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Editorial

The Use of Antidepressant Drugs in Climacteric Syndrome

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Vasomotor symptoms (VMS) or hot flashes interfere with women's quality of life and are the probable cause of sleep disorders, lack of energy, depression and tiredness in the peri- and postmenopausal period. These symptoms normally last between 2 and 10 years, with an average of 7.4 years or more.[1,2] Estrogen therapy is the treatment of choice for VMS and reduces both the weekly frequency and the severity of these symptoms.[3,4] For hot flashes relief, hormone treatment lasts 3 to 5 years and discontinuity may lead to recurrence in up to 50% of symptoms. On the other hand, by considering the benefits of hormone therapy for osteoporosis prevention, quality of life improvement and treatment of persistent VMS,[1] there is a current trend to extend treatment until the age of 60 or 65 years old.

Other drug therapies are suggested for women who do not wish to undergo estrogen therapy, usually for fear of cancer, and those with contraindications to hormone treatment, although the results of these therapies are far lower than conventional estrogen therapy. These include selective serotonin reuptake inhibitors (SSRIs) and selective serotonin and norepinephrine reuptake inhibitors (SNRIs). Despite the inferior therapeutic results, after estrogen, these are the most used drugs for the treatment of VMS,[5] and they have a very fast action (in days) in reducing hot flashes, while their antidepressant action will occur later (in weeks).[6]

The efficacy of this treatment is hard to evaluate, because the symptom reduction may be caused by the placebo effect of these drugs.[5] Furthermore, clinical trials have no long-term follow up of patients, and most studies evaluate treatment efficacy by comparing with placebo at 4 to 12 weeks and the effect at 12 to 24 weeks after drug discontinuation.[7] Both SSRIs and SNRIs bring mild to moderate improvement in symptoms and 25% to 69% reduction in hot flashes.[7–9] For the treatment of VMS, the North America Menopause Society (NAMS) recommends paroxetine (recommendation level I), citalopram, escitalopram, venlafaxine and desvenlafaxine (level II),[7] although only paroxetine has been approved by the FDA and is recommended by the American College of Obstetrics and Gynecology (ACOG).[10]

Regarding fluoxetine and sertraline, publications present conflicting results. Some authors argue that these medications are less effective and should be considered as a second line treatment.[11] In some studies, less consistent results were observed with no statistically significant improvement in hot flashes.[7] In contrast, other studies have shown a reduction in VMS,[12,13] including in women with breast cancer.[14,15] For these reasons, prescriptions are recommended in various services,[16] and in Brazil these medications are provided for free by the Ministry of Health; hence, they are more accessible to the entire population, especially those with low purchasing power.

Regarding adverse events, in a systematic review and meta-analysis published in 2014, no difference was found between the most cited side effects when comparing SSRIs with the placebo group.[17] However, in several other studies, nausea, dry mouth, constipation, headache, and loss of appetite were the most frequently reported side effects with the use of SSRI/SNRI.[9,18,19] Anorexia, vomiting, sexual dysfunction and insomnia[19] or improved sleep were also reported with use of with paroxetine.[20–22]

Sexual dysfunction caused by SSRIs/SNRIs occurs in 32.5% to 73% of patients.[23,24] According to some authors, sexual dysfunction appears to be more related to medication dose or prior depression.[25] Since increased blood pressure is a side effect that may arise with the use of SNRIs, there should be caution in the use by hypertensive patients,[26] and these drugs are not recommended as a first line treatment in hypertensive women.[27] Nowadays, the rise in antidepressant prescriptions has been a cause for much concern worldwide. In France, the overall prevalence of prescriptions increased from 6.5% in 1999–2000 to 10.4% in 2009–2010[28] and in the US, from 5.84% in 1996 to 10.52% in 2005.[29] In the Netherlands, the use of these drugs almost doubled between 1996 and 2012[30] and in the United Kingdom, from 1995 to 2011, prescriptions increased from 61.9% to 129.9% per 1,000 people-year.[31]

In the Netherlands, between 1996 and 2012, long-term therapy was higher among women than men (two thirds of patients) with predominance in the age group of 45 to

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64 years old (45% of them).[30] Selective serotonin reuptake inhibitors accounted for 52% of prescribed antidepressants, and among prescriptions in general, 47% were for depression, 23% for anxiety and ~ 25% for somatic reasons (ill defined).[31] This age group covers both peri- and postmenopausal patients and it is very likely that women with vasomotor and neurovegetative symptoms characteristic of this climacteric phase were included, as anxiety and depression are often associated with hot flashes.

However, the prolonged and justified use for estrogen therapy does not apply to alternative SSRI or SNRI therapy for the treatment of postmenopausal women. According to some authors, there is no conclusive evidence on the safety of antidepressants over time and their use could be more dangerous than beneficial, because it could interfere with the adaptive processes regulated by serotonin.[32] The menopausal transition is an adaptive process of physiological mechanisms exerted by serotonergic neurons that are "poorly regulated" in this period, as a result of the estrogen level drop.[33] After some period of hormonal instability, there is a re-adaptation of the organism to the new hypoestrogenic level, and hot flash symptoms and its repercussions on the female organism disappear. As SSRIs would be indicated to restore that balance, they should be prescribed for the shortest possible time.

Therefore, some questions arise: how often do doctors offer the discontinuation of SSRI or SNRI therapy when the patient reports being well after the start of medication? Is there any control over the duration of the use of these drugs? The literature on the use of SSRIs/SNRIs in climacteric women addressing this aspect of therapy is scarce. Prolonged use of these drugs may result in ineffectiveness and possible risks. In women, especially older women, are reported higher risks for falls and fractures, stroke, suicide attempts, epileptic seizures and digestive bleeding.[34] According to the literature, two thirds of outpatients with anxiety and/or depression receive treatment with psychotherapy, notably antidepressants, and these are generally used for long periods.[35] In the Netherlands, 30% of patients taking antidepressants do so for at least one year; in England, half of patients and in the USA, two thirds use the medication for at least two years. Only 10% of the patients discontinue the use of these drugs each year.[36–39]

With regard to climacteric symptoms, information on overprescription of these drugs is not conclusive. Literature data specifically focused on the time of use and monitoring of patients receiving this treatment for climacteric VMS are frustrating. Side effects of antidepressants are underreported in the literature because they result from short-term studies. Thus, gynecologists who treat women in the climacteric period should be alert to common and persistent side effects with long-term use.[40] When treating climacteric VMS, the most rational should be the use for short periods of time. When SSRIs or SNRIs are prescribed, patients should return in short time intervals for an initial assessment of therapeutic outcomes and side effects.

According to international consensus, the discontinuity of antidepressants should be addressed at six to 18 months after symptom remission in case of anxiety and four to 12 months in case of depressive disorders. Unnecessary continuation of antidepressant use may result in severe side effects and harm the health of patients.[34] Therefore, the recommendation is an individualized treatment based on international guidelines.[41,42]

For the treatment of hot flashes, unfortunately, there are no protocols that clearly determine how long SSRIs/SNRIs can or should be used in climacteric women. In the absence of evidence, patients who would eventually benefit from relief of depressive symptoms in the perimenopause may be reluctant to discontinue therapy for fear of symptom recurrence. Thus, many patients with transient episodes of depression or anxiety resulting from vasomotor phenomena receive antidepressant therapy at the beginning of treatment and prolong it beyond the necessary time, thereby becoming dependent on this therapy, which is often unnecessary and dispensable.[43,44]

This question is not intended to restrict the prescription of such drugs, as they are relatively safe products. In Brazil, they are not even included in the group of controlled drugs; hence, far from controlled, addictive drugs, which facilitates the use and prescription. However, the increasing use of antidepressants is worrisome, not because of the increase in indications and prescriptions for new patients, but mainly due to the prolonged use by those already taking the drug. Long-term use is advisable only in cases of chronicity or in patients who experience recurrence of symptoms after withdrawal. In such situations, and if associated with complaints of depression and anxiety, support from psychiatric specialists is advised for the benefit of the patient.

Despite much controversy, SSRIs/SNRIs are yet another therapeutic option for treating hot flashes, although the results are not exciting in most patients. For women who cannot or do not wish to take estrogens, non-hormonal management, such as SSRI or SNRI is a realistic and safe therapeutic option[45] as long as proper precautions are taken to avoid unnecessary prolonged use.

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

References

1 Pinkerton JV, Sánchez Aguirre F, Blake J, Cosman F, Hodis HN, Hoffstetter S et al. The NAMS 2017 Hormone Therapy Position Statement Advisory Panel. The 2017 hormone therapy position statement of The North American Menopause Society. Menopause. 2017;24(7):728–753. doi: 10.1097/GME.0000000000000921
2 Avis NE, Crawford SL, Greendale G, Bromberger JT, Everson-Rose SA, Gold EB et al. Study of Women's Health Across the Nation. Duration of menopausal vasomotor symptoms over the menopause transition. JAMA Intern Med. 2015;175(4):531–539. doi: 10.1001/jamainternmed.2014.8063
3 Santen RJ, Allred DC, Ardoin SP, Archer DF, Boyd N, Braunstein GD et al; Endocrine Society. Postmenopausal hormone therapy: an Endocrine Society scientific statement. J Clin Endocrinol Metab. 2010;95(7, Suppl 1):s1–s66. doi: 10.1210/jc.2009-2509
4 Maclennan AH, Broadbent JL, Lester S, Moore V. Oral oestrogen and combined oestrogen/progestogen therapy versus placebo for hot flushes. Cochrane Database Syst Rev. 2004;(4):CD002978. doi: 10.1002/14651858.CD002978.pub2
5 Santen RJ, Loprinzi CL, Casper RF. Menopausal hot flashes [Internet]. UpToDate; 2016 [2019 May 12]. Available from: https://www.uptodate.com/contents/menopausal-hot-flashes

6 Freeman EW, Guthrie KA, Caan B, Sternfeld B, Cohen LS, Joffe H et al. Efficacy of escitalopram for hot flashes in healthy menopausal women: a randomized controlled trial. JAMA. 2011;305(3): 267–274. doi: 10.1001/jama.2010.2016

7 [No authors listed]. Nonhormonal management of menopause-associated vasomotor symptoms: 2015 position statement of The North American Menopause Society. Menopause. 2015;22(11): 1155–1172, quiz 1173–1174. doi: 10.1097/GME.0000000000000546

8 Stearns V, Ullmer L, López JF, Smith Y, Isaacs C, Hayes D. Hot flushes. Lancet. 2002;360(9348):1851–1861. doi: 10.1016/s0140-6736(02)11774-0

9 Wiśniewska I, Jochymek B, Lenart-Lipińska M, Chabowski M. The pharmacological and hormonal therapy of hot flushes in breast cancer survivors. Breast Cancer. 2016;23(2):178–182. doi: 10.1007/s12282-015-0655-2

10 Committee on Practice Bulletins-Gynecology. ACOG Practice Bulletin N. 126: Management of gynecologic issues in women with breast cancer. Obstet Gynecol. 2012;119(3):666–682. doi: 10.1097/AOG.0b013e31824e12ce

11 Handley AP, Williams M. The efficacy and tolerability of SSRI/SNRIs in the treatment of vasomotor symptoms in menopausal women: a systematic review. J Am Assoc Nurse Pract. 2015;27(1): 54–61. doi: 10.1002/2327-6924.12137

12 Loprinzi CL, Sloan JA, Perez EA, Quella SK, Stella PJ, Mailliard JA, et al. Phase III evaluation of fluoxetine for treatment of hot flashes. J Clin Oncol. 2002;20(6):1578–1583. doi: 10.1200/JCO.2002.20.6.1578

13 Takahashi TA, Johnson KM. Menopause. Med Clin North Am. 2015;99(3):521–534. doi: 10.1016/j.mcna.2015.01.006

14 Kimmick GG, Lovato J, McQuellon R, Robinson E, Muss HB. Randomized, double-blind, placebo-controlled, crossover study of sertraline (Zoloft) for the treatment of hot flashes in women with early stage breast cancer taking tamoxifen. Breast J. 2006;12(2):114–122. doi: 10.1111/j.1075-122X.2006.00218.x

15 Irarrázaval O ME, Gaete G L. [Antidepressants agents in breast cancer patients using tamoxifen: review of basic and clinical evidence]. Rev Med Chil. 2016;144(10):1326–1335. doi: 10.4067/S0034-98872016001000013

16 Leon-Ferre RA, Majithia N, Loprinzi CL. Management of hot flashes in women with breast cancer receiving ovarian function suppression. Cancer Treat Rev. 2017;52:82–90. doi: 10.1016/j.ctrv.2016.11.012

17 Shams T, Firwana B, Habib F, et al. SSRIs for hot flashes: a systematic review and meta-analysis of randomized trials. J Gen Intern Med. 2014;29(1):204–213. doi: 10.1007/s11606-013-2535-9

18 L'Espérance S, Frenette S, Dionne A, Dionne JY; Comité de l'évolution des pratiques en oncologie (CEPO). Pharmacological and non-hormonal treatment of hot flashes in breast cancer survivors: CEPO review and recommendations. Support Care Cancer. 2013;21(5):1461–1474. doi: 10.1007/s00520-013-1732-8

19 Al-Safi ZA, Santoro N. Menopausal hormone therapy and menopausal symptoms. Fertil Steril. 2014;101(4):905–915. doi: 10.1016/j.fertnstert.2014.02.032

20 Simon JA, Portman DJ, Kaunitz AM, et al. Low-dose paroxetine 7.5 mg for menopausal vasomotor symptoms: two randomized controlled trials. Menopause. 2013;20(10):1027–1035. doi: 10.1097/GME.0b013e3182a66aa7

21 Pinkerton JV, Joffe H, Kazempour K, Mekonnen H, Bhaskar S, Lippman J. Low-dose paroxetine (7.5 mg) improves sleep in women with vasomotor symptoms associated with menopause. Menopause. 2015;22(1):50–58. doi: 10.1097/GME.0000000000000311

22 Capriglione S, Plotti F, Montera R, Luvero D, Lopez S, Scaletta G, et al. Role of paroxetine in the management of hot flashes in gynecological cancer survivors: Results of the first randomized single-center controlled trial. Gynecol Oncol. 2016;143(3): 584–588. doi: 10.1016/j.ygyno.2016.10.006

23 Williams VS, Baldwin DS, Hogue SL, Fehnel SE, Hollis KA, Edin HM. Estimating the prevalence and impact of antidepressant-induced sexual dysfunction in 2 European countries: a cross-sectional patient survey. J Clin Psychiatry. 2006;67(2):204–210

24 Montejo AL, Llorca G, Izquierdo JA, Rico-Villademoros F. Incidence of sexual dysfunction associated with antidepressant agents: a prospective multicenter study of 1022 outpatients. Spanish Working Group for the Study of Psychotropic-Related Sexual Dysfunction. J Clin Psychiatry. 2001;62(Suppl 3):10–21

25 Basson R, Schultz WW. Sexual sequelae of general medical disorders. Lancet. 2007;369(9559):409–424. doi: 10.1016/S0140-6736(07)60197-4

26 Stubbs C, Mattingly L, Crawford SA, Wickersham EA, Brockhaus JL, McCarthy LH. Do SSRIs and SNRIs reduce the frequency and/or severity of hot flashes in menopausal women. J Okla State Med Assoc. 2017;110(5):272–274.

27 Heerlein A. Antidepressant pharmacologic treatments. Rev Chil Neuro-psiquiatr. 2002;40(Suppl 1):21–45. doi: 10.4067/S0717-92272002000500003

28 Olié JP, Elomari F, Spadone C, Lépine JP. [Antidepressants consumption in the global population in France]. Encephale. 2002;28(5 Pt 1):411–417.

29 Olfson M, Marcus SC. National patterns in antidepressant medication treatment. Arch Gen Psychiatry. 2009;66(8):848–856. doi: 10.1001/archgenpsychiatry.2009.81

30 Verhaak PFM, de Beurs D, Spreeuwenberg P. What proportion of initially prescribed antidepressants is still being prescribed chronically after 5 years in general practice? A longitudinal cohort analysis. BMJ Open. 2019;9(2):e024051. doi: 10.1136/bmjopen-2018-024051

31 Mars B, Heron J, Kessler D, Davies NM, Martin RM, Thomas KH, Gunnell D. Influences on antidepressant prescribing trends in the UK: 1995-2011. Soc Psychiatry Psychiatr Epidemiol. 2017;52(2): 193–200. doi: 10.1007/s00127-016-1306-4

32 Andrews PW, Thomson JA Jr, Amstadter A, Neale MC. Primum non nocere: an evolutionary analysis of whether antidepressants do more harm than good. Front Psychol. 2012;3:117. doi: 10.3389/fpsyg.2012.00117

33 Stearns V, Beebe KL, Iyengar M, Dube E. Paroxetine controlled release in the treatment of menopausal hot flashes: a randomized controlled trial. JAMA. 2003;289(21):2827–2834. doi: 10.1001/jama.289.21.2827

34 Coupland C, Dhiman P, Morriss R, Arthur A, Barton G, Hippisley-Cox J. Antidepressant use and risk of adverse outcomes in older people: population based cohort study. BMJ. 2011;343:d4551. doi: 10.1136/bmj.d4551

35 Alonso J, Angermeyer MC, Bernert S, Bruffaerts R, Brugha TS, Bryson H, et al. Use of mental health services in Europe: results from the European Study of the Epidemiology of Mental Disorders (ESEMeD) project. Acta Psychiatr Scand Suppl. 2004;(420):47–54. doi: 10.1111/j.1600-0047.2004.00330.x

36 Meijer WEE, Heerdink ER, Leufkens HGM, Herings RMC, Egberts AC, Nolen WA. Incidence and determinants of long-term use of antidepressants. Eur J Clin Pharmacol. 2004;60(1):57–61. doi: 10.1007/s00228-004-0726-3

37 Johnson CF, Macdonald HJ, Atkinson P, Buchanan AI, Downes N, Dougall N. Reviewing long-term antidepressants can reduce drug burden: a prospective observational cohort study. Br J Gen Pract. 2012;62(604):e773–e779. doi: 10.3399/bjgp12X658304

38 Petty DR, House A, Knapp P, Raynor T, Zermansky A. Prevalence, duration and indications for prescribing of antidepressants in primary care. Age Ageing. 2006;35(5):523–526. doi: 10.1093/ageing/afl023

39 Mojtabai R, Olfson M. National trends in long-term use of antidepressant medications: results from the U.S. National Health and Nutrition Examination Survey. J Clin Psychiatry. 2014;75(2): 169–177. doi: 10.4088/JCP.13m08443

40 Bet PM, Hugtenburg JG, Penninx BW, Hoogendijk WJ. Side effects of antidepressants during long-term use in a naturalistic setting. Eur Neuropsychopharmacol. 2013;23(11):1443–1451. doi: 10.1016/j.euroneuro.2013.05.001

41 Bandelow B, Zohar J, Hollander E, Kasper S, Möller HJ; WFSBP Task Force on Treatment Guidelines for Anxiety, Obsessive-Compulsive and Post-Traumatic Stress Disoders. World Federation of Societies of Biological Psychiatry (WFSBP) guidelines for the pharmacological treatment of anxiety, obsessive-compulsive and post-traumatic stress disorders - first revision. World J Biol Psychiatry. 2008;9(4):248–312. doi: 10.1080/15622970802465807

42 Cleare A, Pariante CM, Young AH, Anderson IM, Christmas D, Cowen PJ, et al. Evidence-based guidelines for treating depressive disorders with antidepressants: a revision of the 2008 British Association for Psychopharmacology guidelines. J Psychopharmacol. 2015;29(5):459–525. doi: 10.1177/0269881115581093

43 Johnson CF, Williams B, MacGillivray SA, Dougall NJ, Maxwell M. 'Doing the right thing': factors influencing GP prescribing of antidepressants and prescribed doses. BMC Fam Pract. 2017;18(1):72. doi: 10.1186/s12875-017-0643-z

44 Bosman RC, Huijbregts KM, Verhaak PF, Ruhé HG, van Marwijk HW, van Balkom AJ, Batelaan NM. Long-term antidepressant use: a qualitative study on perspectives of patients and GPs in primary care. Br J Gen Pract. 2016;66(651):e708–e719. doi: 10.3399/bjgp16X686641

45 Mintziori G, Lambrinoudaki I, Goulis DG, Ceausu I, Depypere H, Erel CT et al. EMAS position statement: non-hormonal management of menopausal vasomotor symptoms. Maturitas. 2015;81(3):410–413. doi: 10.1016/j.maturitas.2015.04.009

High Incidence of Herpes Simplex Virus-1 in Cord Blood and Placenta Infection of Women in Southern Brazil

Alta incidência do vírus herpes simplex 1 em sangue de cordão e infecção na placenta de mulheres no sul do Brasil

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Abstract

Objective Estimate the prevalence of human herpesvirus type 1 HSV-1 DNA in placental samples, its incidence in umbilical cord blood of newborns and the associated risk factors.

Methods Placental biopsies and umbilical cord blood were analyzed, totaling 480 samples, from asymptomatic parturients and their newborns at a University Hospital. Nested polymerase chain reaction (PCR) and gene sequencing were used to identify the virus; odds ratio (OR) and relative risk (RR) were performed to compare risk factors associated with this condition.

Results The prevalence of HSV-1 DNA in placental samples was 37.5%, and the incidence in cord blood was 27.5%. Hematogenous transplacental route was identified in 61.4% from HSV-1$^+$ samples of umbilical cord blood paired with the placental tissue. No evidence of the virus was observed in the remaining 38.6% of placental tissues, suggesting an ascendant infection from the genital tract, without replication in the placental tissue, resulting in intra-amniotic infection and vertical transmission, seen by the virus in the cord blood. The lack of condom use increased the risk of finding HSV-1 in the placenta and umbilical cord blood.

Conclusion The occurrence of HSV-1 DNA in the placenta and in cord blood found suggests vertical transmission from asymptomatic pregnant women to the fetus.

Keywords
- HSV-1
- vertical transmission
- placenta
- umbilical cord
- herpesvirus

Resumo

Objetivo Estimar a prevalência do DNA do vírus herpes humano 1 (HSV-1) em amostras de placenta, sua incidência no sangue do cordão umbilical de recém-nascidos e fatores de risco associados.

Métodos Biópsias de placenta e de sangue de cordão umbilical foram analisadas, totalizando 480 amostras de parturientes assintomáticas e seus recém-nascidos em um hospital universitário. Reação de cadeia de polimerase (RCP) *nested* e sequenciamento

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gênico foram usados para identificar o vírus; odds ratio (OR) e risco relativo (RR) foram realizados para comparar os fatores de risco associados à essa condição.

Resultados A prevalência do DNA do HSV-1 em amostras de placenta foi de 37,5%, e a incidência no sangue do cordão foi de 27,5%. A via transplacentária hematogênica foi identificada em 61,4% das amostras de HSV-1 + do sangue do cordão umbilical, pareadas com o tecido placentário. Nenhuma evidência do vírus foi observada nos restantes 38,6% dos tecidos placentários, sugerindo uma infecção ascendente do trato genital. A falta de uso do preservativo aumentou o risco de encontrar o HSV-1 na placenta e no sangue do cordão umbilical.

Conclusão A ocorrência de DNA do HSV-1 na placenta e no sangue do cordão umbilical sugere uma transmissão vertical de gestantes assintomáticas para o feto.

Palavras-chave
- HSV-1
- transmissão vertical
- placenta
- cordão umbilical
- herpesvírus

Introduction

Human herpesvirus type 1 (HSV-1) is a ubiquitous neurotropic virus in humans. The main characteristics are the lifelong latent/persistent infection in the sensory ganglia innervating the primary infection site, and the production of vesicular lesions upon reactivation.[1,2] Typically associated with orofacial lesions, HSV-1 has emerged as a pathogen of genital infections, especially in the Americas among people between 15 and 49 years old, which is the reproductive age group.[2–4]

Genital HSV-1 is the main cause of the first episode of genital herpes in women in high income countries, as its seroprevalence is declining during childhood as a cause of oral lesions.[5,6] Consequently, adolescents and young adults have their first exposure to the virus with the initiation of sexual activity.[1,7] All over the world, $\sim$ 132 million women have incident or prevalent HSV infection during pregnancy.[8] The first estimate of global neonatal herpes infection incidence predicts that the Americas have the highest regional rate due to genital HSV-1 infection.[7] Overall seroprevalence of antibodies against HSV-1 of 67.2% was identified among young people during a study managed in Brazil.[9] Another study investigated the prevalence of HSV-1 and HSV-2 by polymerase chain reaction (PCR) in cervical samples of 261 Brazilian women and found the occurrence of HSV-1 in 23% of the samples, while 5.4% had the HSV-2 DNA detected.[3]

During the asymptomatic virus shedding, the virus can be transmitted to the partner or even to the newborn during labor.[10–12] The infant can become infected during pregnancy, labor or in the postnatal period.[12] Congenital infections, not resulting in miscarriage, may affect the infant in several ways, including skin or eye lesions (cataracts, chorioretinitis or microphthalmia), neurological calcifications, microcephaly, seizures, delayed growth, and psychomotor developmental problems.[11,13]

The present study aimed to simultaneously investigate the incidence of HSV-1 in neonatal cord blood and the prevalence of HSV-1 DNA in placental tissue of parturient women by correlating risk factors associated with infection and vertical transmission.

Methods

The present work was carried out as an observational study designed to evaluate the prevalence of HSV-1 in placental samples of parturient women and the incidence of HSV-1 in cord blood samples from their newborns. Specimens were collected between March 2011 and March 2014, using a convenience sampling strategy. All of the parturients who agreed to participate voluntarily by a signed informed consent were included in the study. Patients $<$ 18 years old were allowed to participate by the consent of the legal guardian. Patients with mental disabilities or unable to express their wishes were automatically excluded from the study. The sample size was calculated on the basis of a presumed 3.3–28.0% HSV-1 prevalence in the placenta, with associated 95% confidence intervals (CIs) using Epi-Info 7.0 (Centers for Disease Control and Prevention, Atlanta, GA, USA).[14–18] The sample consisted of 160 women under medical care at the Obstetric ward from the Hospital Universitário Dr. Miguel Riet Correa Jr. (HU/UFRG, in the Portuguese acronym), a University hospital in Rio Grande, southern Brazil. Clinical examination was performed in all women when they were admitted to the obstetric ward.

Collection of umbilical cord blood samples and placental tissue biopsies were performed as previously described by Finger-Jardim et al.[19] Subsequently, placenta samples were stored in TE buffer (10mM Tris-HCl pH 8.0; 1mM EDTA) at - 20°C, and the umbilical cord blood at - 4° C until further processing.

DNA extraction from umbilical cord blood was performed with the PureLink Genomic DNA Mini kit (Invitrogen - Life Technologies, Carlsbad, CA, USA), according to the specifications of the manufacturer. DNA extraction from placental tissue was performed using an adapted protocol of the mentioned commercial kit as previously described.[18] DNA samples were stored at - 20°C until used, and its quality was assessed by amplification of the human *CCR2* gene. Polymerase chain reaction products were visualized by UV light after electrophoresis on 1.5% agarose gels stained with Blue Green Loading Dye (LGC Biotecnologia, São Paulo, SP, Brazil).

Detection of HSV-1 in placental tissue and blood samples was determined by nested PCR using an adapted version of

specific protocols to detect the virus.[18,20–22] The two consecutive PCR reactions used 5uL of the DNA template in the first round and 1.5 uL product from the first round in the second round, respectively. The reagents used in the reaction were: 1X PCR buffer, 2 mM MgCl2, 0.5 mM dNTPs, 1U Platinum Taq DNA polymerase enzyme (Life Technologies, Carlsbad, CA, USA), Milli-Q H_2O q.s.p. and HSV-1. Previously described primers by Aurelius et al[20] were employed to amplify a fragment of 138pb of the HSV-1 *gD* gene. Samples were processed with positive and negative controls in each reaction, and with a blank reaction (no DNA added). The positive control was obtained from a dead cell suspension containing the virus (Vero cell DNA, simian DNA virus-positive, Virology Laboratory of the Universidade Federal do Rio Grande do Sul). Polymerase chain reaction products were subjected to electrophoresis on 2% agarose gels, stained with Blue Green and visualized by UV illumination in an LPIX Transilluminator (Loccus, São Paulo, Brazil). Positive samples were repeated at a new reaction with positive and negative controls and a blank reaction. The positive samples were purified with Illustra GFX PCR DNA and Gel Band Purification Kit (GE Healthcare Life Sciences, Piscataway, NJ, USA) and sequencing was performed using an ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, CA, USA) in an automated ABI 3130XL analyzer (Thermo Scientific, Waltham, MA, USA). The sequences found in this study were compared to HSV-1 sequences available in the GenBank database, using the BLASTn algorithm.

Data on risk factors for HSV-1 infection were obtained by a self-reported questionnaire and hospital database. Clinical, gynecological, laboratory and sociodemographic variables were evaluated for each participant. An active search was performed on the charts of the neonates presenting positive HSV-1 by PCR from umbilical cord blood samples. The Chi-squared test was used to compare categorical variables: age, educational attainment, skin color, marital status, income, age at onset of sexual intercourse, number of lifetime partners, contraception method, comorbid STDs (sexual transmitted diseases), number of gravidity, mode of delivery, history of abortion, time between rupture of membranes and delivery, and gestational time. The OR for each variable was calculated, potential risk factors and protective factors were investigated, and frequency distributions and percentages were determined. Differences were considered statistically significant when $p < 0.05$. Multivariate analysis with Poisson regression was also performed, followed by the construction of a hierarchical linear model, which incorporated variables with $p \leq 0.20$ in the crude analysis. The first level consisted of demographic and socioeconomic variables, while in the second included the variables comprising risk factors for HSV-1 infection. All analyzes were performed using SPSS Statistics for Windows, Version 12.0 (SPSS Inc., Chicago, IL, USA) and Epi Info v.7.0.

The present study was approved by the Research Ethics Committee at the Health Area (CEPAS, in the Portuguese acronym) of the University Federal do Rio Grande (UFRG, in the Portuguese acronym) (CEPAS N° 54/2011). All of the participants (or their legal guardians, when appropriate) provided written informed consent for participating in the study

Results

Along the whole study, 480 specimens were analyzed, comprising 160 placentas (maternal and fetal sides = 320 samples) and 160 newborns cord blood samples. All of the samples were tested. The prevalence of HSV-1 found in the placenta was 37.5% ($n = 60$) (maternal, fetal, or both interfaces infected, showing tissue permissiveness to the virus) and the incidence in cord blood was 27.5% ($n = 44$). Vertical hematogenous transplacental transmission was identified in 27 (61.4%) umbilical cord blood samples. HSV-1 was present in cord blood, without evidence of virus in the 17 (38.6%) corresponding placentas, suggesting intra-amniotic infection without placental involvement (►**Table 1**).

Regarding the gynecological history, the only variable significantly associated with HSV-1 infection in cord blood was the use of hormonal contraception. Women who used hormonal contraception or othercontraceptive method, except for condoms, had almost 4 times more chances to present the virus in the umbilical cord blood of their neonates than those associated with condom use or another method (95%CI: 1.30–9.04; $p = 0.009$; ►**Table 1**). There were no significant associations between the presence of HSV-1 and obstetric variables.

The presence of placental HSV-1 increased the chance of this viral infection in the umbilical cord blood (95%CI: 1.92–8.27; $p < 0.001$; OR = 3.99). Also, the presence of HSV-1 in the cord blood increased the chance of placental infection (95%CI: 1.48–3.124.43; $p < 0.001$; RR = 2.15).

A total of 15 subjects (34%) were born with alterations, such as ocular inflammation and pustules in the genital region after vaginal delivery, limb bruising, decreased reflexes, hypotonia and thrombocytopenia. There was one case of hydrocephaly and one case of congenital syphilis. None had herpes diagnosis or were investigated for this infection at birth, and the mothers did not return after an active search for pediatrics care. Clinical findings such as low birthweight (data not shown) and prematurity had no significant association with cord blood incidence.

