL-10 ($p < 0.001$), which were significantly higher in the group of patients with EOC than in the control group. Another important difference between the groups was the lower level of cytokine IL-17a in the group of EOC patients ($p = 0.027$).

This significant difference between the groups was also observed in relation to endothelial cell-derived MPs. Their

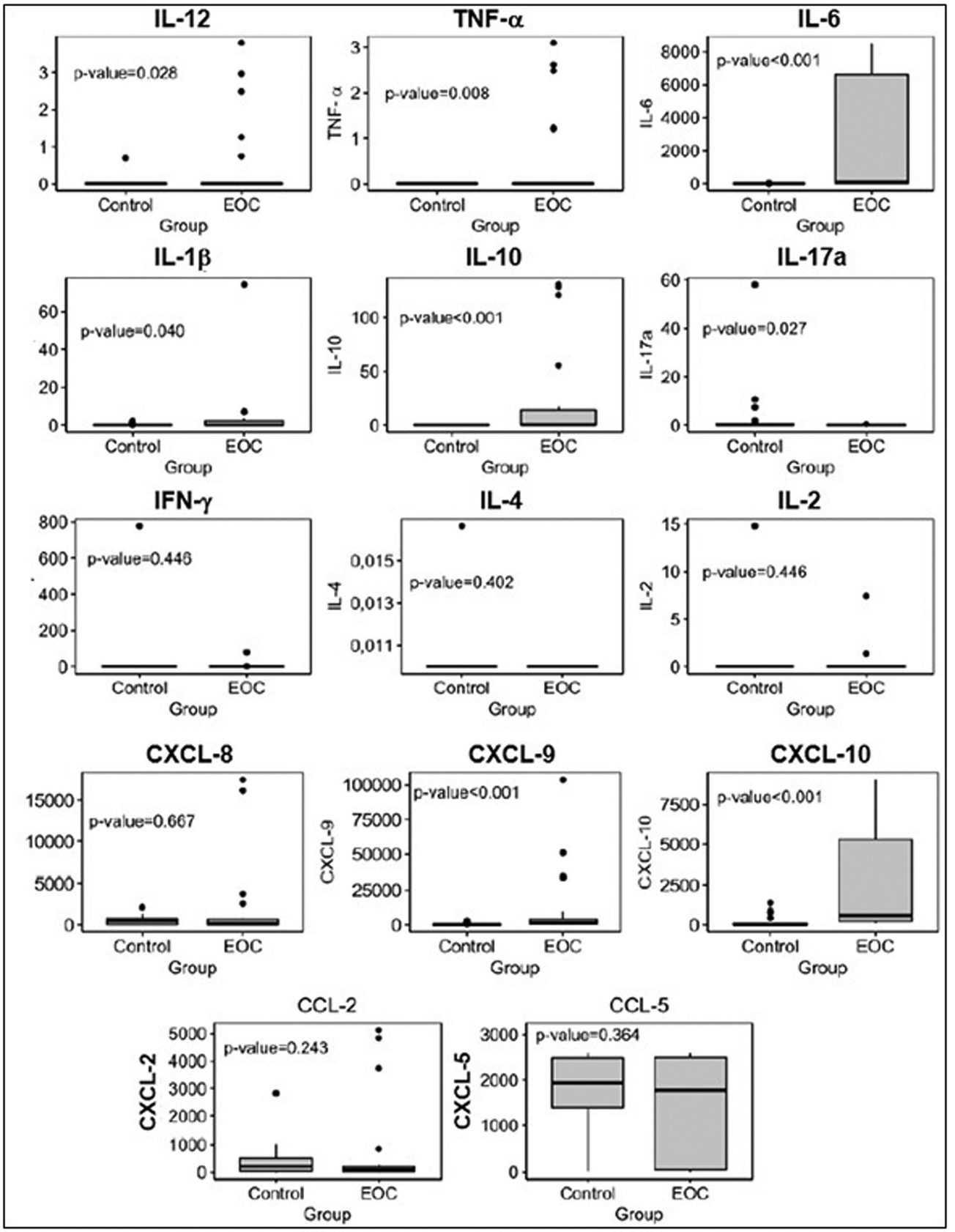

Fig. 1 Boxplot – plasma biomarkers (pg/mL). Comparison of proinflammatory cytokines (IL-1β, IL-6, TNF-α IL-12, and IFN-γ), regulatory cytokines (IL-2, IL-10, and IL-17a), and chemokines (CCL-2, CCL5, CXCL8, CXCL9, and CXCL10) in the control group and in ovarian cancer patients. Abbreviation: EOC, epithelial ovarian cancer. Notes: Data were expressed as median with interquartile range values. Differences between groups were evaluated using the Kruskal-Wallis test.

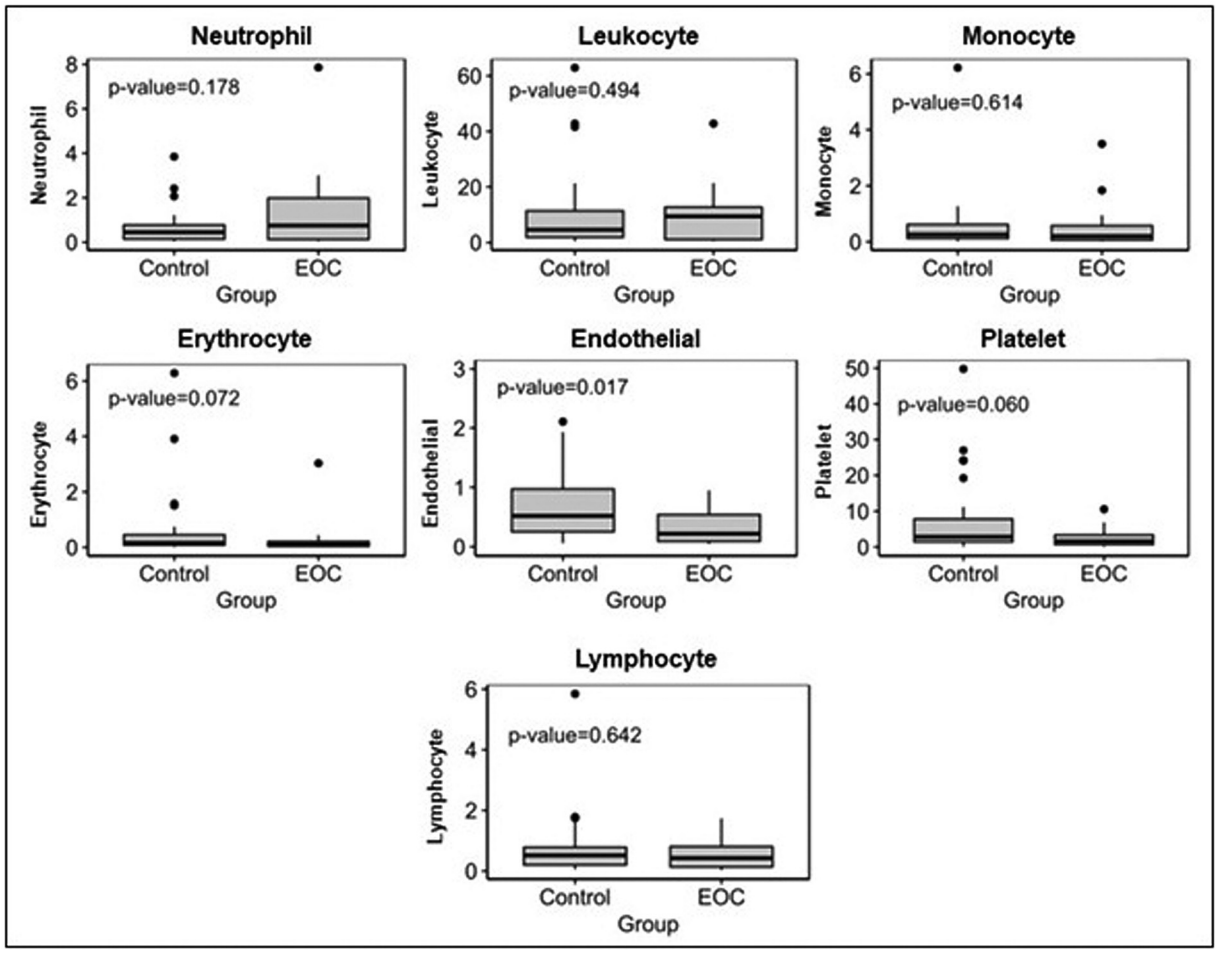

Fig. 2 Boxplot – circulating microparticles. Comparison of the circulating microparticles (MPs) between the control group and ovarian cancer patients according to the specific cellular origin. Abbreviation: EOC, epithelial ovarian cancer. Notes: Data were expressed as median and interquartile range values. Differences between groups were evaluated using the Kruskal-Wallis test.

levels were lower in EOC patients than in the control group ($p = 0.017$) (►**Fig. 2**).

The percentage of circulating cytokines, chemokines, and MPs in patients with type-1 and -2 tumors, according to their specific cellular origin, was evaluated and is shown in ►**Fig. 3**. There were no differences in the plasma levels of cytokines and chemokines between type-1 and -2 tumors. However, the frequency of leukocytes and MPs derived from endothelial cells was higher in type-2 tumors than in those without malignancy ($p < 0.005$).

To evaluate potential relationships among cytokines, chemokines, and MPs in the EOC and control groups, all data obtained in the present study were used to develop the biological networks, in which the nodes represented the cytokines, chemokines, and MPs that were evaluated, and the lines represented positive or negative correlations and strong, weak, or moderate levels (►**Fig. 4**). It was possible to observe that there was a balance among cytokines, chemokines, and MPs in the control group, with fewer and weaker connections between the biomarkers. In EOC patients, the first cluster was characterized by strong and moderate correlations between the MPs, and the cytokine and chemokine networks were moderately correlated to the MP network. When comparing the network of the EOC patients to that of the control group, the former presented a larger number of inflammatory/regulatory cytokines and chemokines, as well as both negative and positive correlations between them, which led to a higher complexity of these networks.

We also established a cellular interaction network between both types of tumors, observing many strong and moderate correlations involving cytokines, chemokines, and MPs, and only a negative interconnection in the networks. Additionally, type-2 tumors presented more correlations than type-1 tumors (►**Fig. 5**).

Discussion

Ovarian cancer is a heterogeneous group of malignancies, and EOC is its most fatal type. Due to their non-specific symptoms, they are usually diagnosed at an advanced stage. To date, there are no reliable screening tests and diagnostic methods to detect the disease at an early stage. Therefore, studying the carcinogenesis of ovarian cancer and developing effective screening detection strategies to detect the disease in its early stages is of utmost importance. This is believed to be the best method to develop a successful

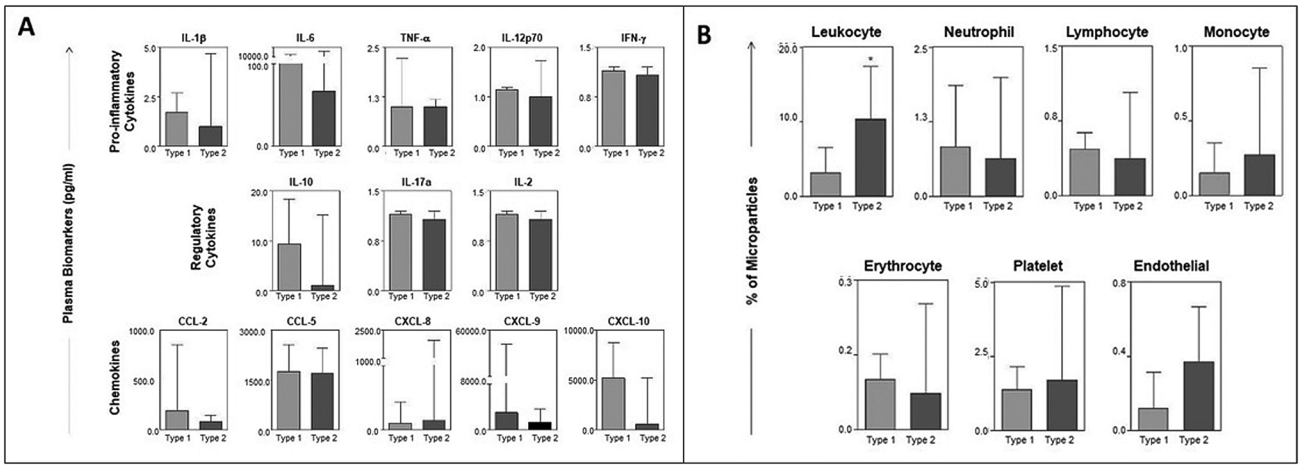

Fig. 3 (**A**) Levels of cytokines and chemokine in ovarian cancer patients according to tumor type. The results were presented in a column-chart format and were expressed as median and interquartile range values in pg/mL. Statistical differences were considered significant when $p < 0.05$. (**B**) Percentage of circulating MPs in patients with type-1 and type-2 tumors according to the specific cellular origin. The results were presented in a column-chart format and were expressed as the median and interquartile range values in pg/mL. Statistical differences were considered significant when $p < 0.05$.

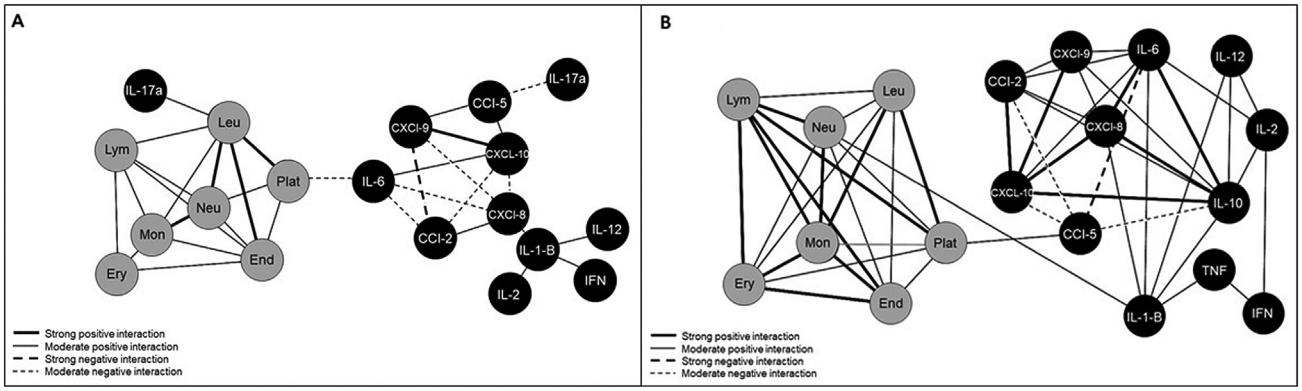

Fig. 4 (**A**) Biomarker networks in the control group. (**B**) Biomarker networks in EOC patients according to their specific cellular origin. Notes: MP nodes were assembled, and biomarker correlation indices were established between groups. The strength of the interactions was represented by different line styles according to the following ranges: negative ($r < 0$–dotted line), positive ($r > 0$–continuous line); weak (r ranging from 0 to 0.36–thinner line), moderate (r ranging from 0.36 to 0.67), and strong ($r > 0.68$–thicker line).

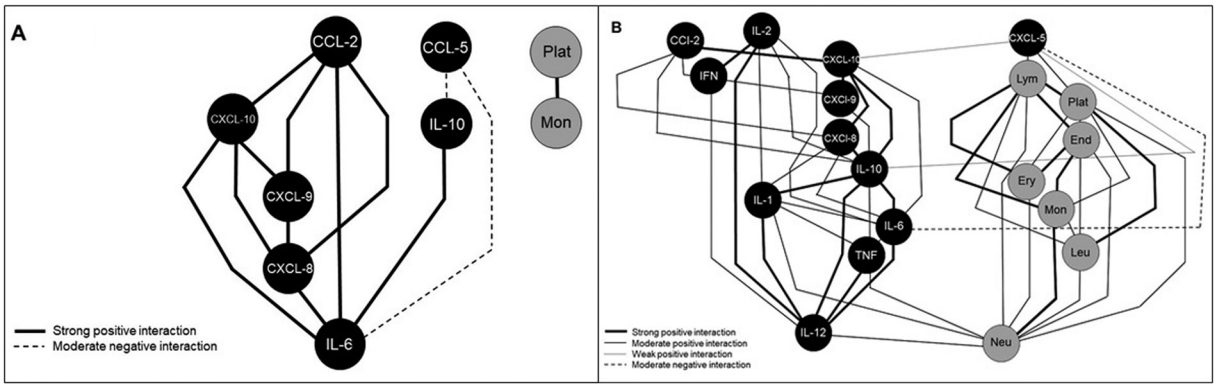

Fig. 5 (**A**) Biomarker networks in type-1 tumors. (**B**) Biomarker networks in type-2 tumors. Note: chemokine, cytokine, and MP nodes were assembled, as well as the biomarker correlation indices among groups (negative, moderate, and strong-positive correlation).

treatment and ensure improved survival for patients with ovarian cancer.[19]

In recent years, the role of cytokines in carcinogenesis and their participation in intercellular communication has been well established by several authors.[7,9,16] However, despite the known proinflammatory or regulatory effects of inflammation, it is unclear whether cytokines have any application in cancer treatment, especially in epithelial tumors. In 1996, a study[20] on ovarian tissue showed that it contained several proinflammatory growth factors, cytokines, and chemokines. Subsequently, other studies[21–23] showed a predominantly humoral immune response and an immunosuppressive pattern with IL-6, IL-10, and IFN-γ associated with EOC. In the present study, we investigated plasma cytokines, chemokines, and MP levels in both women with EOC and a control group. EOC patients showed higher levels of proinflammatory cytokines (IL-6, TNF-α, and IL-12), regulatory cytokines (IL-10), and chemokines (CXCL-9 and CXCL-10), which corroborated this environmental proinflammatory/regulatory mechanism for the development of ovarian cancer.

Some cytokines and chemokines have a protagonist role in literature, such as IL-6, whose signaling seems to play a leading role in the inflammatory process, and it is one of the major immunoregulatory cytokines found in the EOC microenvironment. Therefore, it has been proposed that IL-6 is a central cytokine that promotes ovarian cancer progression, although its exact role during disease development has not been well established. In ovarian cancer, IL-6 antagonist signaling has been accepted as having a therapeutic potential through inhibition of the cytokine network.[11,24] In another study,[25] which analyzed the level of cytokines in the peritoneal fluid of patients with ovarian cancer, higher levels of IL-6 were related to shorter disease-free survival and overall survival. In the present study, we evaluated this cytokine and found a significant difference in its expression in the EOC group when compared with the control group (►**Fig. 2**), highlighting its importance for ovarian cancer, and possibly for carcinogenesis.

In addition, IL-12 is known to increase the antitumor activity of natural killer (NK) cells, and its activity is antagonized primarily by IL-10, with its immunosuppressive or immunostimulatory action.[26–28] The present study showed that these cytokines not only played an important role in ovarian cancer, but that they also interacted in the process.. Further studies on this may lead to potential strategies against ovarian cancer.

Various types of cell secrete CXCL10, including endothelial cells stimulated by IFN-γ wh h h IL-12 cytokine family.[29] This revealed that, compared with the control group, the EOC patients presented increased levels of CXCL10 and decreased levels of MPs derived from endothelial cells, which could be explained by the increased activity of endothelial cells in ovarian cancer, and the related increased production of CXCL10 and lower release of microparticles.

In the present study, we found increased percentages of leukocyte-derived and endothelial-derived MPs in type-2 tumors compared with type-1 tumors, although endothelial-derived MPs were less frequent in the EOC group than in the control group. These results corroborate the dualistic model that categorizes EOC into two types, and suggest a difference in susceptibility to carcinogenesis in both tumors. In contrast, it does not provide an explanation for the decreased levels of endothelial-derived microparticles in the EOC group.

Interactions involving cytokines, chemokines, and MPs and their isolated effects have been reported in literature,[6,7] including those related to carcinogenesis. Among these are the correlations regarding proinflammatory cytokines IL-6 and TNF-α with the immunosuppressive cytokine IL-10 for a poor prognosis of EOC, the correlation between elevated levels of IFN-γ and increased survival, and the correlation between elevated levels of IL-6 and IL-10 with lower survival rates.[21] However, the complex network of interactions involving these structures has not been clearly elucidated, and a better understanding may lead to the development of potential cancer therapies. To better understand the correlations involving cytokines, chemokines, and MPs and, consequently, find possible diagnostic or prognostic tumor markers, hierarchical networks were used to simulate the inflammatory environment of the studied groups. Noteworthy, the supposed global relationships regarding cytokines, chemokines, and MPs were found in clusters. Using the Cytoscape software, we could create complex networks, which graphically showed the inflammatory profile of each group, as well as the correlations involving different parameters and the characteristics of each correlation. Women with ovarian malignancy presented a greater number of strong interactions between inflammatory and immune factors, especially ones involving CXCL-8, and greater complexity in all interactions. This may reflect a greater systemic inflammatory response in ovarian cancer and the involvement of a higher number of possible tumor markers and different interactions among them. This result can be explained by the specific location of ovarian epithelial cells in the peritoneal cavity, where they are clearly exposed to various proinflammatory agents.[30] These results are in agreement with those of studies[26,31] that show that, in EOC, there is a larger proinflammatory microenvironment and a more complex communication pathway, with the exchange of signaling factors that together can support tumor growth and progression. Another noteworthy finding was the substantial difference between networks from type-1 and type-2 tumors, with a greater number of correlations present in type-2 tumors. This result may reflect the discrepancies in the carcinogenesis of both tumors. Our data suggest that there is an interaction involving these soluble factors which are crucial for tumor growth and may validate this network as a key therapeutic target in ovarian cancer.

The present study was not limited to the quantification of cytokines, chemokines, and MPs to evaluate the inflammatory response involved in ovarian carcinogenesis. Interaction networks developed among these biomarkers demonstrated the greater complexity involved in the inflammatory response to EOC. Another strength of the present study is the molecular comparison of type-1 and type-2 EOCs, which shows a different pattern of interactions involving the

biomarkers although it did not present quantitative differences between the groups. The present study had several limitations. The EOC group was not divided according to the tumor histology, which did not enable the analysis of the inflammatory response pattern in each histological type. In addition, the study had a limited number of patients, which reflected the low prevalence of the disease.

Conclusion

The results of the present study enable us to conclude that there are different patterns of systemic inflammatory response assessed by the levels of cytokines, chemokines, and MPs in women with EOC and without evidence of malignancy, and a greater systemic inflammatory response in patients with EOC was observed. The study also showed that type-2 tumors present more complex correlations regarding these biomarkers than type-1 tumors. Since tumor markers are potential tools for screening, diagnosis, prognosis, and posttherapy follow-up in cancer treatment, these molecules are important targets for further studies. Additionally, this may lead to the prescription of specific types of targeted therapies to patients depending on the inflammatory response profile of their disease. Therefore, a full understanding of cancer immunobiology will stimulate the development of more effective immunotherapeutic approaches against these tumors.

Contributions

All of the authors contributed with the project and data interpretation, the writing of the article, the critical review of the intellectual content, and with the final approval of the version to be published.

Conflict of Interests

The authors have no conflict of interests to declare.

Acknowledgments

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Underestimated Cervical Cancer among Women over 65 Years Old: Is It Time to Revise the Screening Target Age Group?

Câncer cervical subestimado entre mulheres com mais de 65 anos: É hora de rever a faixa etária alvo do rastreamento?

Renata Alfena Zago[1] Deolino João Camilo-Júnior[2] Solange Correa Garcia Pires D'Ávilla[1,3] José Cândido Caldeira Xavier-Júnior[1,2,4]

[1] School of Medicine, Centro Universitário Católico Unisalesiano Auxilium, Araçatuba, SP, Brazil
[2] Instituto de Patologia de Araçatuba, Araçatuba, SP, Brazil
[3] Faculdade de Medicina de São José do Rio Preto, São Paulo, SP, Brazil
[4] Faculdade de Medicina de Botucatu, Universidade Estadual Paulista, Botucatu, SP, Brazil

Address for correspondence José Cândido Caldeira Xavier-Júnior, MD, PhD, Pathology Institute of Araçatuba (Private Clinic), Floriano Peixoto Street no. 808, Araçatuba, São Paulo 16015-000, Brazil (e-mail: josecandidojr@yahoo.com.br).

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Abstract

Objective To compare cytological and histological results from women > 64 years old who followed the Brazilian national cervical cancer screening guidelines with those who did not.

Methods The present observational retrospective study analyzed 207 abnormal cervical smear results from women > 64 years old in a mid-sized city in Brazil over 14 years. All results were reported according to the Bethesda System. The women were divided into those who followed the screening guidelines and those who did not.

Results Atypical squamous cells of undetermined significance and low-grade squamous intraepithelial lesion cytology results were found in 128 (62.2%) cases. Of these, 112 (87.5%) had repeated cytology with positive results. The other 79 (38.1%) with abnormal results should have been referred to colposcopy and biopsy. Out of 41 (51.9%) biopsied women, 23 (29.1%) had a confirmed diagnosis of neoplasia or precursor lesion. In contrast, among the 78 (37.7%) biopsied patients, 40 (51.3%) followed the guideline recommendations, with 9 (22.5%) positive biopsies. Of the 38 (48.7%) women who did not follow the guidelines, there were 24 (63.1%) positive results. Women who did not follow the guidelines demonstrated higher chances of cancer and precursor lesions (odds ratio [OR]: 5.904; 95% confidence interval [CI]: 2.188–15.932; $p = 0.0002$).

Conclusion Women > 64 years old who did not follow the national screening protocol showed significant differences in the frequency of abnormal results and severity of diagnosis compared with those who followed the protocol.

Keywords
- aging health
- cytology
- papanicolaou test
- screening
- uterine cervical neoplasms

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Resumo

Objetivo Comparar os resultados citológicos e histológicos de mulheres > 64 anos que seguiram as diretrizes nacionais brasileiras de rastreamento do câncer do colo do útero com aquelas que não as seguiram.

Método O presente estudo observacional retrospectivo analisou 207 resultados anormais de esfregaço cervical de mulheres > 64 anos de idade em uma cidade de médio porte no Brasil durante 14 anos. Todos os resultados foram relatados de acordo com o Sistema Bethesda. As mulheres foram divididas entre as que seguiram as diretrizes de rastreamento e as que não o fizeram.

Resultados Resultados citológicos com células escamosas atípicas de significado indeterminado e lesão intraepitelial escamosa de baixo grau foram encontrados em 128 (62,2%) casos. Destes, 112 (87,5%) repetiram a citologia com resultados positivos. Os outros 79 (38,1%) com resultados anormais deveriam ter sido encaminhados para colposcopia e biópsia. Das 41 (51,9%) mulheres biopsiadas, 23 (29,1%) tiveram diagnóstico confirmado de neoplasia ou lesão precursora. Em contrapartida, entre as 78 (37,7%) pacientes biopsiadas, 40 (51,3%) seguiram as recomendações da diretriz, com 9 (22,5%) biópsias positivas. Entre as 38 (48,7%) mulheres que não seguiram as orientações, houve 24 (63,1%) resultados positivos. As mulheres que não seguiram as diretrizes demonstraram maiores chances de câncer e lesões precursoras (odds ratio [OR]: 5,904; intervalo de confiança [IC] de 95%: 2,188–15,932; $p = 0,0002$).

Conclusão Mulheres > 64 anos que não seguiram a diretriz nacional de rastreamento apresentaram diferenças significativas na frequência de resultados anormais e gravidade do diagnóstico em comparação com aquelas que seguiram a diretriz.

Palavras-chave

- saúde do idoso
- citologia
- teste de papanicolau
- programas de rastreamento
- neoplasias do colo do útero

Introduction

Despite national guidelines for screening and treatment,[1] cervical cancer (CC) is the fourth most deadly cancer in Brazilian women.[2] Worldwide, the incidence of CC in 2020 was 13.3 per 100,000 women, and the mortality was 7.3 per 100,000.[3] In Brazil, the mortality was 6.12 deaths per 100,000 women in 2022,[2] and among women > 65 years old (screened or not), it was 22.1% between 1996 and 2015.[4]

The Brazilian CC screening program targets women aged 25 to 64 years old based on conventional cytology.[1] The first two tests should be performed yearly, and if both results are negative, the tests should be performed every 3 years. However, almost half of the tests occur within a year,[1,5] that is, some women were overscreened. In contrast, others are unscreened because all cytological tests are spontaneous; thus, only women who seek health services undergo cytologic examinations.[5]

In this context, there are various explanations for the high incidence and mortality rates, including the low coverage rate of cytology, the opportunistic nature of the program, and the fact that there are no testing intervals or age group restrictions.[5,6] Also, there is almost no control over the amount or quality of the latest tests performed on older women who reach 64 years old when screening stops.[7,8] According to the guidelines, a patient should not reach the age limit without considering her screening history; it is critical to have at least two negative tests in the previous 5 years and no prior history of preinvasive neoplastic disease before ceasing cytological collections.[1] In this context, the present study compared the follow-up of cytological results from women > 64 years old and biopsied patients who did or did not adhere to the Brazilian national CC screening guidelines.

Methods

The present observational, retrospective and analytical study compared the prevalence of abnormal cervical smears in women > 64 years old who did or did not follow the screening protocol. Our cohort came from Araçatuba, a mid-sized city in the southeastern countryside of the state of São Paulo, Brazil, and its region. The sample consisted of conventional cervical smears obtained from the records of the Instituto de Patologia de Araçatuba from January 1, 2002, to December 31, 2015 (14 years). This laboratory receives tests collected for CC screening from patients of the Brazilian Unified Health System (SUS, in the Portuguese acronym). Smears were collected from private clinics in Araçatuba and surrounding areas.

The results were reported according to the Bethesda System: atypical squamous cells of undetermined significance (ASC-US); atypical squamous cells cannot exclude high-grade squamous intraepithelial lesion (ASC-H); low-grade squamous intraepithelial lesion (LSIL); high-grade squamous intraepithelial lesion (HSIL); squamous cell carcinoma (SCC); atypical glandular cells of undetermined significance (AGC-US); atypical glandular cells favor neoplastic (AGC); endocervical carcinoma in situ; invasive cervical

adenocarcinoma; invasive endometrial adenocarcinoma; and adenocarcinoma not otherwise specified.[9] There are additional categories in the Brazilian national guidelines: atypical undetermined cells of undetermined significance and atypical undetermined cells, which cannot exclude high-grade intraepithelial lesions. Both refer to results in which it is impossible to determine if the atypical cells are glandular or squamous.[1]

Patients with abnormal results were compared in a subsequent step: repeat cytology in 6 months or go to colposcopy and biopsy, depending on the first abnormal cytology result. Then, those who were biopsied were divided into two groups: those who had at least two consecutive negative cytopathological tests in the previous 5 years (that is, those who followed the national CC screening guidelines and those who did not).[1] The magnitude of association was analyzed using the odds ratio (OR) with a 95% confidence interval (CI). Data were expressed as absolute (*n*) and relative (%) frequencies to assess the association between diagnostic categories. The significance level was set at 5%. Our research ethics committee approved the study under protocol CAAE: 83847517.10000.5379.

Results

Over these 14 years, there were 207 abnormal cytological results among women > 64 years old. Of these, 120 (58.0%) were classified as ASC-US and 8 (3.9%) were LSIL. According to the national screening program,[1] these patients should undergo repeat cytology in 6 months: 112 (87.5%) repeated the cytology and only 33 (25.7%) showed an abnormal result in the second exam. Finally, 33 (25.7%) biopsies were performed in this group, of which 7 (5.5%) demonstrated some abnormality: 1 cervical intraepithelial neoplasia (CIN) I, 3 CIN II, 1 CIN III, 1 SCC and 1 endometrial carcinosarcoma. The other 79 (38.1%) patients with abnormal results should have been referred to colposcopy and biopsy; however, 43 (54.4%) repeated the cytology, with 22 abnormal results (1 ASC-US, 3 AGUS, 9 ASC-H, 1 AGCH, 1 LSIL, 5 HSIL, 1 SCC and 1 atypical undetermined cells that cannot exclude high-grade intraepithelial lesions). Biopsy was performed in 41 (51.9%) of the women in this group, and 23 (29.1%) were positive (2 endometrial adenocarcinomas, 9 SCC, 5 CIN II, 5 CIN III and 2 adenocarcinomas). The results of the first cytology and their follow-up are shown in ►**Table 1**.

Table 1 The frequency of abnormal cytology and follow-up among women over 64 years old compared with following national guidelines

Cytologic results	First cytology	Repeated cytology	Abnormal results in second cytology	Biopsies	Abnormal biopsies
ASC-US	120 (58.3%)	105 (87.5%)	23 ASC-US 9 ASC-H 1 LSIL	29 (24.2%)	8 (6.6%)
ASC-H	28 (13.6%)	12 (42.8%)	6 ASC-H 2 HSIL	14 (50.0%)	8 (28.6%)
Atypical undetermined cells of undetermined significance	16 (7.8%)	12 (75%)	1ASCUC 1 AGUS 1 Atypical undetermined cells cannot exclude high-grade intraepithelial lesions	7 (43.7%)	2 (12.5%)
AGUS	9 (4.4%)	7 (7,8%)	1 AGCH 1 AGUS	3 (33.3%)	2 (22.2%)
LSIL	8 (3.9%)	7 (87.5%)	3 ASC-US	6 (75%)	1 (12.5%)
HSIL	8 (3.9%)	6 (75%)	2 ASC-H 3 HSIL	5 (62.5%)	2 (25%)
SCC	7 (3.4%)	2 (28.6%)	1 SCC 1 ASC-H	7 (100%)	6 (85.7%)
Atypical undetermined cells cannot exclude high-grade intraepithelial lesions	5 (2.4%)	3 (60%)	1 HSIL	3 (60%)	1 (20%)
AGC	3 (1.5%)	1 (33.3%)	1 AGUS	2 (66,7%)	1 (33.3%)
Adenocarcinoma not otherwise specified	1 (0.5%)	–	–	1 (100%)	1 (100%)
Invasive endometrial adenocarcinoma	1 (0.5%)	–	–	1 (100%)	1 (100%)
Invasive cervical adenocarcinoma	1 (0.5%)	–	–	–	–
Total	207 (100%)	155 (75.2%)	56 (27.1%)	78 (37.7%)	33 (15.9%)

Abbreviations: AGC: atypical glandular cells favor neoplastic; AGC-US: atypical glandular cells of undetermined significance; ASC-H: atypical squamous cells cannot exclude HSIL; ASC-US: atypical squamous cells of undetermined significance; CI: confidence interval; HSIL: high-grade squamous intraepithelial lesion; SCC: squamous cell carcinomas; LSIL: low-grade squamous intraepithelial lesion.
*All frequencies are relative to the total of the first cytology

Table 2 Frequency of abnormal biopsies among women over 64 years old compared with following national guidelines

Histological subtypes	Followed guideline	Not followed guideline	OR (95% CI)	Total
Negative	31 (77.5%)	14 (36.8%)	1	45 (57.7%)
Premalignant lesions (CIN I, II, and III)	7 (17.5%)	11 (28.9%)	3.479 (1.114–10.864)	18 (23.1%)
SCC	1 (2.5%)	9 (23.7%)	NA	10 (12.8%)
Cervical adenocarcinoma	–	2 (5.3%)	NA	2 (2.6%)
Others	1 (2.5%, endometrial adenocarcinoma)	2 (5.3%, endometrial carcinosarcoma and endometrial adenocarcinoma)	NA	3 (3.8%)
Total	40	38	5.904 (2.188–15.932) p = 0.0002	78 (100%)

Abbreviations: CI: confidence interval; CIN: cervical intraepithelial neoplasia; OR: odds ratio; SCC: squamous cell carcinoma.

Over the entire period, 78 (37.7% of the first abnormal results) biopsies were performed. Of these, 40 (51.3%) had at least 2 negative tests consecutively in the previous 5 years, following the national protocol: 31 (77.5%) negative biopsies and 9 (22.5%) positive results (1 endometrial adenocarcinoma, 1 SCC, 2 CIN I, 2 CIN II, 3 CIN III). Among the 38 (48.8%) women who did not follow the guidelines, 14 (36.8%) biopsies were negative and 24 (63.1%) were positive (1 endometrial carcinosarcoma, 1 endometrial carcinoma, 9 squamous cell carcinomas, 2 cervical adenocarcinomas, 5 CIN II, and 6 CIN III). The biopsy results are shown in ►**Table 2**. Then, women who did not follow the guidelines demonstrated higher chances of cancer and precursor lesions (OR: 5.904; 95%CI: 2.188–15.932; $p = 0.0002$).