Discussion

Vertical transmission is the passage of a pathogen from mother to child that can occur still in the uterus (hematogenous transplacental), peripartum or during the postnatal period.[23]

In the present study, the prevalence of HSV-1 DNA found in placenta samples was 37.5% ($n = 60$), which is considered high when compared with other studies that also identified the occurrence of HSV only in the placenta and reported prevalence rates between 2.6 and 28%.[14–18] These findings demonstrate that the virus prevalence in placental tissue is frequent among the pregnant women who joined the study. Therefore, the monitoring and tracking of the virus during pregnancy is quite relevant, considering the possible neonatal complications that might occur. However, it is important to note that most studies have investigated placental tissue from unsuccessful pregnancies and, differently from what was analyzed in the present study, few of them investigated the

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Table 1 Sociodemographic, Obstetrical and Gynecological Profile of Parturient Women, Stratified by HSV-1 Positivity in the Sample

Variable/ Category (*n*)	*n* (%)*	HSV-1+ placenta *n* (%)*	Prevalence Ratio	95%CI	*p-value;*[c,b]	HSV-1+ cord blood n (%)*	Odds Ratio	95% CI	*p-value*[b]
Age (years old) (133)									
≤20	35 (26.3)	14 (40)	1.0		0.39	7 (20)	1.0		*0.22*
21-30	71 (53.4)	34 (47.9)	1.19	0.74–1.92		25 (35.2)	2.17	0.83–5.68	
31-40	27 (20.3)	9 (33.3)	0.83	0.42–1.62		10 (37)	2.35	0.75–7.34	
Educational attainment (years) (150)									
≥9	83 (55.3)	30 (36.1)	1.0		*0.81*	23 (27.7)	1.0		*0.45*
≤8	67 (44.7)	23 (34.3)	0.94	0.61–1.47		15 (22.4)	0.75	0.35–1.59	
Skin color (self-reported) (155)									
White	99 (63.9)	38 (38.4)	1.0		*0.58*	29 (29.3)	1.0		*0.41*
Non-white	56 (36.1)	19 (33.9)	1.13	0.72–1.76		13 (23.2)	0.72	0.34–1.55	
Marital Status (155)									
Stable Partner	117 (75.5)	43 (36.8)	1.0		*0.74*	35 (29.9)	1.0		*0.16*
No Stable Partner	38 (24.5)	14 (37.8)	1.0	0.62–1.61		7 (18.9)	1.89	0.76–4.69	
Income (in minimum wages)[a] (146)									
≥2	50 (34.2)	20 (40)	1.0		*0.42*	14 (28)	1.0		*0.59*
≤1	96 (65.8)	32 (33.3)	0.83	0.53–1.29		23 (24)	0.81	0.37–1.75	
Age at onset of sexual intercourse (years) (128)									
≥16	63 (49.2)	28 (44.4)	1.0		*0.49*	23 (36.5)	1.0		*0.09*
≤15	65 (50.8)	25 (38.5)	1.15	0.76–1.74		15 (23.1)	1.91	0.88–4.14	
Number of lifetime partners (124)									
1	39 (31.5)	18 (46.2)	1.0		*0.53*	15 (38.5)	1.0		*0.27*
2– 4	56 (45.1)	20 (35.7)	0.77	0.47–1.26		16 (28.6)	0.64	0.26–1.52	
≥ 5	29 (23.4)	13 (44.8)	0.97	0.57–1.64		6 (20.7)	0.41	0.13–1.26	
Contraception (130)									
Condom and/or other methods	39 (30)	13 (33.3)	1.0			6 (15.4)	1.0		
Contraception (excluding condom)	91 (70)	42 (46.2)	1.38	0.84–2.27	*0.17*	35 (38.5)	3.43	1.30–9.04	***0.009***
Comorbid STDs (153)									

Table 1 (*Continued*)

Variable/ Category (*n*)	*n* (%)*	HSV-1+ placenta *n* (%)*	Prevalence Ratio	95%CI	*p-value*;[c,b]	HSV-1+ cord blood n (%)*	Odds Ratio	95% CI	*p-value*[b]
No	143 (93.5)	51 (35.9)	1.0			37 (26.1)	1.0		*0.77*
Yes	10 (6.5)	4 (40)	1.11	0.50–2.45	*0.72*	3 (30)	1.22	0.30–4.99	
Gravidity (129)									
1st pregnancy	53 (41.1)	21 (39.6)	1.0		*0.46*	14 (26.4)	1.0		*0.34*
≥ 2	76 (58.9)	35 (46.1)	1.16	0.76–1.75		26 (34.2)	1.44	0.66–3.13	
Mode of Delivery (159)									
Cesarean	86 (54.1)	28 (32.6)	1.0		*0.19*	28 (32.6)	1.0		*0.13*
Vaginal	73 (45.9)	31 (42.5)	1.30	0.87–1.95		16 (21.9)	0.58	0.28–1.18	
History of abortion (148)									
No	121 (81.8)	44 (36.4)	1.0		*0.43*	32 (26.4)	1.0		*0.73*
Yes	27 (18.2)	12 (44.4)	1.22	0.75–1.98		8 (29.6)	1.17	0.46–2.93	
Time between rupture of membranes and delivery (minutes) (122)									
≤360 minutes	82 (67.2)	31 (37.8)	1.0		*0.39*	21 (25.6)	1.0		*0.94*
≥360 minutes	40 (32.8)	12 (30)	0.79	0.45–1.37		10 (25)	0.96	0.40–2.31	
Gestational time (weeks)[c] (143)									
≥38 (full-term)	117 (81.8)	51 (43.6)	1.0		*0.40*	35 (29.9)	1.0		*0.63*
≤37 (preterm)	26 (18.2)	9 (34.6)	1.79	0.45–1.40		9 (34.6)	1.24	0.50–3.04	

Abbreviations: CI, confidence interval; HSV1, human herpesvirus type 1.
*All respondents.
[a]Calculed as equivalence to the Brazilian minimum wage at the time of the study (approximately US$ 350.00);
[b]Chi-squared test.
[c]Calculated by means of the Capurro method; statistically significant result is indicated in bold.

difference between HSV-1 and HSV-2.[15,17] Finger-Jardim et al[18] found a 29.9% prevalence of HSV-1 in the fetal side of the placental tissue, using the same methodology applied in the present work, and women who reported no use of condom during sexual intercourse had two times more chances to be HSV-1-positive in the placental tissue. Therefore, the placental tissue seems to play an important role as a reservoir for the virus. Burgos et al[24] simulated HSV-1 vertical transmission in mice and investigated the presence of viral DNA in fetuses, newborns and adult models in the offspring of mothers with latent infection. HSV-1 DNA was identified and quantified by PCR, and all subjects presented detectable levels of the virus in the central nervous system (CNS), in the placenta, and in the blood. That study demonstrated that viral DNA in the blood is common, and it reaches the placental tissue through the maternal bloodstream, clearly confirming vertical transmission of the virus in animal models.[24] Similarly, our study presented the hematogenous transplacental route as an important pathway for HSV-1 transmission.

The present work reported an incidence of 27.5% ($n = 44$) for HSV-1 DNA in cord blood and, among these, 61.4% ($n = 27$) had the viral DNA also detected in the placenta, indicating vertical transmission from placenta through the blood. Tavakoli et al[25] investigated the incidence of HSV DNA in newborn umbilical cord blood and found 2% for HSV-1 and 4% for HSV-2. Thirty percent of them were born by cesarean section and mothers were asymptomatic seropositive for the virus.[25] During pregnancy, pathogens can be transmitted to the placenta by ascending infection from decidual cells (maternal side of the placenta) to the villi (fetal side).[16]

Regarding genital HSV lesions, all of the parturients were asymptomatic during clinical examination and denied genital herpetic lesions. HSV-1 induces less recurrent infections in the genital tract when compared with HSV-2, but apparently infects the newborn more easily later. However, $\sim$ 70% of the infected newborns have normal development if they are not affected by the disseminated form, which can be lethal.[10] In the present study, 38.6% from the positive cord blood samples presented no evidence of virus in the respective placentas and no genital lesions, suggesting an ascending origin of the virus. In this scenario, we consider that pregnant women with asymptomatic viral shedding can transmit the virus to the newborn, as previously reported in other studies.[10,11,18]

The presence of the virus in the blood nourishing the fetus during pregnancy is facilitated by its occurrence in the placenta, which is a tissue shown to be permissive to HSV infection *in vitro*.[23,26] There are few studies investigating HSV vertical transmission using the cord blood as a target tissue. Finger-Jardim et al[19] studied the prevalence of HSV-2 in the placenta, and found viral DNA in 9% of the analyzed tissues and a 1.1% incidence in the umbilical cord blood of newborns. Considering the mentioned study, membrane rupture time was significantly associated with infection, unlike the observed for HSV-1 in the present work. Another report using the same methodology applied in the present study found 28% and 29.9% of prevalence of HSV-1 DNA in maternal and fetal placental samples, and lack of condom use and vaginal delivery were identified as independent risk factors for HSV-1 infection in the maternal side of the placenta.[18] Lack of condom use is a risk factor in the present study, increasing vertical transmission by almost four times, thus reassuring HSV-1 as a sexually transmitted infection (STI) agent and supporting the idea of ascendant infection transmission, and this association has been previously observed in other studies.[7,12,18]

HSV is an STI, and the use of condom during sexual intercourse is a protective method. In the present study, the use of methods for contraception (oral or injectable) lacking the physical barrier method such as condoms, was a significant variable related to detection of HSV-1 DNA in the umbilical cord blood of newborns. However, among the positive umbilical blood samples for viral DNA, almost 40% were viral-negative in the placentas, suggesting an asymptomatic ascending genital tract infection. This result showed that women who were exposed to the virus without using condoms had a risk almost four times higher of presenting viral DNA in the umbilical cord blood of their newborns. These findings support previous studies that project HSV-1 as an emergent STI.[3,4]

The high incidence of HSV-1 DNA found in the umbilical cord blood of newborns suggests vertical transmission in the uterus. An active search was performed on the charts of newborns presenting HSV-1 in the umbilical cord blood, and 13.6% ($n = 6$) of them were born from mothers who underwent preeclampsia protocol. No significant association with low birthweight, prematurity and cord blood HSV-1 incidence was observed. Alterations such as vaginal postpartum ocular inflammation, limb bruising, decreased reflexes, hypotonia and pustules were reported in the medical records, but no clinic or laboratorial investigation for HSV was realized. Neonatal herpes infection can cause newborn skin and eye lesions, and these symptoms can be caused by the HSV-1 virus.[13,27] Identification of HSV-exposed infants allows resources to be focused on those at highest risk, but it is hard to identify the disease in asymptomatic or HSV-1 recurrent mothers.

Some limitations should be considered when interpreting the results of the present work. The present study was conducted in pregnant women from a single university hospital (medium-sized hospital, 185 beds) who serves only patients depending on the Brazilian public health system. Another limitation is related to the self-administered questionnaire, since patients sometimes did not answer to all questions. Also concerning this matter, there were no questions about oral injuries caused by herpes, information that would provide more rich details for the obtained results.

Conclusion

In summary, the present study described a high prevalence of HSV-1 in placenta tissue samples, as well as a resulting high incidence in cord blood. The presence of HSV-1 in the placenta represents a risk for vertical transmission. This observation, in association with the investigated risk factors, shows the importance of virus transmission and the possibility of asymptomatic ascending infection of the genital tract. The occurrence of this virus was high in the group of women studied and, consequently, in the newborns, with these born with signs suggesting viral infection. This research contributes to better

understand HSV-1 as a major infection, also being regarded as an STI that can lead to diseases in newborns. The high occurrence of vertical transmission deserves greater attention, since this virus can cause serious complications to newborns.

Contributions

AMMB, VPH, ECA and FF-J, conceived the study. All authors planned the study. AMMB, MAS, VPH and CGV obtained the funding for the study. ECA and Finger-Jardim F. collected and processed the samples. ECA and FF-J analyzed the data and drafted the manuscript. ECA, Finger-Jardim F. and CGV contributed to the data interpretation. All of the authors reviewed the manuscript, and gave input at all stages of the study. All of the authors have approved the final version of the manuscript for submission.

Conflict of Interests

The authors have no conflict of interests to declare.

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References

1 Johnston C, Corey L. Current concepts for genital herpes simplex virus infection: diagnostics and pathogenesis of genital tract shedding. Clin Microbiol Rev. 2016;29(1):149–161. doi: 10.1128/CMR.00043-15
2 James SH, Sheffield JS, Kimberlin DW. Mother-to-child transmission of herpes simplex virus. J Pediatric Infect Dis Soc. 2014;3 (Suppl 1):S19–S23. doi: 10.1093/jpids/piu050
3 Pereira VS, Moizeis RN, Fernandes TA, Araújo JM, Meissner RV, Fernandes JV. Herpes simplex virus type 1 is the main cause of genital herpes in women of Natal, Brazil. Eur J Obstet Gynecol Reprod Biol. 2012;161(2):190–193. doi: 10.1016/j.ejogrb.2011.12.006
4 Looker KJ, Magaret AS, May MT, Turner KM, Vickerman P, Gottlieb SL, et al. Global and regional estimates of prevalent and incident herpes simplex virus type 1 infections in 2012. PLoS One. 2015;10 (10):e0140765. doi: 10.1371/journal.pone.0140765
5 Ryder N, Jin F, McNulty AM, Grulich AE, Donovan B. Increasing role of herpes simplex virus type 1 in first-episode anogenital herpes in heterosexual women and younger men who have sex with men, 1992-2006. Sex Transm Infect. 2009;85(6):416–419. doi: 10.1136/sti.2008.033902
6 Kaspersen MD, Höllsberg P. Seminal shedding of human herpesviruses. Virol J. 2013;10:226. doi: 10.1186/1743-422X-10-226
7 Looker KJ, Magaret AS, May MT, Turner KME, Vickerman P, Newman LM, et al. First estimates of the global and regional incidence of neonatal herpes infection. Lancet Glob Health. 2017;5(3): e300–e309. doi: 10.1016/S2214-109X(16)30362-X
8 Kimberlin DW. Why neonatal herpes matters. Lancet Glob Health. 2017;5(3):e234–e235. doi: 10.1016/S2214-109X(17)30047-5
9 Clemens SAC, Farhat CK. Seroprevalence of herpes simplex 1-2 antibodies in Brazil. Rev Saude Publica. 2010;44(4):726–734. doi: 10.1590/S0034-89102010000400017
10 Gardella C, Brown Z. Prevention of neonatal herpes. BJOG. 2011; 118(2):187–192. doi: 10.1111/j.1471-0528.2010.02785.x
11 Anzivino E, Fioriti D, Mischitelli M, Bellizzi A, Barucca V, Chiarini F, et al. Herpes simplex virus infection in pregnancy and in neonate: status of art of epidemiology, diagnosis, therapy and prevention. Virol J. 2009;6:40. doi: 10.1186/1743-422X-6-40
12 James SH, Kimberlin DW. Neonatal herpes simplex virus infection. Infect Dis Clin North Am. 2015;29(3):391–400. doi: 10.1016/j.idc.2015.05.001
13 Straface G, Selmin A, Zanardo V, De Santis M, Ercoli A, Scambia G. Herpes simplex virus infection in pregnancy. Infect Dis Obstet Gynecol. 2012;2012:385697. doi: 10.1155/2012/385697
14 Satosar A, Ramirez NC, Bartholomew D, Davis J, Nuovo GJ. Histologic correlates of viral and bacterial infection of the placenta associated with severe morbidity and mortality in the newborn. Hum Pathol. 2004;35(5):536–545. doi: 10.1016/j.humpath.2004.01.015
15 Syridou G, Spanakis N, Konstantinidou A, Piperaki ET, Kafetzis D, Patsouris E, et al. Detection of cytomegalovirus, parvovirus B19 and herpes simplex viruses in cases of intrauterine fetal death: association with pathological findings. J Med Virol. 2008;80(10): 1776–1782. doi: 10.1002/jmv.21293
16 McDonagh S, Maidji E, Ma W, Chang HT, Fisher S, Pereira L. Viral and bacterial pathogens at the maternal-fetal interface. J Infect Dis. 2004;190(4):826–834. doi: 10.1086/422330
17 Al-Buhtori M, Moore L, Benbow EW, Cooper RJ. Viral detection in hydrops fetalis, spontaneous abortion, and unexplained fetal death in utero. J Med Virol. 2011;83(4):679–684. doi: 10.1002/jmv.22007
18 Finger-Jardim F, Avila EC, da Hora VP, Gonçalves CV, de Martinez AMB, Soares MA. Prevalence of herpes simplex virus types 1 and 2 at maternal and fetal sides of the placenta in asymptomatic pregnant women. Am J Reprod Immunol. 2017;78(1):e12689. doi: 10.1111/aji.12689
19 Finger-Jardim F, Teixeira LO, de Oliveira GR, et al. Herpes simplex virus: prevalence in placental tissue and incidence in neonatal cord blood samples. J Med Virol. 2014;86(3):519–524. doi: 10.1002/jmv.23817
20 Aurelius E, Johansson B, Sköldenberg B, Staland A, Forsgren M. Rapid diagnosis of herpes simplex encephalitis by nested polymerase chain reaction assay of cerebrospinal fluid. Lancet. 1991; 337(8735):189–192. doi: 10.1016/0140-6736(91)92155-u
21 Aurelius E, Johansson B, Sköldenberg B, Forsgren M. Encephalitis in immunocompetent patients due to herpes simplex virus type 1 or 2 as determined by type-specific polymerase chain reaction and antibody assays of cerebrospinal fluid. J Med Virol. 1993;39 (3):179–186.
22 Schmutzhard J, Merete Riedel H, Zweygberg Wirgart B, Grillner L. Detection of herpes simplex virus type 1, herpes simplex virus type 2 and varicella-zoster virus in skin lesions. Comparison of real-time PCR, nested PCR and virus isolation. J Clin Virol. 2004;29 (2):120–126. doi: 10.1016/S1386-6532(03)00113-6
23 Bhatta AK, Keyal U, Liu Y, Gellen E. Vertical transmission of herpes simplex virus: an update. J Dtsch Dermatol Ges. 2018;16(6): 685–692. doi: 10.1111/ddg.13529
24 Burgos JS, Ramirez C, Guzman-Sanchez F, Alfaro JM, Sastre I, Valdivieso F. Hematogenous vertical transmission of herpes simplex virus type 1 in mice. J Virol. 2006;80(6):2823–2831. doi: 10.1128/JVI.80.6.2823-2831.2006
25 Tavakoli A, Monavari SH, Bokharaei-Salim F, Mollaei H, Abedi-Kiasari B, Fallah FH, et al. Asymptomatic herpes simplex virus infection in Iranian mothers and their newborns. Fetal Pediatr Pathol. 2017;36(1):27–32. doi: 10.1080/15513815.2016.1229368
26 Avanzi S, Leoni V, Rotola A, Alviano F, Solimando L, Lanzoni G, et al. Susceptibility of human placenta derived mesenchymal stromal/stem cells to human herpesviruses infection. PLoS One. 2013;8 (8):e71412. doi: 10.1371/journal.pone.0071412
27 Pinninti SG, Angara R, Feja KN, Kimberlin DW, Leach CT, Conrad DA, et al. Neonatal herpes disease following maternal antenatal antiviral suppressive therapy: a multicenter case series. J Pediatr. 2012;161(1):134–8.e1,3. doi: 10.1016/j.jpeds.2011.12.053

Gestational Diabetes in the Population Served by Brazilian Public Health Care. Prevalence and Risk Factors

Diabetes gestacional na população atendida pelo sistema público de saúde no Brasil. Prevalência e fatores de risco

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Abstract

Objective To assess the prevalence of gestational diabetes mellitus and the main associated risk factors in the population served by the Brazilian Unified Health System in the city of Caxias do Sul, state of Rio Grande do Sul.

Materials and Methods A descriptive, cross-sectional and retrospective study was conducted. Maternal variables were collected from the medical records of all pregnant women treated at the basic health units in 2016. Hyperglycemia during pregnancy (pregestational diabetes, overt diabetes and gestational diabetes mellitus) was identified by analyzing the results of a 75-g oral glucose tolerance test, as recommended by the Brazilian Ministry of Health. Based on the data, the women were allocated into two groups: the gestational diabetes group and the no gestational diabetes group.

Results The estimated prevalence of gestational diabetes among 2,313 pregnant women was of 5.4% (95% confidence interval [95%CI]: 4.56–6.45). Pregnant women with 3 or more pregnancies had twice the odds of having gestational diabetes compared with primiparous women (odds ratio [OR] = 2.19; 95%CI: 1.42–3.37; $p < 0.001$). Pregnant women aged 35 years or older had three times the odds of having gestational diabetes when compared with younger women (OR = 3.01; 95%CI: 1.97–4.61; $p < 0.001$). Overweight pregnant women were 84% more likely to develop gestational diabetes than those with a body mass index lower than 25 kg/m^2 (OR = 1.84; 95%CI: 1.25–2.71; $p = 0.002$). A multivariable regression analysis showed that being overweight and being 35 years old or older were independent variables.

Conclusion In this population, the prevalence of gestational diabetes mellitus was of 5.4%. Age and being overweight were predictive factors for gestational diabetes.

Keywords
- gestational diabetes
- public health
- prevalence
- maternal health

Resumo

Objetivo Avaliar a prevalência de diabetes mellitus gestacional, e dos principais fatores de risco associados, em população usuária do Sistema Único de Saúde em Caxias do Sul-RS.

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Métodos Um estudo descritivo, transversal e retrospectivo foi feito. As variáveis maternas foram coletadas de registros de prontuários de todas gestantes atendidas nas Unidades Básicas de Saúde do município em 2016. A identificação de hiperglicemia na gestação (diabetes pré-gestacional, diabetes identificado durante a gestação e diabetes mellitus gestacional) foi feita pela avaliação dos resultados do teste oral de tolerância com 75 g glicose, conforme preconizado pelo Ministério da Saúde. Com base nesses dados, as gestantes foram separadas em dois grupos: o grupo com diabetes gestacional e o grupo sem diabetes gestacional.

Resultados A prevalência estimada de diabetes gestacional em 2.313 gestantes foi de 5,4% (intervalo de confiança de 95% [IC95%]: 4,56–6,45). Gestantes com 3 ou mais gestações apresentaram chance 2 vezes maior para a ocorrência de diabetes gestacional, quando comparadas às primigestas (razão de possibilidades [RP] = 2,19; IC95%: 1,42- 3,37; $p < 0,001$). Gestantes com idade de 35 anos ou mais apresentaram chance três vezes maior do que as mais jovens (RP = 3,01; IC95%: 1,97–4,61; $p < 0,001$). A chance de desenvolver diabetes gestacional em gestantes com sobrepeso foi 84% maior do que a das com índice de massa corporal inferior a 25 kg/m^2 (RP = 1,84; IC95%: 1,25–2,71; $p = 0,002$). A análise de regressão multivariada mostrou sobrepeso e idade materna como variáveis com associação independente.

Conclusão Nesta população, a prevalência de diabetes mellitus gestacional foi de 5,4%. Idade materna e sobrepeso pré-gestacional foram fatores preditivos para diabetes gestacional.

Palavras-chave
- diabetes gestacional
- saúde pública
- prevalência
- saúde materna

Introduction

In the past 20 years, the global epidemic of diabetes and obesity has reached the population of women of reproductive age; in parallel, there was an increase in the incidence of hyperglycemia during pregnancy.[1,2] The International Diabetes Federation estimated that, in 2017, 21.3 million (16.2%) live births were from pregnancies with hyperglycemia; 86.4% of these were due to gestational diabetes mellitus (GDM), 6.2% were due to diabetes detected before pregnancy, and 7.4% were due to other types of diabetes (including type-1 and type-2 diabetes) detected for the first time during pregnancy.[3]

This broad variation results from multiple of methodological issues, such as the absence of universal criteria for GDM screening and different population characteristics.[2] In addition, there are little data available regarding estimates of the global prevalence of GDM, especially in developing countries.

In 2016, Zhu and Zhang[4] reported a large variation in the prevalence of GDM in different regions of the world, including a higher prevalence in the Middle East and North Africa (12.9%), Southeast Asia (11.7%) and regions of the Western Pacific (11.7%), and a lower prevalence in Europe (5.8%). In Central and South America, the prevalence was of 11.2% (95% confidence interval [95%CI]: 7.1–16.6); however, this figure was derived based on data from only two countries: Brazil (5.7%) and Cuba, (16.6%). Brazil has few studies regarding this issue,[5–7] the most relevant being that of Schmidt et al (2001),[5] which showed an estimated prevalence between 2.4% and 7.2%, depending on the criteria used to diagnose GDM.

Transitional hyperglycemia during pregnancy complicated by GDM occurs primarily due to the functional incapacity of maternal β-pancreatic cells to meet the insulin needs for adequate fetal development; this insufficiency is accentuated starting in the second gestational trimester.[8] This metabolic complication is associated with long-term perinatal and long-term outcomes for the maternal-fetal pair,[9,10] such as excessive fetal growth and consequent complications during labor.[9] A history of GDM in pregnancy is associated with a higher risk of metabolic syndrome, type-2 diabetes mellitus (DM2) and cardiovascular diseases in postchildbirth follow-ups.[10] Approximately 50% of women with GDM progress to DM2 after 10 years.[10]

Gestational diabetes mellitus can have a bigger impact on the health of the mother and her offspring, and it is suggested that it plays a significant role in the global diabetes epidemic. While its prevalence has increased in different populations throughout the world in recent decades, individual reports of this global trend cannot be compared because of the variety of methodological issues. Nonetheless, GDM is an important public health problem today, and it affects the heterogeneous Brazilian population. Considering the relevance of this topic, the present study was developed to estimate the prevalence of GDM and evaluate the associated risk factors among the users of the Brazilian Unified Health System in the city of Caxias do Sul, state of Rio Grande do Sul.

Materials and Methods

A cross-sectional, retrospective, prevalence study was performed from January 1st to December 31st, 2016, in a population of pregnant women who were users of the Unified Health

System (UHS) and attended prenatal follow-up visits at the 47 basic health care units (BHCU) in the city of Caxias do Sul. The present study was approved and supported by the Municipal Health Department and by the Research Ethics Committee of Universidade de Caxias do Sul (number: 2,048,666).

A total of 3,411 medical records were searched for the selected period, which were registered in the SisPreNatal-Datasus software of the Brazilian Ministry of Health and filed in their respective health care units. The medical records that were not found after numerous attempts throughout the entire period of data collection were considered lost. All of the evaluated medical records were from pregnant women residing in Caxias do Sul. Demographic, clinical, and laboratory data were collected and transferred to a database designed for the study and handled exclusively by the researcher responsible for it. The following variables were collected: mother's age (years); race/ethnicity (Caucasian, of African descent and other); level of schooling (≤ 8 years and > 8 years); family history of diabetes in first-degree relatives; obstetric history of GDM; previous hypertensive syndrome, defined as hypertension, preeclampsia and eclampsia; previous abortions; smoking during pregnancy; parity (1, 2 or ≥ 3); pregestational weight (kg) obtained at the first prenatal visit; height (centimeters); and pregestational body mass index (BMI). Fasting glycemia, glycated hemoglobin, and/or 75-g oral glucose tolerance test (OGTT) results and/or the use of antihyperglycemic drugs were analyzed to identify the presence of hyperglycemia during pregnancy. To identify GDM in the study population, the OGTT results were analyzed, as recommended by the Brazilian Ministry of Health,[11] and a positive diagnosis was made when one or more of the following criteria were present: glycemia (fasting) ≥ 92 mg/dl and ≤ 125 mg/dl; blood glucose 1 hour after overload ≥ 180 mg/dl; glycemia 2 hours after overload ≥ 153 mg/dl and ≤ 199 mg/dl. Pregestational diabetes or overt diabetes was considered if glycemia (fasting) ≥ 126mg/dl and/or glycemia 2 hours postoverload ≥ 200 mg/dl and/ or glycated hemoglobin $> 6.5\%$.[11]

The statistical analysis of the data was performed through univariate and multivariate logistic regression, using GDM as a variable response. The variables included in the multivariate regression were selected by the backward technique if they presented a p-value < 0.15 in the univariate step and the percentage of missing data was lower than 10%.[11]

The R software (R Foundation, Vienna, Austria) was used in the statistical analysis of the data. The presence of multicollinearity was evaluated by the estimation of variance inflation factors (VIFs); VIF values > 2.5 indicated considerable multicollinearity in the logistic regression analysis. The calibration and discriminatory ability of the final multiple logistic regression model were evaluated using the Hosmer-Lemeshow test. Values of $p \geq 0.05$ for the Hosmer-Lemeshow test indicated which of the models was calibrated.[12]

Results

There were 3,411 medical records registered by the Health Department of those, 2,797 (82%) were filed and therefore available for the study. Of these 2,797 filed records, 484 (17%) had no clinical or laboratory data to identify any type of hyperglycemia or glycemic normality during the gestational period; therefore, they were excluded from the study; 2,313 records contained fasting glycemia information at the first visit; 1,079 had OGTT information; no charts contained information on glycated hemoglobin; 856 had information on another type of fasting glycemia; 1,079 medical records contained fasting glucose at the first visit and OGTT, and 1,234 did not provide OGTT data.

Thus, the study sample consisted of 2,313 medical records. Based on the data collected from these charts, the patients were allocated into two groups: 1) pregnant women without GDM (2,187; 94.6%) and 2) pregnant women with GDM (126; 5.4%).

In group 1, 25 (1.1%) records met the criteria for pregestational DM, but did not meet the criteria for GDM. The 126 charts that composed group 2 met the criteria for GDM based on OGTT results (►**Fig. 1**). The estimated prevalence of GDM was of 5.4% (95%CI: 4.56–6.45).

The analysis of the variables showed that pregnant women aged ≥ 35 years were three times more likely to develop GDM than younger women (OR = 3.01; 95%CI: 1.97–4.61; $p < 0.001$) (►**Table 1**). Pregestational BMI ≥ 25 kg/m^2 doubled the chance of developing GDM compared with a lower BMI (OR = 1.84;

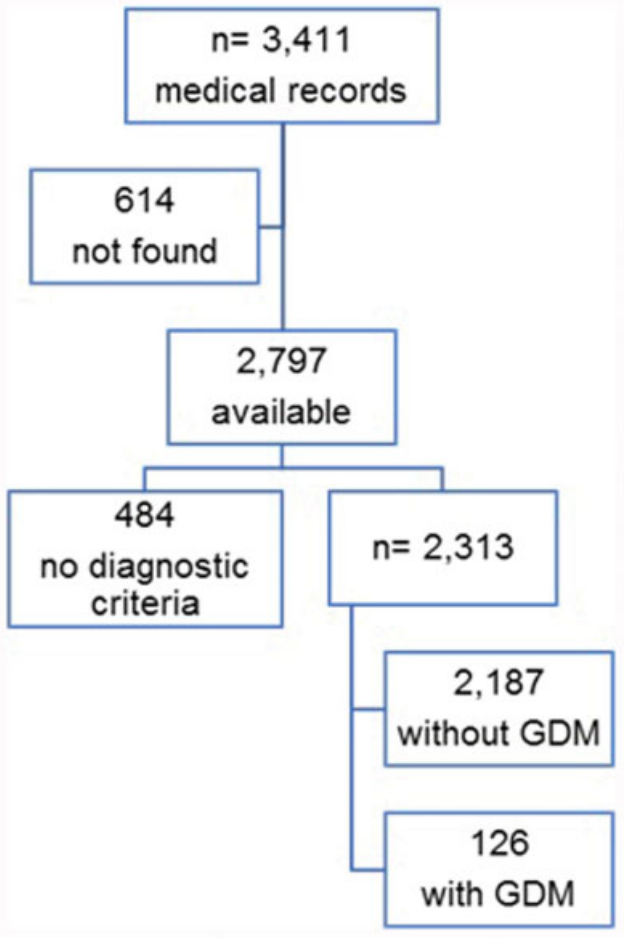

Fig. 1 Flowchart for the composition of the groups with and without gestational diabetes mellitus (GDM) based on 3,411 medical records of pregnant women.

Table 1 Sociodemographic characteristics of pregnant women with and without gestational diabetes mellitus

Variable	Group 1 n (%)	Group 2 n (%)	Odds ratio (95% confidence interval)	*p*-value
Age (years)				
<35[a]	1,939 (95.4)	94 (4.6)		
≥35	219 (87.3)	32 (12.7)	3.01 (1.97–4.61)	< 0.001
Race/Ethnicity				
Caucasian[a]	589 (94.8)	32 (5.2)		
Of African descent	49 (89.1)	6 (10.9)	2.25 (0.90–5.65)	0.083
Other	83 (94.3)	5 (5.7)	1.11 (0.42–2.93)	0.835
Schooling (years)				
≤8[a]	748 (94.9)	40 (5.1)		
> 8	826 (94.1)	52 (5.9)	1.18 (0.77–1.80)	0.451

Notes: [a]Reference category. Group 1: no GDM; group 2: with GDM.

95%CI: 1.25–2.71; $p = 0.002$) (►**Table 2**). Women with 3 or more pregnancies had 2 times higher odds of having GDM than primiparous women (OR = 2.19; 95%CI: 1.42–3.37; $p < 0.001$). The likelihood of women with 2 pregnancies developing GDM was not statistically significant (OR = 1.19; 95%CI: 0.72–1.98; $p = 0.503$) (►**Table 2**).

Women of African descent (OR = 2.25; 95%CI: 0.90–5.65; $p = 0.083$) and those classified as being of another race/ethnicity (OR = 1.11 95%CI: 0.42–2.93; $p = 0.835$), those with higher levels of schooling (< 8 years; OR = 1.18; 95% CI: 0.77–1.80; $p = 0.451$), those who smoked during pregnancy (OR = 1.41; 95%CI: 0.88–2.28; $p = 0.156$), and those with previous hypertensive syndromes (PHSs; OR = 1.34; 95%CI: 0.47–3.76; $p = 0.584$), previous GDM (OR = 3.24; 95%CI: 0.93–11.21; $p = 0.064$), previous abortion [93-2.54), $p = 0.091$], family history of type-2 diabetes mellitus (AF-DM2; OR = 1.44 95%CI: 0.96–2.17; $p = 0.076$) and low height (OR = 1.16 95%CI: 0.53–2.54; $p = 0.707$) did not have an increased likelihood of developing GDM compared with the reference categories (►**Tables 1** and **2**).

Age ≥ 35 years and BMI ≥ 25 kg/m^2 were independent variables with a significance level of 5%. Hosmer-Lemeshow statistics indicated that the logistic model was satisfactorily adjusted, with agreement between the observed and the expected frequencies of the outcome ($p = 0.794$). The area under the receiver operating characteristic (ROC) curve (AUC) associated with the multiple logistic regression model was 0.62. Therefore, the model had an almost perfect performance to discriminate between the categories of the binary outcome (whether someone has or does not have GDM) (►**Table 3**).