Discussion

There is a significant frequency of CC precursor lesions and neoplasm in Brazilian women > 64 years old. A recent screening history influences the frequency and severity of the abnormal diagnosis. Many women in this age group with abnormal cytology did not correctly follow the screening protocols to confirm or treat the abnormality.

When women had indications to repeat the cytology because of their low-grade characteristics, 87.5% of the women did so. Under the Bethesda System, ASC-US suggests LSIL (CIN I); however, with a 10 to 20% possibility of HSIL (CIN II or CIN III).[9] The Brazilian guidelines assume this degree of benign behavior of the alteration and make conservative recommendations; thus, women with ASC-US and LSIL cytology results should undergo repeat cytology in 6 months.[1]

It is essential to highlight that some degree of neoplasia or premalignant lesions was found in 5.5% of biopsied patients. Other studies showed that conventional cytology had an overall sensitivity of 50 to 75% for detecting low-grade lesions and of 55 to 90% for high-grade lesions (CIN II/III).[8,10,11]

Considering women whose cytological results have high-grade characteristics with indications to proceed directly to colposcopy and possible biopsy, 54.4% underwent a second cytological test, not following the current guidelines. Of this group, 29.1% had some type of neoplasm in a later biopsy. This finding suggests an underestimated number of CC diagnoses and a higher accumulated risk of CC in women who did not undergo screening as recommended, primarily among those with a high-grade lesion possibility.[12,13] In India, the frequency of abnormal biopsies in women > 65 years old was also high (47.3%), demonstrating a higher frequency of cervical alterations among older women who continue the screening, corroborating the present study.[14]

When analyzing biopsies from 15 women with premalignant lesions, only 18.7% had followed the guidelines; among 10 cases of SCC, only 1 had followed the protocols, although all cases of adenocarcinomas had improper screening history. For glandular lesions, the difficulty in representing endocervical cells, especially among older women with some grade of retraction, may explain the screening not being performed appropriately.[1,8,9] The Brazilian guidelines consider any atypical glandular cell high-risk and associated with CIN II/III or cancer.[1]

Inadequate cervical screening in older women is a possible reason for delayed diagnosis and poor prognosis.[15] On the other hand, adequate screening can reduce the incidence of cervical cancer by 75%, as well as mortality.[16] Therefore, in agreement with the present study, women > 64 years old with inadequate screening had a higher risk of CC and worse outcomes.[17,18]

Other studies showed that few women who reached the age of exiting screening programs had been adequately screened during the preceding years.[19,20] Indeed, among women in the target group, there was poor follow-up, low frequency, and precarious cellular representation in samples, which may lead to underestimation of the prevalence of CC and premalignant lesions during screening of women at the target age.[21] In the context of inadequate cervical screening program performance, the frequency of cervical cancer could be more significant than expected.

In countries that implemented screening using DNA testing, high-risk human papillomavirus was present in smears of women > 70 years old, and there were premalignant lesions in 45% of them even after their exit from

screening.[22] This finding indicates the importance of screening these women later in life, especially if they had an abnormal screening history or were not screened. These findings reinforce the relevance of reassessing the age of exit of the protocol, the quality of smears and the frequency of previous screening.

We identified (63.1%) severe abnormalities in older women who did not follow the guidelines (endometrial carcinosarcoma, SCC, adenocarcinomas, CIN II and CIN III). This finding is similar to the American scenario, where lesions in advanced stages may be explained by irregular screening history despite the guidelines.[23] The decreasing interest in screening with advancing age also explains why older women have higher incidences of CC, especially where screening programs have an opportunistic character, as is the case in Brazil.[24,25] These explanations were also advanced in Australia[26] and Finland[27] to explain the frequency of abnormal tests in older women with a history of inadequate screening.

The suboptimal screening performance among this group can be explained by the level of patient education regarding the disease and limited access to the test.[28] The lack of knowledge of health professionals in Brazil (and worldwide) about the target ages and subsequent steps in national protocols for diagnosing, monitoring, and treating precursor lesions and neoplasm can also explain the results.[29,30]

A limitation of the present study is that we analyzed data from a medium-sized city, which might not represent all Brazilian populations. Nevertheless, the present study illustrates the prevalence of abnormal cervical smear results in our community since our laboratory is the only pathology laboratory in the city.

Conclusion

Because CC mortality in Brazil is high, the frequency of abnormal cytological results among women $>$ 64 years old is not insignificant. The present study demonstrated that women who did not follow the national guidelines had higher rates of true precursor lesions (CIN II/III) and invasive neoplasms (SCC, adenocarcinomas, and others) than those who followed the guidelines. These findings suggest revising the screening exit age in Brazil to reduce the incidence of CC.

Contributions

Substantial contributions to the design, data collection or analysis, and interpretation of data: Zago R. A., José C. C. X-V., Deolino J. C-J., Solange C. G. P. D.. Writing of the article or relevant critical review of intellectual content: Zago R. A., José C. C. X-V.,. Final approval of the version to be published: José C. C. X-V.

Conflict of Interests

The authors have no conflict of interests to declare.

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Incidence and Outcomes Associated with Menopausal Status in COVID-19 Patients: A Systematic Review and Meta-analysis

Incidência e resultados associados ao estado da menopausa em pacientes com COVID-19: Uma revisão sistemática e metanálise

Abolfazl Akbari[1] Ahmadreza Zarifian[1,2] Alireza Hadizadeh[3] Ezat Hajmolarezaei[4]

[1] Mashhad University of Medical Sciences, Mashhad, Iran
[2] University Hospital Lewisham, King's College London, London, United Kingdom
[3] Tehran University of Medical Sciences, Tehran, Iran
[4] Mashhad University of Medical Sciences, Mashhad, Iran

Address for correspondence Ezat Hajmolarezaei, Mashhad, 91778 99191Razavi Khorasan Province, Iran (e-mail: hajmollarezaeie@mums.ac.ir).

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Abstract

Objective Menopause causes several changes in the body that may affect the response to COVID -19. We aimed to investigate the possible association between menopausal status and incidence and outcomes in COVID-19 patients.

Methods Combinations of keywords*COVID-19*, *menopause*, and *estrogen* were used to search the PubMed, Embase, Web-of-Science, and Scopus databases for articles reporting the incidence and outcomes of COVID-19 (discharge, length-of-admission, intensive care, or mortality) in premenopausal women, available through December 29, 2022. Data from studies comparing the incidence of COVID-19 infection with the age-matched male population were pooled and meta-analyzed using a random-effects model.

Results Overall, 1,564 studies were retrieved, of which 12 were finally included in the systematic review to compare disease outcomes, and 6 were meta-analyzed for the incidence of COVID-19 in premenopausal and postmenopausal women. All studies reported better COVID-19-associated outcomes in premenopausal women compared with postmenopausal women. After adjusting for confounding factors, three studies found better outcomes in postmenopausal women, and two found no association between menopausal status and COVID-19 outcomes. Our meta-analysis found a higher incidence of COVID-19 infection among premenopausal women than postmenopausal women, when compared with age-matched men (odds ratio = 1.270; 95% confidence interval: 1.086–1.486; $p = 0.003$).

Keywords
- COVID-19
- menopause
- estrogen
- climacteric

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Conclusion The incidence of COVID-19 was significantly higher in premenopausal women than in postmenopausal women when compared with age-matched men. Although premenopausal women may have more favorable COVID-19-associated outcomes, the presumed preventive effect of estrogens on the incidence and related outcomes of COVID-19 in premenopausal women cannot be proven at present. Further longitudinal studies comparing pre- and post-menopausal women are required to provide further insight into this matter.

Resumo

Objetivo A menopausa causa diversas alterações no corpo que podem afetar a resposta ao COVID-19. Nosso objetivo foi investigar a possível associação entre o status da menopausa e a incidência e os resultados em pacientes com COVID-19.

Métodos Combinações de palavras-chave COVID-19, menopausa e estrogênio foram usadas para pesquisar os bancos de dados PubMed, Embase, Web-of-Science e Scopus para artigos relatando a incidência e os resultados do COVID-19 (alta, tempo de internação, tratamento intensivo cuidados ou mortalidade) em mulheres na pré-menopausa, disponível até 29 de dezembro de 2022. Dados de estudos comparando a incidência de infecção por COVID-19 com a população masculina da mesma idade foram agrupados e meta-analisados usando um modelo de efeitos aleatórios.

Resultados No geral, 1.564 estudos foram recuperados, dos quais 12 foram finalmente incluídos na revisão sistemática para comparar os resultados da doença e 6 foram meta-analisados para a incidência de COVID-19 em mulheres na pré e pós-menopausa. Todos os estudos relataram melhores resultados associados ao COVID-19 em mulheres na pré-menopausa em comparação com mulheres na pós-menopausa. Após o ajuste para fatores de confusão, três estudos encontraram melhores resultados em mulheres na pós-menopausa e dois não encontraram associação entre o status da menopausa e os resultados do COVID-19. Nossa meta-análise encontrou uma maior incidência de infecção por COVID-19 entre mulheres na pré-menopausa do que mulheres na pós-menopausa, quando comparadas com homens da mesma idade (odds ratio = 1,270; intervalo de confiança de 95%: 1,086–1,486; p = 0,003).

Conclusão A incidência de COVID-19 foi significativamente maior em mulheres na pré-menopausa do que em mulheres na pós-menopausa quando comparadas com homens da mesma idade. Embora as mulheres na pré-menopausa possam ter resultados mais favoráveis associados ao COVID-19, o efeito preventivo presumido dos estrogênios na incidência e nos resultados relacionados ao COVID-19 em mulheres na pré-menopausa não pode ser comprovado no momento. Mas estudos longitudinais comparando mulheres pré e pós-menopausa são necessários para fornecer mais informações sobre este assunto.

Palavras-chave
- COVID 19
- menopausa
- estrogênio
- climatério

Introduction

The emergence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)-induced Coronavirus Disease 2019 (COVID-19) has caused serious illness and death around the world. The World Health Organization (WHO) reported > 546 million confirmed cases and > 6 million deaths as of August 17, 2022.[1] Males reportedly have higher rates of disease severity,[2] hospitalization, readmission,[3] and mortality[4] compared with females with COVID-19. Following the recent reports on this sex difference,[5] researchers have tried to investigate the possible causes. In comparison with premenopausal women, postmenopausal women have considerably decreased plasma sex hormone concentrations (for example, estrogen and progesterone depletion).[6] It has long been known that estrogen plays a role in the immune response and regulates both the innate and adaptive immune systems.[7] Previous reviews have put forward possible effects of estrogen on the entrance and replication of viruses, innate/adaptive immune responses, and thrombosis.[8] The protective role of estrogen is reportedly linked to downregulating the expression of angiotensin-converting enzyme 2 (ACE-2), which acts as the SARS-CoV-2 receptor on target cells, by estradiol and modulation of the immune response. In vivo studies have also shown that estrogen treatment can reduce morbidity and mortality in mice infected with the Influenza A virus,[9,10] where higher levels of estrogen administration were associated with increased

survival and lower pulmonary cytokine production after influenza infection.[10,11]

Despite the growing body of evidence addressing predisposing factors for COVID-19 (for example, older age, gender, comorbidities), little is known about the association between menopausal status and COVID-19 outcomes and the role of sex hormones in COVID-19. In the present study, we systematically reviewed the available literature on the link between menopausal status and COVID-19 incidence and outcomes, comparing pre- and postmenopausal women. We also pooled data from premenopausal or postmenopausal groups that had an age-matched control group to reduce the effects of confounding factors.

Methods

The present study followed the Preferred Reporting Items for Systematic Review and Meta-Analysis Protocols (PRISMA) standards.[12] The study was approved by the Ethics Committee of Mashhad University of Medical Sciences, Mashhad, Iran (code: IR.MUMS.IRH.REC.1402.050).

The PubMed, SCOPUS, Web of Science, and Embase databases were searched for studies investigating the relationship between menopausal status and COVID-19 infection up to December 29, 2022. We manually searched Google Scholar and the reference lists of the included papers to find other studies that might meet our inclusion criteria. The search terms *COVID-19*, *menopause*, and *estrogen* were used in various combinations.

Initially, all studies in the English language that reported information on menopausal status and COVID-19 patients were included with no restrictions on publication date.[13] After removing the duplicates, all articles comparing the incidence and outcomes of premenopausal and postmenopausal females with COVID-19 were included in the systematic review. Studies comparing the variables with age-matched males were included in the meta-analysis. Review articles, case reports, non-human studies, letters, reports based on Web sites, and government regulatory documents were all excluded.

The study selection was performed by two reviewers (Akbari A. and Hadizadeh A.) based on the title, abstract, and full text of the papers. When there was no agreement, the decision was made by a third reviewer (Zarifian A.), who checked eligibility to make the final decision. Two reviewers (Akbari A. and Hadizadeh A.) independently assessed the quality of the included papers. The Joanna Briggs Institute (JBI) assessment tools were used to assess the included papers.[14] Any disagreement was resolved by discussion between the authors.

Study characteristics including the first author's surname, publication date, title, study design, site of study (country), sample size, menopausal criteria, and patient recruitment date were extracted from the included articles. COVID-19 outcomes (discharge, intensive care unit [ICU] admission, length of hospitalization, and mortality) as well as further analysis of the initial findings were extracted and summarized in ►**Chart 1**. Search strategies used in different databases are listed in supplementary ►**Chart 2**.

Quantitative analyses were conducted on studies reporting the incidence of COVID-19 infection among premenopausal females, postmenopausal females, and age-matched males. The incidence of COVID -19 in premenopausal women was compared with that in men of the same age, and a similar comparison was made for postmenopausal women. The odds ratio (OR) of these comparisons was calculated and reported with the 95% confidence interval (CI) in brackets. A *p-value* < 0.05 was considered statistically significant. Interstudy heterogeneity was quantitatively calculated and presented using the I^2 index. Due to high heterogeneity (Cochran $Q < 0.05$), we used the random-effects model for our meta-analysis. Sensitivity analysis was performed using fixed-effects model analyses. Potential publication bias was investigated using funnel plots, as well as the Begg and Egger test. Statistical analyses were performed using Comprehensive Meta-Analysis Software (CMA v.3, Biostat Inc., Englewood, NJ, USA).

Results

A total of 1,564 studies were found by searching the databases, of which 775 were duplicates. Of the 789 remaining papers, 34 were reviewed in full text. Finally, 12 studies were included in the present systematic review[15–26] (►**Fig. 1**) and 6 were included in the meta-analysis.[15–25] The total number of patients in the 12 included studies was 331,821, ranging from 147 to 152,637. Seven studies were conducted in Asia (five in China[15,16,20,22,23] and two in India[17,21]), one each in Canada,[18] United States,[25] Italy,[24] and United Kingdom,[26] and one was a multicenter study.[19] All but one cross-sectional study[15] had a retrospective cohort design.

Incidence of COVID-19

The meta-analysis of six studies comparing premenopausal and postmenopausal females with age-matched males showed a higher incidence of COVID-19 in premenopausal females than in postmenopausal females (OR = 1.270; 95%CI [1.086–1.486]; $p = 0.003$) (►**Fig. 2**). A sensitivity analysis for this comparison was done on two studies that used > 1 year of amenorrhea as menopausal criteria (21, 23), which showed a significantly higher incidence of COVID-19 in the premenopausal group compared with postmenopausal women (fixed effect model: OR = 1.345; 95%CI: 1.164–1.555; $p < 0.0001$). In these 6 studies, the total number of COVID-19 cases was 19,861 in the premenopausal group and 18,610 in the postmenopausal group.

Outcomes of COVID-19

The study characteristics of 12 included studies are summarized in ►**Table 1**. All studies reported better COVID-19-associated outcomes in premenopausal women than in postmenopausal women. However, after adjusting for confounding factors, premenopausal women had more favorable COVID-19 outcomes in only three studies (15, 16, 22), while two others found postmenopausal women to have better COVID-19 outcomes (20, 24), and three found no significant difference in this regard (17, 21, 23), while three studies did

Chart 1 Characteristics and outcomes of included studies

First author	Study design	Country	Patients	Date	Menopausal criteria	Sample size	Age (years old)	Crude findings	Further analysis	Corrected confounding variables
Ding et al.	Cross-sectional	China	Patients hospitalized at 3 branches of Tongji Hospital	28 January - 8 March, 2020	Amenorrhea > 1 year	1,730	60.33 ± 14.36	Severity (premenopause: 46% versus postmenopause: 58%) and clinical outcomes including discharge (premenopause: 23% versus postmenopause: 6%), remained in hospital (premenopause: 77% versus postmenopause: 92%), and death (premenopause: 0% veersus postmenopause: 2%) were significantly different between premenopausal women and postmenopausal women.	**Age-match comparison** Severity ($p = 0.83$) and clinical outcomes ($p = 0.49$) including discharge, remained in hospital, and death did not differ significantly between menopausal women and age-matched men, whereas premenopausal women had significantly better clinical outcomes and fewer severely ill patients than age-matched men ($p < 0.01$ for both).	Age and comorbidities
Wang XW. et al.	Retrospective cohort	China	COVID-19 inpatients at the Taikang Tongji Hospital	15 February - 30 April, 2020	Amenorrhea > 1 year	300	65.3 ± 14.8	Postmenopausal women had higher rates of severe disease (41.7 versus 0%), bilateral pulmonary infiltration (91.7 versus 64.7%), and mortality (2.0 versus 6.0%) than premenopausal women.	**Age-match comparison** Men had higher rates of severe disease (23.7% versus 0%; $p = 0.003$) and bilateral pulmonary infiltration (86.1% versus 64.7%; $p = 0.04$) than premenopausal women. However, there was no significant difference in mortality (2.0% versus 0%; $p = 1.00$) between the 2 groups. Men and postmenopausal women had the same percentage of severe disease (32.7% versus 41.7%; $p = 0.21$), bilateral lung infiltration (86.1% versus 91.7%; $p = 0.24$), and mortality (2.0% versus 6.0%; $p = 0.25$).	Age, body mass index , comorbidities, treatment, and laboratory results
Mishra et al.	Retrospective cohort	India	Females admitted at tertiary care dedicated COVID hospital	May – August, 2020	Amenorrhea > 1 year	147	39.05 ± 15.42	Length of hospital stay (premenopause: 8.6 ± 3.9; postmenopause: 14.1 ± 8.9; $p < 0.01$), severe disease (premenopause: 7.3%; postmenopause: 23.5%; $p < 0.01$), and mortality (premenopause: 0%; postmenopause: 9.8%; $p < 0.01$) were significantly different between groups.	**Multivariate logistic regression:** Menopausal status is not associated with length of hospital stay ($p = 0.057$) or severity progression ($p = 0.262$).	Age, obesity, comorbidities, oxygen/ventilator requirement, hemoglobin, and neutrophil to lymphocyte ratio
O'Brien et al.	Retrospective cohort	Canada	Canadian COVID-19 dataset	Up to 27 July 2020	More than 60 years/o	101,121	NR	Women had a lower COVID -19 incidence rate than men unless they were 80 years of age or older. Premenopausal women had a lower incidence rate than men of the same age.	–	
Seeland et al.	Retrospective cohort	17 countries	Electronic health records in a TriNetX Real-World database	Up to 16 July 2020	> 50	68,466	NR	Premenopausal women had higher incidence rates than men of the same age. Mortality rates increased steadily with age in both sexes, but the increase was steeper in men at 50 years old.	–	
Sha et al.	Retrospective cohort	China	Jinan Infectious diseases Hospital in Shandong, Shandong	31 January - 17 April, 2020	> 55	413	57.25 ± 3.75	Postmenopausal women had a higher mortality rate than	**Age-match comparison** Premenopausal women had the same in-hospital mortality rate as	Age and comorbidities

(*Continued*)

Chart 1 (*Continued*)

First author	Study design	Country	Patients	Date	Menopausal criteria	Sample size	Age (years old)	Crude findings	Further analysis	Corrected confounding variables
			Provincial Chest Hospital in Shandong, and Huanggang Central Hospital in Hubei					premenopausal women (5.2% versus 3.8%).	men of the same age (3.8 versus 4.0%, $p = 0.918$). Postmenopausal women had a considerably lower mortality rate than men in the same age group (5.2 versus 21.0%, $p = 0.007$).	
Garg et al.	Retrospective cohort	India	A COVID-19 facility	April – July, 2020	Amenorrhea > 1 year	720	60.15 ± 6.39	Postmenopausal women and men had a higher risk of death than premenopausal women (premenopause: 8.6%; postmenopause: 19.4%)	**Age-match comparison** Risk of death in men ≤ 48 years old was 12.8% and in men > 48 it was 25.9%	Age and comorbidities
Liu et al.	Retrospective cohort	China	Renmin Hospital of Wuhan University (Wuhan, China) and Xiangyang Central Hospital (Xiangyang, China)	20 January - 1 April, 2020	> 55	459	63.25 ± 3.50	Postmenopausal women had a higher risk of death and longer length of hospitalization than premenopausal women (premenopause: 3; postmenopause: 7, premenopause: 18 (9–28); postmenopause: 13 (7–24)	**Age-match comparison** The difference in incidence between men and women was not observed in patients > 55 years old. 141 patients were < 55 years old, of whom 19 died (16 men versus 3 women; $p < 0.005$). Of the 318 cases > 55 years old, 115 died (47 women versus 68 men, $p = 0.149$).	Age and comorbidities
Wang M. et al.	Retrospective cohort	China	The Central Hospital of Wuhan, Wuhan Red Cross Hospital, the Central Hospital of Enshi Tujia and Miao Autonomous Prefecture, and Lichuan People's Hospital in Hubei Province	31 December, 2019–31 March, 2020	Amenorrhea > 1 year	2,501	56.18 ± 4.32	–	**Multivariable logistic regression analysis and age-match comparison** There is no significant differences between premenopausal and postmenopausal females after propensity score matching by age (odds ratio of severe disease: 0.63 [0.32–1.24] and odds ratio of death: 1.83 [0.16–21.5]).	Age and comorbidities
Ferretti et al.	Retrospective cohort	Italy	Patients hospitalized at IRCCS San Matteo Foundation (Pavia, Italy) for COVID-19	February - December, 2020	> 50	1,764	70.10 ± 4.05	Postmenopausal women had higher incidence and mortality rates than premenopausal.	**Age-match comparison** Premenopausal women had higher incidence and mortality rates than men of the same age. However, incidence and mortality rates were higher in age-matched men than in postmenopausal women.	Age and comorbidities
Costeira et al.	Retrospective cohort	United Kingdom	Female users of the COVID Symptom Study application	7 May-15 June, 2020	Amenorrhea > 1 year	152,637	53.8	Menopausal women had higher rates of predicted COVID-19 ($p < 0.01$), but tested COVID-19 patients and severity of disease were not significantly different with postmenopausal women ($p > 0.05$).	–	
Toure et al.	Retrospective cohort	USA	Nonpregnant women admitted to the Hospital System in Rhode Island	March 1-June 30, 2020 and July 1, 2020 - February 28, 2021	> 55	1,863	67.57 ± 18.0	Postmenopause was associated with higher mortality (OR = 8.6 [2.7–27.6]), readmission (OR = 1.5 [1.04–2.2]), severe illness (OR = 5.7 [1.3–23.9]), and longer length of hospitalization (OR 1.6 = [1.2–2.2]).		

Abbreviations: CI, confidence interval; NR, not reported; OR, odds ratio.

Chart 2 Search strategy

PubMed (29 December)		
Search	**Query**	**Results**
#1 (Menopause)	(post-menopausal [title/abstract] OR postmenopausal [title/abstract] OR Postmenopause [title/abstract] OR Post-menopause [title/abstract] OR peri-menopausal [title/abstract] OR perimenopausal [title/abstract] OR peri-menopause [title/abstract] OR perimenopause [title/abstract] OR premenopausal [title/abstract] OR pre-menopausal [title/abstract] OR pre-menopause [title/abstract] OR premenopause [title/abstract] OR Climacteric [title/abstract] OR Menopause[title/abstract] OR menopausal [title/abstract] OR Menstrual [title/abstract] OR Menses[title/abstract] OR Menstruation [title/abstract] OR Hypoestrogenic[title/abstract] OR Hypo-estrogenic [title/abstract] OR Estrogenic[title/abstract])	178,597
#2 (COVID-19)	("severe acute respiratory syndrome coronavirus 2" OR "Wuhan coronavirus" OR "Wuhan seafood market pneumonia virus" OR "COVID19 virus" OR "COVID-19 virus" OR "coronavirus disease 2019 virus" OR "SARS-CoV-2" OR "SARS2" OR "2019-nCoV" OR "2019 novel coronavirus" OR "COVID-19" OR "2019 novel coronavirus infection" OR "COVID19" OR "coronavirus disease 2019" OR "coronavirus disease-19" OR "2019-nCoV disease" OR "2019 novel coronavirus disease" OR "2019-nCoV infection" OR "Coronavirus Infections" OR "Coronavirus Infection" OR "Infection, Coronavirus" OR "Infections, Coronavirus" OR "novel coronavirus" OR Covid* OR "sars 2")	332,066
Final	**#1 AND #2**	**399**
SCOPUS (7 October)		
#1 (Menopause)	TITLE-ABS-KEY(post-menopausal OR postmenopausal OR Postmenopause OR Post-menopause OR peri-menopausal OR perimenopausal OR peri-menopause OR perimenopause OR premenopausal OR pre-menopausal OR pre-menopause OR premenopause OR Climacteric OR Menopause OR menopausal OR Menstrual OR Menses OR Menstruation OR Hypoestrogenic OR Hypo-estrogenic OR Estrogenic)	291,187
#2 (COVID-19)	TITLE-ABS-KEY("severe acute respiratory syndrome coronavirus 2" OR "Wuhan coronavirus" OR "Wuhan seafood market pneumonia virus" OR "COVID19 virus" OR "COVID-19 virus" OR "coronavirus disease 2019 virus" OR "SARS-CoV-2" OR "SARS2" OR "2019-nCoV" OR "2019 novel coronavirus" OR "COVID-19" OR "2019 novel coronavirus infection" OR "COVID19" OR "coronavirus disease 2019" OR "coronavirus disease-19" OR "2019-nCoV disease" OR "2019 novel coronavirus disease" OR "2019-nCoV infection" OR "Coronavirus Infections" OR "Coronavirus Infection" OR "Infection, Coronavirus" OR "Infections, Coronavirus" OR "novel coronavirus" OR Covid* OR "sars 2")	417,588
Final	**#1 AND #2**	**583**
Embase (30 July)		
#1 (Menopause)	(post-menopausal:ti,ab,kw OR postmenopausal:ti,ab,kw OR Postmenopause:ti,ab,kw OR Post-menopause:ti,ab,kw OR peri-menopausal:ti,ab,kw OR perimenopausal:ti,ab,kw OR peri-menopause:ti,ab,kw OR perimenopause:ti,ab,kw OR premenopausal:ti,ab,kw OR pre-menopausal:ti,ab,kw OR pre-menopause:ti,ab,kw OR premenopause:ti,ab,kw OR Climacteric:ti,ab,kw OR Menopause:ti,ab,kw OR menopausal:ti,ab,kw OR Menstrual:ti,ab,kw OR Menses:ti,ab,kw OR Menstruation:ti,ab,kw OR Hypoestrogenic:ti,ab,kw OR Hypo-estrogenic:ti,ab,kw OR Estrogenic:ti,ab,kw)	244,423
#2 (COVID-19)	("severe acute respiratory syndrome coronavirus 2":ti,ab,kw OR "Wuhan coronavirus":ti,ab,kw OR "Wuhan seafood market pneumonia virus":ti,ab,kw OR "COVID19 virus":ti,ab,kw OR "COVID-19 virus":ti,ab,kw OR "coronavirus disease 2019 virus":ti,ab,kw OR "SARS-CoV-2":ti,ab,kw OR "SARS2":ti,ab,kw OR "2019-nCoV":ti,ab,kw OR "2019 novel coronavirus":ti,ab,kw OR "COVID-19":ti,ab,kw OR "2019 novel coronavirus infection":ti,ab,kw OR "COVID19":ti,ab,kw OR "coronavirus disease 2019":ti,ab,kw OR "coronavirus disease-19":ti,ab,kw OR "2019-nCoV disease":ti,ab,kw OR "2019 novel coronavirus disease":ti,ab,kw OR "2019-nCoV infection":ti,ab,kw OR "Coronavirus Infections":ti,ab,kw OR "Coronavirus Infection":ti,ab,kw OR "Infection, Coronavirus":ti,ab,kw OR "Infections, Coronavirus":ti,ab,kw OR "novel coronavirus":ti,ab,kw OR Covid*:ti,ab,kw OR "sars 2":ti,ab,kw)	295,859

(*Continued*)

Chart 2 (*Continued*)

PubMed (29 December)		
Search	**Query**	**Results**
Final	**#1 AND #2**	**390**
WOS (30 July)		
#1 (Menopause)	(TI = (post-menopausal OR postmenopausal OR Postmenopause OR Post-menopause OR peri-menopausal OR perimenopausal OR peri-menopause OR perimenopause OR premenopausal OR pre-menopausal OR pre-menopause OR premenopause OR Climacteric OR Menopause OR menopausal OR Menstrual OR Menses OR Menstruation OR Hypoestrogenic OR Hypo-estrogenic OR Estrogenic) OR AB = (post-menopausal OR postmenopausal OR Postmenopause OR Post-menopause OR peri-menopausal OR perimenopausal OR peri-menopause OR perimenopause OR premenopausal OR pre-menopausal OR pre-menopause OR premenopause OR Climacteric OR Menopause OR menopausal OR Menstrual OR Menses OR Menstruation OR Hypoestrogenic OR Hypo-estrogenic OR Estrogenic))	158,610
#2 (COVID-19)	(TI = ("severe acute respiratory syndrome coronavirus 2" OR "Wuhan coronavirus" OR "Wuhan seafood market pneumonia virus" OR "COVID19 virus" OR "COVID-19 virus" OR "coronavirus disease 2019 virus" OR "SARS-CoV-2" OR "SARS2" OR "2019-nCoV" OR "2019 novel coronavirus" OR "COVID-19" OR "2019 novel coronavirus infection" OR "COVID19" OR "coronavirus disease 2019" OR "coronavirus disease-19" OR "2019-nCoV disease" OR "2019 novel coronavirus disease" OR "2019-nCoV infection" OR "Coronavirus Infections" OR "Coronavirus Infection" OR "Infection, Coronavirus" OR "Infections, Coronavirus" OR "novel coronavirus" OR Covid* OR "sars 2") OR AB = ("severe acute respiratory syndrome coronavirus 2" OR "Wuhan coronavirus" OR "Wuhan seafood market pneumonia virus" OR "COVID19 virus" OR "COVID-19 virus" OR "coronavirus disease 2019 virus" OR "SARS-CoV-2" OR "SARS2" OR "2019-nCoV" OR "2019 novel coronavirus" OR "COVID-19" OR "2019 novel coronavirus infection" OR "COVID19" OR "coronavirus disease 2019" OR "coronavirus disease-19" OR "2019-nCoV disease" OR "2019 novel coronavirus disease" OR "2019-nCoV infection" OR "Coronavirus Infections" OR "Coronavirus Infection" OR "Infection, Coronavirus" OR "Infections, Coronavirus" OR "novel coronavirus" OR Covid* OR "sars 2"))	277,897
Final	**#1 AND #2**	**190**

not use multivariate analysis (►**Chart 1**). The confounding factors adjusted for in each study are described in ►**Chart 1**, with all studies adjusted for age and comorbidities. All included studies were of adequate quality (►**Chart 3**).

Publication Bias

The Egger and Begg tests revealed no significant publication bias for the reported incidence rates in the included studies. ►**Fig. 2** shows the funnel plot for the COVID-19 incidence, which also indicates no significant publication bias.

Discussion

The meta-analysis of studies comparing premenopausal and postmenopausal females with age-matched males showed a higher incidence of COVID-19 in premenopausal women than in postmenopausal women, and the sensitivity analysis of studies that used > 1 year of amenorrhea as menopausal criteria confirmed it. We have shown that premenopausal women have better COVID-19-associated outcomes than postmenopausal women. Our findings revealed that the available literature could not still yield conclusive evidence on whether menopausal status (that is, serum estrogen levels) has a significant association with outcomes of COVID-19. Therefore, we are not able to determine if the sex-based disparities in COVID-19 incidence and outcomes is associated with estrogen levels, or if other potential effects may be influential as well. Consistent with the main finding of our study, Mishra et al. reported that most premenopausal women were more likely to have mild symptoms than postmenopausal women.[17]

The differences in COVID-19-associated outcomes between premenopausal women and postmenopausal women can be explained by several factors such as age, estrogen depletion, sedentary lifestyle, and comorbidities, which are more common in postmenopausal women.[27] Some studies reported that patients affected by COVID-19 were predominantly men,[28] while some others reported both sexes as being equally affected, or women to be predominant.[29] In addition, disease severity and mortality rates were reported to be higher in men than in women.[29] However, sex differences in morbidity and mortality were less evident in patients > 70 years old when women are in postmenopausal status.[30,31] One possible justification for these findings arises from the fact that estradiol downregulates the expression of

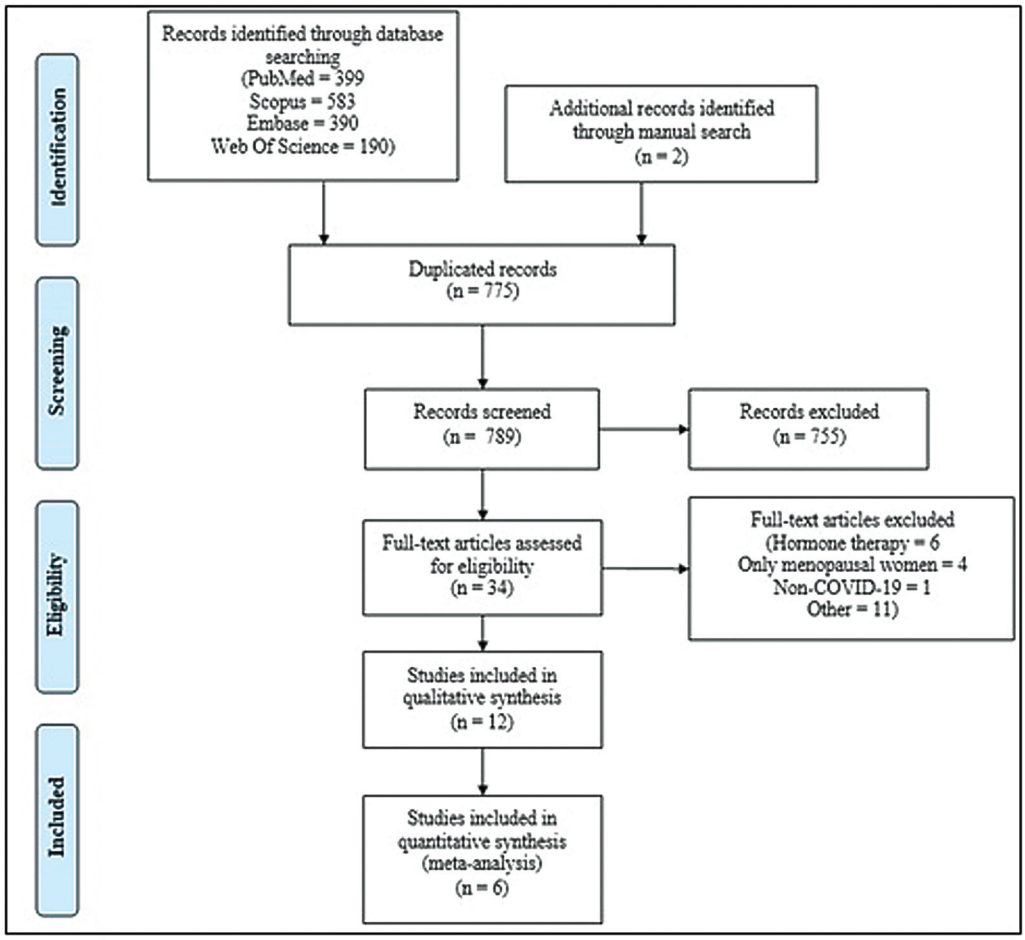

Fig. 1 PRISMA flow chart depicts the flow of information through the different phases of the study.