Discussion

The present study showed that 5.4% of pregnant women cared for in 2016 by the Unified Health System in Caxias do Sul had GDM. In this population, women who became pregnant and were overweight/obese had the most frequent metabolic complications during pregnancy. Despite the methodological differences, the results of the present study showed a certain similarity to those described by Schmidt et al[5] in 2001, who estimated the prevalence of GDM based on data from six Brazilian capitals (Porto Alegre, São Paulo, Rio de Janeiro, Salvador, Fortaleza and Manaus). In that study, the authors concluded that the prevalence of GDM was of 2.4% (95%CI: 2.0–2.9) according to the American Diabetes Association (ADA) 2000 diagnostic criteria and of 7.2% (95%CI: 6.5–7.9) according to the World Health Organization (WHO) 1999 criteria.

Studies show that the number of pregnant women with GDM has been increasing in recent decades in a proportion parallel to that of DM2.[2,3] This scenario requires effective commitment from all health areas involved with women's health during pregnancy. When analyzing the prevalence of GDM in different global regions, the results vary according to ethnic/racial, socioeconomic and cultural characteristics and screening criteria.[13,14] India has observed a significant increase in the prevalence of GDM, with large differences among regions.[15] This situation led the WHO (2016),[16] to implement the pilot project "The Women in India with GDM Strategy (WINGS)" with the aim of developing a suitable model of care for women with GDM in low-and middle-income countries.

We have scarce scientific literature showing the epidemiological reality of GDM in Brazil. The most relevant and comprehensive study on GDM was published in 2001.[5] During this period of nearly 20 years, population, health and socioeconomic indicators underwent significant changes, and epidemiological transitions occurred in a peculiar way.[17] National epidemiological studies designed with a targeted objective could provide evidence-based information about the current reality and trends of GDM. The present study was developed at Universidade de Caxias do Sul in partnership with the Municipal Health Department to obtain a more in-depth picture of pregnant women with GDM. These patients are referred from the BHCUs to the High-Risk Pregnancy Clinic of the university, and GDM is the main cause for referral to this secondary/tertiary care unit. In addition to providing assistance, this clinic offers long-term, multidisciplinary follow-up for these women and their offspring, and has both academic and care provision goals.

Table 2 Clinical characteristics of the groups of pregnant women with and without gestational diabetes mellitus

Variables	Group 1 n (%)	Group 2 n (%)	Odds ratio (95% confidence interval)	*p*-value
Pregestational BMI (kg/m^2)				
≤24.9[a]	1,043 (96)	43 (4)		
>25	949 (92.9)	72 (7.1)	1.84 (1.25- 2.71)	0.002
Parity				
1	886 (92.2)	35 (3.8)		
2	596 (95.5)	28 (4.5)	1.19 (0.72- 1.98)	0.503
3	670 (92)	58 (8)	2.19 (1.42- 3.37)	< 0.001
Smoking				
No[a]	1539 (95)	81 (5)		
Yes	309 (93.1)	23 (6.9)	1.41 (0.88- 2.28)	0.156
PHS				
No[a]	1945 (94.6)	110 (5.4)		
Yes	53 (93)	4 (7)	1.34 (0.47- 3.76)	0.584
Previous GDM				
No[a]	1981 (94.8)	108 (5.2)		
Yes	17 (85)	3 (15)	3.24 (0.93–11.21)	0.064
Previous abortion				
No[a]	1792 (94.9)	97 (5.1)		
Yes	240 (92.3)	20 (7.7)	1.54 (0.93- 2.54)	0.091
AF-DM2				
No[a]	1321 (95.3)	65 (4.7)		
Sim	563 (93.4)	40 (6.6)	1.44 (0.96- 2.17)	0.076
Height (cm)				
≤150[a]	140 (6.7)	7 (5.8)		
>150	1945 (93.3)	113 (94.2)	1.16 (0.53- 2.54)	0.707

Abbreviations: AF-DM2, family history of type-2 diabetes mellitus; BMI, body mass index; GDM, gestational diabetes mellitus; PHS, previous hypertensive syndromes (hypertension, preeclampsia and eclampsia).
Notes: [a]Reference category. Group 1: no GDM; group 2: GDM.

Table 3 Results of the binary multiple logistic regression analysis

Variable	Odds ratio (95% confidence interval)	*p*-value[b]
Age (years)		
<35[a]		
≤35	3.124 (1.904–5.125)	< 0.001
Body mass index (kg/m^2)		
≤24.9[a]		
≥25	1.498 (0.966–2.324)	0.0711

Notes: N = 1785. [b]$p = 0.794$ according to the Hosmer-Lemeshow test (calibration); [a]reference category; area under the curve (AUC) = 0.62 (discrimination).

The racial/ethnic characteristics of the local population are quite homogeneous; 82.52% are Caucasians,[18] the majority of whom are descendants of Italian immigrants who settled in the state of Rio Grande do Sul in the second half of the 19th century. Our results are pertinent to a specific reality of the southern region of Brazil, and therefore they might differ from those of other regions. The results did not show significant differences in the likelihood of developing GDM among ethnic subgroups (Caucasians, those of African descent, and those of other ethnicities); however, our findings were discordant with those presented by Hedderson et al,[13] who identified a variation in the risk of developing GDM among different racial groups within and outside the United States, in a retrospectively examined multiethnic population of 216,089 pregnant women.

Women with the most advanced maternal age (≥35 years) had twice as much chance of developing GDM than younger women. Concordant results were found in a study conducted in the city Pelotas, state of Rio Grande do Sul, in 2009, in which a population of 4,243 women older than 35 years showed an OR of 6.09 for GDM in late pregnancy, compared with younger pregnant women (age: < 20 years).[6] In this context, we highlight a study by Lao et al[19] in a population of 15,827 primiparous women, who showed a progressive increase in the risk of developing GDM with increasing maternal age, starting at 25 years. In recent years, there has been a significant increase in the number of women who become pregnant at the age of 35 years or older. This increase was of 28% between 2010 and 2016 in Brazil; in the region of

the present study, the increase was of 27%.[20] The surveillance system for risk factors and protection for chronic diseases based on telephone survey (VIGITEL, in Portuguese), estimated in 2017 that among women aged > 18 years, there was an increase in BMI as age advanced.[21] In addition, it is worth noting the lack of knowledge among the female population regarding the risks of gestation at a later age.[22] This combination of important risk factors for GDM deserves special attention from public health managers to implement prevention programs.

A high percentage (48%) of overweight (BMI $\geq 25\ kg/m^2$) women was identified in the present study, which is in agreement with the 2017 VIGITEL report,[21] which stated that 51.2% of women aged ≥ 18 years were overweight.[21] This finding corroborates other results that describe a linear increase in the cases of GDM as the maternal BMI increases.[6,23,24]

The number of pregnancies has been evaluated as a non-traditional risk factor for the development of GDM. Parity ≥3 resulted in a greater chance of developing GDM compared with primiparous women ($p < 0.001$), but this association lost significance in the adjusted analysis. Jesmin et al,[25] in a study with 3,447 pregnant women in Bangladesh, reported a higher risk of GDM with increased gestation numbers.[25] However, Seghieri et al[26] did not identify a direct association of this variable with the progression of pancreatic cell dysfunction and the onset of GDM, and suggested obesity and maternal age as possible mediating factors.

A family history of DM2, level of schooling, smoking, previous hypertension and low maternal height showed no association with the outcome in the present study. However, careful interpretation of these results is necessary due to the methodological limitations inherent to the retrospective design. Data collection from medical records can be challenging because numerous clinical data are not filed despite being fundamental to qualified medical care and for scientific research. This serious problem has existed for decades, but could be improved by the introduction of standardized, digitalized medical records containing a minimum number of mandatory information fields. In addition, there is a need for permanent monitoring, which could be performed using sample surveys on six-month basis. The Brazilian Ministry of Health and the state and municipal health departments have engaged in dialogue regarding the adoption of measures to improve their data records.

Despite the limitations of the present study, the extensive bibliography sometimes corroborated our findings; however, at other times, the literature refuted our results, which were sometimes controversial.[5,6,23,27–30] The different results indicate that more studies are needed to establish the real association of various factors with GDM.

Conclusion

The present study analyzed maternal age as predictive factor for GDM in the population of pregnant women who are users of the Brazilian Unified Health System in the city of Caxias do Sul. Despite the limitations described, the collected data came from a sample that represents about ~ 50% of the population of pregnant women who attended health care services in the city in 2016. This epidemiological study provided a qualitative and quantitative evaluation of the information contained in medical records, and highlighted the insufficient logistics involved in filing such records, which could be used by the city hall managers and staff. The results of the present study describe a complication of pregnancy, and may be a starting point for more comprehensive prospective studies in the near future. To conclude, population-based scientific research in accordance with regional needs should be promoted because the university-community partnership tends to strengthen the production of knowledge and integrate the theoretical content of academic disciplines with practical reality, which can result in substantial scientific development.

Contributors

All authors contributed with the project and the interpretation of data, 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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References

1 World Health Organization. Global Report on Diabetes. Geneva: WHO; 2016
2 Ferrara A. Increasing prevalence of gestational diabetes mellitus: a public health perspective. Diabetes Care. 2007;30(Suppl 2): S141–S146. doi: 10.2337/dc07-s206
3 International Diabetes Federation. IDF Diabetes Atlas. 8. ed. Brussels: IDF; 2017
4 Zhu Y, Zhang C. Prevalence of gestational diabetes and risk of progression to type 2 diabetes: a global perspective. Curr Diab Rep. 2016;16(1):7. doi: 10.1007/s11892-015-0699-x
5 Schmidt MI, Duncan BB, Reichelt AJ, Branchtein L, Matos MC, Costa e Forti A, et al; Brazilian Gestational Diabetes Study Group. Gestational diabetes mellitus diagnosed with a 2-h 75-g oral glucose tolerance test and adverse pregnancy outcomes. Diabetes Care. 2001;24(7):1151–1155. doi: 10.2337/diacare.24.7.1151
6 Dode MAS, Santos IS. [Risk factors for gestational diabetes mellitus in the birth cohort in Pelotas, Rio Grande do Sul State, Brazil, 2004]. Cad Saude Publica. 2009;25(5):1141–1152. doi: 10.1590/S0102-311×2009000500021. Portuguese.
7 Massucatti LA, Pereira RA, Maioli TU. Prevalência de diabetes gestacional em unidades de saúde básica. Rev Enferm Atenção Saúde. 2016;1:70–79.
8 Buchanan TA. Glucose metabolism during pregnancy: normal physiology and implications for diabetes mellitus. Isr J Med Sci. 1991;27(8-9):432–441.

9 Wang Z, Kanguru L, Hussein J, Fitzmaurice A, Ritchie K. Incidence of adverse outcomes associated with gestational diabetes mellitus in low- and middle-income countries. Int J Gynaecol Obstet. 2013; 121(1):14–19. doi: 10.1016/j.ijgo.2012.10.032

10 Damm P, Houshmand-Oeregaard A, Kelstrup L, Lauenborg J, Mathiesen ER, Clausen TD. Gestational diabetes mellitus and long-term consequences for mother and offspring: a view from Denmark. Diabetologia. 2016;59(7):1396–1399. doi: 10.1007/s00125-016-3985-5

11 Organização Pan-americana da Saúde. Ministério da Saúde. Federação Brasileira das Associações de Ginecologia e Obstetrícia. Sociedade Brasileira de Diabetes. Rastreamento e Diagnóstico de Diabetes Mellitus Gestacional no Brasil. Brasília: Opas; 2017. Doi: 10.1080/09638280600756372

12 Vittinghoff E, Glidden DV, Shiboski SC, McCulloch CE. Regression methods in biostatistics linear, logistic, survival, and repeated measures models. New York: Springer; 2012

13 Hedderson MM, Darbinian JA, Ferrara A. Disparities in the risk of gestational diabetes by race-ethnicity and country of birth. Paediatr Perinat Epidemiol. 2010;24(5):441–448. doi: 10.1111/j.1365-3016.2010.01140.x

14 Buckley BS, Harreiter J, Damm P, Corcoy R, Chico A, Simmons D et al; DALI Core Investigator Group. Gestational diabetes mellitus in Europe: prevalence, current screening practice and barriers to screening. A review. Diabet Med. 2012;29(7):844–854. doi: 10.1111/j.1464-5491.2011.03541.x

15 Seshiah V, Balaji V, Balaji MS, Paneerselvam A, Arthi T, Thamizharasi M, Datta M. Prevalence of gestational diabetes mellitus in South India (Tamil Nadu) – a community based study. J Assoc Physicians India. 2008;56:329–333.

16 Kayal A, Mohan V, Malanda B, Anjana RM, Bhavadharini B, Mahalakshmi MM, et al. Women in India with Gestational Diabetes Mellitus Strategy (WINGS): Methodology and development of model of care for gestational diabetes mellitus (WINGS 4). Indian J Endocrinol Metab. 2016;20(5):707–715. doi: 10.4103/2230-8210.189230

17 Duarte EC, Barreto SM. Transição demográfica e epidemiológica: a Epidemiologia e Serviços de Saúde revisita e atualiza o tema. Epidemiol Serv Saude. 2012;21(4):529–532. doi: 10.5123/s1679-49742012000400001

18 Instituto Brasileiro de Geografia e Estatística. Censo Demográfico 2010: Características Gerais da População, Religião e Pessoas com Deficiência. Rio de Janeiro: IBGE; 2011

19 Lao TT, Ho LF, Chan BCP, Leung WC. Maternal age and prevalence of gestational diabetes mellitus. Diabetes Care. 2006;29(4): 948–949. doi: 10.2337/diacare.29.04.06.dc05-2568

20 Ministério da Saúde. Departamento de Informática do SUS. Datasus [Internet]. Sisprenatal: sistema de acompanhamento da gestante. [cited 2019 Sep 16]. Available from: http://datasus.saude.gov.br/sistemas-e-aplicativos/epidemiologicos/sisprenatal. Accessed September 16, 2019.

21 Ministério da Saúde. Secretaria de Vigilância em Saúde. Departamento de Vigilância de Doenças e Agravos Não Transmissíveis e Promoção da Saúde. Vigitel Brasil 2015: Vigilância de Fatores de Risco e Proteção para Doenças Crônicas por Inquérito Telefônico. Brasília: Ministério da Saúde; 2015

22 Cooke A, Mills TA, Lavender T. Informed and uninformed decision making'–women's reasoning, experiences and perceptions with regard to advanced maternal age and delayed childbearing: a meta-synthesis. Int J Nurs Stud. 2010;47(10):1317–1329. doi: 10.1016/j.ijnurstu.2010.06.001

23 Chu SY, Callaghan WM, Kim SY, Schmid CH, Lau J, England LJ, Dietz PM. Maternal obesity and risk of gestational diabetes mellitus. Diabetes Care. 2007;30(8):2070–2076. doi: 10.2337/dc06-2559a

24 Zhu WW, Yang HX, Wang C, Su RN, Feng H, Kapur A. High prevalence of gestational diabetes mellitus in Beijing: effect of maternal birth weight and other risk factors. Chin Med J (Engl). 2017;130(9):1019–1025. doi: 10.4103/0366-6999.204930

25 Jesmin S, Akter S, Akashi H, Al-Mamun A, Rahman MA, Islam MM et al. Screening for gestational diabetes mellitus and its prevalence in Bangladesh. Diabetes Res Clin Pract. 2014;103(1):57–62. doi: 10.1016/j.diabres.2013.11.024

26 Seghieri G, De Bellis A, Anichini R, Alviggi L, Franconi F, Breschi MC. Does parity increase insulin resistance during pregnancy? Diabet Med. 2005;22(11):1574–1580. doi: 10.1111/j.1464-5491.2005.01693

27 Egan AM, Vellinga A, Harreiter J, Simmons D, Desoye G, Corcoy R, et al. DALI Core Investigator group. Epidemiology of gestational diabetes mellitus according to IADPSG/WHO 2013 criteria among obese pregnant women in Europe. Diabetologia. 2017;60(10): 1913–1921. doi: 10.1007/s00125-017-4353-9

28 Moore Simas TA, Szegda KL, Liao X, Pekow P, Markenson G, Chasan-Taber L. Cigarette smoking and gestational diabetes mellitus in Hispanic woman. Diabetes Res Clin Pract. 2014;105(1): 126–134. doi: 10.1016/j.diabres.2014.04.026

29 Yang X, Hsu-Hage B, Zhang H, Yu L, Dong L, Li J et al. Gestational diabetes mellitus in women of single gravidity in Tianjin City, China. Diabetes Care. 2002;25(5):847–851. doi: 10.2337/diacare. 25.5.847

30 Zhang C, Ning Y. Effect of dietary and lifestyle factors on the risk of gestational diabetes: review of epidemiologic evidence. Am J Clin Nutr. 2011;94(6, Suppl):1975S–1979S. doi: 10.3945/ajcn.110.001032

Self-care and Health Care in Postpartum Women with Obesity: A Qualitative Study

Autocuidado e atenção à saúde em puérperas com obesidade: Um estudo qualitativo

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Abstract

Objective To explore the experiences of women with obesity regarding self-care and the care provided by their families and health team after childbirth.

Methods A clinical qualitative study performed at the Postnatal Outpatient Clinic of Hospital da Mulher, Universidade Estadual de Campinas, Brazil. The sample was selected using the saturation criteria, with 16 women with obesity up to 6 months after childbirth.

Results The analysis comprised three categories: 1) postnatal self-care; 2) family support for woman after childbirth; and 3) postnatal health care service for women with obesity.

Conclusion Women with obesity need support from the health team and from their families after childbirth, when they are overwhelmed by the exhausting care for the newborn. The present study reveals how important it is for health care professionals to broaden their perception and care provided after childbirth for women with obesity so they may experience an improvement in their quality of health and of life.

Keywords
- self-care
- health care
- postpartum
- childbirth
- obesity
- psychologycal issues

Resumo

Objetivo Explorar as vivências de mulheres com obesidade sobre o autocuidado e os cuidados recebidos da família e da equipe de saúde após o parto.

Métodos Estudo clínico-qualitativo realizado no Ambulatório de Revisão Puerperal do Hospital da Mulher da Universidade Estadual de Campinas, Brasil. A amostra foi selecionada de acordo com os critérios de saturação, com 16 mulheres com obesidade até 6 meses após o parto.

Resultados A análise de conteúdo compreendeu três categorias: 1) autocuidado pós-natal; 2) apoio familiar para a mulher após o parto; e 3) atenção do serviço de saúde à mulher com obesidade no pós-parto.

Conclusão As mulheres com obesidade necessitam de acolhimento e do apoio da equipe de saúde e de suas famílias após o parto, quando são absorvidas pelo cuidado exaustivo do recém-nascido. Este estudo revela o quão importante é para os profissionais de saúde ampliar sua percepção e cuidado após o parto às mulheres com obesidade para que estas possam melhorar sua qualidade de saúde e de vida.

Palavras-chave
- autocuidado
- cuidados de saúde
- pós-parto
- parto
- obesidade
- aspectos psicológicos

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Introduction

The period after birth is a critical moment of transition and of physiological and psychological adaptations, which leaves women more susceptible to physical and emotional intercurrences.[1,2] The new maternity brings many challenges: besides her physiological recovery, the woman has to deal with the routine of caring for her baby and for herself. During this period, significant changes in her life demand new challenges, such as the acceptance of a new body image, sleep deprivation, adjustmentments in family relationships, as well as changes in her professional life, and in her health care and dietary care.[3]

A continuum of maternal and newborn care is essential to guarantee maternal and neonatal physical and mental health, irrespective of any complications at birth. This care requires a support network on which these women have relied throughout their lives: family, community and healthcare services.[4]

The postpartum period is considered by some authors[3] as the 4th gestational trimester. It is a perspective that considers the woman and her baby as still being a mutually-dependent unit, linked both physiologically and behaviorwise. The intention behind insisting on the postpartum period as the "4th trimester" is to encourage actions to support women and their families during this critical period.[3,5]

The innumerable physiological, psychological and social changes that take place in the lives of women after childbirth constitute a learning process of changes in lifestyle. The development of public health interventions for this specific population, at this point in their lives, when they are so busy, remains a challenge.[6]

Modern families are not receiving sufficient or quality support from family members or friends. Home visits by relatives and friends can help improve the mother's emotional wellbeing and self-esteem, as well as competency, family functioning, father-son/daughter relationship, and problem-solving.[7]

The mother's well-being after childbirth is greatly influenced by her psychosocial state, as well as by family support and by her environment.[8] Having a baby brings emotional experiences to a woman's life. New psychological elaborations are needed, and some women feel more vulnerable to psychological problems during this period. Feelings of being overburdened and insecure about their ability to be a mother are linked to distress in the postnatal period.[8]

Self-care is an important component of motherhood.[9] Time, limited resources and difficulty to accept help have been identified as obstacles to women's ability to care for themselves.[10]

The present study endeavors to explore the experiences of women with obesity vis-à-vis their self-care and the care they receive during the postnatal period, both from family members and the healthcare team. We define self-care here in its broadest meaning, as any care an individual takes towards him/herself. Thus, we try to identify aspects that can enable healthcare professionals to offer comprehensive care suited to women with obesity after childbirth.

Methods

The clinical qualitative method was used,[11,12] which enables us to understand the emotional experiences of people involved in a healthcare setting. A fundamental part of this methodological structure is the interviewee's discourse. In this case, the scientific investigation is made based on the significance the interviewee attributes to the experiences, based on the premise that this is an efficient way of learning and inferring results that reveal the nexus of meanings.[12] The clinical qualitative methodhas three particularities that define it: a) existentialist attitude: appreciation of the angst and anxiety arising from falling ill; b) clinical attitude: appreciation of the reception of the emotional suffering of a person and the desire to provide help; c) psychoanalytic attitude: appreciation of the elements underlying the interview, also admitting that unconscious elements are present in the interviewer-interviewee relationship.

Setting

The present research was performed at the Postnatal Outpatient Clinic of Hospital da Mulher, Universidade Estadual de Campinas, a tertiary public teaching hospital, in Southeastern Brazil, which is a national benchmark in public care for women's and neonatal health. To this end, it relies on a multi-professional and interdisciplinary team, and it also promotes teaching, research and further education. The Postnatal Outpatient Clinic monitors postpartum women. The initial stage in the clinical qualitative research is acculturation,[12] through which the researcher establishes a direct relationship with the population to be studied. The main researcher went to the Postnatal Outpatient Clinic for three months (between January and April 2016). The information gathered in this stage (the perceptions of the researcher and the reports of dialogues with the professionals or women after childbirth) were recorded in a field diary and used to formulate the questions initially proposed for the interviews.

Participants

The selection of the sample was intentional: women over 18 years of age; up to 6 months after delivery; and with body mass index (BMI) $\geq$ 30 Kg/m^2 before pregnancy were included. Women who were not breastfeeding were excluded. The sample was selected using the information saturation criteria,[13] after discussion and validation with two research groups. The participants were women from the Postnatal Outpatient Clinic, and they were selected according to data recorded on the same day as their medical consultation. They were approached face to face by the interviewer (the main researcher), and were invited to take part in the study by means of an interview.

Data Collection

The data was collected at the Outpatient Clinic, and the interviews took place between April and August 2017. All of the participants signed an informed consent form before the interviews, which were held in a private room, thus guaranteeing confidentiality. A single, semi-directed interview was

performed with each participant, with open-ended questions allowing for depth,[14] developed based on a script that was not rigid, thus enabling the interviewer to make the necessary adaptations based on the information provided by the interviewee. We have selected the questions pertaining to the theme of the present article that were made during the interview.

Trigger question: Tell me a little about how you have been feeling since your baby was born.

- Are you taking care of yourself?
- In what ways do you feel cared for?
- Do you have anyone to take care of you at home?
- How do you think the healthcare team can help you at this time?

Data Analysis

Data analysis followed the seven steps described in the analysis of clinical qualitative content: 1) editing of the material: transcription of recorded interviews and convergence with material recorded in the field diary; 2) free-floating reading of the collected material: reading of material while suspending directed attention; 3) comments and impressions: taking notes and highlighting on the right hand margin of the transcript; 4) subcategorization and categorization: group and name significant speech within the same theme; however, the different categories contain heterogeneous ideas; 5) discussion with academic peers about the analyzed material; 6) category definition: refinement of the categories; and 7) validation of the analyzed material together with peers.

For the content analysis of the field research, the transcriptions of the interviews were performed by one of the co-authors. The editing of the written material, based on the transcriptions of the interviews and field analysis, was performed by the main researcher and author of the present study. At a later date, all of the material was read separately by the two independent researchers. Both completed the first stages of content analysis and comments individually. Following this, together they definedthe categories, which were also discussed with the research advisors and then presented and validated by two research groups.

The research was approved by the Ethics Committee of the Universidade Estadual de Campinas and the Brazilian National Board of Health in February 2017 (under the number CAAE62565116.3.0000.5404). The COREQ Checklist was also used for the present study.

Results

The 16 women approached agreed to take part in the study. There were no refusals (►**Table 1**).

The clinical qualitative content analysis revealed three categories: 1) postnatal self-care; 2) support from the family; and 3) postnatal healthcare services for women with obesity.

Postnatal Self-care

The interviewees revealed a desire to take care of themselves, but the lonely routine with the baby made it difficult for them to think of doing anything for themselves. Caring for the newborn was a priority, which is perfectly natural at this stage in which the helplessness of the baby demands a huge, intense effort:

> I don't take much care of myself. I have to admit I am not very vain, and now, less than ever, but I would like to [...] the weight problem is something I would like to [deal with] because weight brings a lot of problems: it brought hypertension, it brought gestational diabetes, it brings, could bring me other problems that I don't want to deal with, I want to be healthy so she will be, you understand? *(Participant 11)*

> Ah, I'm more worried about the baby [...]. Not so much about myself, more about him. *(Participant 2)*

The breastfeeding routine and other care measures for the newborn can aggravate this situation and constitute an excuse for not thinking about or caring for themselves. We perceive that the issue of self-care, for some of these women, was not part of their routine long before pregnancy. The interviewees associated the word self-care with vanity rather than a health issue.

> Ah, it's complicated, you see, I just let myself go: it's hair, nails, and now I don't even go out. I just stay at home with him [the baby]. And there's no way I can go to the beauty parlor; you have to have time to have your nails done, have your hair done, right? And up until now, I haven't managed that, his feeding time is on demand, right? *(Participant 4)*

Table 1 Characteristics of women with pre-pregnancy obesity after childbirth

Participants	Age (years)	Postpartum month	Weight (Kg)	Body mass index
P1	22	5	93	36.3
P2	34	2	118	45.5
P3	33	5	99	33.5
P4	23	2	94	31.8
P5	26	1	97	32.8
P6	23	4	105	37.6
P7	34	2	91	34.7
P8	29	3	94	32.9
P9	27	1	79	31
P10	29	4	96	31
P11	43	2	99	34.3
P12	39	3	84	35.4
P13	23	5	142	52.2
P14	29	2	79	31.2
P15	36	3	85	32.8
P16	20	2	82	30.5

From the health point of view, we perceived that despite identifying the postnatal period as a maturing process, they reveal a sense of negation of themselves in favor of the baby's emotional state, a feeling of their non-existence as 'beings' at this moment. They experience this process as something natural, because they feel that in some way they were already abnegating themselves in favor of pleasing others.

[…] We are trapped in a corner over there and you say, no, you are going to live for others and not for yourself. We have to understand ourselves and then the others, because if we are not well, we can't help others. Do you understand? Especially when we are mothers, there are times when we have to help, but if we are well, our child is well. During this 4-month phase [of the baby], they feel everything you have, normally I'm well, but the day when I was ill, the baby became ill, the day I'm feeling poorly, the baby feels a bit poorly. *(Participant 6)*

The interviewees reveal the importance of this stage after childbirth and how powerful they are to bring about changes, as long as they have the support of family members, friends and/or healthcare professionals. Encouraging the positive changes arising from pregnancy, so that these accomplishments continue after childbirth, is a way of guaranteeing the health of the woman. The subjective experiences, the sensation of maturing, and the intense care for the baby are important factors in insuring they are conscious of the need for self-care.

> So I got it into my head, I myself am going to change, at the right moment, so I had planned the change so when he came, he helps me too and encourages me, because it is good to have an incentive in life, isn't it? *(Participant 9)*

> Ah, how people talk, my God! How you are prettier, you lost weight, see! And I feel better, something like tiredness, that kind of thing, it's much better, [your] disposition *(Participant 14)*

Support from the Family

The postpartum period marks a change in the women's attachment to healthcare services and family relationships. They reported a feeling of loss of the attention and care that they had received during pregnancy. This is experienced as a sudden and violent disruption, which corroborates the feeling of loneliness and helplessness.

> Things that happen after pregnancy that make us feel so out of it that we think that nobody is helping us, but they are, you see? We don't feel cared for but they are taking care [of us]. *(Participant 6)*

Family members, in addition to helping with the routine at home and with the baby, can also provide support and care, while embracing the insecurities and helplessness felt by the woman during the postpartum period.

> My father wanted to come and spend time with me, and I said "please come, dad"; then, my friend said "but your dad will not change diapers," but I know that my father, he is caring […]. And that he will take care of me. *(Participant 4)*

Participants with strong family support reported the ability to organize meals in a more routine fashion, while those who did not have this support sought more practical and often unhealthy solutions.

> I don't have much time to go to the supermarket, as fruit and vegetables have to be bought at least weekly […]. So, we are eating lots of tinned, fried, or preserved food; it's bad, but unfortunately… *(Participant 4)*

It is a great challenge for the woman after childbirth and those surrounding her to balance support and care for this woman, without depriving her of her autonomy. The family can be more aware and available to meet the physical and emotional needs of the woman: a caring gesture, the preparation of food, listening to her, holding her, always taking care not to see her as being fragile or less capable of making decisions about her life and that of her child(ren). As the woman finds herself in a period of greater emotional vulnerability, the care provided by the family can be seen as invasive or a threat to her autonomy.

> Yes. I know that they are being excessively zealous towards me, I am grateful because few families, few pregnant women or new mothers get the opportunity of having their family close by, to take care of a newborn child; I'm lucky but it bothers me that I can't be in charge of my own life. *(Participant 11)*

The relationships with partners were approached many times during the interviews. The women spoke of the changes in their relationships and of how they identified that their partners' behavior in relation to childcare was very different from their own, which was reinforced culturally, and often by themselves, with feelings of guilt about delegating the care of their children to their partners.

> It's difficult to find a father who helps a lot, who accepts the routine, who bathes and dresses the baby, wow! It is difficult to find, most do not want to know, but I think it is cultural. *(Participant 6)*

> There is no more conversation […] we love each other and we get on well, but that's the way it is […] [my partner] cared for me and suddenly stopped, we miss it, it's the same as when something is taken away from you. *(Participant 3)*

Postnatal Healthcare Service for Women with Obesity

The interviewees reported strong affective bonds with their antenatal team, with difficulties in disengaging themselves from their care. This caused an impact on the eating habits of these women, who reported that it would have been easier to lose weight and maintain a healthy eating pattern under the constant supervision of the team.

> Here [at this healthcare service] I was losing weight without realizing it; it was my dream. I tried to keep this going, as my intention was to lose weight […] when you already have a tendency to enjoy eating, over the course of days I went back to my previous pattern. Eating a lot. *(Participant 11)*

During the postpartum period, follow-up at the healthcare service becomes less frequent and focused on contraception and breastfeeding. This was considered a negative experience, as they had to deal with the loss of this bond. They reported the need for a support network, which should include both family members and the healthcare service. The development of not only consultations, but also approaches that enable a discussion about subjectivity, self-care, food and weight are required.

> I really needed dietary follow-up, an incentive with someone saying you are doing everything right […] because when you look at yourself, you see that you really have that willpower, you are putting faith in yourself that you will achieve it and improve your self-esteem. *(Participant 8)*

> […] The healthcare team needs to provide this help to the mother, as it really is too much for the mother. *(Participant 11)*

We perceived in the analysis of the interviews that women were keen to talk about their lives, showing interest in the possibility of being heard and welcomed. During the interviews, the moment in which the participants were most frequently emotional was when they were asked if they felt cared for. They revealed experiences of great solitude and a very strong desire that their relatives and health team perceive, understand and care for them without taking away their autonomy (►**Fig. 1**).

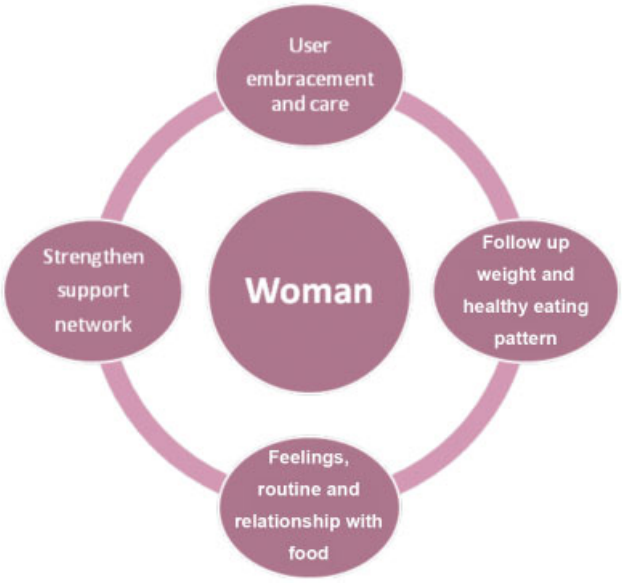

Fig. 1 Postnatal care for women with obesity.