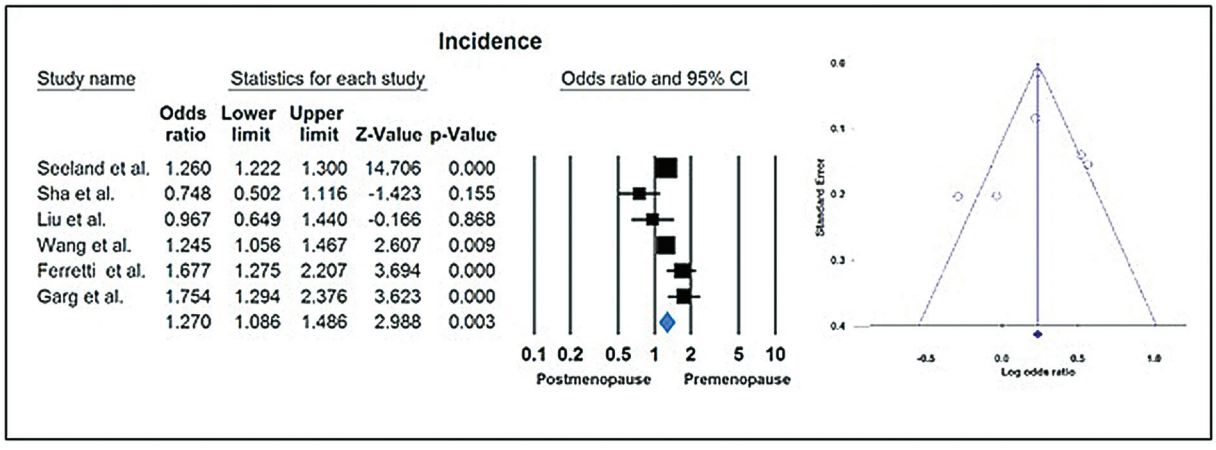

Fig. 2 Meta-analysis of studies comparing premenopausal and postmenopausal females with age-matched males.

ACE-2 mRNA in bronchial epithelial cells, the host-cell receptor which has been proven to be used by SARS-CoV-2 virions for viral uptake.[32] However, our results did not confirm these findings.

The literature suggests that cytokine storm leads to adverse clinical manifestations or even acute deterioration and mortality in critically ill patients with COVD-19.[33] Impaired acquired immune responses and uncontrolled innate inflammatory responses may be associated with the mechanism of cytokine storm in COVID -19. Early control of cytokine storm by anti-inflammatory treatments may improve the survival rate of patients with COVID-19.[34] It is well-known that pretreatment of human macrophages with estrogen can reduce tumor necrosis factor alpha (TNF-α) expression by inhibiting nuclear factor-kappa B (NFk-B) and JAK2 signaling pathways.[35] Estrogen also attenuates

Table 1

Study	1.Were the two groups similar and recruited from the same population?	2. Were the exposures measured similarly to assign people to both exposed and unexposed groups?	3. Was the exposure measured in a valid and reliable way?	4.Were confounding factors identified?	5. Were strategies to deal with confounding factors stated?	6. Were the groups/ participants free of the outcome at the start of the study (or at the moment of exposure)?	7. Were the outcomes measured in a valid and reliable way?	8. Was the follow-up time reported and sufficient to be long enough for outcomes to occur?	9. Was follow-up complete, and if not, were the reasons to loss to follow-up described and explored?	10. Was appropriate statistical analysis used?
Wang et al.	Y	Y	Y	N	N	Y	Y	Y	Y	Y
Mishra et al.	N	Y	Y	N	N	Y	Y	Y	Y	Y
O'Brien et al.	Y	Y	N	Y	Y	Y	Y	Y	N	Y
Seeland et al.	Y	Y	N	Y	Y	Y	Y	Y	Y	Y
Sha et al.	Y	Y	N	Y	Y	Y	Y	Y	Y	Y
Garg et al.	Y	Y	Y	N	N	Y	Y	Y	Y	Y
Liu et al.	Y	Y	N	Y	Y	Y	Y	Y	Y	Y
Wang et al.	Y	Y	Y	Y	Y	Y	Y	Y	N	Y
Ferretti et al.	Y	Y	N	Y	Y	Y	Y	Y	Y	Y
Costeira et al.	U	Y	Y	N	N	Y	Y	Y	N	Y
Toure et al.*	U	U	U	N	N	U	Y	Y	U	U

*only the abstract was available.

Chart 3 (B) Quality assessment table for cross-sectional studies based on JBI Critical Appraisal

Study	Were the criteria for inclusion in the sample clearly defined?	Were the study subjects and the setting described in detail?	`Was the exposure measured in a valid and reliable way?	Were objective, standard criteria used for measurement of the condition?	Were confounding factors identified?	Were strategies to deal with confounding factors stated?	Were the outcomes measured in a valid and reliable way?	Was appropriate statistical analysis used?
Ding et al.	Y	Y	Y	Y	Y	Y	Y	Y

monocyte and macrophage recruitment by downregulating the expression of chemokine ligand 2 during inflammation and dampening toll-like receptor 4-mediated NFk-B activation.[36] Along with its immunomodulatory properties, estrogen alters the expression of T helper 1 (Th-1) and Th-2 type cytokines, inhibits overactive inflammatory processes, and restores homeostatic conditions, thereby averting cytokine storm syndrome.[37,38] In a recent review, estrogens were shown to have remarkable anti-inflammatory and immunomodulatory effects on COVID-19 infections.[39] Another study showed that SARS-CoV-2 induces stress in the endoplasmic reticulum that exacerbates the infection, and estrogen may play a role in reducing the endoplasmic reticulum stress through stimulating estrogen-mediated signaling pathways.[40] An in-vivo study by Channappanavar et al. showed a protective effect of estrogen against COVID-19 death. They demonstrated that female mice given an estrogen receptor antagonist had a higher mortality rate due to SARS-COV-2 infection. Additionally, they noted that ovariectomized and gonadectomized female mice had a poor prognosis and considerable lung involvement with proinflammatory cytokines and chemokines.[41] Pirhadi et al. have also reported several antiviral effects for estrogen therapy through immunomodulatory and nonimmune mechanisms.[42] Improving the hydration of the oral cavity by stimulating hyaluronic acid production and enhancing the lower airway function can also be other probable mechanisms by which estrogen can lead to increased production of mucus-containing antiviral compounds.[43,44] In addition, estrogen therapy has been shown to decrease viral titers.[31] It may also decrease neutrophil recruitment, edema, and inducible nitric oxide synthase in the lungs. All of these have been associated with lower disease intensity.[45]

The results of our meta-analysis were not inconsistent with the previous large cohort of 44,268 postmenopausal and 108,369 premenopausal women, which showed that there was no significant difference between postmenopausal and premenopausal women in terms of COVID-19 incidence.[26] Also, a cross-sectional study by Ding et al. showed a higher prevalence of COVID -19 in postmenopausal women compared with premenopausal women.[15] Costeira et al. also showed that COVID-19 patients who used oral contraceptive pills (85% of whom were premenopausal) had a lower rate of hospitalization. According to a retrospective cohort study involving 5,451 women with COVID-19, those who underwent hormone replacement therapy (HRT) had a reduced mortality risk compared with women not receiving HRT.[46] Furthermore, an important finding in the study by Seeland et al. was the strong positive effect of regular estradiol therapy on the survival of postmenopausal women with COVID-19.[19] We recommend future meta-analyses examine the role of oral contraceptive pills and hormone replacement therapy in association with COVID-19 infections. In addition, previous studies suggest that poorer COVID -19 outcome in obese patients may also be related to the level of estradiol produced by the fat mass.[47]

In general, it is believed that estrogens have protective cardiovascular and metabolic effects. Studies have shown that females have a lower risk of cardiovascular events compared with males of the same age, while this risk roughly levels off after menopause.[29,48,49] Activation of G-protein-coupled receptor 30 (GPR-30) by estrogen has been shown to reduce the extent of ischemia and reperfusion injuries.[50] Reducing the low-density lipoprotein (LDL) oxidation and subsequently the oxidative stress is another reported mechanism.[51] It has also been shown that women who received HRT early after menopause had a considerably lower risk of cardiovascular events.[52] However, HRT is associated with venous thromboembolism (VTE),[53] which occurs in $\sim$ 15% of severe to critical COVID -19 patients.[54] Future studies should compare symptoms of COVID-19 between premenopausal and postmenopausal women to decipher the role of menopausal status and hormonal changes in COVID -19 severity.

The present study had several limitations. First, the effects of estrogen on COVID-19 outcomes may be dose-dependent, which cannot be investigated because the available studies have not assessed the serum sex hormone concentrations in COVID-19 patients. Second, postmenopausal women reportedly have higher concentrations of inflammatory cytokines compared with premenopausal women,[55–57] which can be a confounding factor that cannot be incorporated in our analyses. Third, the observed differences between premenopausal and postmenopausal groups are mainly due to factors such as age and comorbidities.[27] To determine the effects of sex hormones, we performed an age-matched analysis, which, however, cannot remove all confounding effects. Another limitation of the present review is the limited number of well-designed studies found with our strict inclusion criteria. Finally, some of the included studies have not used precise criteria to determine menopause in women, which can add to heterogeneity of the results.

Conclusion

Premenopausal women have better COVID-19-associated outcomes than postmenopausal women. In addition, the incidence of COVID-19 was considerably higher in premenopausal women than in postmenopausal women when compared with age-matched men. However, the presumed preventive effects of estrogen on the incidence and outcomes of COVID-19 in premenopausal women cannot be proven at present, as other well-known risk factors that are more common in older women must also be considered. Further longitudinal studies comparing pre- and postmenopausal women are required to provide further insight into this matter.

Conflict of Interests
The authors have no conflict of interests to declare.

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Efficacy, Safety, and Acceptability of Misoprostol in the Treatment of Incomplete Miscarriage: A Systematic Review and Meta-analysis

Eficácia, segurança e aceitabilidade do misoprostol no tratamento do aborto incompleto: Uma revisão sistemática e metanálise

Thiago Menezes da Silva[1] Moema Alves Guerra de Araujo[1] Ana Carolina Zimmermann Simões[1] Ronnier de Oliveira[1] Kleyton Santos de Medeiros[2,3] Ayane Cristine Sarmento[2] Robinson Dias de Medeiros[4] Ana Paula Ferreira Costa[2] Ana Katherine Gonçalves[2,4]

[1] Maternidade Escola Januário Cicco, Universidade Federal do Rio Grande do Norte, Natal, RN, Brazil
[2] Centro de Ciências da Saúde, Universidade Federal do Rio Grande do Norte, Natal, RN, Brazil
[3] Instituto de Ensino, Pesquisa e Inovação, Liga Contra o Câncer, Natal, RN, Brazil
[4] Department of Obstetrics and Gynecology, Universidade Federal do Rio Grande do Norte, Natal, RN, Brazil

Address for correspondence Ana Katherine Gonçalves, PhD, Professor, Campus Universitário, 59078-970, Lagoa Nova, Natal, RN, Brazil (e-mail: anakatherine@ufrnet.br).

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Abstract

Objective To assess the efficacy, safety, and acceptability of misoprostol in the treatment of incomplete miscarriage.

Data sources The PubMed, Scopus, Embase, Web of Science, Cochrane Library, and Clinical Trials databases (clinicaltrials.gov) were searched for the relevant articles, and search strategies were developed using a combination of thematic Medical Subject Headings terms and text words. The last search was conducted on July 4, 2022. No language restrictions were applied.

Selection of studies Randomized clinical trials with patients of gestational age up to 6/7 weeks with a diagnosis of incomplete abortion and who were managed with at least 1 of the 3 types of treatment studied were included. A total of 8,087 studies were screened.

Data collection Data were synthesized using the statistical package Review Manager V.5.1 (The Cochrane Collaboration, Oxford, United Kingdom). For dichotomous outcomes, the odds ratio (OR) and 95% confidence interval (CI) were derived for each study. Heterogeneity between the trial results was evaluated using the standard test, I^2 statistic.

Keywords
- Abortion
- Miscarriage
- Misoprostol
- Curettage

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Data synthesis When comparing misoprostol with medical vacuum aspiration (MVA), the rate of complete abortion was higher in the MVA group (OR = 0.16; 95%CI = 0.07–0.36). Hemorrhage or heavy bleeding was more common in the misoprostol group (OR = 3.00; 95%CI = 1.96–4.59), but pain after treatment was more common in patients treated with MVA (OR = 0.65; 95%CI = 0.52–0.80). No statistically significant differences were observed in the general acceptability of the treatments.

Conclusion Misoprostol has been determined as a safe option with good acceptance by patients.

Resumo

Objetivo Avaliar a eficácia, segurança e aceitabilidade do misoprostol no tratamento do aborto incompleto.

Fontes de dados Os bancos de dados PubMed, Scopus, Embase, Web of Science, Cochrane Library e bancos de dados de Ensaios Clínicos (clinicaltrials.gov) foram pesquisados para os artigos relevantes, e estratégias de busca foram desenvolvidas usando uma combinação de termos temáticos de Medical Subject Headings e palavras de texto. A última pesquisa foi realizada em 4 de julho de 2022. Nenhuma restrição de idioma foi aplicada.

Seleção dos estudos Foram incluídos ensaios clínicos randomizados com pacientes com idade gestacional até 6/7 semanas com diagnóstico de aborto incompleto e que foram manejadas com pelo menos um dos três tipos de tratamento estudados. Um total de 8.087 estudos foram selecionados.

Coleta de dados Os dados foram sintetizados usando o pacote estatístico Review Manager V.5.1 (The Cochrane Collaboration, Oxford, United Kingdom). Para resultados dicotômicos, o odds ratio (OR, na sigla em inglês) e o intervalo de confiança (IC) de 95% foram derivados para cada estudo. A heterogeneidade entre os resultados do ensaio foi avaliada usando o teste padrão, estatística I^2.

Síntese dos dados Ao comparar misoprostol com aspiração a vácuo médico (MVA, na sigla em inglês), a taxa de aborto completo foi maior no grupo MVA (OR = 0,16; IC95% = 0,07–0,36). Hemorragia ou sangramento intenso foi mais comum no grupo do misoprostol (OR = 3,00; 95%CI = 1,96–4,59), mas a dor após o tratamento foi mais comum em pacientes tratados com MVA (OR = 0,65; 95%CI = 0,52–0,80). Não foram observadas diferenças estatisticamente significativas na aceitabilidade geral dos tratamentos.

Conclusão O misoprostol tem se mostrado uma opção segura e com boa aceitação pelos pacientes.

Palavras-chave
- Aborto
- Aborto espontâneo
- Misoprostol
- Curetagem

Introduction

According to estimates from the World Health Organization (WHO), ~ 55 million abortions occurred between 2010 and 2014 worldwide, with 45% considered unsafe abortions. Africa, Asia, and Latin America account for 97% of the unsafe abortions.[1] The WHO defines unsafe abortion as a procedure for the termination of pregnancy performed by people without the necessary skills. Alternatively, it is defined as a procedure performed in an environment not standardized to perform medical procedures, or a combination of these two factors. Despite scientific advances that allow safe abortions for patients, unsafe abortions continue to occur, causing increased healthcare costs, complications, and maternal deaths.[2] In Brazil, abortion is a public health problem because of its magnitude and persistence.[3]

Additionally, several procedures have been performed in the face of abortion, such as pharmacological, surgical, or expectant procedures.[4] Not all forms of treatment are widely available in public services, and not all are accepted by patients. A previous meta-analysis comparing the possible management of first-trimester miscarriages found that one other success was achieved for every three patients treated surgically instead of medically. In contrast, expectant management showed variable efficacy depending on the clinical presentation.[5]

The WHO defines abortion as the expulsion or extraction of a conceptus before reaching 22 weeks of gestational age or weighing < 500 grams.[6] Additionally, abortion can be classified and approached in different ways, including gestational age (early or late), clinical presentation (threatened, inevitable, infected, incomplete, complete, or missed), and origin (spontaneous or provoked).[7]

Incomplete abortion, the subject of the present study, has an eminent clinical diagnosis and is characterized by transvaginal bleeding associated with an open uterine cervix upon physical examination when the products of conception have not been wholly discharged.[5] This is the most frequent clinical presentation of this condition.[7–9]

Currently, misoprostol (prostaglandin E2 analog), along with mifepristone, is the reference drug for medicated uterine emptying in cases of spontaneous or induced abortion, both in the first gestational trimester and at more advanced gestational ages. However, mifepristone is unavailable in Brazil.[4,5] Misoprostol works by inducing the uterus to contract and expel the remaining tissues, with no immediate necessity for operating theatres, sterile equipment, or skilled personnel. Thus, it is even more relevant in low-resource settings. Therefore, the present systematic review and meta-analysis aimed to assess the efficacy, safety, and acceptability of misoprostol in the treatment of incomplete miscarriage. Managing these patients using a less invasive option may be essential.

Methods

The present systematic review was designed and reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA).[10] As this study is based on published studies, no ethical approval or patient consent was required.

The study protocol was registered in the PROSPERO International Prospective Register of Systematic Reviews (registration number: CRD42018116776).

The design of the present study followed the PICOS strategy for systematic reviews as follows: Population (P): patients with incomplete miscarriage diagnosed up to 6/7 weeks of gestation; Intervention (I): treatment with misoprostol; Comparator (C): a manual vacuum aspiration (MVA) or curettage; Outcomes (O): Efficacy: complete evacuation of the uterus, with no need for additional intervention; Safety: adverse effect profile (frequency and severity); Acceptability: patient's overall satisfaction and if she would choose the same method again; Study design (S): randomized controlled trials (RCTs).

The articles were searched in the PubMed/MEDLINE, SCOPUS, EMBASE, Web of Science, Cochrane Library, and Clinical Trials databases using the following combinations of Medical Subject Heading terms and Boolean operators: "(*pregnancy* OR *pregnant women* OR *abortion* OR *miscarriage*) AND (*misoprostol* OR *dilatation* OR *curettage* OR *vacuum* OR *aspiration*) AND (*Randomized controlled trial* OR *blind method* OR *trial* OR *RCT*)." The final search was conducted on January 15, 2021, and updated on July 4, 2022. No language restrictions were applied. The keyword details and complete search strategy are provided in Supplemental file 1.

The inclusion criteria were randomized clinical trials with patients of gestational age up to 13 weeks and 6 days, diagnosed with incomplete abortion and managed with at least one of the three types of treatment studied. Nonrandomized studies and those that analyzed nonspontaneous abortions or patients not in the first trimester were excluded from the review.