Discussion

Our results show that women with postnatal obesity tend to neglect care for themselves as caring for the newborn takes priority. Our interviewees report an experience associated with mourning for themselves, for their life up to that point, and how they lose themselves in a kind of 'temporary depersonalization.' These results are similar to those of other studies with postnatal women, irrespective of their BMI.[10,15] In their experience of motherhood, even women considered psychologically healthy experience a psychological withdrawal, giving up part of their interests as well as themselves to guarantee the baby's care. Mother and child become an autonomous unit that makes it possible for the mother to identify her baby's needs, something that is impossible to be identified by other people or in other circumstances.[16]

However, in this discussion we would like to highlight that women with obesity deserve greater attention in the postnatal period because, as shown by the interviewees, before becoming pregnant these women had a behavior pattern of prioritizing the needs of others over their own. This could reveal a difficulty to perceive themselves in a positive manner. A woman with obesity already feels that she is seen in a bad light, both by herself and by others. Our results are compatible with those in the literature, which show that obesity is correlated with low self-esteem and low self-control, social stigma, and shame.[17,18] As a consequence, obesity and excess weight do not affect only the health, but also the individual's sociability. Obesity has a stigma, a form of social discrimination that can cause many negative psychological effects in an individual.[11,19]

Another aspect to be emphasized in our study of the experiences of women vis-à-vis their self-care is that they show that this is an opportune moment for interventions, since they feel that the experiences of motherhood bring an important maturing, and that, since pregnancy, they have become more inclined to acquire new habits. In the literature, we find that interventions by women's healthcare teams should be present during the pregnancy and continue throughout the puerperium to ensure that new habits be maintained.[15,20–22] Price et al[22] (2012) state that new mothers were more disposed and interested in talking about behavior and goals for their children, but that months after the birth, especially when returning to work, women begin to focus again on themselves, and there is a window of opportunity to talk about their goals and behavior.

Our study also points to a psycho-educational need on the part of the interviewees, since they limit self-care to esthetic issues, and do not perceive it as being linked to health care. Promoting health is linked to strengthening the subjects' autonomy; self-care, appreciation of subjective experiences, as well as of the sociocultural contexts in which the individuals find themselves.[23,24] The advances in health care can guarantee an improvement in women's quality of life, and are one of the greatest challenges of this century. We perceive that this ignorance may be associated with psychological and cultural issues, and that health care professionals play an important role in this process of awareness as to the importance of self-care in health.[25]

Bearing in mind the experiences related by women with obesity after childbirth in relation to themselves, their families and the health care team, a discussion about the network of care for these women would be relevant to develop strategies to provide support and care for them. The postpartum period is a critical moment, and it demands a continuum of maternal care in its different human dimensions.[26]

The intense care regarding the physical and emotional needs of the baby and his/her primitive psychological states also awakens/evokes states of primitive anxiety and a sense of internal solitude in the mother, as well as the mourning process a woman must face vis-à-vis her pregnancy and life prior to maternity. This concept of solitude was described by Klein[27] (1984) as a feeling of loneliness irrespective of the external context, irrespective of being among beloved people and surrounded by love and attention.

Family relationships should be encouraged and strengthened at this time. It is important that families are aware of what constitutes this moment in a woman's life and her needs. The interviewees reported that these relationships can be conflictual and disrespectful, and cause further emotional overload to the mother. The literature describes how much a good family relationship can help these women have a better quality of life in the postnatal period. Price et al[22] (2012) state that, during this period, women find many barriers to eating healthy, and when there is the constant presence of a family member, they are able to eat better and lead a healthier life.

It is very important that health care teams be aware of the family relationships of these women to identify failures in the family support network as well as when the care offered takes away their autonomy.

In order for women with obesity to become aware of the need for self-care, it is important for the team to begin with assertive comments and offer alternatives as to how this woman can care for herself, by showing that this is both an external and internal task. Our results correspond to those presented by Chugh et al[28] (2013) in that the disposition to lose weight depends as much on self-motivation as on the incentive of the health professional. The study[28] showed that the perception of the health professionals regarding the lowest level of motivation for these women to lose weight can make this process even more difficult.

The content of the care offered after childbirth must be developed in such a way as to include more priorities in women's health studies.[29,30] New mothers have multiple unmet needs, and health institutions should be aware of these needs and provide them with support by means of clear and precise information, so they do not feel alone, sheltering them in their search for information and rights.[3] Moreover, women have different ideas, experiences and expectations about losing weight in the postnatal period. Health care professionals can take care of the needs of each woman to promote their autonomy and better results in their health and lifestyle.[31]

We have proposed a plan directed at healthcare professionals who care for women with postnatal obesity (►**Fig. 1**). Our study also sought to provide tools for healthcare professionals during the follow-up of these women. The follow-up should make the women feel a sense of belonging and care; in it, weight and nutrition should be monitored, and the support network, whether from family, friends, or other women going through the postpartum period, must be strengthened, and environments in which these women are encouraged to talk about their feelings, routine and relationship with food should be created. The idea is that the health service provides centrality for the women and their experiences. The women showed throughout the interviews that more important than talking was the feeling that they were being heard.

►**Fig. 1** proposes a model of care for the psychological aspects of women with obesity. We emphasize the care already included in the gynecological and obstetric teams' routines of breastfeeding, contraception and clinical care.

Conclusion

The postnatal period is a landmark in the physiology, the social life and psychological state of women, which is capable of transforming their subjectivity and identity. This period requires interventions by health professionals. The women with obesity already feel discriminated against, both by themselves and by others, which also brings risks of psychopathological disorders and risks regarding their physical health and weight, which are more evident after childbirth. The women with obesity need attention as well as the presence of the health team and of family members in order to take better care of their health, at a moment when women are already overwhelmed by the exhausting care for the newborn. Effective strategies are needed for women with obesity in the postnatal period, thus guaranteeing their quality of life.

Contributions
DBFS, ERT and FGS conceived and designed the study. DBFS collect the data. All of the authors were involved in data analysis and interpretation. DBFS, LR, DSMP and FGS were involved in writing. All authors approved the final version of the manuscript.

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

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References

1 Ghiasvand F, Riazi H, Hajian S, Kazemi E, Firoozi A. The effect of a self-care program based on the teach back method on the postpartum quality of life. Electron Physician. 2017;9(4):4180–4189. doi: 10.19082/4180

2 Woolhouse H, Gartland D, Perlen S, Donath S, Brown SJ. Physical health after childbirth and maternal depression in the first 12 months post partum: results of an Australian nulliparous pregnancy cohort study. Midwifery. 2014;30(3):378–384. doi: 10.1016/j.midw.2013.03.006

3 Tully KP, Stuebe AM, Verbiest SB. The fourth trimester: a critical transition period with unmet maternal health needs. Am J Obstet Gynecol. 2017;217(1):37–41. doi: 10.1016/j.ajog.2017.03.032

4 Jasienska G, Bribiescas RG, Furberg AS, Helle S, Núñez-de la Mora A. Human reproduction and health: an evolutionary perspective. Lancet. 2017;390(10093):510–520. doi: 10.1016/S0140-6736(17)30573-1

5 Simonetta J, Klerman JA, Daley K, Pozniak A. Family and medical leave in 2012: technical report. 2014 [cited 2018 Sep 12]. Available from: https://www.dol.gov/asp/evaluation/fmla/fmla-2012-technical-report.pdf

6 Ostbye T, Peterson BL, Krause KM, Swamy GK, Lovelady CA. Predictors of postpartum weight change among overweight and obese women: results from the Active Mothers Postpartum study. J Womens Health (Larchmt). 2012;21(2):215–222. doi: 10.1089/jwh.2011.2947

7 Byrne F, Grace R, Tredoux J, Kemp L. Structured social relationships: a review of volunteer home visiting programs for parents of young children. Aust Health Rev. 2016;40(3):262–269. doi: 10.1071/AH15057

8 He H, Zhu L, Chan SWC, Chong YS, Jiao N, Chan YH, et al. The effectiveness and cost-effectiveness of web-based and home-based postnatal psychoeducational interventions for first-time mothers: randomized controlled trial protocol. JMIR Res Protoc. 2018;7(1):e35. doi: 10.2196/resprot.9042

9 Coates R, Ayers S, de Visser R. Women's experiences of postnatal distress: a qualitative study. BMC Pregnancy Childbirth. 2014;14:359. doi: 10.1186/1471-2393-14-359

10 Barkin JL, Wisner KL. The role of maternal self-care in new motherhood. Midwifery. 2013;29(9):1050–1055. doi: 10.1016/j.midw.2012.10.001

11 Faria-Schützer DB, Surita FGC, Alves VLP, Vieira CM, Turato ER. Emotional experiences of obese women with adequate gestational weight variation: a qualitative study. PLoS One. 2015;10(11):e0141879. doi: 10.1371/journal.pone.0141879

12 Turato ER. Tratado da metodologia da pesquisa clínico-qualitativa: construção teórico-epistemológica, discussão comparada e aplicação às áreas da saúde e humanas. 5. ed. Petrópolis: Vozes; 2011

13 Glaser BG, Strauss AL. Discovery of grounded theory. New York: Routledge; 1999

14 Morse JM, Field PA. Qualitative research methods for health professionals. 2. ed. Thousand Oaks: Sage; 1995

15 Christenson A, Johansson E, Reynisdottir S, Torgerson J, Hemmingsson E. Women's perceived reasons for their excessive postpartum weight retention: a qualitative interview study. PLoS One. 2016;11(12):e0167731. doi: 10.1371/journal.pone.0167731

16 Winnicott DW. Through pediatrics to psychoanalysis. London: Hogarth Press; 1958

17 Keith L, Gillanders D, Simpson S. An exploration of the main sources of shame in an eating-disordered population. Clin Psychol Psychother. 2009;16(4):317–327. doi: 10.1002/cpp.629

18 Brañas-Garza P, Espín AM, Lenkei B. BMI is not related to altruism, fairness, trust or reciprocity: Experimental evidence from the field and the lab. Physiol Behav. 2016;156:79–93. doi: 10.1016/j.physbeh.2016.01.008

19 Oliver MD, Datta S, Baldwin DR. A sympathetic nervous system evaluation of obesity stigma. PLoS One. 2017;12(10):e0185703. doi: 10.1371/journal.pone.0185703

20 van der Pligt P, Olander EK, Ball K, Rawford D, Hesketh KD, Teychenne M, et al. Maternal dietary intake and physical activity habits during the postpartum period: associations with clinician advice in a sample of Australian first time mothers. BMC Pregnancy Childbirth. 2016;16:27. doi: 10.1186/s12884-016-0812-4

21 Moran LJ, Flynn AC, Louise J, Deussen AR, Dodd JM. The effect of a lifestyle intervention on pregnancy and postpartum dietary patterns determined by factor analysis. Obesity (Silver Spring). 2017;25(6):1022–1032. doi: 10.1002/oby.21848

22 Price SN, McDonald J, Oken E, Haines J, Gillman MW, Taveras EM. Content analysis of motivational counseling calls targeting obesity-related behaviors among postpartum women. Matern Child Health J. 2012;16(2):439–447. doi: 10.1007/s10995-011-0746-4

23 Delormier T, Frohlich KL, Potvin L. Food and eating as social practice–understanding eating patterns as social phenomena and implications for public health. Sociol Health Illn. 2009;31(2):215–228. doi: 10.1111/j.1467-9566.2008.01128.x

24 Poland B, Krupa G, McCall D. Settings for health promotion: an analytic framework to guide intervention design and implementation. Health Promot Pract. 2009;10(4):505–516. doi: 10.1177/1524839909341025

25 Compean-Ortiz LG, Quintero-Valle LM, Del Ángel-Pérez B, Reséndiz-González E, Salazar-González BC, González-González JG. Educación, actividad física y obesidad en adultos con diabetes tipo 2 desde la perspectiva del autocuidado de Orem. Aquichan. 2013;13:347–362.

26 Lipsky LM, Strawderman MS, Olson CM. Maternal weight change between 1 and 2 years postpartum: the importance of 1 year weight retention. Obesity (Silver Spring). 2012;20(7):1496–1502. doi: 10.1038/oby.2012.41

27 Klein M. On the sense of loneliness. In: Klein M. Envy and Gratitude: and Other Works, 1946–1963. New York: The Free Press; 1984:300–313

28 Chugh M, Friedman AM, Clemow LP, Ferrante JM. Women weigh in: obese African American and White women's perspectives on physicians' roles in weight management. J Am Board Fam Med. 2013;26(4):421–428. doi: 10.3122/jabfm.2013.04.120350

29 Schmied V, Mills A, Kruske S, Kemp L, Fowler C, Homer C. The nature and impact of collaboration and integrated service delivery for pregnant women, children and families. J Clin Nurs. 2010;19(23-24):3516–3526. doi: 10.1111/j.1365-2702.2010.03321.x

30 Declercq ER, Sakala C, Corry MP, Applebaum S, Herrlich A. Listening to mothers III: new mothers speak out [Internet]. New York (NY): Childbirth Connect; 2013 [cited 2018 Oct 10]. Available from: https://www.nationalpartnership.org/our-work/resources/health-care/maternity/listening-to-mothers-iii-new-mothers-speak-out-2013.pdf

31 Montgomery KS, Aniello TD, Phillips JD, et al. Experiences accompanying postpartum weight loss: benefits, successes, and well-being. Health Care Women Int. 2013;34(7):577–591. doi: 10.1080/07399332.2012.736568

Construct and Criterion Validity of the Postmenopause Sexuality Questionnaire – PMSQ

Validação de construto e critério do questionario para avaliação da sexualidade feminina após a menopausa – QSFM

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Abstract

Objective To assess the construct and criterion validity of the Postmenopause Sexuality Questionnaire (PMSQ).

Methods The present methodological questionnaire validation study included postmenopausal women. The construct validity was tested by factor analysis and the criterion validity was performed using the correlation between the PMSQ and the Female Sexual Function Index (FSFI). The ROC curve was used to verify sensitivity, specificity and to determine the cutoff point of the PMSQ.

Results A total of 181 women with a mean age of 56.4 ± 5.7 years old were evaluated. The exploratory factor analysis showed that the PMSQ presented Kaiser test $= 0.88$ and $\chi^2 = 3293.7$ ($p < 0.001$), commonalities ≥ 0.5, and extraction of 9 factors with eigenvalue ≥ 1; explaining 66.3% of the total variance. The PMSQ presented factor loadings between 0.4 and 0.8. A strong correlation between the 2 questionnaires ($r = 0.79$; $p = 0.000$) was shown. The cutoff point of the PMSQ was ≤ 55.5, assuming 87.9% sensitivity and 78.9% specificity ($p < 0.001$).

Conclusion Since the PMSQ showed a strong correlation with the FSFI questionnaire, it presented good psychometric properties to assess the sexuality in postmenopausal women. Based on these results, the PMSQ could be widely tested as a specific instrument to examine the sexual function in postmenopausal women. Future studies, designed to examine the PMSQ instrument in different populations, are needed.

Keywords

- menopause
- sexuality
- physiological sexual dysfunction
- psychometric
- sensitivity
- specificity

Resumo

Objetivos Validar o construto e o critério do Questionário para Avaliação da Sexualidade Feminina após a Menopausa (QSFM).

Métodos Estudo metodológico de validação de questionário incluiu mulheres na pós-menopausa. A validade de construto foi testada por meio da análise fatorial e a validade de critério foi realizada por meio da correlação entre o QSFM e o Índice de Função

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Sexual Feminina (FSFI). A Curva ROC foi utilizada para verificar sensibilidade, especificidade e determinar o ponto de corte do QSFM.

Resultados Foram avaliadas 181 mulheres, com idade média de 56,4 ± 5,7 anos. A análise fatorial exploratória mostrou que o QSFM apresentou teste de Kaiser = 0,88 e $\chi^2 = 3293,7$ ($p < 0,001$), comunalidades ≥ 0,5 com extração de nove fatores com autovalor ≥ 1; explicando 66,3% da variância total. O QSFM apresentou cargas fatoriais entre 0,4 e 0,8. Uma forte correlação entre os dois questionários (r = 0,79; $p = 0,000$) foi demonstrada. O ponto de corte do QSFM foi ≤ 55,5, assumindo sensibilidade de 87,9% e especificidade de 78,9% ($p < 0,001$).

Conclusão Como o QSFM demonstrou uma forte concordância com o questionário FSFI, ele apresentou boas propriedades psicométricas para avaliar a sexualidade em mulheres na pós-menopausa. Com base nesses resultados, o QSFM pode ser amplamente utilizado como um instrumento específico para examinar a função sexual em mulheres na pós-menopausa. Estudos futuros são necessários para examinar o instrumento QSFM em diferentes populações.

Palavras-chave
- menopausa
- sexualidade
- disfunção sexual fisiológica
- psicometria
- sensibilidade
- especificidade

Introduction

The climacteric period is a biological phase of life and not a pathological process.[1] Menopause is defined as the last menstrual period, recognized 12 months after its occurrence.[2] The mean age of menopause ranges from 48 to 52 years globally.[3] In this period, hormonal variations and progressive estrogen deficiency may sometimes result in debilitating short, medium and long term conditions.[4] Decreased pelvic support, genital atrophy, and decreased lubrication may result in dyspareunia and finally in sexual dysfunction.[5] Female sexual desire is not spontaneous and the sexual response includes intimacy and emotional satisfaction as goals.[6] Any disruptions in the response cycle, such as dyspareunia or difficulties with arousal, reduce motivation and the search for intimacy with the partner. Sexual dysfunction can affect women of various ages, colors, social status and ethnicities. It is characterized by psychophysiological changes in the sexual response, including sexual desire, arousal, orgasm, and even pain.[7] Hormonal changes in the menopause, particularly hypoestrogenism and decreased testosterone levels, associated with biological, cultural and social processes may have a direct impact on sexuality, well-being, and quality of life.[8–10]

The prevalence of sexual dysfunction after menopause has shown to increase from 12.1% to 48.0%.[11] Worldwide, the prevalence of sexual dysfunction in postmenopausal women using questionnaires that do not include a specific menopause domain has been reported to vary between 61% and 86%.[12,13] In sexually active postmenopausal Brazilian women, it seems that 70% suffer from sexual dysfunction, especially those > 50 years old.[14,15] Among the studies on sexual dysfunction in older women, the most commonly used instrument is the FSFI and its short form FSFI–6, but other instruments have also been used.[16–19] Even though these instruments measure sexual dysfunction in menopausal, perimenopausal and postmenopausal women, they do not have a menopause specific domain, and they are not quite suitable for measuring sexual dysfunction in postmenopausal women. They do not have questions linking sexual dysfunction to the menopause condition.

The PMSQ tested in the current study was previously and partially validated in Portuguese to measure the different domains of sexual function in Brazilian postmenopausal women.[20] Therefore, the primary objective of the present study was to assess the construct and criterion validities of this questionnaire and to determine its cutoff level to identify postmenopausal women with or without sexual dysfunction.

Methods

This methodological study enrolled postmenopausal women who were selected using accessibility sampling at the General Gynecology and Climacteric Outpatient Clinics of a teaching and research hospital, between November 2017 and June 2018. According to the current recommendations, the sample size criterion for factor analysis was 5 subjects per item.[21–28] A total of 181 postmenopausal women with stable and regular sexual activity, regardless of marital status or sexual orientation, were examined. Natural menopause was defined as 12 consecutive months of absence of menstruation. Women with hysterectomy before menopause were included if age ≥ 48 years old and follicle stimulating hormone (FSH) ≥ 25 mIU/mL.[3] Other hormones such as estradiol, total testosterone, free thyroxin, and thyroid stimulating hormone were also measured. Women with an earlier diagnose of menopause, already using estrogen-progestin hormone therapy, were also included. Women with severe hypertension, decompensated diabetes, severe heart disease, musculoskeletal diseases with movement disabilities, current or past cancer diagnosis, bilateral oophorectomy, vulvodynia or using medications that could interfere with the libido were excluded.

Data were collected during a single interview, after signing the free and informed consent form. Sociodemographic characteristics, body weight, and height were obtained with

the woman standing barefoot. The body mass index (BMI) (weight / height^2) was calculated following the Brazilian guidelines.[22,23] The waist circumference was verified using an inelastic tape, positioned at the smallest circumference between the final costal arch and the iliac crest. The PMSQ and the FSFI questionnaires were both applied in this sequence and face to face. Despite the referred formal educational level, most of the patients had very little schooling and little ability to read and answer the questionnaires without help. So, a single researcher, the main author, carefully read the questions and the participant pointed out the item that corresponded to the answer she had chosen. Approval of the project was obtained from the local Ethics and Research Committee.

The PMSQ initially contained 43 items distributed into nine domains, namely: self-image (5), sexual quality of life (6), sexual intimacy (6), desire (7), arousal (5), orgasm (4), dyspareunia/vaginism (2), satisfaction (5) and influence of menopause (3). The questions were answered on an ordinal Likert scale (0–5). The scores (0–100) were standardized by the formula $(X / 215) \times 100$, where: X is the answer for each item and 215 is the maximum possible gross score ($5 \times 43 = 215$); 0 indicates the worst sexual function and 100 indicates the best sexual function. The items were designed based on sexual domains validated in other questionnaires, and all of them were evaluated by specialists in sexology and submitted to the test-retest method.[20] For the publication in English, the PMSQ instrument was translated from Portuguese into English as follows: a native English speaker and a native Brazilian Portuguese speaker translated the questionnaire independently. Finally, a bilingual author confronted the two versions, keeping the most appropriate terms. As the instrument was applied to women of native Portuguese language, the English version of the instrument was not yet validated in any English speaking population.

The FSFI, a gold standard questionnaire designed to evaluate female sexual function, was previously validated in Brazilian Portuguese.[24] This instrument contains 19 items in six domains: sexual desire (2), sexual arousal (4), vaginal lubrication (4), orgasm (3), sexual satisfaction (3) and pain (3). The items are answered on an ordinal Likert scale (0–5), with increasing scores according to the presence of the function questioned, with total scores varying from 2 to 36. Based on validation studies, a cutoff point of 26.5 was proposed.[25] However, its cutoff point to discriminate menopause women with or without sexual dysfunction was established as 23.[26] Because the FSFI has been used in populations of all ages, including menopausal women, and already presented a cutoff considering the age, it was chosen for validating the PMSQ questionnaire.

Descriptive analyses of data included the variable ages, family income in minimum wages, education, self-declared color, occupation, number of previous pregnancies, menarche age, sexarche age, menopause age, BMI, waist-hip ratio, clinical comorbidities and use of menopause hormone therapy. The data distribution was verified using the Shapiro-Wilk test. The Cronbach α was used to verify the internal consistency of both PMSQ and FSFI questionnaires. The Pearson coefficient correlation was used to verify the possible correlation between PMSQ and FSFI questionnaires. Exploratory Factor Analyses were used to examine the construct validity of the PMSQ. The Kaiser-Mayer-Olkin test measured the fitness of the sample and the Bartlett sphericity test verified whether the data were adequate for the analysis; Varimax orthogonal rotation principal component analysis was used, and any factor loading > 0.40 was retained for interpretation of the instrument structure.[27,28]

The criterion validity was performed using the Pearson correlation coefficient (r), between the PMSQ and FSFI as gold standard.[29] The cutoff point of the PMSQ questionnaire was established using the ROC curve with a 95% confidence interval (CI). The scores ≤ 23, suitable for women > 50 years old, were used as cutoff points of the FSFI.[26,30] Proportions between two variables estimated by the FSFI and PMSQ were compared using the chi-squared test (χ^2). The data and this exploratory factorial analysis were performed using the SPSS Statistics for Windows, version 17 (IBM Corp., Armonk, NY, USA). The ROC curve was calculated using the Medcalc Statistical Software version 18.9.1 (MedCalc Software, Ostend, Belgium). Any p-value < 0.05 was considered statistically significant in a two-tailed test.

Results

Most participants were married (85.1%) and more than half (60.8%) self-declared as catholic. As for self-declared color, 58.0% (105) were mixed, 28.2% (51) white, and 13.8% (25) black. More than half (53.0%) had a maximum of 8 years of schooling. Almost two-thirds of them (61.3%) gained a family income of 2 minimum wages, that is, about £400 per month. The BMI was 29.1 ± 5.0 kg/m^2. A total of 96 (53.0%) subjects reported regular physical activity, with a mean of 3.3 ± 1.2 times a week. Smoking was reported by 8.8% (16/181) and 21.0% (38/181) reported drinking socially; $\sim$ 1 beer (500ml) per week. The mean ages of menarche and sexarche were 13.3 ± 1.7 years old and 19.3 ± 3.9 years old, respectively. Women with previous hysterectomy (27.1%) reported surgery at 41.8 ± 7.0 years old. The age of natural menopause in 132 participants (72.9%) was 48.4 ± 5.2 years.

The descriptive analysis of the PMSQ questionnaire with 43 items yielded a mean score of 54.9 ± 15.1 and total α coefficient of 0.93; in the domains self-image and dyspareunia, the α coefficients were 0.44 and 0.33, respectively. The correlation matrix of the PMSQ with 43 items showed significant correlations between all items, but the item 34 (I can put my finger in my vagina without feeling pain) presented poor, but still significant correlations ($r < 0.30$), and a sampling adequacy measure of 0.42 in the anti-image matrix; therefore, this item was removed from the exploratory factor analyses. The remaining 42 items presented Kaiser's test $= 0.88$, $\chi^2 = 4006$, $p < 0.001$, indicating that the sample and the correlation matrix were adequate to carry out the exploratory analyses. Almost all items presented commonalities ≥ 0.5 and 10 factors were extracted with an eigenvalue ≥ 1, explaining 66.06% of the total variance.

Afterwards, rotated analyses of the matrix enabled the exclusion of 6 items: item 29 (it is very difficult for me to get aroused, loadings ≤ 0.4); item 22 [(I get aroused just by thinking about having sex) loading in the same factor as item 25 (I get excited just thinking about sex)]; item 3 (I am sexually desirable), item 8 (my partner's sexual performance satisfies me), item 11 (I feel frustrated about my sexual life) and item 17 (I like my partner to caress my genitals [vagina, clitoris]) because they loaded in more than one factor. The item 19 (I like to have sex/make love), despite presenting cross loadings in three factors, was maintained because it is clinically important to assess the postmenopausal sexuality.

After deleting the previously mentioned items, a new exploratory analysis of the PMSQ with 36 items was performed. The analysis of this 36-item version showed Kaiser test = 0.88 and $\chi^2 = 3293$ ($p < 0.001$) and commonalities $\geq$0.5 (►**Table 1**). Nine factors were extracted with eigenvalue ≥ 1, explaining 66.3% of the total variance. After the rotation, item 23 (I feel pleasure during sexual intercourse) and 32 (I get easily aroused when I am touched), despite having crossed loads, they were maintained because they are clinically important in evaluating postmenopausal sexuality.

The items had factor loadings between 0.4 and 0.8, and total α of 0.92; among the domains, the α coefficients ranged between 0.63 and 0.87 (►**Table 2**).

The PMSQ, with 36 items, yielded a total mean score of 54.5 ± 15.4, whereas the specific menopause influence domain showed the lower mean (37 ± 24.5). The self-image domain presented the highest mean (65.9 ± 24.6). The average score obtained with the FSFI was 22.6 ± 6.5. The lowest mean was found in the desire domain (3.1 ± 1.2), and the highest one was found in the pain domain (4.2 ± 1.7). The FSFI presented a total α coefficient of 0.93 and, among the domains, the Cronbach α varied between 0.76 and 0.94. As shown in ►**Fig. 1**, the Pearson coefficient correlation between the two questionnaires was r = 0.788 ($p < 0.001$).

The association between the PMSQ and FSFI scores in the orgasm domain was strong (r = 0.70; $p < 0.001$). The influence of menopause showed moderate (r = 0.40–0.59; $p < 0.001$) correlations with all FSFI domains, except with the pain domain. The self-image domain, which was not evaluated in the FSFI, presented weak (r = 0.15–0.19) but significant correlations with all other domains of this questionnaire ($p < 0.05$ for all comparisons).

The total score of ≤ 23 obtained in the FSFI was used as the classification variable. In the ROC curve analysis, an area under the curve of 0.90 (95% CI: 0.85–0.94) and cutoff point of ≤ 55.6 was observed in the PMSQ, and sensitivity of 87.9% and specificity of 78.9% ($p < 0.001$) was detected using this instrument (►**Fig. 2**, panel A). Using the cutoff point of ≤ 23, the FSFI identified 91/181 (50.3%) women with sexual dysfunction. When the PMSQ questionnaire was used, 99/181 (54.7%) women reported sexual dysfunction. Therefore, regarding the ability to identify sexual dysfunction, no difference was found between the two questionnaires (4.42%; 95% CI: 5.82–14.53; $\chi^2 = 0.71$; $p = 0.400$). The areas of the ROC curves of the FSFI and PMSQ questionnaires were similar (difference of 4%; 95% CI: - 0.003–0.08; $p = 0.07$) (►**Fig. 2**, panel B).

The comparison between proportions of menopausal women with sexual dysfunction and menopausal women without sexual dysfunction, either in the total or in a particular domain score in the PMSQ is shown in ►**Table 3**. It is worth noticing that because the primary objective was to validate this questionnaire, the analysis was not stratified by any patient characteristic.

Discussion

The demographic profile of the study participants is similar to the profile already performed in other studies in Brazil and other countries.[12,31,32] More than half (62.4%) of the participants were overweight or obese. About half of them exercised regularly and had concluded their fourth grade education, and more than half (61.3%) had a monthly family income of £400. The vast majority (85.0%) were married. Natural menopause occurred in 72.9% of the women and the mean age of the menopause was 48.4 ± 5.2 years. The PMSQ with 36 items demonstrated that this questionnaire is an adequate instrument to evaluate sexual dysfunction in menopausal women. The correlation between the FSFI, used as a gold standard, and the PMSQ was high (r = 0.79; $p < 0.001$). The PMSQ cutoff point was established as ≤ 55.6, assuming a sensitivity of 87.9% and specificity of 78.9% ($p < 0.001$). The PMSQ identified 54.7% of the women with sexual dysfunction and, when the FSFI was used, that proportion was 50.3%.

The current study has several strengths. Factor analysis assured that the PMSQ fit the theoretical concepts of Basson female sexual response cycle.[28,33,34] In addition, the factor loading of the individual items met the expected standard, supporting the factorial validity of this instrument. The results met the statistical requirements of the factorial structure and the internal consistency of the total instrument and its domains were high. Another aspect to be considered is that the criterion validity was verified using the gold standard FSFI questionnaire.[35] The PMSQ also has the ability of measuring both peripheral (lubricating) and central sexual response (arousal, desire), important domains for assessing sexual response, such as sexual intimacy and self-image.

Among the potential limitations of the present study, the number of participants of five per item was close to the average that is recommended.[28] Another drawback was the low socioeconomic level of the population included in the study, and the low level of education. In addition, the researcher needed to conduct the interviews face to face. Therefore, for external validation, the authors are aware of the need to examine the applicability of the instrument to other populations with different socioeconomic levels and different levels of education.