Two researchers, TMS and ACZS, independently screened the studies according to their titles and abstracts. Duplicate studies were excluded, and the full text was reviewed to determine whether they met the selection criteria. A third researcher, ACAS, resolved the disagreements between the reviewers regarding the inclusion of an article. Once the studies were selected, the data of each were summarized in a single spreadsheet to standardize all the results obtained; the latter process was conducted by two researchers, MAGA and RO. Any missing data would have been retrieved by contacting the corresponding author or their coauthors through phone or e-mail, but there was none.

The following data were included in the spreadsheet: author, year of study, country in which the study was conducted, number of patients enrolled, age of patients, gestational age, number of patients assigned to each treatment method, main adverse effects reported, satisfaction with the technique, and follow-up time.

One of the researchers, AKG, used the Cochrane risk-of-bias analysis tool[11] to assess randomization, participant allocation, participant-practitioner blinding, and outcome assessment. Incomplete data and possible conflicts of interests were also considered. The risk of bias was assessed according to the predetermined criteria: low, high, or uncertain.

Data were synthesized by another researcher, APFC, using the statistical package in Review Manager V.5.1. For dichotomous outcomes, the OR and 95%CI were derived for each study. The heterogeneity between the trial results was evaluated using a standard test with $p = 0.1$ and the I^2 statistic, which is a quantitative measure of inconsistency across studies, with 0% indicating no observed heterogeneity and values of 50% indicating substantial heterogeneity. When heterogeneity was measured ($I^2 = 75\%$), a random-effects model was used to combine the trials and calculate the relative risk (RR) and 95%CI using the DerSimonian and Laird algorithm in a meta-analysis package for R. The other study characteristics and results have been summarized.

The quality of evidence of the studies was evaluated by one of the researchers, KSM, according to the Grading of Recommendations Assessment, Development, and Evaluation (GRADE).[12]

Results

Study Selection

Database searches identified 8,087 articles (►**Fig. 1**). From this initial amount, 67 were excluded due to duplication, 7,985 were excluded after title and abstract review, 1 study could not be retrieved, and 21 were excluded because they did not meet the eligibility criteria. Case reports and series were excluded from the present study. Ultimately, nine studies met the eligibility criteria and were included in the final review (►**Chart 1**).

Throughout the search and selection of studies, seven articles,[13–19] which compared mifepristone and misoprostol,

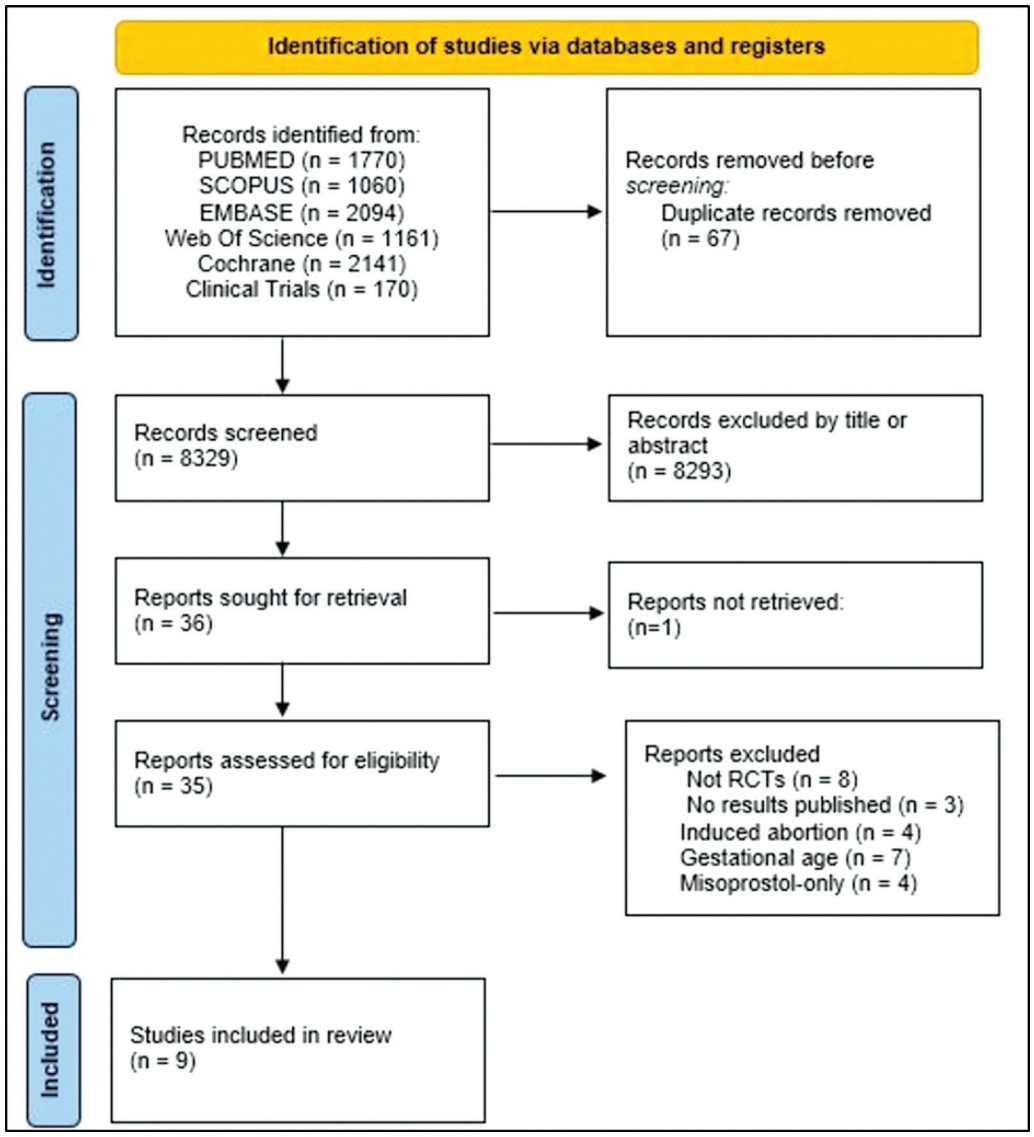

Fig. 1 PubMed search strategy.

were analyzed. However, these were discarded because they did not meet the inclusion criteria. The studies included patients with a gestational age of 5 to 12 weeks but excluded incomplete abortion from their analysis. These studies concluded that the treatment with mifepristone and misoprostol was more effective than misoprostol alone for the management of missed miscarriages, which was not the objective of our analysis. Nonetheless, given the relevance of this drug in current studies and its future potential as another accessible treatment option, the meta-analysis in question will be addressed in the "Discussion" section.

Study Characteristics

Of these studies, eight were RCTs comparing treatment with misoprostol and MVA. One multicentric trial compared misoprostol with "surgical evacuation," which could be MVA or curettage, according to the usual practice of the service. However, there was no information regarding the number of patients allocated to each procedure. A total of 2,992 patients were enrolled, with a mean age of 28.1 years old and gestational age up to 13 6/7 weeks. The follow-up time ranged from 24 hours to 28 days in the study protocols. Eight studies involving 1,950 patients who underwent treatment with either misoprostol or MVA were included for meta-analysis.[20–27]

Outcomes of Misoprostol

When comparing misoprostol with MVA, the rate of complete abortion was higher in the MVA group (OR = 0.16; 95% CI = 0.07–0.36) (►**Fig. 2**). Hemorrhage or heavy bleeding was more common in the misoprostol group (OR = 3.00; 95% CI = 1.96–4.59), but pain after treatment was more frequent in patients treated with MVA (OR = 0.65; 95%CI = 0.52–0.80) (►**Figs. 3** and **4**). Regarding the general acceptability of the treatment (in relation to overall satisfaction and if the same method would be chosen again), misoprostol showed an OR of 0.67 with a 95%CI of 0.38–1.19 (►**Fig. 5**).

Studies that could not be included in the meta-analysis were analyzed individually and showed conflicting results. Shochet et al.[28] compared 465 patients who were given

Chart 1 Characteristics of the studies included in the systematic review

Author, year	Country	Study sesign	Sample	Average age of participants (years old)	Gestational age inclu ded (weeks)	Intervention	Groups of comparison (n° of patients)		Follow-up time (days)	Outcomes
							Misoprostol	**Manual vacuum aspiration (MVA)**		
Dabash R. et al, 2010[24]	Egypt	RCT	695	28	0–12	400mcg sublingual x MVA	348	347	7	Favours MVA in safety.
R. Montesinos et al., 2011[22]	Ecuador	RCT	203	18-33	0–12	600 mcg oral x MVA	106	97	7	Favours MVA in safety.
Taylor J. et al, 2011[26]	Ghana	RCT	218	26	0–12	600 mcg oral x MVA	108	110	7–14	Favours misoprostol in acceptability.
Chigbu B. et al, 2012[25]	Nigeria	RCT	320	29	0–12	600 mcg oral x MVA	160	160	7–14	Favours misoprostol in acceptability.
Shochet T. et al, 2012[28]	Nigeria, Niger, Senegal, Burkina Faso, Mauritania	RCT	839	28	0–12	400 mcg sublingual x surgical evacuation	465	374 allocated to "surgical evacuation" (Curettage or MVA)	7–14	Favours "surgical evacuation" in efficacy and safety.
Das CM. et al., 2014[20]	Pakistan	RCT	222	28	0–12	600 mcg x MVA	111	111	7	No difference between groups.
Ibyiemi KF. et al, 2019[21]	Nigeria	RCT	198	28	0–13	600 mcg oral x MVA	100	98	7	Favours MVA in efficacy and safety (except for "pain").
Ani VC. et. al, 2022[27]	Nigeria	RCT	203	29	First trimester (9.1 ± 2.0)	400mcg sublingual x MVA	102	101	7	Favours MVA in efficacy, favours misoprostol in safety (specifically in pain).
Nwafor et al, 2021[23]	Nigeria	RCT	94	18–45	0–13	600 mcg oral x MVA	48	46	7	Favours misoprostol in acceptability.

Abbreviation: MVA, manual vacuum aspiration.

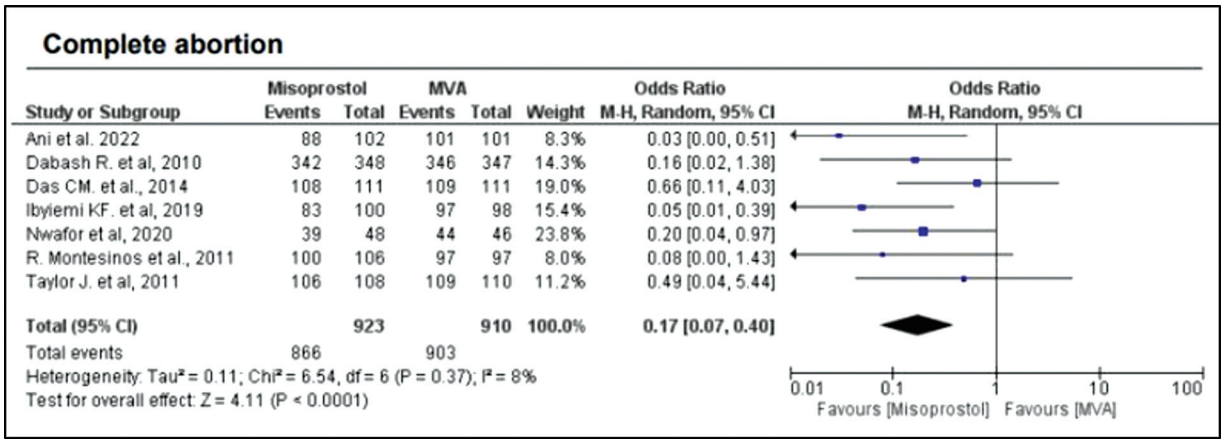

Fig. 2 Complete abortion.

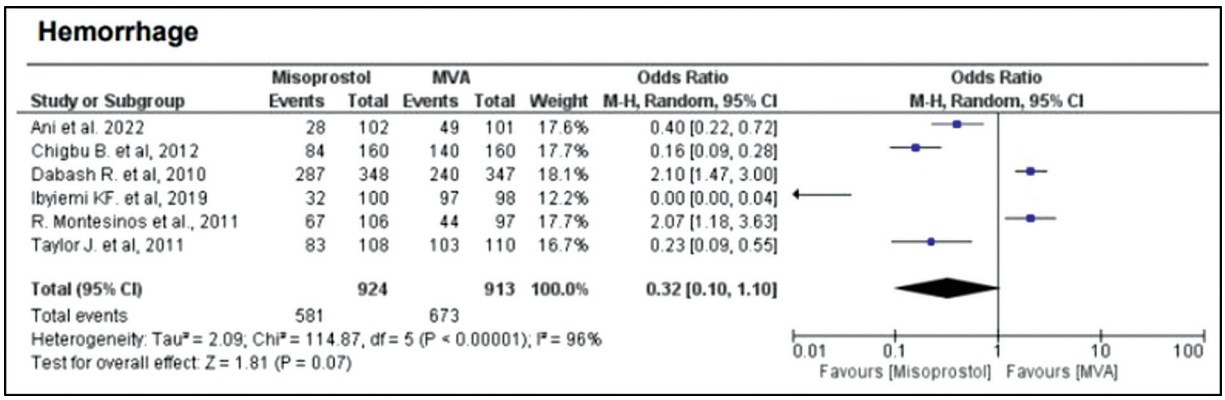

Fig. 3 Hemorrhage.

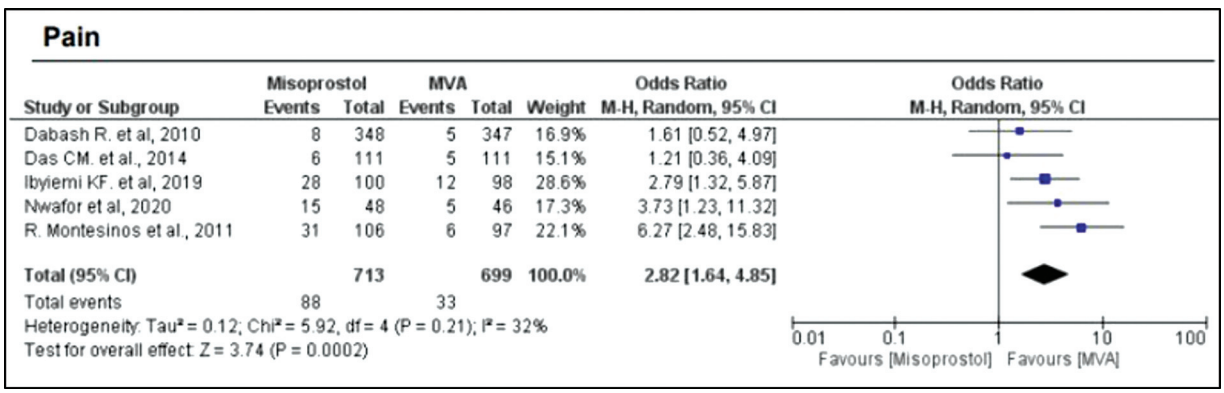

Fig. 4 Pain.

400 µg of sublingual misoprostol with 374 patients undergoing surgical evacuation (MVA or curettage) and observed higher efficacy (risk ratio [RR] = 0.90; CI = 0.88–0.92) and lower rates of hemorrhage (0.6 versus 11.6%) and pain (24.4 versus 54.8%) in the surgical group ($p < 0.001$). Nonetheless, a higher number of patients in the misoprostol group said that they would choose the same treatment again if needed (97.6 versus 87.8%; $p < 0.001$). No significant difference was noted in overall satisfaction with the method (98.5 versus 98.1% in the misoprostol and surgical groups, respectively; $p = 0.78$).

Risk of Bias

In general, the studies presented a low risk of bias. The clinical trials were conducted safely. However, Shochet et al. (2012), Ibiyemi et al. (2019), and Ani et al. (2022) showed some risk of bias in the randomization process (►**Fig. 6**).

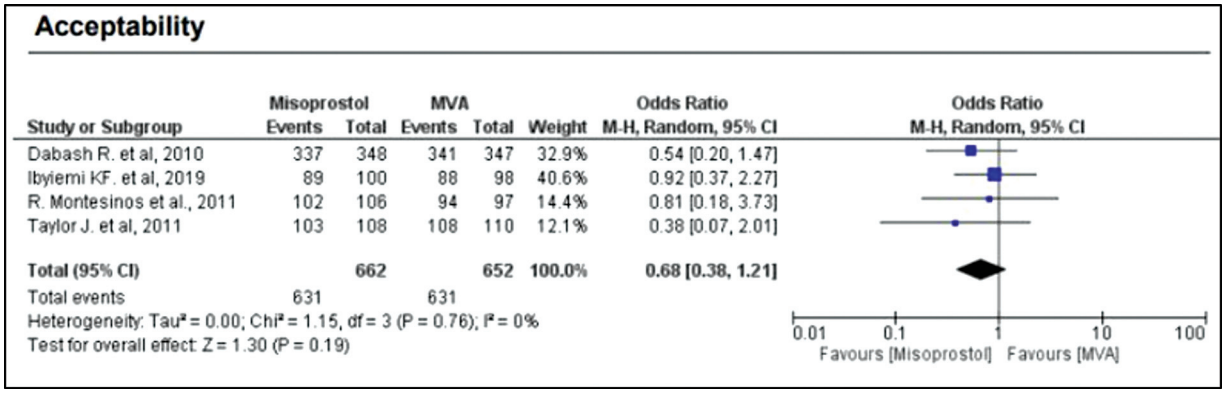

Fig. 5 Acceptability.