Moderate correlation was found between the domain influence of menopause in the PMSQ and all FSFI domains, except for the pain domain, which showed weak correlations with almost all other domains. The low correlation between the FSFI pain domain and the other PMSQ domains suggests that pain during sex may be only slightly related to the sexual response components. These results are in accordance with

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Table 1 Values of factor load and commonalities of the 36-item PMSQ[a]

	Items	Factors									
		1	2	3	4	5	6	7	8	9	h^2
Orgasm	I only get an orgasm with great effort	0.84	0.11	0.12	0.10	0.04	0.25	0.09	0.12	0.02	0.82
	It is difficult for me to get an orgasm	0.78	0.20	0.20	0.14	0.11	0.23	0.14	0.12	−0.01	0.80
	I get an orgasm easily	0.74	−0.03	0.30	0.15	−0.05	0.04	0.23	0.16	0.09	0.73
	It is impossible for me to have an orgasm	0.65	0.13	0.14	0.27	0.13	0.21	0.07	0.08	0.12	0.62
Sexual intimacy	I hug and caress my partner's body during the intercourse	0.25	0.72	0.14	0.21	−0.02	−0.06	0.11	0.02	0.09	0.67
	I like to caress the penis of my partner	0.09	0.72	−0.13	0.31	0.22	0.09	0.02	0.00	−0.03	0.69
	I get emotionally involved with my partner during the sexual intercourse	0.15	0.69	0.21	0.14	0.19	0.09	0.03	0.17	0.06	0.64
	I like to be caressed by my partner	−0.01	0.69	0.26	−0.01	0.05	−0.02	0.19	−0.06	0.07	0.59
	I am concerned with my sexual life	−0.14	0.48	−0.12	−0.15	0.35	0.06	0.03	−0.35	0.06	0.54
	I am satisfied with my sentimental life	0.07	0.42	0.25	−0.10	0.03	0.05	−0.04	0.26	0.40	0.48
Satisfaction	The feeling of sex is good	0.16	0.12	0.72	0.25	0.22	0.11	0.19	0.04	0.06	0.72
	Sex makes me feel accomplished	0.32	0.21	0.67	0.26	0.07	0.13	0.02	0.12	0.13	0.71
	I feel satisfied with sex	0.24	0.20	0.63	0.32	0.18	0.19	0.17	0.09	0.10	0.71
	Considering the frequency of the relations with my current partner, I am	0.23	0.38	0.53	−0.13	0.03	0.16	−0.03	0.28	0.13	0.61
	I get easily aroused when I'm touched	0.17	0.10	0.46	0.43	0.19	0.22	0.17	−0.08	0.21	0.59
	I feel uncomfortable during the sexual intercourse	0.25	−0.07	0.44	0.08	−0.21	0.08	0.31	0.10	−0.23	0.48
Arousal	I get wet during the intercourse	0.25	0.01	0.14	0.69	0.01	0.07	0.01	0.06	−0.06	0.57
	I want to have sex	0.29	0.14	0.14	0.64	0.20	0.05	0.24	0.22	0.09	0.69
	I like to have sex/make love	0.14	0.39	0.18	0.61	0.22	0.13	0.30	0.05	0.04	0.74
	I feel like having sex when I am caressed	−0.07	0.24	0.33	0.51	0.17	0.16	0.39	−0.01	0.08	0.63
	I feel pleasure during sexual intercourse	0.43	0.18	0.34	0.47	−0.03	0.26	0.21	0.08	0.07	0.67
Desire	I get excited just by thinking about sex	0.08	0.03	0.10	0.27	0.77	0.19	−0.03	0.09	0.11	0.74
	I think, fantasize, dream of having sex/making love	−0.01	0.26	0.10	0.25	0.74	0.07	−0.05	0.04	0.10	0.70
	I really want to get sexually excited	0.11	0.15	0.21	0.01	0.66	−0.10	0.18	−0.10	−0.01	0.57
	I have less sexual intercourse than I would like to	0.01	0.01	−0.16	−0.23	0.51	−0.09	0.31	−0.27	−0.03	0.52
Menopause	The fact that I no longer menstruate increased the frequency of my sexual intercourse	0.18	−0.06	0.13	0.06	0.04	0.80	0.05	0.09	−0.08	0.71
	As a result of the menopause I feel less willing to have sex	0.24	0.16	0.09	0.08	0.03	0.77	0.18	0.08	−0.09	0.74
	How has your desire to have sex after menopause	0.23	0.02	0.24	0.22	0.02	0.65	0.23	0.20	0.01	0.68
	I want to have sex	0.12	0.12	0.09	0.27	0.24	0.21	0.65	0.03	0.13	0.65

Table 1 (*Continued*)

	Items	Factors									
		1	2	3	4	5	6	7	8	9	h^2
Importance of sexual life	I am not interested in sex	0.30	0.16	0.25	0.21	0.14	0.15	0.63	0.15	0.05	0.68
	I feel sexually cold	0.37	0.12	0.18	0.11	−0.08	0.29	0.59	0.26	0.01	0.69
Quality of sexual life	I am unhappy with my sexual activity	0.11	0.11	0.17	0.04	0.04	0.03	0.27	0.77	−0.02	0.71
	I want to improve my sensuality	0.10	−0.15	−0.16	0.06	−0.14	0.21	−0.09	0.66	0.09	0.57
	I feel dissatisfied with my sexual activity	0.24	0.19	0.35	0.13	0.02	0.13	0.12	0.63	0.00	0.65
Self-image	I am still a sensual, charming woman	−0.01	0.15	0.08	−0.11	0.04	−0.04	0.23	−0.08	0.83	0.80
	I feel well with my body image	0.15	0.00	0.05	0.24	0.11	−0.12	−0.09	0.11	0.77	0.72

[a]Extraction method: principal component analysis; Rotation method: Varimax with Kaiser normalization.
h^2 communality.

Table 2 Alpha coefficients of the total instrument and its specific domains (PMSQ)

Domain	Number of questions	Questions	Score	Cronbach α**
Self-image	2	1–2	0–5	0.63
Quality of sexual life	3	3–5	0–5	0.65
Sexual intimacy	6	6–11	0–5	0.75
Desire	4	12–15	0–5	0.68
Importance of sexual life	3	16–18	0–5	0.77
Arousal	5	19–23	0–5	0.82
Orgasm	4	24–27	0–5	0.87
Satisfaction	6	28–33	0–5	0.80
Influence of menopause	3	34–36	0–5	0.79
Total	36	–	–	0.92

**Cronbach α, measure of the internal consistency for each domain and the total questionnaire.

Table 3 Comparison of the scores total and by domain of the PMSQ between women with and without sexual dysfunction

Domains	Without SD (82)	With SD (99)	T***	
	(sd)	(sd)		*p-value*
Orgasm	69.21 (20.99)	30.15 (18.74)	13.08	0.000
Menopause	51.63 (23.02)	24.85 (18.29)	8.54	0.000
Sexual intimacy	73.58 (15.63)	51.38 (18.93)	8.64	0.000
Quality sexual life	53.01 (20,84)	34.41 (19.28)	6.18	0.000
Self-image	72.68 (22.72)	60.20 (24.83)	3.53	0.001
Desire	53.66 (21.84)	38.99 (17.50)	4.91	0.000
Satisfaction	79.96 (8.60)	53.23 (17.86)	13.16	0.000
Arousal	71.95 (16.64)	40.69 (17.77)	12.20	0.000
Importance sexual life	77.97 (17.43)	45.86 (22.03)	10.94	0.000
Total	68.49 (7.45)	42.87 (9.36)	20.50	0.000

Abbreviations: SD, sexual dysfunction; sd, standard deviation.
***Student's *t*-test.

the theoretical framework adopted for the construct, in which the sexual response involves a coordinated sequence of several phases, including desire, arousal, orgasm, and sexual intimacy.[6]

A study conducted in Brazil with 540 women, at the ages between 45 and 60 years old with sexual dysfunction has shown association between lubrication condition and sexual dysfunction, but those who presented satisfaction in the relationship to their partners had lower sexual complaints.[36] Sexual intimacy domain in the questionnaire assesses intimacy with the partner during sexual intercourse and many older women maintain sexual satisfaction because of the

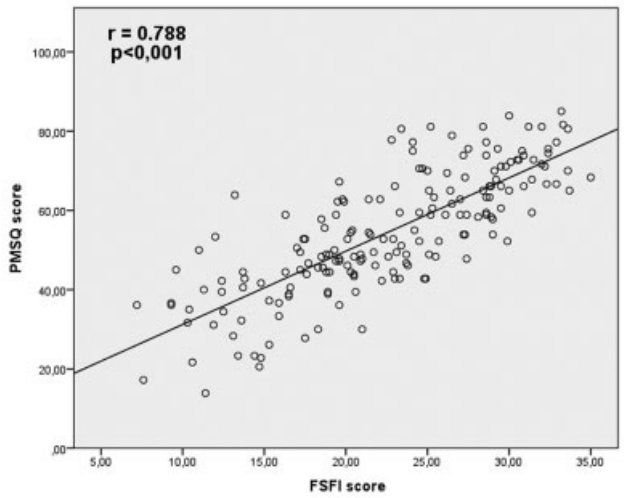

Fig. 1 Correlation between FSFI and PMSQ scores.

protective role of the psychosocial factors clearly associated with a happy relationship. In a postal survey conducted in Australia, the relationship factors had a more negative impact on desire than the age or menopause condition. This same study showed that physiological and psychological factors may be more significant for low genital arousal and low orgastic function.[37] The moderate but significant correlations between sexual intimacy ($r = 0.40$) with desire, satisfaction, arousal and a weak correlation with menopause ($r = 0.20$), orgasm ($r = 0.30$) and self-image ($r = 0.30$) in the current study support the knowledge that sexual intimacy is an important factor and should weigh among the domains of any instrument designed to evaluate the female sexual function.

A general decline in postmenopausal self-esteem and well-being may also contribute to the loss of sexual intimacy with the partner.[38] The physical and psychological symptoms of menopause and the simultaneous decline in sexual function may result in an inferiority sensation and negative body image in postmenopausal women, thus reducing their quality of life.[39] The self-image domain, being directly related to self-esteem, showed a better correlation with sexual intimacy in the present study, but no significant correlation with sexual quality of life and menopause domains. In the group of women classified with sexual dysfunction, the PMSQ showed the lowest mean score in the menopause domain, indicating that the menopause condition itself has a negative impact on the sexual response cycle, sexual quality of life, arousal and desire. In the analysis of the FSFI scores, except for the menopause domain, the domains that obtained low scores the most in this population were the desire and arousal domains.[12]

Conclusion

The psychometric validity of the PMSQ, including construct and criterion validity, responded satisfactorily to the tests performed. The current instrument showed adequate factor loadings, good internal consistency, and high coefficient correlation with the gold standard instrument. Therefore, the evaluation of sexual dysfunctions during menopause has a valid and reliable instrument that includes specific domain.

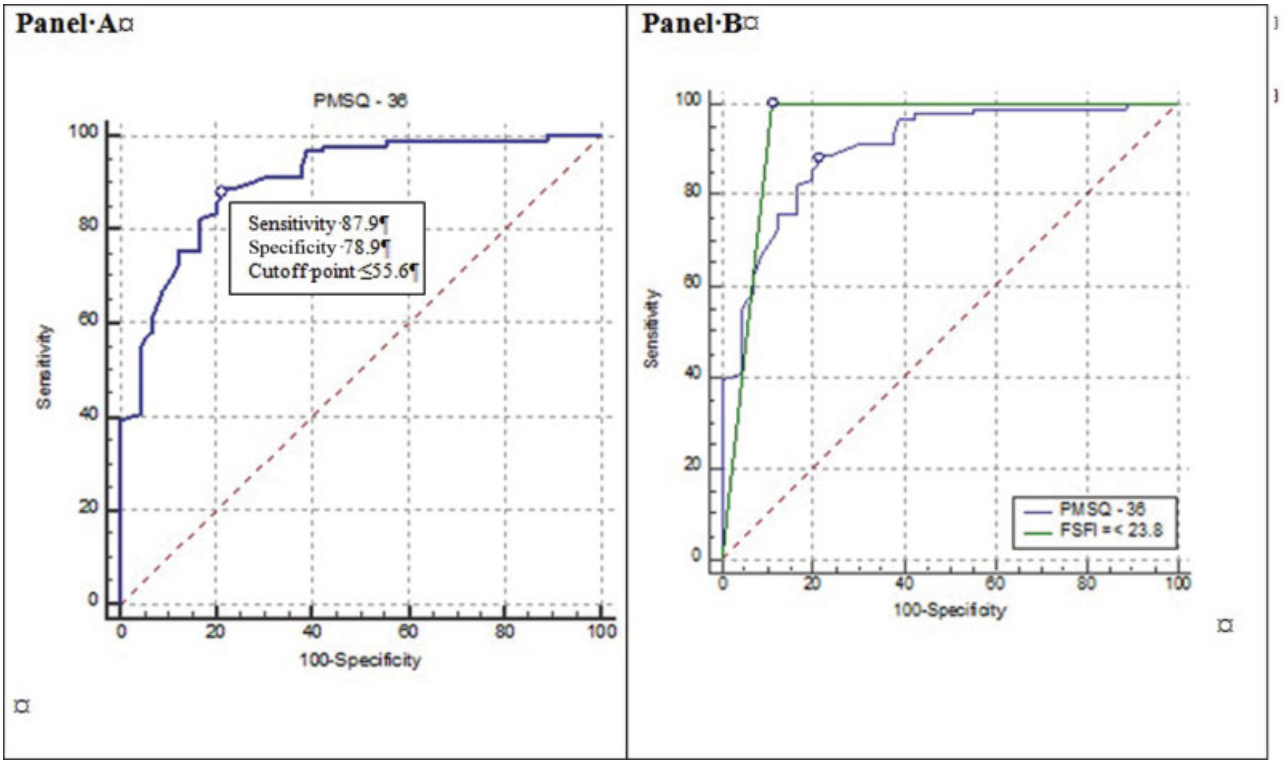

Fig. 2 Panel A - ROC curve with cutoff point of PMSQ questionnaire. Panel B - Comparison between total areas of FSFI and PMSQ questionnaire.

The PMSQ can be used to examine sexual function in postmenopausal women, but further studies in other populations with different social levels and lifestyles are needed.

Contributions

All authors were involved in the design and interpretation of the analyses, contributed to the writing of the manuscript, read and approved the final manuscript.

Conflict of Interests

The authors have no conflict of interests to declare.

References

1 Crema IL, De Tilio R, Campos MTA. Repercussões da menopausa para a sexualidade de idosas: revisão integrativa da literatura. Psicologia (Cons Fed Psicol). 2017;37(3):753–769. doi: 10.1590/1982-3703003422016

2 Jenabi E, Shobeiri F, Hazavehei SMM, Roshanaei G. Assessment of questionnaire measuring quality of life in menopause women: a systematic review. Oman Med J. 2015;30(3):151–156. doi: 10.5001/omj.2015.34

3 Schoenaker DAJM, Jackson CA, Rowlands JV, Mishra GD. Socioeconomic position, lifestyle factors and age at natural menopause: a systematic review and meta-analyses of studies across six continents. Int J Epidemiol. 2014;43(5):1542–1562. doi: 10.1093/ije/dyu094

4 Davis SR, Lambrinoudaki I, Lumsden M, et al. Menopause. Nat Rev Dis Primers. 2015;1:15004. doi: 10.1038/nrdp.2015.4

5 Cabral PUL, Canário ACG, Spyrides MH, Uchôa SAC, Eleutério Júnior J, Amaral RLG, et al. [Influence of menopausal symptoms on sexual function in middle-aged women]. Rev Bras Ginecol Obstet. 2012;34(7):329–334. doi: 10.1590/S0100-72032012000700007

6 Basson R. The female sexual response: a different model. J Sex Marital Ther. 2000;26(1):51–65. doi: 10.1080/00926230027 8641

7 McCool ME, Zuelke A, Theurich MA, Knuettel H, Ricci C, Apfelbacher C. Prevalence of female sexual dysfunction among premenopausal women: a systematic review and meta-analysis of observational studies. Sex Med Rev. 2016;4(3):197–212. doi: 10.1016/j.sxmr.2016.03.002

8 Chedraui P, Pérez-López FR, Mezones-Holguin E, San Miguel G, Avila C; Collaborative Group for Research of the Climacteric in Latin America (REDLINC). Assessing predictors of sexual function in mid-aged sexually active women. Maturitas. 2011;68(4):387–390. doi: 10.1016/j.maturitas.2010.12.004

9 De Lorenzi DRS, Saciloto B. Frequência da atividade sexual em mulheres menopausadas. Rev Assoc Med Bras. 2006;52(4):256–260. doi: 10.1590/S0104-42302006000400027

10 Brown L, Bowden S, Bryant C, et al. Validation and utility of the attitudes to ageing questionnaire: links to menopause and well-being trajectories. Maturitas. 2015;82(2):190–196. doi: 10.1016/j.maturitas.2015.06.042

11 Cuerva MJ, Gonzalez D, Canals M, et al; SMS Young Experts Group. The sexual health approach in postmenopause: The five-minutes study. Maturitas. 2018;108:31–36. doi: 10.1016/j.maturitas.2017.11.009

12 Nazarpour S, Simbar M, Ramezani Tehrani F, Alavi Majd H. Sexual function and exercise in posmenopausal women residing in Chalous and Nowshahr, Northern Iran. Iran Red Crescent Med J. 2016;18(5):e30120. doi: 10.5812/ircmj.30120

13 Ambler DR, Bieber EJ, Diamond MP. Sexual function in elderly women: a review of current literature. Rev Obstet Gynecol. 2012;5(1):16–27.

14 Dombek K, Capistrano EJM, Costa ACC, Marinheiro LPF. Risk factors associated with sexual dysfunction in Brazilian postmenopausal women. Int J Impot Res. 2016;28(2):62–67. doi: 10.1038/ijir.2016.4

15 Valadares ALR, Lui-Filho JF, Costa-Paiva L, Pinto-Neto AM. Middle-aged female sexual dysfunction and multimorbidity: a population-based study. Menopause. 2016;23(3):304–310. doi: 10.1097/GME.0000000000000533

16 Rosen R, Brown C, Heiman J, Leiblum S, Meston C, Shabsigh R, et al. The Female Sexual Function Index (FSFI): a multidimensional self-report instrument for the assessment of female sexual function. J Sex Marital Ther. 2000;26(2):191–208. doi: 10.1080/009262300278597

17 Chedraui P, Pérez-López FR, Sánchez H, Aguirre W, Martínez N, Miranda O, et al. Assessment of sexual function of mid-aged Ecuadorian women with the 6-item Female Sexual Function Index. Maturitas. 2012;71(4):407–412. doi: 10.1016/j.maturitas.2012.01.013

18 Isidori AM, Pozza C, Esposito K, Giugliano D, Morano S, Vignozzi L, et al. Development and validation of a 6-item version of the female sexual function index (FSFI) as a diagnostic tool for female sexual dysfunction. J Sex Med. 2010;7(3):1139–1146. doi: 10.1111/j.1743-6109.2009.01635.x

19 Jara D, Fuenzalida A, Figueroa R, del Prado M, Flores D, Blümel JE, et al. Is the Menopause Rating Scale accurate for diagnosing sexual dysfunction among climacteric women? Maturitas. 2009;62(3):321–323. doi: 10.1016/j.maturitas.2009.01.008

20 Borges VLF, Medeiros SF. [Validation of a questionnaire to evaluate the female sexual function in postmenopausal women]. Rev Bras Ginecol Obstet. 2009;31(6):293–299. doi: 10.1590/S0100-72032009000600005

21 Terwee CB, Bot SD, de Boer MR, van der Windt DA, Knol DL, Dekker J, et al. Quality criteria were proposed for measurement properties of health status questionnaires. J Clin Epidemiol. 2007;60(1):34–42. doi: 10.1016/j.jclinepi.2006.03.012

22 Associação Brasileira para o Estudo da Obesidade e da Síndrome Metabólica [Internet]. Diretrizes brasileiras de obesidade. 4. ed. São Paulo: Abeso; 2016 [cited 2018 Oct 07]. Available from: http://www.abeso.org.br/uploads/downloads/92/57fccc403e5da.pdf

23 Ministério da Saúde. Secretaria de Atenção à Saúde. Departamento de Ações Programáticas Estratégicas [Internet]. Caderneta de saúde da pessoa idosa. 4. ed. Brasília: Ministério da Saúde; 2017 [cited 2018 Oct 07]. Available from: https://portalarquivos2.saude.gov.br/images/pdf/2017/setembro/27/CADERNETA-PES-SOA-IDOSA-2017-Capa-miolo.pdf

24 Pacagnella RC, Vieira EM, Rodrigues OM Jr, Souza C. Adaptação transcultural do Female Sexual Function Index. Cad Saude Publica. 2008;24(2):416–426. doi: 10.1590/S0102-311X2008000200021

25 Wiegel M, Meston C, Rosen R. The female sexual function index (FSFI): cross-validation and development of clinical cutoff scores. J Sex Marital Ther. 2005;31(1):1–20. doi: 10.1080/00926230590475206

26 Castroviejo Royo C, Conde Redondo C, Rodriguez Toves LA, Garcia-Tunon CM, Gonzalez Tejero C, Oceja JMMS. Female sexual function in Castilla Y Leon (Spain): normal ranges. Gynecol Obstet (Sunnyvale). 2014;4:9. doi: 10.4172/2161-0932.1000240

27 Psicometria PasqualiL. Rev Esc Enferm USP. 2009;43:992–999. doi: 10.1590/S0080-62342009000500002

28 Anthoine E, Moret L, Regnault A, Sébille V, Hardouin JB. Sample size used to validate a scale: a review of publications on newly-developed patient reported outcomes measures. Health Qual Life Outcomes. 2014;12:176. doi: 10.1186/s12955-014-0176-2

29 Hutz CS, Bandeira DB, Trentini CM. Psicometria. Porto Alegre: Artmed; 2015

30 Fawcett T. An introduction to ROC analysis. Pattern Recognit Lett. 2006;27(8):861–874. doi: 10.1016/j.patrec.2005.10.010

31 Pasquali L. Instrumentação psicológica: fundamentos e práticas. Porto Alegre: Artmed; 2010

32 Oliveira VN, Fontes CJF, Medeiros SF. Correlação entre a idade da menopausa e a idade da menarca em mulheres climatéricas do distrito centro-oeste de Cuiabá-MT. Reprod Clim. 2001;16(3): 207–210.
33 Prince MJ, Acosta D, Guerra M, Huang Y, Jimenez-Velazquez IZ, Llibre Rodriguez JJ, et al. Reproductive period, endogenous estrogen exposure and dementia incidence among women in Latin America and China; A 10/66 population-based cohort study. PLoS One. 2018;13(2):e0192889. doi: 10.1371/journal.pone.0192889
34 Basson R, Berman J, Burnett A, Derogatis L, Ferguson D, Fourcroy J, et al. Report of the international consensus development conference on female sexual dysfunction: definitions and classifications. J Urol. 2000;163(3):888–893. doi: 10.1016/S0022-5347(05)67828-7
35 Wong ELY, Huang F, Cheung AWL, Wong CKM. The impact of menopause on the sexual health of Chinese Cantonese women: A mixed methods study. J Adv Nurs. 2018;74(7):1672–1684. doi: 10.1111/jan.13568
36 Lett C, Valadares ALR, Baccaro LF, Pedro AO, Filho JL, Lima M, et al. Is the age at menopause a cause of sexual dysfunction? A Brazilian population-based study. Menopause. 2018;25(1):70–76. doi: 10.1097/GME.0000000000000952
37 Hayes RD, Dennerstein L, Bennett CM, Sidat M, Gurrin LC, Fairley CK. Risk factors for female sexual dysfunction in the general population: exploring factors associated with low sexual function and sexual distress. J Sex Med. 2008;5(7):1681–1693. doi: 10.1111/j.1743-6109.2008.00838.x
38 Monteleone P, Mascagni G, Giannini A, Genazzani AR, Simoncini T. Symptoms of menopause - global prevalence, physiology and implications. Nat Rev Endocrinol. 2018;14(4):199–215. doi: 10.1038/nrendo.2017.180
39 Nazarpour S, Simbar M, Tehrani FR, Majd HA. The relationship between menopausal symptoms and sexual function. Women Health. 2018;58(10):1112–1123. doi: 10.1080/03630242.2017.1414100

Is Surgical Treatment an Option for Locally Advanced Cervical Cancer in the Presence of Central Residual Tumor after Chemoradiotherapy?

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Abstract

Objective To evaluate the outcomes of surgical treatment in patients with chemoradiotherapy (CRT)-resistant and locally advanced cervical cancer (LACC).

Methods Patients with LACC who underwent surgery due to resistance to CRT between 2005 and 2015 were reviewed retrospectively. Disease-free survival (DFS) and overall survival (OS) related factors were analyzed.

Results A total of 23 patients were included in the study and the median age was 51 years old. A total of 14 patients (60.8%) experienced recurrence; among these recurrences, 8 of them were local, 5 were distant, 1 was both distant and local. A total of 9 patients (39%) died. The Median DFS and OS durations were 15 and 32 months, respectively. A total of 17 patients (74%) had undergone simple hysterectomy, 4 (17%) radical hysterectomy, and 2 (9%) total pelvic exenteration. Postoperative grade 3 and 4 complications were seen in 12 patients (52%). Macroscopic tumor presence in the pathology specimen was associated with distant recurrence and positive surgical margins with local recurrence (Log-Rank test $p = 0.029$ and $p = 0.048$, respectively). The only factor associated with OS was surgical margin positivity (Log-Rank test $p = 0.008$). The type of surgery, grades 3 and 4 postoperative complications, brachytherapy, and tumor histology were not associated with recurrence.

Conclusion In patients with LACC, hysterectomy is an option in the presence of a central residual tumor after CRT. However, the risk of grades 3 and 4 complications of performed surgery is high. The presence of macroscopic tumor in the pathology specimen and positive surgical margins are poor prognostic factors. The goal of the surgeon should be to achieve a negative surgical margin. It does not seem important if the surgery is simple or radical.

Keywords

- cervical cancer
- brachytherapy
- chemoradiotherapy
- recurrence
- resistant tumor

Introduction

The incidence of invasive cervical cancer is declining, especially in developed countries, due to successful screening programs and HPV vaccination. Introduction of chemoradiotherapy instead of radiotherapy alone ~ 20 years ago, which was shown to be beneficial to survival, is a major advance in locally advanced cervical cancer (LACC) therapy.[1] However, the relative survival rate of these patients seems to have remained unchanged over the last 40 years, according to The Surveillance, Epidemiology, and End Results (SEER) Program of the National Cancer Institute data.[2]

The standard treatment approach in LACC (stage 1B2–4A) is external beam radiation therapy (EBRT) followed by intracavitary brachytherapy (ICBT) and concomitant cisplatin-based chemotherapy.[3–5] Studies showed that a response between

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80 and 90% is obtained with this treatment.[6–8] Survival rates in these patients were reported as between 67 and 83%.[9–11] A small group of patients with cervical cancer can be unresponsive to CRT. In these patients, a residual tumor is detected in the cervix after chemoradiotherapy (CRT) and is considered to be a CRT-resistant tumor. These patients are considered to have a poor prognosis, such as patients with recurrent cervical cancer. According to some studies, 90% of relapses occur within 3 years, and the 5-year survival rate is $< 5\%$.[12,13] There are no strong recommendations and evidence for the optimal treatment for patients with resistance to CRT. Possible options are simple hysterectomy, radical hysterectomy, or extended surgeries such as pelvic exenteration; moreover, when surgery is not feasible, other treatment approaches are chemotherapy and reirradiation issue. Lack of sufficient knowledge due to the small patient population is a limitation on this topic. According to the Dindo et al[14] classification, grade 3 complication has been defined as any postoperative complication requiring surgical, endoscopic or radiological intervention under general anesthesia.

In the present study, we reviewed the clinical and survival outcomes of the patients with LACC who had undergone surgery due to resistance to CRT. Clinicopathological factors such as tumor size in the pathology specimen (as macroscopic, microscopic or tumor-free), the presence of tumor in surgical margin, operation type, grade-3 complication, brachytherapy, and histology of the tumor were analyzed.

Methods

Patients with LACC who were operated for CRT resistance at the Oncology Center of the Istanbul University between 2005 and 2015 were included in the study. Patients had received CRT for LACC. Due to lack of complete response to CRT, the patients had been referred to surgery. A total of 25 patients who had been operated with a diagnosis of residual tumor following CRT were identified. One patient was excluded from the study due to a postoperative pathological diagnosis of endometrial carcinoma and due to loss to follow-up. Demographic, clinicopathological, and follow-up data of 23 patients were recorded.

Initial Evaluation and Chemoradiation Protocol

All of the patients had a pathological diagnosis of invasive cervical cancer with cervical biopsy. After diagnosis, the patients were classified as stage 1B2 to 4A according to the International Federation of Gynecology and Obstetrics (FIGO) staging system by gynecological examination, magnetic resonance imaging (MRI), and positron emission tomography (PET). These patients were defined as having LACC. The patients diagnosed as having LACC had been started on a general treatment protocol including pelvic EBRT, 1.8–2 Gy per fraction, total 45–50 Gy with cisplatin 40 mg/m2/week and '3D conformal HDR brachytherapy 5 Gy once weekly $\times$ 5 weeks to high-risk clinical target volume' in the Department of Radiation Oncology. These patients had been evaluated with gynecological examination and MRI at the end of EBRT, before brachytherapy. In the absence of expected tumor regression in the cervix according to postCRT MRI, the CRT-resistance of tumors was accepted. The patients with clinical and/or radiological presence of residual tumor in the cervix had been defined as patients with CRT-resistant tumors and therefore had undergone surgery. The patients were offered to have surgery if the brachytherapy could not be applied or completed. The evaluation of these patients had been performed in weekly organized gynecological oncology meetings of the faculty, with the participation of medical oncology, radiation oncology, gynecologic oncology, radiology, nuclear medicine and gynecopathology teams.

Evaluated Data

External beam radiation therapy (EBRT) doses and fractions, doses of external boost radiotherapy given to patients unable to receive brachytherapy, as well as doses and fractions of brachytherapy were recorded separately for the study.

Age, height, weight, parity, coitarche, smoking status of the patients, as well as the presence of chronic disease, were recorded. Duration between the last radiotherapy dose and surgery, type of the surgery, and postoperative grades 3 and 4 complications were also recorded.

Several data were collected from pathology reports, including presence of tumor in the specimen (macroscopic, microscopic or tumor-free), histological subtypes, and presence of tumor in surgical margins. Sites of recurrence, duration until recurrence, and recurrence treatment were recorded in patients with relapse. Recurrences were classified as local if they were detected in the pelvis, cervix, or vagina and as distant if they were detected in extrapelvic locations. Disease-free survival (DFS) and overall survival (OS) analyses were performed. The influence of the following criteria on recurrence was analyzed: 1–tumor size in the pathology specimen; 2–surgical margin; 3–operation type; 4–presence of grade 3 complication; 5–whether brachytherapy was administered or not; and 6–tumor histology. According to the Dindo et al[14] classification, grade 3 complication has been defined as any postoperative complication requiring surgical, endoscopic or radiological intervention under general anesthesia. The association between these criteria and DFS or OS was examined using the Kaplan-Meier survival analysis. The period between the date of operation and the date of last visit or death of the patient was recorded as follow-up time. Disease-free survival was defined as the period between the time of surgery and the observation of the recurrence. Overall survival was the time between the surgery and death, and follow-up time was evaluated as the time between the surgery and the time that the patient was last examined (death or last visit).

Statistical Analysis

IBM SPSS for Windows, Version 21 (IBM Corp., Armonk, NY, USA) was used to perform all analyses. When evaluating the study data, besides descriptive statistical methods (mean, standard deviation [SD], median, frequency, percentage, minimum, and maximum), the Fisher exact test was used to compare two groups. To assess survival, the Kaplan-Meier survival analysis was performed. A p-value < 0.05 was considered statistically significant. Because the present study is a retrospective review, permission of the local ethics

committee was not sought. However, all of the patients signed an informed consent form that allowed our center to use their clinical data for scientific trials.

Results

►**Table 1** shows age, height, weight, body mass index (BMI), parity, coitarche, smoking status, presence of chronic disease, and brachytherapy administration data. A total of 9 patients had not received brachytherapy (7 due to gross residual tumor, 2 due to closed cervical canal). Of these 9 patients, 8 had received between 10 and 20 Gray external boost radiotherapy. One patient had received neither brachytherapy nor external boost (only EBRT had been administered). As shown in ►**Table 2**, 73.9% ($n = 17$) of the patients had undergone simple hysterectomy, 17.4% ($n = 4$) radical hysterectomy, and 8.7% ($n = 2$) total exenteration. Grade 3 surgical complications had been seen in 52.1% ($n = 12$) of the patients. These included gastrointestinal system (GIS) injuries requiring colostomy / ileostomy (2 patients), infection requiring relaparotomy (2 patients), urinary tract injury requiring nephrostomy (2 patients), rectovaginal fistula (2 patients) and vesicovaginal fistula (4 patients). A total of 10 out of 12 cases of grade 3 complications occurred after hysterectomy, and 2 of them were after radical surgery. The complications rate was 10/17 for simple hysterectomy, and 2/6 for radical surgery.

Table 1 Characteristics of the patients

Variables		
Age (years old) Min-Max ; median	31–68	51
Parity Min-Max ; median	0–8	2
Height (m) Min-Max ; mean ± SD	1.50–1.76	1.61 ± 0.07
Weight (kg) Min-Max ; mean ± SD	55–105	67.1 ± 11.2
BMI (kg/m^2) Min- Max ; mean ± SD	20.2–41.0	25.9 ± 4.5
Smoker (n, %)	12	52.1
Coitarche (years) Min-Max ; mean ± SD	15–27	20.1 ± 3.6
Brachytherapy administered (n, %)	14	60.8
not (n, %)	9	39.2
Reason for no brachytherapy, (n)		
gross tumor in cervix	7	
closed cervical canal	2	
Brachytherapy dose (Gray/fraction)	10–25/2–5	
External boost radiotherapy, (n) yes no	8 1	
External boost radiotherapy dose (Gray)	10–20	
Time between last radiotherapy and operation min-max; median	1–12	3

The Relation between the Rate of Complication and the Period from the Last Radiotherapy until Surgery

Fewer complications were seen in patients who were operated within the first 2 months (33.3% versus 64.3%). A total of 9 patients in the first 2 months and 14 patients after 2 months were operated. A total of 3 (33.3%) out of 9 patients operated within the first 2 months and 9 (%64.3) out of 14 patients operated after 2 months had experienced grade 3 complications.

Pathological Evaluation Results

The tumor had been detected microscopically in 17.3% ($n = 4$) of the patients and macroscopically in 60.8% ($n = 14$). The pathological evaluation had revealed no tumor in 21.7% ($n = 5$). The surgical margins had been positive in 43.4% ($n = 10$) of the patients and negative in 56.5% ($n = 13$). The histological diagnosis had been squamous in 69.5% ($n = 16$) and nonsquamous in 30.5% ($n = 7$; adenocarcinoma

Table 2 Clinicopathological features

Feature	*n* (%)
Operation type	
Simple hysterectomy	17 (73.9)
Radical hysterectomy	4 (17.4)
Exenteration	2 (8.7)
Surgical margin	
Positive	10 (43.5)
Negative	13 (56.5)
Grade-3 complication*	
Yes	12 (52.2)
No	11 (47.8)
Pathology specimen	
Tumor-free	5 (21.7)
Microscopic	4 (17.3)
Macroscopic	14 (60.8)
Histology	
Squamous	16 (69.5)
Other*	7 (30.5)
Recurrence	
Yes	14 (60.8)
No	9 (39.2)
Site of recurrence	
Local	8 (34.7)
Distant	5 (21.7)
Local + distant	1 (4.3)
Final status	
Dead	9 (39.1)
Living with disease	5 (21.7)
Healthy	9 (39.1)

*5 patients with adenocarcinoma, 1 patient with glassy cell carcinoma, 1 patient with small cell carcinoma.

Table 3 The outcomes of 5 patients with distant recurrence

Site of recurrence	Treatment for recurrence	Final status
Lung	Chemotherapy	deceased
Adrenal gland	Adrenalectomy + chemotherapy	alive
Extensive intraabdominal implantation	Chemotherapy	deceased
Brain + local	Supportive care	deceased
Brain + lung + bone	Radiotherapy and chemotherapy	deceased
Brain + lung	Radiotherapy and chemotherapy	deceased

in 5 patients, glassy cell carcinoma in 1, and small cell carcinoma in 1) (►**Table 2**).

Clinical Outcomes

Relapse had occurred in 14 (60.8%) patients, 9 of whom had deceased (39.1% of all patients). No patient had died due to causes other than the disease. Five patients had been classified as living with disease (21.7% of all patients) and 9 (39.1%) as healthy (►**Table 2**). At the time of diagnosis of recurrence, 8 patients had local, 5 had distant and 1 had both local and distant recurrence. The approach to 8 patients with local recurrence had been surgical treatment (total exenteration) in 2, supportive care in 1, and medical treatment (systemic chemotherapy) in others. One patient had refused treatment. The findings of the patients with distant recurrence are shown in ►**Table 3**. Positive surgical margins, operation type, presence of macroscopic tumor in pathology specimen, occurrence of grade 3 complication, whether brachytherapy had been administered or not, and tumor histology were analyzed in terms of relation to recurrence. The presence of macroscopic tumor in the pathology specimen was found to be related to recurrence ($p = 0.029$) (►**Table 4**). When the factors associated only with local recurrence were investigated separately, the only factor that was found to be related to local recurrence was positive surgical margins ($p = 0.048$) (►**Table 5**). The comparison of simple hysterectomy and radical hysterectomy in terms of micro- or macroscopic tumors, surgical margins and complications is presented in ►**Table 6**.

Survival Analysis

The median follow-up period of 23 patients was 20.0 months (6–118 months) and the median OS was 32.0 ± 8.1 months (95% confidence interval [CI]: 16.0–48.0). The 2-year OS ratio was calculated as 63.3%. A total of 14 patients (60.8%) had disease recurrence. The median DFS was 15.0 ± 4.4 months (95% CI: 6.3–23.6). The 2-year DFS rate was 29.1%.

Disease-free Survival and Overall Survival with Regard to Surgical Margin

Recurrence had occurred in 8 (80%) out of 10 patients with positive surgical margins and in 6 (46.2%) out of 13 patients with negative surgical margins. The median DFS was 7.0 ± 1.58 (95% CI: 3.9–10.0) months with positive surgical margins and 24.0 ± 3.8 (95% CI: 16.4–31.5) with negative surgical margins (log rank test $p = 0.048$) (►**Fig. 1**).

A total of 4 (40.0%) out of 10 patients with positive surgical margins and 10 (76.9%) out of 13 patients with negative surgical margins were alive. The median OS was estimated as 20.0 months with positive surgical margins and as 36.0 months with negative surgical margins (log rank test $p = 0.008$) (►**Fig. 2**).

Disease-free Survival and Overall Survival with Regard to the Presence of Macroscopic Tumor

Recurrence had occurred in 12 (85.7%) out of 14 patients with macroscopic tumor and in 2 (22.2%) out of 9 patients with microscopic or no tumor. The median DFS was 11.0 months with macroscopic tumor and 16.0 months with microscopic or no tumor (log rank test $p = 0.029$) (►**Fig. 3**).

The number of patients was not adequate to calculate the median overall survival duration by Long Rank test analysis with regard to the presence of macroscopic tumor (►**Fig. 4**).

Table 4 Analysis of factors affecting distant recurrence

Variables		*p-value*	OR	95.0%CI for OR	
				Lower	Upper
Pathology specimen	Tumor-free/Microscopic Macroscopic	0.029	7.750	1.226	48.984
Surgical margin	Negative Positive	0.203	2.669	0.589	12.086
Operation type	Simple hysterectomy Radical surgery	0.067	4.991	0.894	27.857
Grade-3 complication	Yes No	0.659	1.353	0.354	5.173
Brachytherapy	Yes No	0.821	1.208	0.234	6.238
Histology	Squamous Non-squamous	0.485	1.737	0.369	8.183

Abbreviations: CI, confidence interval; OR, odds ratio.

Table 5 Analysis of factors affecting only local recurrence

Variables		*p-value*	OR	95.0%CI for OR	
				Lower	Upper
Pathology specimen	Tumor-free/Microscopic Macroscopic	0.297	3.350	0.345	32.571
Surgical margin	Negative Positive	0.048	15.635	1.027	237.966
Operation type	Simple hysterectomy Radical surgery	0.453	1.989	0.330	11.976
Grade-3 complication	Yes No	0.111	3.998	0.726	22.025
Brachytherapy	Yes No	0.210	5.147	0.397	66.803
Histology	Squamous Non-squamous	0.497	2.096	0.248	17.723

Abbreviations: CI, confidence interval; OR, odds ratio.

Table 6 Comparison of simple hysterectomy and radical hysterectomy in terms of micro- or macroscopic tumor, surgical margins, and complications

Variables		Type of surgery		
		Simple	Radical	*p-value*
Grade3 complication	No	7	4	0.371
	Yes	10	2	
Surgical margin	Negative	10	3	1.000
	Positive	7	3	
Tumor specimen	Microscopic/ tumor-free	8	1	0.340
	Macroscopic	9	5	

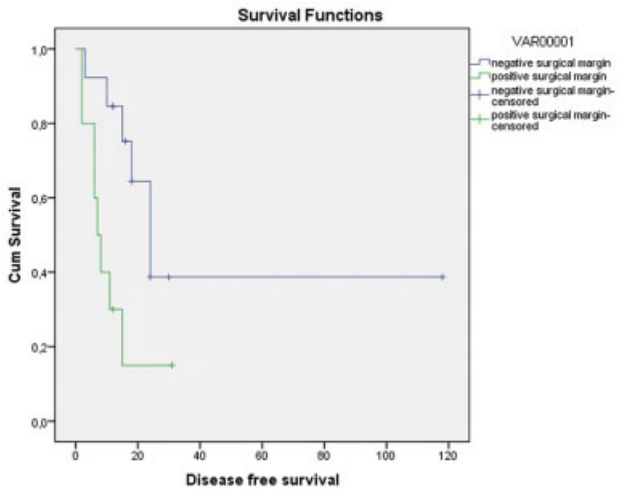

Fig. 1 Disease-free survival graph with regard to surgical margin (months).

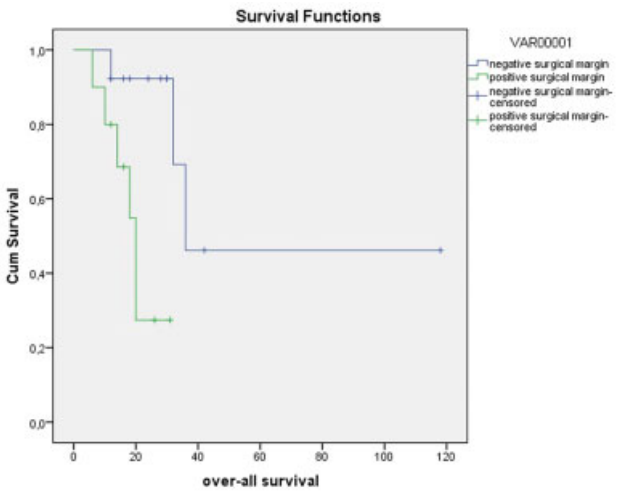

Fig. 2 Overall survival graph with regard to surgical margin (months).

Discussion

Cotreatment with radiotherapy and surgery as primary approach is avoided in patients with cervical cancer in order not to increase morbidity. However, studies published in recent years have suggested that surgical approach following radiotherapy was acceptable and had no negative impact on morbidity and survival.[13,15–18] These studies suggested surgical approach either for the purpose of completion of hysterectomy, when brachytherapy could not be performed optimally, or as an alternative to brachytherapy.

In the present study, we analyzed the findings in LACC patients who had undergone surgery due to persistence after CRT. Out of 23 cases assessed in the present study, 14 (60.8%) had relapsed. The median DFS duration was 15.0 ± 4.4 (95% CI: 6.3–23.6) months. All of the recurrences had happened within the first 2 years.

Size of tumor in the pathology specimen, histological subtype of tumor, surgical margin, operation type, whether brachytherapy had been administered or not, and occurrence of grade 3 complication were analyzed in terms of relation to recurrence (►**Table 4**). Among these, surgical margins positivity was related to local recurrence only and the presence of macroscopic tumor in the pathology specimen to overall recurrence ($p = 0.048$ and $p = 0.029$, respectively). In the

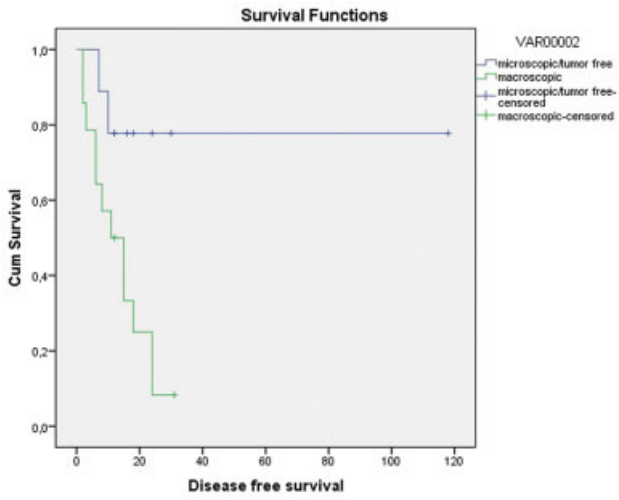

Fig. 3 Disease-free survival graph with regard to presence of macroscopic tumor in the pathologic specimen (months).

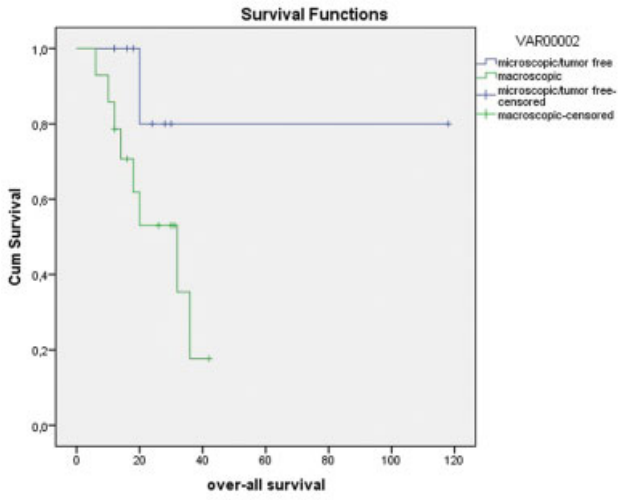

Fig. 4 Overall survival graph with regard to presence of macroscopic tumor in the pathologic specimen (months).

presence of gross tumor, distant recurrences could not be prevented even if negative surgical margins were reached. At this point, adjuvant systemic chemotherapy can be considered to be useful at preventing distant recurrences. When we analyzed for local recurrence, local control could be achieved even in the presence of gross tumor if surgical margin negativity could be achieved.

Simple Hysterectomy? Radical Surgery?

In patients with central residual tumor detected in the evaluations after CRT, there is no certain view about the preference between simple hysterectomy and more radical operation as the required surgical treatment. In the present study, there was no statistical difference between patients who had undergone simple hysterectomy or more radical surgery, in terms of both distant and local recurrences (►**Tables 5** and **6**). According to this result, it might be important to avoid radical surgery not to increase morbidity unless a survival benefit is obtained. However, when simple hysterectomy is performed to avoid the morbidity of radical surgery, the risk of surgical margin positivity, which is a negative determinant of survival, might be augmented. In the present study, surgical margin positivity was found to be 70% (7 out of 10 patients) in radical surgery and 50% (3 out of 6 patients) in simple hysterectomy. Statistical analysis was not performed due to the limited number of patients. We observed in the present study that surgical margin negativity was quite significant for survival. The median DFS was estimated to be 7.0 months with positive surgical margins and 24.0 months with negative surgical margins (log rank test $p = 0.048$) (►**Fig. 1**). Accordingly, it can be suggested that it is best to perform surgery as extended as possible to attain negative surgical margins and to improve survival. Attaining surgical margin negativity is more likely with extended surgery. On the other hand, detecting surgical margin positivity intraoperatively is not possible, especially in irradiated tissues. In a study by Boers et al,[19] surgical margin negativity could be achieved in 53 out of 61 patients in spite of performing radical surgery. In this respect, frozen section evaluation may be guiding; however, it is not practical and can be misleading.

Our purpose in surgical treatment should be to achieve negative surgical margins; however, ensuring it intraoperatively is difficult in this patient population. The surgeon may perform simple hysterectomy if surgical margin negativity can be ensured. Boers et al[19] reported that radical surgery did not improve survival in patients with central residual tumor and they did not recommend radical surgery for these patients. To sum up, we believe that the type of surgery should be decided according to the intraoperative evaluation. Moreover, initial stage of disease, imaging findings after CRT, and clinical examination findings should be definitely considered. In the present study, attaining negative surgical margins, rather than the operation type, appeared to be important. Surgical marginal positivity that may be encountered while avoiding radical surgery may result in poor prognosis.

Complications

In the present study, this surgical treatment following CRT was not quite pleasing in terms of postoperative complications. Several studies reported acceptable levels of postoperative complication rate, but in the present study, grade 3 complications were observed in 52% of the patients.[16] By examining further, we analyzed the period between the last session of radiotherapy and the surgery administered to the patients. Grade 3 complications had occurred in 3 (33.3%) out of 9 patients when operated within the first 2 months and in 9 (64.3%) out of 14 patients when operated after 2 months. Due to the small number of patients, statistical analysis could not be done; however, the complication rate had increased almost twofold in patients operated after the first 2 months. This might be attributed to the timing of surgery after the development of radiation fibrosis. Fibrosis, defined as the overaccumulation of collagen in tissues due to radiation injury, is a late-term complication of radiotherapy.[20] Complications include difficulty of bleeding control in fibrotic tissue, injury to neighboring organs due to inability to discern tissue planes,

delay in wound healing, and increase of fistula development. Some publications suggested that pentoxifylline or vitamin E were beneficial in the treatment of fibrosis.[21–24]

Depending on the improvements in radiotherapy techniques, a better tissue-radiation dose relationship may result in less tissue damage, especially in disease-free tissues. This may be an explanation for studies in which surgical approach following radiotherapy was acceptable and had no negative impact on morbidity. The frequency of grade 3 complications was not low in our study; however, these complications had no effect on survival. Even though survival was not affected, performing surgery as soon as possible (i.e., before the development of radiation fibrosis) when planned in these patients might be important to prevent complications. In the present study, complication rates were similar in patients with simple hysterectomy and in those with a radical surgery. Grade 3 complications had occurred in 10 out of 17 patients with simple hysterectomy and in 2 out of 4 patients with radical surgery. We believe that a statistical difference could not be demonstrated due to the limited number of our patients. A generally accepted view is that complications increase as surgery is extended. In 34 patients who underwent radical hysterectomy after primary radiotherapy (15 patients for persistent and 19 patients for recurrent disease), Maneo et al[25] estimated the rate of grades 3 and 4 complications to be 44%, the 5-year OS rate to be 49%, and the median survival to be 22 months; they suggested radical surgery as an alternative procedure to exenteration in selected patients.

In the present study, the presence of macroscopic tumor in the pathology specimen was found to be another determinant of recurrence. When evaluating the response to treatment after CRT, residual tumor volume was considered. A large volume of residual tumor is an indicator of poor response and negatively influences survival. In the present study, the median DFS was found to be 11.0 months in the presence of gross tumor after CRT and 16.0 months with microscopic/no tumor (Log-Rank test $p = 0.029$) (►**Fig. 3**). The present study shows that, if surgical margin negativity was attained, the presence of gross tumor was not a risk factor for local recurrence; however, it seemed to be a risk factor for distant recurrences. In our opinion, distant recurrences were not associated with surgery treatment. We think that local recurrences could be linked to surgical treatment. Postoperative systemic chemotherapy to prevent distant recurrences might be argued for. There has been no study on this issue.

In the present study, the pathologic examination had reported no tumor in 21.7% of the patients, tumor cells at microscopic scale in 17.3%, and macroscopic tumor in 60.8%. In spite of being operated due to residual tumor, the pathologic examination had revealed no tumor in 21.7% of the patients. In a similar study, tumor could not be demonstrated in 28% of hysterectomy materials even though preoperative biopsy had revealed it.[19] This fact could be attributed to the fallibility of diagnostic techniques but also to the continuation of tumor regression even until the day of surgery because of the long-term ongoing therapeutic effect of radiotherapy. Indeed, the early phase after radiotherapy is a period in which diagnostic methods including both biopsy and imaging might be misleading, making an accurate diagnosis troublesome. Tumor regression in the course of time is possible. However, during this waiting time, treatment may be delayed in the presence of a real residual tumor, radiation fibrosis may develop, and postoperative complications may increase. We believe that studies about the best method to make the diagnosis most accurately in this period are needed.

Brachytherapy, which is part of CRT, might not be administered for various reasons. Some studies pointed out that hysterectomy could be performed as a completion surgery in this situation.[16–18] In our study, 9 patients could not receive brachytherapy (residual tumor in 7 and closed cervical canal in 2). Out of these patients, 8 had received external boost therapy. In our patient group, whether brachytherapy had been administered or not was found to be insignificant with regard to recurrence (►**Tables 4** and **5**).

The main limitation of the present study included the absence of a comparative group (i.e., patients with a suspicious residual tumor who did not undergo hysterectomy). However, this problem may be overcome by comparing two groups with randomized prospective studies, but such a study is currently absent.

Conclusion

To conclude, clinical experience in the population of LACC patients with CRT-resistance is not sufficient and there is no recommended standard treatment. We think that the treatment should be personalized. Simple hysterectomy and radical hysterectomy are options. However, grades 3 and 4 complication rate of performed surgery is high. Diagnostic techniques (imaging or biopsy) may be more misleading in the period following radiotherapy; therefore, the selection of patients who will be referred to surgery is not clear. The presence of macroscopic tumor in the pathology specimen and positive surgical margins are poor prognostic factors. The most important determinant of survival is to achieve negative surgical margins rather than radical surgery or simple hysterectomy.

Contributors
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.

References

1 Chemoradiotherapy for Cervical Cancer Meta-analysis Collaboration. Reducing uncertainties about the effects of chemoradiotherapy for cervical cancer: a systematic review and meta-analysis of individual patient data from 18 randomized trials. J Clin Oncol. 2008;26(35):5802–5812. doi: 10.1200/JCO.2008.16.4368
2 Siegel R, Naishadham D, Jemal A. Cancer statistics, 2013. CA Cancer J Clin. 2013;63(1):11–30. doi: 10.3322/caac.21166

3 American College of Obstetricians and Gynecologists. ACOG practice bulletin. Diagnosis and treatment of cervical carcinomas. Number 35, May 2002. Int J Gynaecol Obstet. 2002;78(1):79–91. doi: 10.1016/s0020-7292(02)90092-5

4 Trimble EL, Harlan LC, Gius D, Stevens J, Schwartz SM. Patterns of care for women with cervical cancer in the United States. Cancer. 2008;113(4):743–749. doi: 10.1002/cncr.23682

5 Koh WJ, Greer BE, Abu-Rustum NR, Apte SM, Campos SM, Cho KR, et al. Cervical Cancer, Version 2.2015. J Natl Compr Canc Netw. 2015;13(4):395–404, quiz 404. Doi: 10.6004/jnccn.2015.0055

6 Kalaghchi B, Abdi R, Amouzegar-Hashemi F, Esmati E, Alikhasi A. Concurrent chemoradiation with weekly paclitaxel and cisplatin for locally advanced cervical cancer. Asian Pac J Cancer Prev. 2016; 17(S3):287–291. doi: 10.7314/apjcp.2016.17.s3.287

7 Varghese SS, Ram TS, Pavamani SP, Thomas EM, Jeyaseelan V, Viswanathan PN. Concurrent chemo-irradiation with weekly cisplatin and paclitaxel in the treatment of locally advanced squamous cell carcinoma of cervix: a phase II study. J Cancer Res Ther. 2014;10(2):330–336. doi: 10.4103/0973-1482.136621

8 Pignata S, Frezza P, Tramontana S, Errone F, Tambaro R, Casella G, et al. Phase I study with weekly cisplatin-paclitaxel and concurrent radiotherapy in patients with carcinoma of the cervix uteri. Ann Oncol. 2000;11(4):455–459. doi: 10.1023/a:1008379922120

9 Keys HM, Bundy BN, Stehman FB, Muderspach LI, Chafe WE, Suggs CL 3rd, et al. Cisplatin, radiation, and adjuvant hysterectomy compared with radiation and adjuvant hysterectomy for bulky stage IB cervical carcinoma. N Engl J Med. 1999;340(15):1154–1161. doi: 10.1056/NEJM199904153401503

10 Eifel PJ, Winter K, Morris M, Levenback C, Grigsby PW, Cooper J, et al. Pelvic irradiation with concurrent chemotherapy versus pelvic and para-aortic irradiation for high-risk cervical cancer: an update of radiation therapy oncology group trial (RTOG) 90-01. J Clin Oncol. 2004;22(5):872–880. doi: 10.1200/JCO.2004.07.197

11 Chung YL, Jian JJM, Cheng SH, Hsieh CI, Tan TD, Chang HJ, et al. Extended-field radiotherapy and high-dose-rate brachytherapy with concurrent and adjuvant cisplatin-based chemotherapy for locally advanced cervical cancer: a phase I/II study. Gynecol Oncol. 2005;97(1):126–135. doi: 10.1016/j.ygyno.2004.12.039

12 Moore DH, Blessing JA, McQuellon RP, Thaler HT, Cella D, Benda J, et al. Phase III study of cisplatin with or without paclitaxel in stage IVB, recurrent, or persistent squamous cell carcinoma of the cervix: a gynecologic oncology group study. J Clin Oncol. 2004; 22(15):3113–3119. doi: 10.1200/JCO.2004.04.170

13 Cetina L, Garcia-Arias A, Candelaria M, Cantú D, Rivera L, Coronel J, et al. Brachytherapy versus radical hysterectomy after external beam chemoradiation: a non-randomized matched comparison in IB2-IIB cervical cancer patients. World J Surg Oncol. 2009;7:19. doi: 10.1186/1477-7819-7-19

14 Dindo D, Demartines N, Clavien PA. Classification of surgical complications: a new proposal with evaluation in a cohort of 6336 patients and results of a survey. Ann Surg. 2004;240(2): 205–213. doi: 10.1097/01.sla.0000133083.54934.ae

15 Walji N, Chue AL, Yap C, Rogers LJ, El-Modir A, Chan KK, et al. Is there a role for adjuvant hysterectomy after suboptimal concurrent chemoradiation in cervical carcinoma? Clin Oncol (R Coll Radiol). 2010;22(2):140–146. doi: 10.1016/j.clon.2009.11.006

16 Rema P, Suchetha S, Kumar A, Ahmed I. The role of adjuvant hysterectomy after radiotherapy in cervical cancer. Indian J Surg. 2015;77(Suppl 3):902–905. doi: 10.1007/s12262-014-1060-3

17 Sarraf Z, Hamedi B, Hooshmand S, Mosalaie A, Robati M, Momtahan M, et al. The effect of extrafascial hysterectomy after completion of external beam radiotherapy for treatment of locally advanced stages (IIB-III) of cervical cancer. Iran Red Crescent Med J. 2013;15(12):e10758. doi: 10.5812/ircmj.10758

18 Ota T, Takeshima N, Tabata T, Hasumi K, Takizawa K. Adjuvant hysterectomy for treatment of residual disease in patients with cervical cancer treated with radiation therapy. Br J Cancer. 2008; 99(8):1216–1220. doi: 10.1038/sj.bjc.6604619

19 Boers A, Arts HJ, Klip H, et al. Radical surgery in patients with residual disease after (chemo)radiation for cervical cancer. Int J Gynecol Cancer. 2014;24(7):1276–1285. doi: 10.1097/IGC.0000000000000171

20 Coia LR, Myerson RJ, Tepper JE. Late effects of radiation therapy on the gastrointestinal tract. Int J Radiat Oncol Biol Phys. 1995;31(5): 1213–1236. doi: 10.1016/0360-3016(94)00419-L

21 Chiao TB, Lee AJ. Role of pentoxifylline and vitamin E in attenuation of radiation-induced fibrosis. Ann Pharmacother. 2005;39 (3):516–522. doi: 10.1345/aph.1E186

22 Hamama S, Gilbert-Sirieix M, Vozenin MC, Delanian S. Radiation-induced enteropathy: molecular basis of pentoxifylline-vitamin E anti-fibrotic effect involved TGF-β1 cascade inhibition. Radiother Oncol. 2012;105(3):305–312. doi: 10.1016/j.radonc.2012.08.023

23 Jacobson G, Bhatia S, Smith BJ, Button AM, Bodeker K, Buatti J. Randomized trial of pentoxifylline and vitamin E vs standard follow-up after breast irradiation to prevent breast fibrosis, evaluated by tissue compliance meter. Int J Radiat Oncol Biol Phys. 2013;85(3):604–608. doi: 10.1016/j.ijrobp.2012.06.042

24 Okunieff P, Augustine E, Hicks JE, Cornelison TL, Altemus RM, Naydich BG, et al. Pentoxifylline in the treatment of radiation-induced fibrosis. J Clin Oncol. 2004;22(11):2207–2213. doi: 10.1200/JCO.2004.09.101

25 Maneo A, Landoni F, Cormio G, Colombo A, Mangioni C. Radical hysterectomy for recurrent or persistent cervical cancer following radiation therapy. Int J Gynecol Cancer. 1999;9(4):295–301. doi: 10.1046/j.1525-1438.1999.99037.x

The Effect of Testosterone Replacement on Intramedullary, Inguinal and Visceral Fat in Ovariectomized Rats

Efeito da reposição de testosterona na gordura intramedular, inguinal e visceral em ratas ovariectomizadas

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Abstract

Objective The present article aims to evaluate the impact of testosterone treatment on the expansion of visceral, subcutaneous and intramedullary adipose tissue of ovariectomized rats and the visceral and subcutaneous fat expression of peroxisome proliferator-activated receptors (PPARs) gamma.

Methods In total 48 female Wistar rats were castrated and randomly divided into 6 treatment groups: group E2 was submitted to estradiol 5 µg/day; group T, to testosterone 5 µg/day; group E2 + T, to estradiol 5 µg/day + testosterone 5 µg/day; group TT, to testosterone 30 µg/day; group E2 + TT, to estradiol 5 µg/day + testosterone 30 µg/day; and placebo was administered to group P. After 5 weeks, the rats were euthanized, the inguinal and visceral adipose tissues were harvested, weighted, and had their PPAR gamma expression evaluated by reverse transcription quantitative polymerase chain reaction (RT-qPCR). The right femurs were harvested and histologically prepared to perform the number count of the intramedullary adipocytes.

Results The expansion of visceral fat tissue was much higher in the TT group when compared with other treated groups ($p < 0.001$). The TT group also showed a higher expansion of inguinal fat ($p < 0.01$), and groups E2 + T and E2 + TT presented lower growth compared to the P group ($p < 0.01$). The number of femur intramedullary adipocytes only showed significant differences between groups TT and E2 + TT ($p < 0.05$). The expression of PPAR gamma showed no differences among the groups.

Conclusion The use of testosterone in high doses leads to an important expansion in both visceral and inguinal adipose tissues. Association with estradiol exerts an expansion-repressive effect on the visceral and inguinal adipose tissues.

Keywords
- testosterone
- estradiol
- adipose tissue
- postmenopause

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Resumo

Objetivo Este artigo tem como objetivo avaliar o impacto do tratamento com testosterona na expansão dos tecidos adiposos visceral, subcutâneo e intramedular de ratas ovariectomizadas e a expressão de receptores ativados por proliferadores de peroxissoma (RAPPs) gama nas gorduras visceral e subcutânea.

Métodos No total, 48 ratas Wistar foram castradas e divididas aleatoriamente em 6 grupos de tratamento: o grupo E2 recebeu estradiol 5 µg/dia; o grupo T, testosterona 5 µg/dia; o grupo E2 + T, estradiol 5 µg/dia + testosterona 5 µg/dia; o grupo TT, testosterona 30 µg/dia; o grupo E2 + TT, estradiol 5 µg/dia + testosterona 30 µg/dia; e o grupo P recebeu placebo. Após 5 semanas, as ratas foram submetidas a eutanásia, o tecido adiposo inguinal e visceral foi coletado, pesado, e se avaliou a expressão dos RAPP gama por reação em cadeia da polimerase via transcriptase reversa quantitativa (RCP-TRq). Os ossos do fêmur direito foram colhidos e processados histologicamente para contagem de números de adipócitos intramedulares.

Resultados A expansão do tecido adiposo visceral foi muito maior no grupo TT quando comparado a outros grupos tratados ($p < 0{,}001$). O grupo TT também apresentou maior expansão da gordura inguinal ($p < 0{,}01$), e os grupos E2 + T e E2 + TT apresentaram menor crescimento em relação ao grupo P ($p < 0{,}01$). O número de adipócitos intramedulares no fêmur mostrou apenas diferenças significativas entre os grupos TT e E2 + TT ($p < 0{,}05$). A expressão de RAPP gama não mostrou diferenças entre os grupos.

Conclusão O uso de testosterona em altas doses leva a uma importante expansão nos tecidos adiposos visceral e inguinal. A associação com o estradiol exerce um efeito repressivo de expansão nos tecidos adiposos visceral e inguinal.

Palavras-chave

- testosterona
- estradiol
- tecido adiposo
- pós-menopausa

Introduction

Sexual hormones are involved in the balance of energy, and they play essential roles in the control of food intake, energy metabolism and body weight.[1] Body changes observed during the transition to menopause and in the postmenopausal period, with increasing body weight and a different pattern of fat distribution (transfer of the main fat storage from the femoral-gluteal region to the abdominal region) are examples of this involvement.[2,3]

The cell mechanisms implicated in this kind of change are not yet completely clear.[4,5] What has been revealed is that estrogen affects energy metabolism in a genomic manner via the estrogen receptor (ER) or G protein-coupled estrogen receptors (GPERs).[6] Modulation of these receptors determines the action of anti-lipogens, the increase in insulin sensitivity, glucose tolerance, and the decrease in body weight and visceral mass.[1,6] Androgen receptors (ARs) are also present in the fat tissue, but there is less evidence on their effect.[7,8] Some evidences associate androgens to lipogenesis stimulation and lipolysis inhibition on white visceral fat.[1,8]

Estrogen replacement during the postmenopausal period is associated with the reduction in visceral fat, the distribution of android fat mass and lower body mass index (BMI).[9,10] These effects determine a favorable metabolic profile with less risk of diabetes and mortality.[11,12]

Different from estrogen, the impact resulting from androgen replacement on fat tissue is less often applied. Few clinical trials,[8,13,14] most of them using heterogeneous methods, have evaluated the outcomes of this therapy among women during the postmenopausal period.

Some concerns regarding the impacts of androgen replacement therapy in women's health consider that supraphysiological doses may determine an inflammatory response on the fat tissue. Such response, especially at a visceral site, is associated with diseases such as resistance to insulin, dyslipidemia, diabetes, cardiovascular diseases and stroke.[1,6]

The presents paper has the aim of studying the effects on fat tissue of doses of physiological and supraphysiological testosterone associated or nor with estradiol. The authors aim to show that high doses of testosterone define an unhealthy expansion of the visceral, subcutaneous and intramedullary fat tissues.

Methods

Trial Design

In total 48 Wistar female rats (*Rattus norvegicus albinus*) were offered and cared for by the animal research facility of Faculdade de Medicina do ABC, Brazil. The animals were fed using Nuvilab CR1 (NuVital Health, Long Beach, NY, US) and water "ad libitum," properly filtered in a feeding bottle. Artificial lighting was controlled to obtain light/dark cycles of 12 hours each, and temperature between 20 and 28°C, between 60% and 85% of air exchange/hour.

The animals underwent bilateral ovariectomy surgeries (OVXs). After the OVX, the animals underwent vaginal colpocytology on a daily basis for two months. Colpocytology was used to assess the cessation of the estrous cycle to evaluate the possibility of hypoestrogenism.

To confirm the possibility of hypoestrogenism, colpocytology was considered in the diestrus phase for five days in a row.