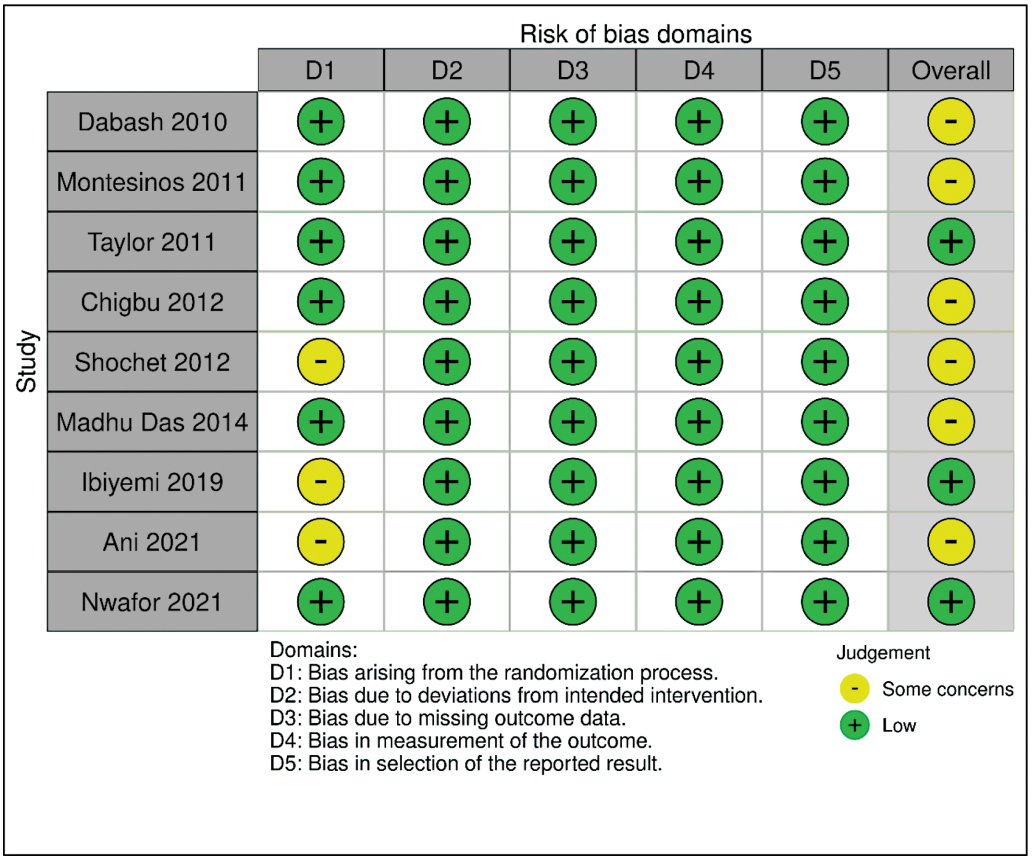

Fig. 6 Risk of bias.

Quality of Evidence

The efficacy and satisfaction of outcomes presented high quality evidence. However, the bleeding/hemorrhage and pain outcomes showed low-quality evidence, mainly due to high heterogeneity between the studies and high CI of the results (►**Chart 2**).

Discussion

Since miscarriage is still a complex health problem, often neglected by health policies, discussion on management options and application of scientific evidence to provide humanized, effective, and safe care that patients will accept is fundamental to alleviate the physical and psychological burden of this event.

In this scenario, misoprostol is a suitable option, although the present meta-analysis revealed that it has a slightly lower efficacy than MVA or curettage, with a higher rate of heavy bleeding. However, the patients reported less pain when using misoprostol and presented similar acceptability as surgical treatments. In addition, misoprostol can be more accessible in low-resource settings, as not all areas have tertiary hospitals with operating rooms where the patients can have proper treatment. Moreover, when treated medically, the patient does not need to be hospitalized for the entire treatment period, as outpatient care seems to be an

Chart 2 GRADE quality of evidence

Certainty assessment							N° of patients		Effect		Certainty	Importance
N° of studies	Study design	Risk of bias	Inconsis-tency	Indirect-ness	Imprecision	Other considerations	Misoprostol	Manual vacuum aspiration	Relative (95% CI)	Absolute (95% CI)		
Inducing abortion												
7	randomised trials	not serious	not serious	not serious	not serious	none	923	910	–	**0** (0.07 higher to 0.4 higher)	⊕⊕⊕⊕ High	CRITICAL
Hemorrhage												
5	randomised trials	not serious	serious[a]	not serious	serious[b]	none	713	699	–	**0** (1.64 higher to 4.85 higher)	⊕⊕◯◯ Low	CRITICAL
Pain												
6	randomised trials	not serious	very serious[c]	not serious	not serious	none	924	913	–	**0** (0.1 higher to 1.1 higher)	⊕⊕◯◯ Low	CRITICAL
Acceptability												
4	randomised trials	not serious	not serious	not serious	not serious	none	662	652	–	**0** (0.38 higher to 1.21 higher)	⊕⊕⊕⊕ High	IMPORTANT

Abbreviation: CI: confidence interval.
a. Heterogeneity of 32%; b. 95%CI: [1.96, 4.59]; c. Heterogeneity of 96%.

alternative,[28] which may be better for patients with a good support network at home.

Furthermore, Von Hertzen et al. performed a randomized clinical trial with 2,066 patients who received three doses of misoprostol 800 µg via different administration routes. Their results showed that the commonly reported adverse effects were pain, diarrhea, fever, or chills. In that study, only 0.04% of the patients had vaginal bleeding that required a return to the hospital.[29] Recently, Sheldon et al. also published a randomized clinical trial using only 800 µg of misoprostol for induced abortion. In this study, treatment efficacy ranged from 84 to 87%, and adverse effects (diarrhea, nausea, vomiting, fever, or chills) were self-limiting and well tolerated by patients. Only one woman had vaginal bleeding, which required a return to the hospital for surgical completion.[30]

In the usual practice, some professionals are concerned about the occurrence of infection when surgical treatment is not chosen. In 2006, the Miscarriage Treatment (MIST) Trial[31] randomized 1,200 patients diagnosed with a first-trimester miscarriage to be treated surgically, medically, or expectantly. The results showed no difference in the incidence of infection within 14 days of follow-up. A posterior analysis of fertility rates in the three groups showed that medical treatment is safe from a reproductive future point of view.[32] However, advising patients on the alarm signs, such as persistent fever, heavy bleeding, change in mental state, dizziness, and fainting that may appear with an infection, and consulting a health care provider is essential.[33]

Concerning the rates of complete evacuation of the uterus after treatment, a network meta-analysis showed that all surgical and clinical methods for managing a miscarriage might be more effective than expectant management and a placebo. Surgical techniques were ranked highest for managing a miscarriage, followed by the clinical approach, which ranked above expectant management and a placebo. Suction aspiration after cervical preparation was the highest-ranking surgical procedure. Expectant management and placebo had the highest chance of serious complications, including the need for unplanned or emergency surgery. A subgroup analysis showed that surgical and clinical methods might be more beneficial in patients with missed miscarriages than in those with incomplete miscarriages.[34]

Concerning the limitations of the present study, we can cite: lack of standardization for some of the outcomes considered. For instance, some studies reported "pain" as the number of patients who presented with symptoms after treatment, whereas some reported, using a mean visual analog scale (VAS). In addition, bleeding was sometimes reported according to its intensity and sometimes referred to as any amount of bleeding. Regarding misoprostol, each study had a specific protocol for dosing and route of administration, which may be pointed out as another problem when comparing outcomes in different studies.

To minimize these effects, several strategies were used to test the evidence, such as the assessment of quality and risk of bias.

Once the efficacy, safety, and acceptability of misoprostol in incomplete abortion are well established, future researchers may be interested in finding the ideal route of administration (oral, sublingual, or vaginal), perfect dosage, and intervals of administration when necessary.

Conclusion

Misoprostol has been determined as a safe option with good patient acceptance. This acceptance may be related to the fact that the patient did not need to be hospitalized and reported less pain. Furthermore, misoprostol appears to be more accessible in low-resource settings. However, the quality of the body of evidence for bleeding/hemorrhage and pain outcomes was "low," mainly because of the high heterogeneity between the studies and an increased CI effect. Therefore, the results regarding the efficacy of misoprostol in this meta-analysis cannot be generalized.

Conflict of Interests

The authors have no conflict of interests to declare.

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Combined Oral Contraceptive Use and the Risk of Cervical Cancer: Literature Review

Uso de anticoncepcional oral combinado e o risco de câncer cervical: Revisão da literatura

Adriane Cristina Bovo[1] Priscila Grecca Pedrão[1] Yasmin Medeiros Guimarães[1] Luani Rezende Godoy[1] Júlio César Possati Resende[1] Adhemar Longatto-Filho[1,2,3,4] Ricardo dos Reis[1]

[1] Hospital do Câncer de Barretos, Barretos, São Paulo, Brazil.
[2] Faculdade de Medicia, Universidade de São Paulo, São Paulo, SP, Brazil.
[3] Life and Health Sciences Research Institute, Faculdade de Medicina, Universidade do Minho, Braga, Portugal.
[4] Government Associate Laboratory, Braga, Portugal.

Address for correspondence Adriane Cristina Bovo, Avenida Thyrson de Almeida, 3103, 79085-040, Aeroranchо, Campo Grande, MS, Brazil (e-mail: acbovo67@gmail.com).

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Abstract

Cervical cancer (CC) is caused by persistent infection of human papillomavirus of high oncogenic risk (hr-HPV); however, several cofactors are important in its carcinogenesis, such as smoking, multiparity, and prolonged use of oral hormonal contraceptives (COCs). Worldwide, 16% of women use COCs, whereas in Brazil this rate is of ~ 30%. The safety and adverse effects of COCs are widely discussed in the literature, including the increase in carcinogenic risk. Due to the existence of several drugs, combinations, and dosages of COCs, it is hard to have uniform information in epidemiological studies. Our objective was to perform a narrative review on the role of COCs use in the carcinogenesis of cervical cancer. Several populational studies have suggested an increase in the incidence of cervical cancer for those who have used COCs for > 5 years, but other available studies reach controversial and contradictory results regarding the action of COCs in the development of CC.

Keywords
- Oral hormonal contraceptives
- Cervical cancer
- HPV

Resumo

O câncer cervical (CC) é causado pela infecção persistente pelo papilomavírus humano de alto risco oncogênico (hr-HPV); entretanto, vários cofatores são importantes na sua carcinogênese, como tabagismo, multiparidade e uso prolongado de contraceptivos hormonais orais (COCs). No mundo, 16% das mulheres usam AOCs, enquanto no Brasil essa taxa é de ~ 30%. A segurança e os efeitos adversos dos COCs são amplamente discutidos na literatura, incluindo o aumento do risco carcinogênico. Devido à existência de várias drogas, combinações e dosagens de COCs, é difícil ter informações uniformes em estudos epidemiológicos. Nosso objetivo foi realizar uma revisão narrativa sobre o papel do uso de COCs na carcinogênese do câncer cervical. Vários

Palavras-chave
- Contraceptivos hormonais orais
- Câncer cervical
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estudos populacionais têm sugerido aumento da incidência de câncer de colo uterino para aquelas que usam COCs há mais de 5 anos, mas outros estudos disponíveis chegam a resultados controversos e contraditórios quanto à ação dos COCs no desenvolvimento do CCU.

Introduction

Cervical cancer (CC) is considered a malignant neoplasm with great potential for prevention and early detection. Worldwide, it is the fourth type of cancer in incidence and mortality among women, with an estimated 604,000 new cases and 342,000 deaths in 2020. More than 85% of these deaths occur in low- and middle-income countries, such as Brazil.[1] In the Brazilian population, it represents the third malignant neoplasm for women, and the National Cancer Institute (INCA, in the Portuguese acronym) estimates the occurrence of 17,010 new cases for the three-year period from 2023 to 2025 and the risk of 15,38 new cases for every 100,000 women, with 6,627 deaths recorded in 2020.[2]

Persistent human papillomavirus (HPV) infection is a necessary but not sufficient cause for the development of CC. Types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59 are considered of high oncogenic risk (hr-HPV); thus, they are associated with high-grade intraepithelial and invasive cervical lesions.[3] Several other cofactors have an impact on the development of CC, including sexually transmitted infections, smoking, multiparity, and prolonged use of oral hormonal contraceptives (COCs).[4–7]

After their introduction in the 1960s, COCs have revolutionized the reproductive lives of millions of women worldwide by allowing effective and convenient familiar planning. Since their wider use, a greater concern about potential adverse effects has motivated the emergence of new generations of COCs, combining lower estrogen doses with new and higher potency progestins and, consequently, fewer adverse effects. The benefit of familiar planning is not only limited to the fertility decline itself, but also to its socioeconomic impact due to maternal, newborn, and infant health benefits. Also, the indirect effects on women's education, income, and employment are relevant.[8] Nevertheless, according to a survey published in 2014, 40% of the 85 million pregnancies that occurred in the world in 2012 were unintended.[9]

The widespread use of COCs is due to their well-established high efficacy and safety. According to data from the United Nations Department of Economic and Social Affairs, the COCs are the fourth most used contraceptive method in the world ($\sim$ 151 million women), which represents 16% of the methods. In contrast, in Brazil, COCs are the most used method, representing 29.7% of the contraceptive choice.[10]

Benefits and adverse effects of COCs are widely discussed, including the risk of cancer.[11–13] The association between the use of COCs and oncogenesis in the cervix is also quite controversial in the literature. Factors such as differences in the frequency of screening between users and nonusers of COCs and unreliable information are pointed out as confounding factors in the analysis.[14] In addition, the variability in active ingredients, combinations, and dosages in the presentations of COCs prevents epidemiological studies from having uniform information for consistent analysis.

A study analyzing the effect of COCs on three different patterns of incidence of genital cancer in several countries concluded that the use of COCs may contribute to the number of cases in countries with a high incidence of CC.[15] In Brazil, the wide use of the method demonstrates the need for a more careful evaluation of the likely impact of its use on the incidence of CC, especially due to the long-term use often seen among the Brazilian female population. Thus, the present article aims to perform a narrative review of the role of COCs use in CC carcinogenesis.

Combined Oral Contraceptives and Carcinogenesis

The role of COCs in carcinogenesis has been discussed in the literature since the 1970s.[16–19] Estrogens and progestogens used for contraception and hormone replacement therapy have been also considered in the International Agency for Research on Cancer (IARC) study groups since the 1970s. In 1979, the IARC published a monograph with a general discussion on the subject, stating that steroid hormones are essential for growth differentiation and function of many tissues in animals and humans. Also, it was established by animal experimentation that modification of the hormonal environment by surgical removal of endocrine glands, by pregnancy, or by exogenous administration of steroids can increase or decrease the spontaneous occurrence of tumors. They also concluded that the incidence of tumors in humans can be altered by exposure to various exogenous hormones, individually or in combination, and that hormonal environment and dosage are involved in the carcinogenic effects of estrogens and progestogens.[20]

Recently, in 2012, the IARC published data showing that the use of COCs may increase risks for some cancers and protect against others. Their use was associated with increased risks for breast cancer in women $<$ 35 years old (both for current and recent users), carcinoma in situ and invasive carcinoma of the cervix, and even liver cancer in populations at low risk for hepatitis B virus infection. In addition, for CC, risks increased with the duration of use and decreased after discontinuation. For endometrial cancer, COCs had a protective effect that increased with the duration of use and remained at least 2 decades after discontinuation. There was evidence that the level of the protective effect was

proportional to the progestogen potency of the preparation and inversely proportional to the estrogen potency. Concerning ovarian cancer, a greater risk reduction was observed with the duration of use and was persistent for at least 30 years after discontinuation. It was also suggested that COCs could reduce the risk of colorectal cancer and had the unlikely potential to change the cancer risk in the thyroid, lung, stomach, urinary tract, gallbladder, pancreas, lymph nodes, skin, and central nervous system.[21]

Gierisch et al. published a meta-analysis confirming these findings. They included 44 studies of breast cancer, 12 of cervical cancers, 11 of colorectal cancers, and 9 of endometrial cancers. The incidence of breast cancer was slightly but significantly increased in female users of COCs (odds ratio [OR] = 1.08; 95% confidence interval [CI] = 1.00–1.17), with higher risk associated with recent use. The risk of CC increased with the duration of use in women with hr-HPV infection, but the heterogeneity of the studies prevented the meta-analysis on this topic. The incidences of colorectal cancer (OR = 0.86; 95%CI= 0.79–0.95) and endometrial cancer (OR = 0.57; 95%CI = 0.43–0.77) were significantly reduced by the use of COCs.[22] Similar results were described in a recent literature review published by Kamani et al., recommending that patients seeking family planning be advised of possible increased carcinogenic risk, but also advised of the advantages for sexual health and reduced risk of endometrial, colorectal, and ovarian cancer.[23]

From the standpoint of population impact, a meta-analysis on estimated cancer risk in US women aged 20-54 years old using COCs was performed and showed that for every 100,000 women using COCs for 8 years, the estimated number of additional cases or fewer per 100,000 users is + 151 (breast), + 125 (cervix), - 197 (endometrium), - 193 (ovary), and + 41 (liver), concluding that for public health, the real effect is insignificant.[24]

Combined Oral Contraceptives and Cervical Cancer

Some experimental models have demonstrated the role of exogenous estrogens – such as those used in COCs – in cervical carcinogenesis. Experimental studies with transgenic mice expressing oncogenes for HPV 16 have shown that they rarely develop CC, although they can develop spontaneous skin tumors. However, with chronic exposure to exogenous 17 beta-estradiol combined with persistent oncogenic expression of HPV 16, it was demonstrated that benign epithelial hyperplasia acquired the ability to transform into neoplastic tissue in the cervix and vagina.[25] Also in transgenic mouse models, the removal of exogenous estrogen has been shown to lead to decreased progression or regression of pre-existing cervical neoplastic disease.[26] According to Chung et al. (2008), the estrogen alpha receptor (ER) is one present in the cervix. Transgenic mice with oncogenes for HPV 16 but deficient in ER had an inhibition of CC progression while exposed to exogenous estrogen, therefore proving the impact of exogenous estrogens on cervical carcinogenesis.[27]

In a monography analyzing carcinogenic risk in humans of hormonal contraception and postmenopausal hormone therapy, the IARC listed some confounding and complicating factors for an accurate analysis of the relationship between COCs and CC. Firstly, women exposed to HPV infection are more susceptible to other sexually transmitted infections; thus, knowledge of sexual habits is necessary for correct analysis. In addition, it would be relevant to determine the persistence of hr-HPV infection, considering the hypothesis that COCs could increase the likelihood of the infection becoming persistent; a fact often ignored in studies at the time. It was also mentioned that ethical issues prevented large prospective studies with follow-up for women with HPV infection until the development of CC to assess the influence of COCs use. Finally, the bias of screening was pointed out, in which patients who use COCs undergo screening tests more often than nonusers. In conclusion, the monography analyzed 5 cohort studies and 16 case-control studies that showed a small increase in the relative risk of CC associated with the long-term use of COCs. This association was also observed in studies with analyses restricted to case-control studies with data on HPV infection and biases related to sexual behavior, screening, and other factors that could not be ruled out as possible explanations for the observed associations.[28]