Then, the animals were randomly divided into 6 groups consisting of 8 animals each. Each group underwent the following hormone treatment: group P: placebo; group E2 + T: estradiol (E2) 5 µg/female rat/day + testosterone 5 µg/female rat/day; group T: testosterone 5 µg/female rat/day; group E2 + TT: estradiol (E2) 5 µg/female rat/day + testosterone 30 µg/female rat/day; group TT: testosterone 30 µg/female rat/day; and group E2: estradiol (E2) 5 µg/female rat/day.

The dose of testosterone dose was calculated to be 6 times higher than that of estradiol. The rationality on this is that most commercial estradiol transdermal patches for women release 50 mcg/day, and the only testosterone transdermal patch ever marketed (Intrinsa, Warner Chilcott UK Ltda, Milbrook, Larne, UK) releases 300 mcg/day (6 times lower).

During 5 weeks, each group was submitted to a corresponding hormone dose based on the daily volume of 0,1 mL, which was applied by subcutaneous injections in the dorsal region. The hormones were prepared and then diluted in sesame oil, Sesame oil was also used by itself as a placebo.

The entire experiment was approved by the Animal Experimentation Ethics Committee of Faculdade de Medicina do ABC (CEUA-FMAB, in Portuguese), under number 01/2016.

Material Collection

A few moments before the euthanasia, a blood sample was collected for glycaemia tests. After the euthanasia, the animals had their right femurs collected, as well as their visceral adipose and inguinal tissues. The femurs were kept in a 10% formaldehyde solution for the histological and morphometric analyses. Adipose tissues were weighted and stored in – 80°C for future analysis of peroxisome proliferator-activated receptors (PPARs) gamma data using the real-time polymerase chain reaction (PCR) technique.

Histology

The femurs were fixed with 10% formaldehyde during 24 hours, and then they were decalcified in a 7% ethylenediaminetetraacetic acid (EDTA) solution with 2% paraformaldehyde in a 0.1-M phosphate buffer (pH 7.4) during 160 days at room temperature. The samples were dehydrated in graded concentrations of ethanol, and then they were embedded in paraffin. Serial 7-µm sections were made using a manual Leica RM-2245 (Leica Biosystems, Nussloch, Germany) microtome, and they were stained with hematoxylin and eosin (H&E).

Morphometry

The morphometric analysis for the estimation of the volume density (Vv) of the intramedullary adipocytes selected five photomicrographs of each group at a magnification of 100X . For the evaluation of the adipocyte Vv, crosshairs of points superimposed on photomicrographs were also needed, and the relation proposed by Weibel was used:[15]

Vv = P1/P, where
Vv = volume density of a given component;
P1 = number of incident points on the component studied;
P = total of incident points on the volume unit

PPAR Gamma Gene Expression RT-qPCR

The total RNA was extracted from ~ 1 cm^2 of adipose tissue using QIAzol lysis reagent (Qiagen, Hilden, Germany). The amount of RNA was determined using NanoDrope (Thermo Scientific, Waltham, MA, US) spectroscopy, and diluted to a final concentration of 50 ηg/µl in 20 µl. In total, 1 µl of RNA was used for the synthesis and amplification of complementary DNA (cDNA), which followed the protocol of the high-capacity RNA-to-cDNA kit (Applied Biosystems, Foster City, CA, US). Reverse transcription quantitative polymerase chain reaction (RT-qPCR) was performed using the PPAR gamma gene (*Mm00440940_m1*), and the endogenous control GAPDH (*Mm99999915_g1*) followed the TaqMan Universal PCR Master Mix Kit (Applied Biosystems) using the StepOne Real-Time PCR System (Life Technologies, Foster City, CA, US). Real-time PCR reactions were conducted as follows: after a pre-denaturation and polymerase-activation program (2 minutes at 50°C and 10 minutes at 95°C), 50 cycles, each one consisting of 95°C for 15 seconds and of 60°C for 1 minute. The negative controls consisted of wells in which the cDNA was absent. The relative expression of PPAR gamma/GAPDH was calculated using the equation ΔCt, which expresses the difference between the number of threshold cycles (Cts) of the target genes and the endogenous control.

Statistical Analysis

The results were calculated and analyzed by one-way analysis of variance (ANOVA) test and the Tukey Test using the GraphPad Prism 5 (GraphPad Software, Inc., San Diego, CA, US) software. Results ≤ 0.05 ($p < 0.05$) were considered relevant.

Results

By the end of the fifth week of treatment, we noticed that the average body weight was different among the groups ($p = 0.018$), considering that group TT reached a higher final average weight of 307 ± 11.8 g, and group E2 + TT presented the lowest average, 264 ± 6.9 g (►**Table 1**).

The average visceral fat weight was different among groups ($p < 0.0001$), and was observed to be much higher in group TT when compared with the other groups. Nevertheless, regarding inguinal fat, the only group with a higher average weight than that of group TT was group P. On the other hand, the groups that have were submitted to both estrogen and testosterone presented an average weight in this particular fat region that was considerably smaller than that of group P ($p < 0.001$), as seen in ►**Fig. 1A** and **B**. By using the micrometer, the number of adipocytes expressed in the intramedullary region was shown to differ among groups

Table 1 Comparison of the final weight and the visceral and inguinal fat of the study groups

	Groups (mean ± standard error of the mean)						
	P	E2	T	E2 + T	TT	E2 +TT	*p*-value
Final weight (g)	291 ± 10.1	273 ± 5.3	271 ± 8.6	284 ± 8.5	307 ± 11.8	264.7 ± 6.9	0.018
Visceral fat (g)	6.5 ± 0.7	5.3 ± 0.5	3.8 ± 0.3	3.9 ± 0.7	10.3 ± 1.2*	4.5 ± 0.7	< 0.01
Inguinal fat (g)	2.6 ± 0.22***	2 ± 0.22	1.7 ± 0.13	1.4 ± 0.17	3.1 ± 0.2**	1.4 ± 0.13	< 0.01

Abbreviations: group E2, estradiol 5 µg/day; group E2 + T, estradiol 5 µg/day + testosterone 5 µg/day; group E2 + TT, estradiol 5 µg/day + testosterone 30 µg/day; group T, testosterone 5 µg/day; group TT, testosterone 30 µg/day; group P, placebo.
Notes: Tukey test: * = $p < 0.05$ (TT versus E2, T, E2 + T e E2 + TT); ** = $p < 0.05$ (TT versus T, E2 + T e E2 + TT); *** = $p < 0.05$ (P versus E2 + T e E2 + TT).

($p = 0.012$), presenting a significant difference between groups TT and E2 + TT ($p < 0.05$) (►**Fig. 1C** and ►**Fig. 2**).

The PPAR gamma data did now show any statistical difference among the groups in any of the adipose tissues analyzed. Despite that, a different behavior was noticed in terms of data amongt tissues: regarding the subcutaneous tissues, the groups submitted to isolated testosterone doses presented a higher average number than group; P regarding the visceral tissue, all groups presented lower numbers than group P (►**Fig. 3A** and **B**).

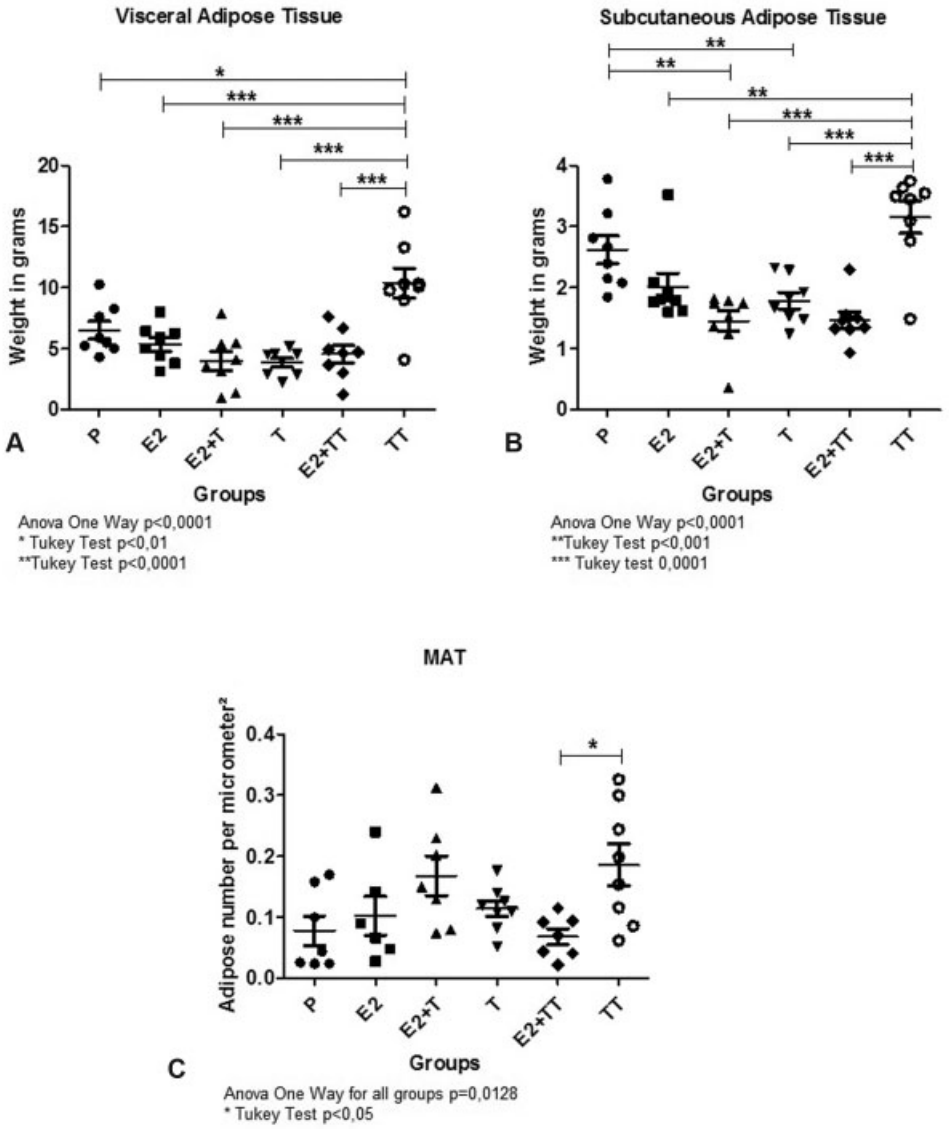

Fig. 1 (**A**) Weight of the visceral and (**B**) subcutaneous fat tissues of all treatment groups. (**C**) Distribution of the number of intramedullary adipocytes in all treatment groups. * Represents the results with significance, that is, $p < 0.05$.

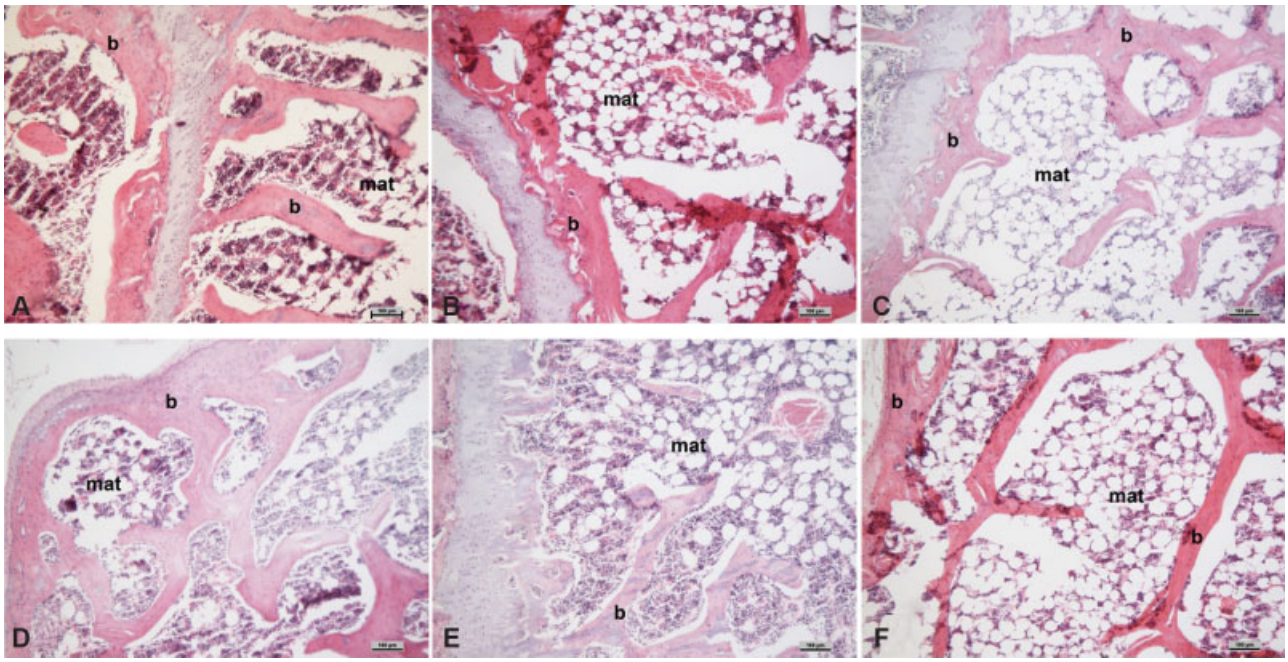

Fig. 2 Photomicrograph of the tibiae (b) with marrow adipose tissue (MAT) stained with hematoxylin and eosin (H&E). (**A**) Group P; (**B**) group E2 + T; (**C**) group T; (**D**) group E2 + TT; (**E**) group TT; (**F**) group E2.

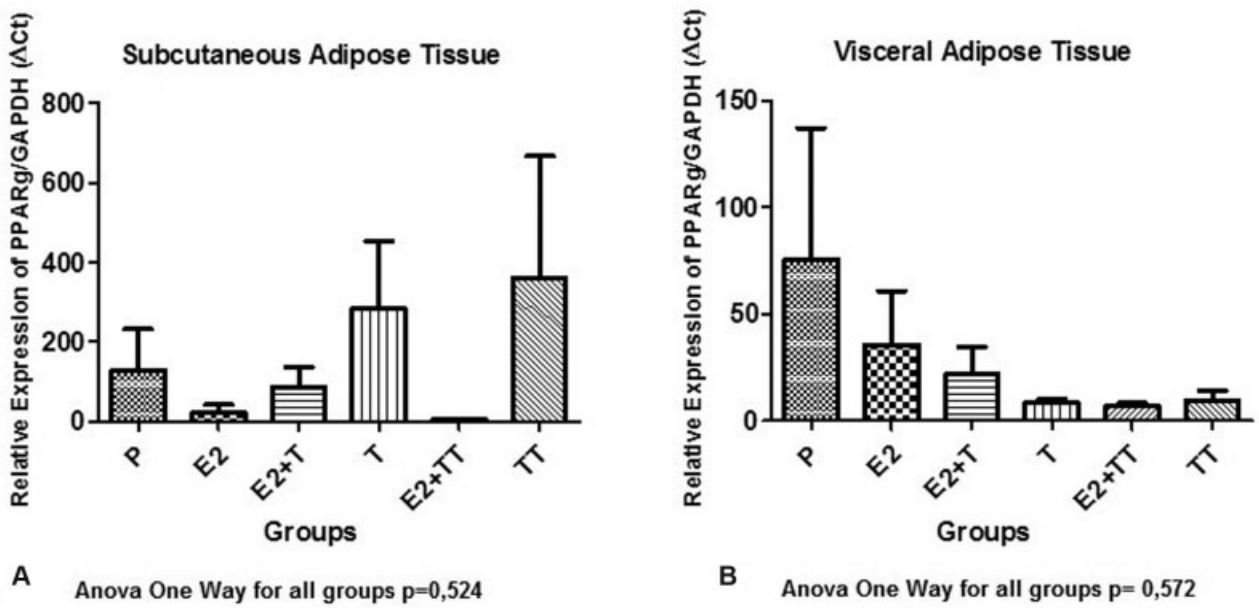

Fig. 3 Expression of the peroxisome proliferator-activated receptor (PPAR) gamma gene in the subcutaneous and visceral adipose tissues in all treatment groups.

Discussion

The present study assessed the impact of the treatment on visceral, subcutaneous and intramedullary fat tissues in 30-week-old female ovariectomized rats.

Two daily doses of testosterone were applied. One of the doses contained an equivalent volume of estradiol (5 µg/day), and the other dose contained a volume of estradiol 6 times higher (30 µg/day) for over 30 days. The group treated with high doses of testosterone showed a relevant visceral and subcutaneous fat growth in comparison to the other groups, which is something that contributed to a heavier final weight in this group. In a different manner, the group submitted to the lower dose showed fat growth similar to the control groups (placebo and isolated estradiol).

The testosterone serum levels show a direct link with the growth in visceral fat tissue in women going through postmenopause.[16–18] However, there are few studies assessing the effects of androgen replacement in fat tissue during this period of a woman's life.[13,14,16–20] Despite applying a heterogeneous

method, evidence confirms the idea that postmenopausal women using androgen have increased lean mass and visceral fat.[1,3,14,19–22]

Androgen supplementation in animals has been observed to increase visceral fat.[23–25] There is a hypothesis that the estrogen serum level influence on this effect.[24] Iwasa et al[25] have evaluated such effect in female rats which have undergone ovariectomy regarding the chronic doses of testosterone (associated or not to estradiol) and its link to food intake, body weight and white fat weight.

Results found by Iwasa et al[25] are conflicting with our results. In comparison with groups treated with testosterone or not and with no association with estrogen, Iwasa et al[25] observed a relevant reduction in the final weight, as well as in the weight of visceral and subcutaneous fat in the group treated with testosterone. Their study concluded that testosterone, once used apart from other elements, had an inhibitory effect on weight gain and adiposity. This conclusion was not in line with the results of the group treated with high doses of testosterone in our study, since the high dose determined a considerable increase in adiposity. Moreover, the group submitted to low doses of testosterone did now show any differences when compared with the control groups.

Iwasa et al[25] also observed that the association of testosterone to estrogen in ovariectomized female rats determined an increase in body weight and in the weight of visceral and subcutaneous fat. Their study concluded that testosterone lowered the inhibitory effect of estrogen on body weight and adiposity. Despite that, our study, using a different method, showed that the groups treated with estrogen had similar gains regarding weight and adiposity, independent of testosterone. However, it is worth nothing that the groups treated with both kinds of hormone have shown a relevant lower subcutaneous fat weight in comparison to group P, which is something that did not occur with the group treated with isolated estrogen.[25]

The difference in results of the study by Iwasa et al[25] and our study can be explained based on the period of treatment (35 days *versus* 16 days respectively) and the testosterone doses used. Considering the results from both studies, it can be said that high doses of testosterone can stimulate excessive adipogenesis, while low doses in association with estradiol can determine its inhibition.

The intramedullary fat tissue represents 70% of the total volume of bone marrow in a healthy young adult,[26] being the third largest fat storage in the human body. The percentage of its contribution to the total volume of body adipose tissue may vary from 1% to 30%.[26–28] Such tissue presents structural characteristics and lineage specific traits, which suggests that marrow adipose tissue (MAT) adipocytes have a source different from that of white and brown fat.[28] Therefore, it is interesting to observe any possible impacts resulting from the testosterone treatment on this fat tissue.

When assessing the number of adipocytes in the bone marrow (which represents the amount of fat in this region), an increase in the average number of adipocytes could be noticed in the group treated with a high dose of testosterone, despite the difference not being statistically relevant in relation to the control groups. Thereby, this fat tissue site showed a behavior pattern similar to that of other fat sites when treated with sex hormones.

It is worth saying, though, that the number of adipocytes was calculated based on the entire femurs, and did not reveal any differences between the proximal and distal regions. Such information is relevant when considering that there may be two forms of MAT: variable (regulated - rMAT) and constant (constitutive - cMAT). The difference between them is based on the bone marrow region and on the different response to external stimuli. Besides, there are different kinds of development pattern, adipocyte size, lipid saturation, and expression of transcription factors. The rMAT form is found inside the red marrow (proximal region of the skeleton), and contains more saturated fat and has a higher sensibility to external stimuli. The cMAT, on the other hand, is located inside the yellow marrow (distal region of the skeleton), and presents more resistance to external stimuli.[27]

The number of intramedullary adipocytes was calculated, and it was diffused in most groups, reducing the possibility to determine the difference between them. Besides that, there is evidence that ovariectomized rats have an increase in MAT,[27,28] something not showed in our study. The accuracy of the methodology for the histological evaluation of the adipocytes was questioned,[29] and could be the reason for such an incoherent result. The coloration using osmium tetroxide and further analysis using micro computed tomography (micro-CT) is considered the standard procedure.[29] However, the risks of the toxicity related to osmium tetroxide and the lack of a microtomograph kept the team from applying such methods.

The PPARs influence the gene network expression involved in adipogenesis, lipidic metabolism, inflammation, and the maintenance of metabolic homeostasis. The PPAR gamma specifically acts as a regulator on adipogenic media, being considered a fundamental regulator for adipocyte differentiation.[30]

Estrogen has an impact on the transcriptional activity of PPRA gamma, and it inhibits its effect on adipocyte differentiation.[31] On the other hand, the effect of androgen over the activity of PPAR gamma on the fat tissue has not been determined. Compatible to what is mentioned in the technical literature, the groups treated with estrogen in the present study revealed a reduced PPAR gamma expression when compared with the control groups in both assessed fat tissues.[31]

The pattern of expression of PPRA gamma varied according to the location of the fat in the groups treated with testosterone. Regarding subcutaneous fat, despite not being statistically significant, the expression was higher than that of the control groups. When considering visceral fat, it was lower in both groups.

Considering that PPRA gamma is involved in adipogenesis, it is expected that its expression would be higher in those groups with higher adipogenesis.[30] This occurred in subcutaneous fat, and the TT group presented a higher fat growth as well as the highest expression. Nevertheless, this did not occur in visceral fat, in which the PPRA gamma expression was inhibited. Dysfunctional visceral adipose expansion

results in an inflammatory state and increases the release of inflammatory cytokines and free fatty acids,[32] which worked as an inhibitor to the activity of PPRA gamma.[33,34]

The present study has some limitations. An individual assessment of each region of the bone marrow could determine different results regarding intramedullary adipose tissue, but this was not possible due to technical reasons. Moreover, the coloration of the bone marrow using osmium tetroxide and further analysis using micro-CT is considered to be the standard procedure for MAT analysis.[29] However, the risks of the toxicity related to osmium tetroxide and the lack of a microtomograph kept the team from applying such methods in the present study.

Conclusion

High doses of testosterone replacement in OVX rats lead to an expansion of visceral, subcutaneous and bone marrow fat. This phenomenon seems to be abrogated by estradiol replacement. The increase in visceral fat is not linked to an increased PPAR gamma expression.

Contributors

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 to interests

The authors have no conflict of interests to declare.

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References

1 Hirschberg AL. Sex hormones, appetite and eating behaviour in women. Maturitas. 2012;71(3):248–256. doi: 10.1016/j.maturitas.2011.12.016
2 Lovejoy JC, Champagne CM, de Jonge L, Xie H, Smith SR. Increased visceral fat and decreased energy expenditure during the menopausal transition. Int J Obes. 2008;32(6):949–958. doi: 10.1038/ijo.2008.25
3 Douchi T, Yamamoto S, Nakamura S, Ijuin T, Oki T, Maruta K, et al. The effect of menopause on regional and total body lean mass. Maturitas. 1998;29(3):247–252. doi: 10.1016/s0378-5122(98)00035-8
4 Zsakai A, Karkus Z, Utczas K, Biri B, Sievert LL, Bodzsar EB. Body fatness and endogenous sex hormones in the menopausal transition. Maturitas. 2016;87:18–26. doi: 10.1016/j.maturitas.2016.02.006
5 Varlamov O, White AE, Carroll JM, Bethea CL, Reddy A, Slayden O, et al. Androgen effects on adipose tissue architecture and function in nonhuman primates. Endocrinology. 2012;153(7):3100–3110. doi: 10.1210/en.2011-2111
6 Blüher M. Importance of estrogen receptors in adipose tissue function. Mol Metab. 2013;2(3):130–132. doi: 10.1016/j.molmet.2013.07.001
7 Dieudonne MN, Pecquery R, Boumediene A, Leneveu MC, Giudicelli Y. Androgen receptors in human preadipocytes and adipocytes: regional specificities and regulation by sex steroids. Am J Physiol. 1998;274(6):C1645–C1652. doi: 10.1152/ajpcell.1998.274.6.C1645
8 Newell-Fugate AE. The role of sex steroids in white adipose tissue adipocyte function. Reproduction. 2017;153(4):R133–R149. doi: 10.1530/REP-16-0417
9 Chen Z, Bassford T, Green SB, Cauley JA, Jackson RD, LaCroix AZ, et al. Postmenopausal hormone therapy and body composition–a substudy of the estrogen plus progestin trial of the Women's Health Initiative. Am J Clin Nutr. 2005;82(3):651–656. doi: 10.1093/ajcn.82.3.651
10 Papadakis GE, Hans D, Rodriguez EG, Vollenweider P, Waeber G, Marques-Vidal P, et al. Menopausal hormone therapy is associated with reduced total and visceral adiposity: the OsteoLaus Cohort. J Clin Endocrinol Metab. 2018;103(5):1948–1957. doi: 10.1210/jc.2017-02449
11 Manson JE, Aragaki AK, Rossouw JE, Anderson GL, Prentice RL, LaCroix AZ, et al. Menopausal hormone therapy and long-term all-cause and cause-specific mortality: The Women's Health Initiative Randomized Trials. JAMA. 2017;318(10):927–938. doi: 10.1001/jama.2017.11217
12 Gartlehner G, Patel SV, Feltner C, Weber RP, Long R, Mullican K, et al. Hormone therapy for the primary prevention of chronic conditions in postmenopausal women: evidence report and systematic review for the US Preventive Services Task Force. JAMA. 2017;318(22):2234–2249. doi: 10.1001/jama.2017.16952
13 Leão LM, Duarte MP, Silva DM, Bahia PR, Coeli CM, de Farias ML. Influence of methyltestosterone postmenopausal therapy on plasma lipids, inflammatory factors, glucose metabolism and visceral fat: a randomized study. Eur J Endocrinol. 2006;154(1):131–139. doi: 10.1530/eje.1.02065
14 Davis SR, Walker KZ, Strauss BJ. Effects of estradiol with and without testosterone on body composition and relationships with lipids in postmenopausal women. Menopause. 2000;7(6):395–401.
15 Fonseca NL Junior, Petri G, Veridiano JM, Rehder JRCL. Alteração do tecido conjuntivo orbitário após aplicação de bimatoprost: estudo experimental em ratos. Rev Bras Oftalmol. 2016;75(4):300–307. doi: 10.5935/0034-7280.20160060
16 Janssen I, Powell LH, Crawford S, Lasley B, Sutton-Tyrrell K. Menopause and the metabolic syndrome: the Study of Women's Health Across the Nation. Arch Intern Med. 2008;168(14):1568–1575. doi: 10.1001/archinte.168.14.1568
17 Guthrie JR, Dennerstein L, Taffe JR, Ebeling PR, Randolph JF, Burger HG, et al. Central abdominal fat and endogenous hormones during the menopausal transition. Fertil Steril. 2003;79(6):1335–1340. doi: 10.1016/s0015-0282(03)00361-3
18 Janssen I, Powell LH, Kazlauskaite R, Dugan SA. Testosterone and visceral fat in midlife women: the Study of Women's Health Across the Nation (SWAN) fat patterning study. Obesity (Silver Spring). 2010;18(3):604–610. doi: 10.1038/oby.2009.251
19 Lovejoy JC, Bray GA, Bourgeois MO, Macchiavelli R, Rood JC, Greeson C, et al. Exogenous androgens influence body composition and regional body fat distribution in obese postmenopausal women–a clinical research center study. J Clin Endocrinol Metab. 1996;81(6):2198–2203. doi: 10.1210/jcem.81.6.8964851
20 Huang G, Basaria S, Travison TG, Ho MH, Davda M, Mazer NA, et al. Testosterone dose-response relationships in hysterectomized women with or without oophorectomy: effects on sexual function, body composition, muscle performance and physical function in a randomized trial. Menopause. 2014;21(6):612–623. doi: 10.1097/GME.0000000000000093

21 Zang H, Rydén M, Wåhlen K, Dahlman-Wright K, Arner P, Lindén Hirschberg A. Effects of testosterone and estrogen treatment on lipolysis signaling pathways in subcutaneous adipose tissue of postmenopausal women. Fertil Steril. 2007;88(1):100–106. doi: 10.1016/j.fertnstert.2006.11.088
22 Zang H, Carlström K, Arner P, Hirschberg AL. Effects of treatment with testosterone alone or in combination with estrogen on insulin sensitivity in postmenopausal women. Fertil Steril. 2006;86(1):136–144. doi: 10.1016/j.fertnstert.2005.12.039
23 Nohara K, Laque A, Allard C, Münzberg H, Mauvais-Jarvis F. Central mechanisms of adiposity in adult female mice with androgen excess. Obesity (Silver Spring). 2014;22(6):1477–1484. doi: 10.1002/oby.20719
24 Iwasa T, Matsuzaki T, Tungalagsuvd A, Munkhzaya M, Yiliyasi M, Kato T, et al. Effects of chronic testosterone administration on body weight and food intake differ among pre-pubertal, gonadal-intact, and ovariectomized female rats. Behav Brain Res. 2016; 309:35–43. doi: 10.1016/j.bbr.2016.04.048
25 Iwasa T, Matsuzaki T, Yiliyasi M, Yano K, Irahara M. The effects of chronic testosterone administration on body weight, food intake, and fat weight were age-dependent. Steroids. 2017;127:18–23. doi: 10.1016/j.steroids.2017.08.014
26 Fazeli PK, Horowitz MC, MacDougald OA, Scheller EL, Rodeheffer MS, Rosen CJ, et al. Marrow fat and bone–new perspectives. J Clin Endocrinol Metab. 2013;98(3):935–945. doi: 10.1210/jc.2012-3634
27 Cawthorn WP, Scheller EL, Learman BS, Parlee SD, Simon BR, Mori H, et al. Bone marrow adipose tissue is an endocrine organ that contributes to increased circulating adiponectin during caloric restriction. Cell Metab. 2014;20(2):368–375. doi: 10.1016/j.cmet.2014.06.003
28 Scheller EL, Cawthorn WP, Burr AA, Horowitz MC, MacDougald OA. Marrow adipose tissue: trimming the fat. Trends Endocrinol Metab. 2016;27(6):392–403. doi: 10.1016/j.tem.2016.03.016
29 Scheller EL, Troiano N, Vanhoutan JN, Bouxsein MA, Fretz JA, Xi Y, et al. Use of osmium tetroxide staining with microcomputerized tomography to visualize and quantify bone marrow adipose tissue in vivo. Methods Enzymol. 2014;537:123–139. doi: 10.1016/B978-0-12-411619-1.00007-0
30 Ahmadian M, Suh JM, Hah N, Liddle C, Atkins AR, Downes M, et al. PPARγ signaling and metabolism: the good, the bad and the future. Nat Med. 2013;19(5):557–566. doi: 10.1038/nm.3159
31 Jeong S, Yoon M. 17β-Estradiol inhibition of PPARγ-induced adipogenesis and adipocyte-specific gene expression. Acta Pharmacol Sin. 2011;32(2):230–238. doi: 10.1038/aps.2010.198
32 Alexopoulos N, Katritsis D, Raggi P. Visceral adipose tissue as a source of inflammation and promoter of atherosclerosis. Atherosclerosis. 2014;233(1):104–112. doi: 10.1016/j.atherosclerosis.2013.12.023
33 Nagy ZS, Czimmerer Z, Szanto A, Nagy L. Pro-inflammatory cytokines negatively regulate PPARγ mediated gene expression in both human and murine macrophages via multiple mechanisms. Immunobiology. 2013;218(11):1336–1344. doi: 10.1016/j.imbio.2013.06.011
34 Ye J. Regulation of PPARgamma function by TNF-alpha. Biochem Biophys Res Commun. 2008;374(3):405–408. doi: 10.1016/j.bbrc.2008.07.068

A Comprehensive Integrative Review of the Factors Associated with Spontaneous Preterm Birth, Its Prevention and Prediction, Including Metabolomic Markers

Uma revisão integrativa abrangente dos fatores associados ao parto prematuro espontâneo, sua prevenção e predição, incluindo marcadores metabolômicos

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Abstract

Preterm birth is a major maternal complication that has a great impact on perinatal and neonatal health, with consequences suffered during childhood and adulthood. Little is known about its etiology and development, resulting in poor screening, prediction and preventive methods. The present integrative review discusses the current knowledge regarding some risk factors for preterm birth, the differences between screening and prediction methods, the limitations of some current preventive interventions, the importance of applying standardized concepts for exposures and outcomes, and why it is important to develop more accurate and reproducible methods to predict preterm birth. In addition, the authors introduce the concept of metabolomics and the technology involved in this technique, and discuss about how it has become a promising approach to identify biomarkers for spontaneous preterm birth.