A possible mechanism to explain the association between the use of COCs and the risk of CC would be the possible interaction between estrogens, progestogens, and hormone receptors in cervical tissue, influencing the natural history of HPV infection. It is postulated that sexual hormones can potentiate the expression of E6 and E7 oncogenes of HPV 16, stimulating the degradation of p53 tumor suppressor genes and increasing the ability of viral DNA to transform cells and induce carcinogenesis.[29]

Studies have pointed out a higher risk of high-grade cervical lesions related to the use of COCs in women with hr-HPV infection, suggesting an interaction between HPV infection and COCs with increased HPV genome expression in neoplasms of COCs users.[30,31] However, although Kjellberg et al. found an association between prolonged use of COCs and high-grade lesions, the association lost significance after considering HPV infection.[32]

A systematic review published in 2003 addressed the relationship between carcinoma in situ or CC and the duration and current use of hormonal contraceptives, with special attention to hr-HPV infection. Twenty-eight eligible studies were identified, including 12,531 women with CC. Compared with women who never used COCs, the relative risks of CC in women who are users increased according to the duration of use: < 5 years, 5 to 9 years, and ≥ 10 years, respectively. Relative risks were 1.1 (95%CI = 1.1–1.2), 1.6 (95%CI = 1.4–1.7), and 2.2 (95%CI = 1.9–2.4) respectively for all women; and 0.9 (95%CI = 0.7–1.2), 1.3 (95%CI = 1.0–1.9), and 2.5 (95% CI = 1.6–3.9) for hr-HPV-positive women. Results were similar when they adjusted the data for in situ and invasive lesions, squamous cell, and adenocarcinoma, HPV status, number of sexual partners, cervical screening frequency, smoking, and barrier contraceptive use.[33]

However, a recent systematic review found no consistent evidence of an association between COCs use and increased risk of pre-neoplastic lesions and CC after considering hr-HPV infection in the analysis.[34]

Vessey et al. compared the incidence of CC in a prospective study with a 10-year follow-up of 6,838 COCs users and 3,154 nonhormonal intrauterine devices (IUD) users.[35] The incidence of preneoplastic lesions and CC ranged from 0.9/1,000 women per year with up to 2 years of COCs use to 2.2/1,000 women per year for those who used it for $\geq$ 8 years. In IUD users, there was no variation in the incidence and the rate was $\sim$ 1/1000 women per year. All cases of invasive cervical neoplasia occurred in the group of COCs users, 9 in women with $>$ 6 years of use. The reduced risk of CC in diaphragm users compared with COCs users and IUD users have been previously reported, as well as the protective effect of sexual partner's vasectomy.[36,37] Moreno et al. published data from a previous IARC multicenter study analyzing CC risk in COCs users with HPV infection, including 8 case-control studies addressing CC and 2 studies on carcinoma in situ.[38] No increase in risk was observed in nonusers or those with up to 5 years of use, but there was a relative risk of 2.82 for those using for between 5 and 9 years and of 4.03 for $>$ 10-year users. Despite these findings showing a more than twofold increase in the risk for women who have used COCs for $>$ 5 years and with persistent hr-HPV infection, no change in contraceptive orientation was recommended when evaluating the risks and benefits of using COCs.[39]

The emergence of some studies demonstrating a significant increase in risk for CC in users of COCs motivated a new publication in 2007 by an IARC advisory group. They analyzed the carcinogenic risk of COCs and combined estroprogestin therapy in postmenopausal women and finally classified COCs as carcinogenic agents.[40] According to this publication, the totality of the evidence at the time indicated that the risk of CC increased with increasing duration of COCs use. They also concluded that the risk was slightly higher for carcinoma in situ than for invasive cancer and that the relative risk seemed to decrease after use cessation. Also, in the same publication, similar results were found regardless of adjustment for the number of sexual partners, frequency of screening, smoking, and barrier contraceptive use. The possibility that the observed association was due to the higher frequency screening bias in COCs users was not excluded but was considered unlikely.[40]

After this classification of COCs as carcinogenic agents by IARC due to their effect on the CC risk, a revision of 24 published epidemiological studies including data from 16,573 women with CC and 35,509 controls was performed by several researchers from the International Collaboration of Epidemiological Studies of Cervical Cancer in the same year. The results confirmed a relative risk of 1.9 for users of $>$ than 5 years for both invasive cancer and carcinoma in situ. It also suggested that 10 years of use around the age of 20 or 30 years old would lead to an increased incidence of CC by age 50 years old from 7.3 to 8.3 per 1,000 women in less developed countries. The risk seems to decrease with use cessation, being similar that of nonusers after 10 years.[41]

The World Health Organization (WHO) has also shown concern about an association between CC and the use of COCs. In 1977, a scientific group was convened to review the possible carcinogenic effect of hormonal contraceptives and identify new studies needed. In that regard, a multicenter case-control study was initiated with data from several countries, mainly developing countries. Preliminary data identified a CC risk of 1.19 for women who used COCs for up to 5 years and 1.53 for $>$ 5-year users.[42] Final data were published in 1993, suggesting a causal relationship between the use of COCs and CC with a relative risk of 1.31 for women who used up to 4 years and a significant increase in the risk for longer-term users, reaching 2.25 for those who have used it for $>$ 10 years. However, the risk returned to the basal status after 8 years of use cessation. In conclusion, the study suggests priority in CC screening in patients who have used COCs for $>$ 4 years.[43] Also, Roura et al. published the results of a prospective cohort of 308,036 women recruited in the European Prospective Investigation in Cancer and Nutrition (EPIC) Study with a mean follow-up of 9 years. They counted 261 cases of CC and 804 cases of grade 3 intraepithelial neoplasia or carcinoma in situ. Duration of COCs use was associated with increased risk of grade 3 intraepithelial neoplasia/carcinoma in situ and CC, hazard ratio (HR) = 1.6 and |HR = 1.8, respectively, for 15-year users versus never-users.[44]

The risk of COCs use and the development of adenocarcinoma specifically has also been investigated. A review of case-control studies for adenocarcinoma alone was published by Castellsagué et al., who found evidence that prolonged use of COCs in hr-HPV positive patients increased the risk of adenocarcinoma (OR = 4.71 for those who had $>$ 5 years of use).[45] Also, a systematic review published in 2020 including 19 studies pointed to a higher association of COCs and cervical adenocarcinoma (1.77; 95%CI: 1.4–2.24), compared with 1.29 (95%CI: 1.18–1.42) in invasive squamous cervical cancer and 1.7 in carcinoma in situ (95%CI: 1.18–2.44).[46]

In addition, an analysis of CC mortality and COCs use was addressed. A prospective study with 25-year follow-up data on 46,000 British women found a 2.5-fold increase in women using COCs, or those who had recently used them (up to 10 years) after adjusting the data for parity, social class, and smoking.[47]

On the other hand, some publications found no association between COCs and CC. Syrjänen et al., analyzing screening data from a cohort study in the former Soviet Union, concluded that COCs are not an independent risk factor for intraepithelial neoplasia or hr-HPV infection. Analysis of data from COCs nonusers, users of nonhormonal methods, and COCs users showed an identical prevalence of hr-HPV infection, cytological abnormalities, and intraepithelial neoplasia histology, but with significant differences ($p < 0.001$) on all sexual behavior variables.[48] Similar results were described by Longatto-Filho et al., analyzing data from a cohort study of over 12,000 Brazilian and Argentinian women. In this study, patients using diverse hormonal contraceptive methods (oral, injectable, patch, implant, vaginal ring, and levonorgestrel IUD) were

included and no evidence was found that hormonal contraceptive use and duration of use are independent risk factors for hr-HPV infection or high-grade cervical intraepithelial neoplasia.[49] In 2017, a meta-analysis of case-control studies including 7,433 cases and 8,186 controls showed no association between COCs use and CC, although an increase was seen in the Asian population.[50] Consistent results were found in a prospective study with a follow-up of 46,022 women who were $\geq$ 44 years old, demonstrating that women who choose to use COCs are not exposed to increased long-term cancer risk.[51]

Despite the tendency of signaling the interaction of COCs and hr-HPV infection in the emergence of cervical lesions, there are evident limitations in the standardization of studies that have aimed to define the impact of their use on cervical carcinogenesis. Retrospective analyses based on different active ingredients, distinct combinations, multiple dosages, and variables in the duration of COCs use are confounding factors that prevent more robust and definitive conclusions. There are gaps in scientific knowledge that deserve to be explored. Can distinct histological types – squamous or glandular lesions – be differently impacted by COCs use? Considering cohorts of women vaccinated against hr-HPV, will COCs users be at higher risk of developing cervical lesions? Which group of women using COCs would be more exposed to a higher risk of developing CC and its precursor lesions? Under what conditions the suspension of the COCs and encouragement of an alternative contraceptive method would be indicated?

The wide long-term use of COCs in Brazilian women requires more studies to define such risk in the Brazilian female population. At least, patients should receive information about the risks and benefits of using COCs for $>$ 5 years, with counseling about the importance of adherence to CC screening. Also, maybe in this population of $>$ 5-year-COC users, there should be discussions about the use of hr-HPV testing for screening.

Considering that the use of combined contraceptives increases the risk of some types of cancer and reduces others, a rigorous analysis of the overall outcome of this equation for the Brazilian female population is needed; especially because the country still has a high incidence of CC with great public health implications.

Conclusion

Despite controversial data in the literature, several populational studies suggest a possible increase in the incidence of CC and its precursor lesions for those who have used COCs for $>$ 5 years. Detailed studies about the impact of this increased risk in the high incidence of CC in Brazilian women who have been using COCs for a long time are still necessary to evaluate risks and benefits, as well as adequate screening coverage for CC in this specific population.

Conflict of Interests
None to declare.

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- **Case reports:** They are of interest if well documented from a clinical and laboratory point of view and should contain new or unexpected aspects in relation to cases already published. Authors should indicate this information in the referral letter. The text of **Introduction** and **Discussion** sections must be based on an up-to-date literature review.
- **Review articles:** Spontaneous contributions are accepted, including integrative, scoping, or systematic reviews with or without meta-analyses. Narrative reviews will only be accepted exceptionally, given the questionable scientific evidence they represent. The methods and procedures adopted to obtain data inserted in the text must be described and based on recent references, including the current year. As this is still subject to controversy, the review should discuss trends and lines of investigation in progress. In addition to the review text, the synthesis and conclusions must be presented.
- **Letters to the Editor:** Must address editorial matters or not, but present relevant information to readers. The letters may be summarized by the editorial board, always keeping the main points. In the case of criticism or comments on published works, the letter is sent to the authors of the cited article so their response can be published simultaneously. All data presented in the letter must be fully citable and cited in the supporting reference list (unpublished data should not be described in the letter).
- **Editorial:** By invitation of the editor only.

OBS. Manuscripts containing results of original clinical or experimental research have priority for publication

Manuscript structure

Title

When writing a scientific article, the researcher must pay attention to the title of the manuscript. The title is the business card of any publication. It should be prepared with great care and preferably be written only after the article is finished. A good title adequately describes the content of the manuscript. It is usually not a sentence, as it does not contain the subject or arranged verbs and objects. **Abbreviations, chemical formulas, excess of adjectives, names of cities and institutions, among others, should be avoided in titles.** The titles of manuscripts submitted to the RBGO must contain a maximum of 18 words.

Abstract

The abstract must provide the context or basis for the study, establish the objectives, basic procedures of the methodology used, main results and main conclusions. It should emphasize new and important aspects of the study or observations. As abstracts are the only substantive part of the article that is indexed in many electronic databases, authors must ensure they accurately reflect the content of the article and highlight the research contribution/innovation to the topic. Abbreviations, symbols and references should not be used in the abstract. In case of original articles from clinical trials, the authors must inform the registration number at the end of the abstract.

1. Abstract: for original articles

Abstracts of original articles submitted to the RBGO must be structured in four sections and contain a maximum of 250 words:

Objective: Retrospective on the topic and the question posed by researchers.

Methods: How it was done; the method employed, including the material used to achieve the objective.

Results: What was found; the main finding and, if necessary, the secondary findings.

Conclusion: What was the conclusion; the answer to the question asked.

2. Abstract: for systematic review articles

Abstracts of systematic review articles submitted to the RBGO must be structured in six sections and contain a maximum of 250 words:

Objective: State the main objective of the article.

Data sources: Describe the data sources examined, including dates, indexing terms and limitations.

Study selection: Specify the number of studies reviewed and criteria used in their selection.

Data collection: Summarize the conduct used in data extraction and how it was used.

Data synthesis: Present the main results of the review and the methods employed to obtain them.

Conclusions: State the main conclusions and their clinical utility.

3. Abstract: for integrative/scoping reviews

It must contain the essence of the article, covering the purpose, method, results and conclusions or recommendations. Expose enough detail so readers can decide on the convenience of reading the entire text (word limit: 150).

NOTE: An abstract in Portuguese may be optionally added by the authors.

Keywords

The keywords of a scientific work indicate the thematic content of the text they represent. The identification of thematic content, the indexing of the work in databases and the quick location and retrieval of the content are considered the main objectives of the mentioned terms. The keyword systems used by the RBGO are DeCS (Health Sciences Descriptors – Lilacs Indexer) and MeSH (Medical Subject Headings – MEDLINE-PubMed Indexer). Five descriptors that represent the work must be chosen on these platforms.

Manuscript body

Manuscripts submitted to the RBGO should have a maximum of 4,000 words. Tables, charts and figures in the **Results** section, as well as references, are not counted.

Introduction

This part of the article prepares the reader to understand the investigation and the justification for its development. It should include the current state of knowledge on the subject, offering only strictly relevant and up-to-date references. The content to be reported in this section should provide context or background for the study, that is, the nature of the problem and its importance, and state the specific purpose, research objective, or hypothesis tested in the study or observation. The research objective is the final part of the introduction and both the main and secondary objectives must be clear and any analyzes in a pre-specified subgroup must be described. The introduction should not include data or conclusions from the work being reported.

Methods

The **Methods** section of a scientific work aims to present the study in a clear and concise way so that it is understandable and can be replicated. It should state how, when and where the study was developed. The

method comprises the material and procedures adopted in the study in order to be able to answer the main question of investigation. The **Methods** section should be structured starting with the type of study design, to show if it is appropriate to achieve the research objective; the research setting (the place and time in which it was developed); the data collection; the intervention to be performed and evaluated (if any) and also the alternative intervention; the statistical methods used and the ethical aspects of research.

NOTE: the RBGO joined the initiative of the International Committee of Medical Journal Editors (ICMJE) and the EQUATOR Network, aimed at improving the presentation of research results. Check related interactive guides:

Randomized clinical trial:

http://www.equator-network.org/reporting-guidelines/consort/

Systematic reviews and meta-analyses:

http://www.equator-network.org/reporting-guidelines/prisma/

Observational studies in epidemiology:

http://www.equator-network.org/reporting-guidelines/strobe/

Qualitative studies:

http://www.equator-network.org/reporting-guidelines/srqr/

Results

The purpose of the **Results** section is to show the findings of the research. These are original data obtained and synthesized by the author in order to provide an answer to the question that motivated the investigation. Results should be presented in a logical sequence in the text, tables and illustrations, mentioning the most important findings first. Whenever appropriate, the statistical significance of results should be indicated. All information in tables or illustrations should not be repeated in the text, and only important observations should be emphasized or summarized. Additional or supplementary materials and technical details may be placed in an appendix, accessible via a link, that will not interrupt the flow of the text. When data are summarized in the **Results** section, numerical results must be presented not only in derived values (e.g. percentages) but also in absolute values from which the derived values were calculated, and specify the statistical methods used to analyze them. Only the tables and figures necessary to explain the argument of the work and to assess its basis should be used. When scientifically appropriate, analyzes of data with variables such as age and sex should be included. The limit of a maximum of five tables, five charts or five figures must not be exceeded. Tables, charts and/or figures must be included in the body of the manuscript and do not account for the requested limit of 4,000 words. For clarification on the resolution of figures, please check https://www.ncbi.nlm.nih.gov/pmc/pub/filespec-images/.

Discussion

In the **Discussion** section, new and important aspects of the study and the conclusions derived from them should be emphasized. Data or other information presented in the **Introduction** or **Results** sections should not be repeated in detail. In experimental studies, it is useful to start the discussion with a brief summary of the main findings, compare and contrast the results with those of other relevant studies, state the limitations of the study and explore the implications of the findings for future research and clinical practice. Claiming precedence and alluding to incomplete works should be avoided, as well as discussing data not directly related to the results of the research presented. New hypotheses may be proposed when justified, but they must be clearly qualified as such. The last paragraph of the **Discussion** section should include the information of the study that relatively contributes to new knowledge.

Conclusion

The **Conclusion** section is intended to relate the conclusions to the objectives of the study. Authors should avoid unsubstantiated statements and conclusions not appropriately supported by their data. In particular, authors should avoid making claims about economic benefits and costs unless their manuscript includes economic analysis and appropriate data.

References

In manuscripts submitted to the RBGO, authors must number references in order of entry in the work and use these numbers for citations in the text. An excessive number of references should be avoided, selecting the most relevant for each statement and giving preference to more recent works. Do not use citations of difficult to access, such as abstracts of works presented at conferences, theses or publications with restricted circulation (not indexed). Cite primary and conventional references (articles in scientific journals and textbooks). References such as “unpublished observations” and “personal communication” should not be used. Authors’ publications (self-citation) should only be used if there is a clear need and they are related to the topic. In this case, include only original works published in regular journals (do not cite chapters or reviews) among the bibliographic references. The number of references should be limited to 35, except for review articles. Citations of references must be placed after the period in superscript, without space after the last word (sequential and numerical citations). Authors are responsible for the accuracy of data contained in the references. To format your references, check **Vancouver:** https://www.ncbi.nlm.nih.gov/books/NBK7256/.

Submission of manuscripts

Articles must be submitted electronically, according to instructions available on the website: http://mc04.manuscriptcentral.com/rbgo-scielo.

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Address: Av. Brigadeiro Luís Antônio, 3.421, sala 903, Jardim Paulista – 01401-001 – São Paulo, SP, Brazil
Telephone: + 55 (11) 5573-4919
E-mail: editorial.office@febrasgo.org.br

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