Keywords
- preterm labor
- premature rupture of fetal membranes
- risk factors
- screening programs
- perinatology
- metabolomics

Resumo

Parto prematuro é uma complicação obstétrica de grande impacto para saúde perinatal e neonatal, tendo consequências também para a infância e a vida adulta. Pouco se sabe sobre sua etiologia e fatores determinantes, o que limita os métodos de rastreamento, predição e prevenção. Esta revisão integrativa traz a discussão sobre o conhecimento atual sobre fatores de risco para parto prematuro espontâneo, as diferenças entre métodos de rastreamento e predição, as limitações das atuais intervenções preventivas, a importância de se aplicar conceitos padronizados para exposição e desfecho na investigação de parto prematuro espontâneo, e porque é importante desenvolver métodos precisos e reprodutíveis para predizer o parto prematuro. Por fim, introduzimos o conceito de metabolômica e da tecnologia envolvida nessa técnica, e discutimos como ela tem se mostrado uma abordagem prosmissora para identificar biomarcadores associados ao parto prematuro espontâneo.

Palavras-chave
- trabalho de parto prematuro
- ruptura prematura de membranas fetais
- fatores de risco
- programas de rastreamento
- perinatologia
- metabolômica

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Introduction

Definition and Impact of Prematurity

The birth of a preterm baby may have diverse negative consequences for the baby him/herself, (his or her) neonatal life, childhood and adulthood, the family, the healthcare system/service, and society as a whole. The present manuscript focuses on the factors associated with preterm birth, the perinatal outcomes and the ways to predict them, supported by the hypothesis that it is possible to better understand and predict the preterm birth process, creating opportunities for increased effectiveness in the prevention of this condition.

It took several decades to consolidate the definition of preterm birth. At the beginning of the 20th century, newborns weighing less than 2,500 g at birth were considered preterm, based primarily on neonatal behavior and progression to neonatal mortality, intracranial hemorrhage and other morbid conditions.[1] In 1950, a group of experts from the World Health Organization (WHO) published a technical report[2] defining preterm newborns as those weighing less than 2,500 g at birth, or those born before 37 weeks of gestation. In this document, the WHO established two priorities for the promotion of research and specific programs aimed at minimizing the consequences of preterm birth: prevention and preterm infant care.

Preterm birth may be classified according to the clinical presentation or the motivator: spontaneous, when due to spontaneous preterm labor (contractions, cervical effacement and dilatation) or preterm premature rupture of the membranes (P-PROM); and therapeutic, when theoretically there is a condition that poses a risk to the mother and/or the fetus, generating sufficient motivation for resolution at a preterm gestational age.[3] Furthermore, iatrogenic preterm birth is defined as birth due to therapeutic intervention without the existence of sufficient risks to justify any intervention, that is, due to convenience, maternal desire or simply without scientific evidence for premature resolution.[4] For each of the three subtypes of preterm births (spontaneous preterm labor, P-PROM, or therapeutic) there are different risk factors and maternal and perinatal associated outcomes.[5–7]

Therefore, at least the distinction between spontaneous and therapeutic preterm birth is highly recommended when studying the determinants and consequences of preterm birth. The recognition that not every preterm birth occurs because of the same determinants was an early step to study the causes and develop preventive strategies. Preterm birth is also categorized according to gestational age at birth, and is divided into: late preterm (between 34 weeks + 0 days and 36 weeks + 6 days), moderately preterm (from 32 + 0 weeks to 33 + 6 weeks), very preterm (from 28 + 0 weeks to 31 + 6 weeks) and extremely preterm ($<$ 28 weeks).[4,8]

Pregnancy of a singleton or multiple fetuses (twins) confers great differences not only in terms of the incidence of preterm birth, but also concerning its associated factors and the maternal and perinatal outcomes.[9] A study evaluating offical data of the Brazilian System of Information on Live Births (Sinasc, in Portuguese) from 2011 to 2014 shows that $\sim$ 53% of twin pregnancies progress to preterm deliveries.[10] Furthermore, there is an increase in complications, such as maternal near-miss events, maternal mortality, perinatal mortality, preeclampsia, and postpartum hemorrhage.[9–11] The increased incidence of complications due to multiple pregnancies associated with a higher rate of twin pregnancies in the last decades denotes the importance of this type of pregnancy in preterm birth and maternal and perinatal health.[9,12] Twin pregnancies are not the focus of the present review, since an adequately designed and appropriate approach would be required for this type of pregnancy in order to evaluate its associated factors, preventive and predictive methods for preterm birth, and the respective perinatal outcomes.[11]

A study[13] by the World Health Organization (WHO) estimated that around 15 million preterm births occur annually worldwide, representing a rate of 10.3% of all deliveries.[3] International data from 1990 to 2010 from 65 countries of Europe, Australasia, and the Americas showed that the absolute number of preterm births and preterm infant rates increased during this period.[3] Countries from North Africa, Sub-Saharan Africa and Asia represent little more than 70% of deliveries and 80% of preterm births across the world. Furthermore, only five countries – India, China, Nigeria, Bangladesh, and Indonesia – account for almost half of preterm births worldwide.[13] Around 17% of preterm births occur in the Americas (North, Central and South America, and the Caribbean), Europe and Australia. However, these regions have the highest proportion of extreme preterm births.[13] Preterm birth represents a huge public health issue in all contexts and countries, be them low-income or high-income.[14,15]

Complications due to preterm births account for more than 1/3 of neonatal deaths worldwide, representing over 1 million newborn infants who died in the first month of life in 2010. The impact of complications due to preterm birth also has repercussions regarding childhood health indicators. It is the second cause of death until age 5 globally, and the first cause of death in mid-income and high-income countries.[3]

Since the 1950s, many advances have been made in the number of options and in the level of scientific evidence-based preventive measures for neonatal complications due to preterm birth. Among those are measures of tertiary prevention, such as the use of tocolytics and corticotherapy for the prevention of hyaline membrane, intraventricular hemorrhage and necrotizing enterocolitis; magnesium sulfate for the prevention of cerebral palsy in cases of imminient preterm delivery; and antibiotic therapy for the prevention of neonatal sepsis and to prolong the latent phase in cases of P-PROM.[16–20] Although these measures have a short- and long-term impact on perinatal morbidity and mortality, they are usually only adopted when the preterm birth has already begun and its ocurrence is imminent. Earlier identification of these cases still in the asymptomatic phase could theoretically increase the window of opportunity for preventive interventions and bring about better perinatal outcomes.[21,22]

There were also advances in the identification and institution of early therapies for neonatal complications such as neonatal sepsis, hypothermia, visual, cerebral (intra- and periventricular

hemorrhage), auditory and/or neuropsychomotor impairments, providing the newborn with the possibility of earlier neonatal follow-up and better long-term results.[23–25] The advent of continuous positive airway pressure (CPAP), for example, mechanical ventilation, the use of exogenous surfactant in the 1970s, and refinement of oxygen saturation targets in neonatal oxygen therapy in the last decade have resulted in significant improvement regarding neonatal survival, especially for extremely preterm infants.[23] Advances in tertiary and quaternary preventions, which correspond to a decrease in complications or adverse events after the emergence of a disease or its sequelae, do not seem to be equally accompanied by primary or secondary interventions. The difficulty is due, in part, to the lack of knowledge of the pathophysiology of preterm birth and its risk factors, which limits the development of preventive measures and effective prediction models.

Risk Factors for Preterm Birth and Prediction

Risk factor is a term used to designate conditions, characteristics, habits or markers that, when present, increase the probability of occurrence of a specific injury. Risk, therefore, is related to the onset of a condition.[26,27] Gender, ethnicity and age are considered fixed risk factors, and weight, body mass index (BMI), smoking, alcoholism or use of a condom, for example, are modifiable risk factors.[26] They may have different strengths of association with the risk of developing a determined condition depending on the combination of other factors, such as time of exposure or even the population studied.[27,28]

An example of a combination of factors is BMI and gestational weight gain. The National Academy of Medicine (NAM), in the United States, formerly known as the Institute of Medicine (IoM), categorized BMI into low weight (BMI $< 18.5\ kg/m^2$), normal (BMI between $18.5\ kg/m^2$ and $24.9\ kg/m^2$), overweight (BMI between $25.00\ kg/m^2$ and $29.9\ kg/m^2$) and obesity (BMI $\geq 30.0\ kg/m^2$).[29] A study[30] evaluating data from a prospective cohort with more than 45 thousand American pregnant women showed that BMI and gestational weight gain seem to have different impacts on the risk of having different subtypes of preterm birth, depending on the category of the initial BMI and the respective weight gain. Nevertheless, in this study, gestational weight gain was calculated by subtracting the initial weight from the last weight before childbirth. This method does not consider that women with preterm delivery had less weeks of gestation to gain weight, mainly in the third trimester, the period in which highest rate of weight gain occurs according to the IoM.[29] This results in a biased comparison of weight gain, for example, between a woman delivering at 28 weeks and another woman delivering at 41 weeks. In this case, the use of weight gain rate per week would be highly recommended. A systematic review[31] evaluating 39 studies including data on almost 1.8 million women highlights the lack of homogeneity in categorizing the initial BMI and defining the outcome according to the subtypes of prematurity and the estimate of the gestational age. Studies with the goal of circumventing these limitations are still scarce, although necessary in order to better understand the role of BMI and gestational weight gain in the risk of the occurrence of different subtypes of prematurity.

Didactically, the risk factors for preterm delivery may be classified as clinical, semiological, microbiological, ultrasonographic and biochemical.[32] Enviromental, social and genetic factors are also included.[33] According to some systematic reviews,[3,7,33] the main clinical risk factors for preterm birth, that is, those that have a higher independent association with preterm delivery, are history of previous preterm delivery, smoking, and multiple pregnancy. A history of previous preterm birth is the most important risk factor for preterm birth. A previous preterm delivery increases 3- to 4-fold the risk of having a new preterm delivery.[7,34–36] Theres is a relative amount of information available, since it is collected from the basic obstetric clinical history, preferentially detailing how and at which gestational age the preterm birth occurred.[34–36] The earlier the preterm delivery, the higher the risk of having a new case of preterm delivery; the number of recurrences was also associated with a 5- to 6-fold increase in the chance of having a new preterm delivery.[36] However, a limitation of this risk marker is that it cannot be applied to nulliparous women.

Smoking is a modifiable risk factor associated with an incidence of preterm birth that is 3- to 4-fold higher in smokers than in non-smokers.[33,37] The risk seems to be dose-dependent: the more cigarettes smoked, the higher the risk. In addition, the risk is also associated with passive smokers, that is, pregnant women exposed to cigarette smoke.[38,39]

It is important to emphasize that a condition that is associated with an outcome may not always be considered a risk factor for the condition. Exposure prior to the appearance of a disease, its removal or reduction is a characteristic associated with a lower incidence of disease; dose-dependence and measure of exposure need to be considered in the relation of risks. These characteristics are preponderant in the application of risk factors as predictors.

The great challenge lies in the limited knowledge of the patophysiology and etiology of preterm birth. There are some propositions concerning the mechanisms involved in preterm birth. A hypothesis by Behrman and Butler[40] highlighted the role of uterine distension, decidual hemorrhage or thrombosis, inflammatory or infectious processes, activation of the hypothalamus-pituitary-adrenal axis and stress, which, alone or in conjunction, may lead to pro-inflammatory activation of the decidua and membranes. Prostaglandins and metalloproteinases, in turn, along with other inflammatory agents, may promote cervical remodeling and/or uterine contractions, leading ultimately to preterm labor and/or P-PROM.[40] In contrast, Menon[41] categorized the risk factors as static and dynamic, also proposing a complex and not fully clear interaction between diverse inflammatory, immunological, environmental, and epigenetic mechanisms, among others, that culminate in senescence and "weakening" of amniotic membranes, decidual and myometrial activation, cervical effacement ,and, finally, preterm birth. Multiple markers involved in these mechanisms are studied as potential predictors of preterm birth.

Systematic reviews have identified many studies evaluating these different biological and biophysical markers, highlighting the fetal fibronectin l (fFN) and the phosphorylated isoform of insulin-like growth factor binding protein (phIGFBP-1), binding proteins present between the chorion of the amniotic membrane and the maternal decidua, and cervical length measurement in the second trimester of pregnancy by transvaginal ultrasound. Systematic reviews have concluded that those markers are not sufficiently accurate to be useful in the clinical prediction of preterm birth, especially in asymptomatic women.[32,42–44]

A Dutch prospective cohort study[45] including nearly 12 thousand women assessed the performance of cervical length measurement in the prediction of preterm birth. The measurement of the cervix was performed between 16 and 22 weeks of gestation. It was shown to be poor and did not vary significantly between nulliparous and multiparous women, as well as among women considered to be at low or high risk. The area under the receiver operating characteristic (ROC) curve ranged from 0.56 to 0.61 for the multiparous and the low-risk nulliparous groups respectively, that is, the method fails to identify around 40% to 50% of women who will have a preterm delivery.

Fetal fibronectin in the vaginal secretion does not show much superior results in asymptomatic women. A cohort study[46] from the United Kingdom analyzed the performance of fFN collected from the cervicovaginal secretion as a predictor of spontaneous preterm delivery at less than 34 weeks of gestation. Almost 1,500 women were included, and the vaginal secretion was collected from 22 to 28 weeks. The study showed that levels above 50 ng/ml have a sensitivity of 46.5% and a specificity of 88.7%. The higher the cut-off point for fFN in the vaginal secretion, the higher the negative predictive value (NPV) and specificity of the fFN. When the cut-off point was 500 ng/ml, the specificity and NPV were higher than 90%. However, its clinical application is still limited, since it is expected that a large part of the population does not have such high levels of fFN during this phase of gestation, and the test has a very low sensitivity with this cut-off point, that is, many women with a preterm delivery do not achieve such high fFN levels in the vaginal secretion during this period.

Other propositions have attempted to address the association between multiple factors involved in the development of preterm birth. A group of experts[4,47] proposed a classification of women at risk for preterm delivery, according to phenotypes. Empirically, those authors defined that the development of preterm birth is not exclusive to a single group of women who necessarily have similar characteristics and risk factors. On the contrary, probably different groups of women have conditions in common that are associated with preterm birth and its different subtypes. Conditions that potentially define the phenotypes of preterm birth were divided into maternal, fetal and placental conditions. These conditions are not based on risk factors, but depend on conditions present in the index pregnancy that determine the occurrence of preterm birth. The application of this new classification could help understand the associations between the determinants of preterm birth, help measure the benefits of preventive measures, help identify conditions most impacted by these measures, and, ultimately, help physicians understand the subgroups of women that are at higher risk of having different subtypes of preterm birth.

The aforementioned group of experts, with the aid of other collaborators, applied this concept through a secondary analysis of an international multicenter cohort study named INTERGROWTH 21st.[48] Slightly more than 50 thousand women had estimates of gestational age calculated by obstetric ultrasound, and 5,828 women had preterm deliveries (10.5%). A cluster analysis of preterm births was conducted, grouped or not, according to one or more of six maternal conditions, seven fetal conditions and three placental conditions. Finally, 12 clusters were identified, drawing attention to cluster 1, in which 1.747 (30%) women had none of the 16 predefined conditions. Over 80% of women from this cluster had preterm births either due to preterm labor or P-PROM. On the other hand, the majority of women were divided into 11 clusters, which were charaterized by major conditions such as preeclampsia/eclampsia, chorioamnionitis, twin pregnancies or bleeding at the beginning of pregnancy etc., showing that it is possible to identify determining factors in subgroups of women with preterm birth, which helps physicians to understand the etiology and identify women at higher risk. Nevertheless, this concept still requires reproducibility. Validation of the cluster determination, along with the predefining conditions in other populations, is necessary. Thus, we are faced with the need to better explore risk models for preterm birth, to identify risk factors and their associations, in order to determine the etiological theories and develop models that are efficient at predicting spontaneous preterm birth.

Prevention of Preterm Birth

According to Geoffrey Rose,[28] there are two prevention strategies: one based on individual preventive measures through the identification of individuals at higher risk of developing the condition; and the other based on measures of the general population, irrespective of the existence of risk factors. Available access to prenatal care, qualified childbirth and postpartum care, incentive programs for healthy lifestyle habits and protection of a woman's right to health care are important strategies that may have an impact on maternal and perinatal health indicators, including preterm birth.[49] A good example of exposure that has preventive measures based on both strategies is smoking. Around 50% of American pregnant women stop smoking in the first trimester of pregnancy.[50] Individual policies such as counseling, stimulation of pharmacologic replacement of nicotine, psychological support, and even financial incentives have an impact on the prevention of adverse perinatal outcomes. Governmental policies such as dissociating the image of the cigarette as a healthy and socially desirable habit through campaigns in the media, increase in taxes for the tobacco industry, and laws that restrict areas where smoking is allowed also demonstrated a beneficial effect.[50] A systematic review[50] including clinical trials testing different strategies for cessation of smoking showed that interventions reduced preterm births by $\sim$ 15%. Although continuous effort and specific public policies are necessary, this is a good example of how identifying the risk associated with prevention strategies may

result in more cost-effective and better maternal and perinatal outcomes.[50–52]

The identification of factors associated with a higher risk of developing spontaneous preterm birth may be useful to help physicians understand its pathophysiology and identify women at higher risk who might benefit from prevention strategies. In the latter case, it may also possible to distinguish between screening for risk and prediction of preterm birth. Although both methods use risk factors as the basis for their models or algorithms, their practical application may be quite distinct.[53]

For example, women with transvaginal ultrasound assessment of cervical length between 20 mm and 25 mm, which was measured in the second trimester by a standardized technique, had an incidence of preterm birth ranging from 22% to 32%.[54,55] This incidence may reach 56% in cases of cervical length shorter than 5 mm.[55] The increased incidence in women with a cervix shorter than 25 mm, in comparison to the general population, confers a 4- to 5-fold higher risk of having a preterm birth. Observational studies[45,54,55] in different populations confirm this inverse association between uterine cervix measurement in the second trimester and the prevalence of spontaneous preterm birth. Therefore, uterine cervices shorter than than 25 mm were considered "short", and those longer than 25 mm were considered "normal".[45,54] Based on the uterine cervix measurement to stratify women at higher risk, several clinical trials[56,57] have tested preventive interventions for spontaneous preterm birth and its association with adverse perinatal events, initially comparing natural micronized progesterone (vaginal tablet) or hydroxyprogesterone caproate (intramuscular injection) with placebo. Systematic reviews with meta-analysis[56,57] showed that the use of vaginal progesterone seems to be beneficial for the reduction of preterm birth before 37, 34 and 28 weeks and of neonatal morbid conditions. However, differences in reduction rates of different morbid conditions or even preterm birth may be attributed to different selection criteria for women included in clinical trials.

The OPPTIMUM study,[58] for example, a British multicenter study including 65 centers in the United Kingdom and 1 in Sweden, published in 2016 (after the systematic review), aimed to evaluate not only the benefit of progesterone in reducing prematurity and neonatal morbidity, but also its long-term effect on the child. The study selected women with singleton pregnancies at high risk of having preterm birth based on: history of previous preterm birth, gestational loss in the second trimester, P-PROM, cervical procedure, and positive vaginal fetal fibronectin. A year after the begining of the clinical trial, the researchers decided to include women at "mid-high" risk, which was defined as women with negative fetal fibronectin, but with a history of spontaneous preterm birth at before 34 weeks, or uterine cervix shorter than 25mm in the second trimester. This double-blinded controlled study randomized more than 600 women in each group (vaginal progesterone 200 mg versus placebo), and demonstrated that progesterone was not beneficial in reducing preterm birth or the majority of perinatal morbid conditions, such as pulmonary bronchodysplasia, neonatal infection, necrotizing enterocolitis, and neurological development and neurocognitive score at 2 years of age. However, the study showed a reduction in neonatal death (non-adjusted odds ratio [OR] of 0.17 [0.06–0.49], *p*-value of 0.0009) and in cerebral alterations on ultrasound (non-adjusted OR of 0.50 [0.31–0.84], *p*-value of 0.008). The authors of this study concluded that the subgroups of women who might benefit from progesterone are not easily identified by the current screening strategies. This should encourage studies on new prevention strategies as well as those aimed at identifying women that may be potentially eligible to undergo this treatment.

Another technique that has been studied for decades is cerclage, which is primarily based on suture of the uterine cervix or isthmus-cervical region to prevent early effacement/dilatation of the cervix. The Shirodkar[59] technique, described in 1953, and a technique by McDonald[60] in 1957 are the basis for all of the subsequently described variations. These techniques were initially proposed for cases with a history of cervical insufficiency, a known cause of late abortion and extreme prematurity. A Cochrane systematic review of 15 clinical trials showed advantages of these techniques in prolonging pregnancy, decreasing the rates of neonatal morbidity and prematurity when indicated to women with a history of cervical insufficiency.[61] The advent of the cervical measurement in the second trimester, associated with a history of preterm birth, seems to have improved the identification of women who will benefit from cerclage to prevent preterm birth, particularly in cases in which there is still no history of recurrent pregnancy loss.[62] This shows that the search for an association of risk factors in the prevention of preterm birth may still be very useful, even in situations in which a good solution was apparently found, as for cervical incompetence and cerclage.

It is also worth mentioning that another intervention was studied for the prevention of preterm birth in high-risk women. A pessary, a device made of firm silicone in the shape of a convex ring, is inserted into the posterior vaginal fornix and fastened to the cervix. The theoretical mechanism for the prevention of preterm birth is based on: 1) a change in the axis of forces resulting from the uterine body and isthmus that act on the cervix; and 2) a potential closure of the cervix with consequent strengthening of the cervical canal and the immunologic barrier of the cervix, preserving the amniotic membranes from contact with the vaginal environment.[63] Although the subject has been studied since the middle of the 20th century, the identification of women who actually benefit from this intervention remains a challenge. The Pesario Cervical para Evitar Prematuridad (PECEP, "Cervical Pessary to Prevent Prematurity") study trial,[63] published in 2012, was the first randomized study using the pessary (*versus* expectant management) to prevent preterm birth. Selecting pregnant women at high risk based on cervical length measurement in the second trimester, with slightly more than 190 women per group, the study showed that the incidence of preterm births before 34 weeks decreased by 80%. Subsequent studies demonstrated conflicting results, and did not confirm such a reduction in the incidence of preterm births observed by Goya et al.[63] However, the selection of eligible women and

the association with other interventions, such as progesterone, is heterogenous among studies.[64–67]

Despite the advances/benefits resulting from a combination of screening for risk and interventions, such as, progesterone, the pessary and cerclage in women selected based on risk factors, there still seem to exist limitations and heterogeneity in screening. Better results from the use of these measures may be potentially hindered. Which women might actually benefit from the use of progesterone during prenatal care? Which women might not benefit from any preventive intervention? Furthermore, there is no consensus over which level of risk estimate determines that a woman should in fact be considered at high risk. Improved identification of women at high (or low) risk of having a preterm birth with the development of prediction models that have good discriminatory performance may be quite relevant to advance the investigation of the benefits of using (or not) progesterone, the pessary or any other form of preterm birth prevention.

Risk Assessment and Prediction of Preterm Birth

The description of these prevention studies and their interpretations are important to highlight the fundamental role of the adequate screening of women who may benefit from prevention strategies. Distinctions must be made regarding the risk assessment model and a predictor model for an outcome. This distinction may actually help physicians understand the clinical application of a screening strategy for women at high risk of having a preterm delivery.

As an example of a risk marker, the cervix is known to be independently associated with a higher risk of having a preterm birth.[54,55] Although this may be useful for the implementation of differentiated care, suggesting screening and interventions for the subgroup of women with a short cervix, this practice is fragile in terms of the population, and has a low impact on prevention.[68] The reason for this is that, despite a higher risk of having a preterm birth, a woman with a short cervix has the highest odds of having a term birth. Furthermore, the shortening process of the cervix may not occur early in the recommended screening phase (the second trimester, between 18 and 24 weeks). In summary, the cervix is a marker of low sensitivity (a considerable proportion of women with a short cervix are likely to deliver at term). At the same time, the marker has a low rate in the general population, since cervices measuring 25 mm and 20 mm correspond respectively to a 5th and a 3rd percentiles in the population curve of cervical measurement.[54] A cohort[54] with almost 3 thousand pregnant women evaluated the performance of 28 markers in the second trimester of pregnancy, and it showed that a short cervix has a sensitivity of 36.8% for preterm birth before 35 weeks. This means that almost two-thirds of women with preterm birth below this gestational age would not be screened using this criterion, resulting in elevated false-negative rates of the method. Therefore, despite the positive association with preterm birth, a short cervix seems to be an inappropriate marker to compose predictive models, resulting in low efficacy when employed in the clinical practice.[45,54,55] Even serial measurements of the cervix, based on the theory that shortening of the cervix over the weeks could be a better predictor of preterm birth, showed a worse predicitive performance than a single measurement.[69]

A prospective observational study[70] included more than 9 thousand nulliparous pregnant women from 8 centers across the United States, and it evaluated the performance of fetal fibronectin and transvaginal measurement of the uterine cervix in predicting spontaneous preterm birth. The area under the ROC curve was of 0.59 for fetal fibronectin $\geq$ 50 ng/dL, and of 0.67 for cervices shorter than 25 mm. The model containing both variables had an area under the ROC curve of 0.67. The authors concluded that the performance was poor and of low clinical utility.

In summary, systematic reviews have concluded that there are no markers in the literature that can be applied in the clinical practice to predict spontaneous preterm birth with a good performance, and that enable new preventive approaches and studies in this area.[71]

Metabolomics and Preterm Birth

The term "omics sciences" is applied to the field of knowledge that focuses on genomic studies, gene identification, DNA sequence polymorphisms, genes and the genome; transcriptomics, which is focused on the study of gene expression – RNAs; proteomics, the large-scale study of proteins; and metabolomics, the scientific study of chemical processes involving metabolites.[72–75] The application of each technique to investigate markers or the pathophysiology of diseases, primarily those involving complex mechanisms that have not yet been fully elucidated, is basically dependent on the objectives and resources available. Actually, an integrated application of the various methods may be the best option.[74] The main advantage of metabolomics is that it seems to be closer to disease phenotype, presenting the result of the final pathway of interactions between genes, RNAm and proteins. According to Dettmer et al,[76] genomics tells what can happen, transcriptomics, what appears to be happening, proteomics, what makes it happen, and metabolomics, what has happened and what is happening.

Metabolomics is the science that studies metabolites, small molecules present in different chains of the metabolism of an organism.[77] These small molecules may be substrates, products and cofactors of intracellular and extracellular chemical reactions such as aminoacids, biliary acids, carbohydrates, lipids, vitamins and others.[78] The group of metabolites in a certain sample or organism is called metabolome. Different techniques are used to identify and quantify metabolites, such as mass spectrometry coupled with liquid or gas chromatography or magnetic resonance imaging. Furthermore, diverse configurations or variants may be used to obtain a better performance, depending on the metabolite of interest, its polarity, the mass spectrum to be studied, or other physicochemical characteristics of the metabolites and samples to be analyzed. Technological advances in instruments for data acquisition and bioinformatics have provided sufficient aid,

so that metabolomics is able to identify and analyze hundreds or even millions of metabolites in a certain biological sample. Studies on diverse applications in biological samples demonstrate a high sensitivity in the detection and measurement of metabolites.[77]

By identifying and quantifying metabolites, this technique is capable of showing the fingerprint of the metabolic interactions of the organism in a certain sample at a certain time. Metabolomics is a technique known as hypothesis-free, that is, it does not require an initial hypothesis. Instead of testing a certain hypothesis, the technique may generate novel hypotheses through its results when elucidating the markers and biological pathways involved in the process of disease development, which may not have been clarified.[74,76,77] It may be a relevant complementary tool for the construction of knowledge in diseases in which the pathophysiology has yet to be fully elucidated and possibly involves multiple complex genetic and environmental interactions, such as preterm delivery, preeclampsia and fetal growth restriction.[77,78]

Metabolomics has been applied in biological samples for the investigation of processes ranging from embryogenesis to the emergence of complex diseases such as cancer, Parkinson disease, diabetes and depression.[79] In the area of maternal and perinatal health care, it has been mainly applied to identify biomarkers, which are clinically useful for the performance of diagnostic or prognostic predictions.[74,78,80]

After identifying 45 metabolites significantly associated with preclampsia in serum samples collected at 15 weeks of gestation from a group of $\sim$ 39 nulliparous pregnant women with a history of preeclampsia (compared with 40 pregnant women without complications), 14 metabolites were selected to compose the final model (validation). The model resulted in an area under the ROC curve of 0.92, and an OR of 23 (95% confidence interval [95%CI]: 7–73).[81] Another study using samples of a similar number of women who progressed to preeclampsia, as well as samples collected a short time earlier (between 11 and 14 weeks), showed more modest results, albeit still promising. The model containing 4 metabolites has a detection rate of only 50%, assuming a false-positive rate of 10%, with an area under the ROC curve of 0.81 for cases of preeclampsia.[82] Fews studies on the identification of biomarkers to compose prediction models for preterm birth have been published until now, and some narrative reviews of the subject have described a great heterogeneity in the methodology employed.[74,78,80] To date, there are no systematic reviews that analyze the performance of metabolomics in predicting spontaneous preterm delivery.

Many aspects regarding the most effective method to investigate preterm birth using metabolomic markers should be discussed. First, there is the type of sample used (urine, blood, amniotic fluid, hair, vaginal secretion). Then, there is the time for sample collection (during the clinical presentation of preterm birth or in the early phase of pregnancy, when there are no symptoms). Furthermore, metabolomics demands a high methodological rigor in the collection and storage of biosamples, since this is a highly sensitive method to identify small low-weight molecules; various types of "noise", or interference in data acquisition, may hinder the identification of these molecules. Heterogeneity in sample collection and storage may be the cause. In addition, a well-delineated study design, with well-defined outcomes, following clear classifications, associated with sequential validations of findings is crucial for the reliability and reproducibility of this technique. Finally, still in the phase of data analysis, caution regarding some important considerations is emphasized. For example, hundreds or even thousands of metabolites are usually analyzed at the same time in a sample. Since the number of variables (metabolites) is much higher than the number of samples (individuals), the analysis is very susceptible to significantly false results. To correct this effect, the Bonferroni correction may be used, attenuating the significance of the *p*-value according to the number of variables (metabolites) analyzed. The false discovery rate (FDR) method may also be applied to control the number of false-positive conclusions, and significance is only assigned to the "most promising" variables.[83] This technique was proposed by Benjamini and Hochberg[83] in the 1990s, and it is based on the proportion between the true null hypothesis (H0) and the rejected null hypothesis, decreasing the possibility of markers considered to be statistically discriminatory. Actually, these markers are not discriminatory. These are only two examples of methodological care required in the phase of data analysis.

Many questions need to be answered concerning the mechanisms involved in the development of preterm birth: why do some women have early cervical remodeling (with an evident short cervix on the transvaginal ultrasound in the second trimester) and others do not? Which and how are the interactions between different risk factors, including infection, vaginal bleeding and body mass index, and how can they determine preterm birth? In theory, metabolomics depicts the final pathway resulting from these interactions, and it seems to be a useful approach not only to predict spontaneous preterm birth, but also to elucidate the many mechanisms involved.

Conclusion

In order to adequately address the investigation of preterm birth, its associated factors and perinatal outcomes, a robust methodological approach is required, using judicious and standardized definitions of exposures and outcomes. Based on this premise, a multifaceted comprehensive approach, albeit integrated, was proposed for data exploration on the factors associated with preterm birth, its prediction and the perinatal outcomes, which may be capable of generating new knowledge regarding this issue. It is expected that the results of this approach may contribute to the prediction of the most effective performance and better understanding of the factors associated with spontaneous preterm birth and consequent adverse perinatal results, collaborating with the development and application of public policies to prevent preterm birth and its perinatal consequences. We acknowledge the fact that the present is an integrative review based on a biased search in the literature and on the interpretation of the studies and respective findings. Although we have not used the standard tools and strategies to measure and report these biases, we consider

that it is a great opportunity to raise the discussion about some of the risk factors associated with sponteaneous preterm birth, how preventive strategies based on these factors have been implemented, and the results so far. We expect that, despite the limitations of the present integrative review, it may contribute to the discussion about recognizing women at a higher risk of having sponteaneous preterm birth and how to prevent it.

Contributors

Souza RT and Cecatti JG conceived and planned the concept of the current manuscript. Souza RT collated material for the first draft of the manuscript. Both authors read, reviewed and approved the final version of the manuscript.

Conflict of Interests

The authors have no conflict of interests to declare.

References

1 Hess JH. A city-wide plan for the reduction of deaths associated with and due to prematurity. J Pediatr. 1936;8(1):104–121. (Proceedings of the 5th Annual Meeting of the American Academy of Pediatrics)

2 EXPERT group on prematurity; final report, Geneva, 17-21 April 1950. World Health Organ Tech Rep Ser. 1950;27:1–11.

3 Blencowe H, Cousens S, Chou D, Oestergaard M, Say L, Moller AB, et al.; Born Too Soon Preterm Birth Action Group. Born too soon: the global epidemiology of 15 million preterm births. Reprod Health. 2013;10(Suppl 1):S2. doi: 10.1186/1742-4755-10-S1-S2

4 Goldenberg RL, Gravett MG, Iams J, Papageorghiou AT, Waller SA, Kramer M, et al. The preterm birth syndrome: issues to consider in creating a classification system. Am J Obstet Gynecol. 2012;206 (2):113–118. doi: 10.1016/j.ajog.2011.10.865

5 Morisaki N, Togoobaatar G, Vogel JP, Souza JP, Rowland Hogue CJ, Jayaratne K, et al; WHO Multicountry Survey on Maternal and Newborn Health Research Network. Risk factors for spontaneous and provider-initiated preterm delivery in high and low Human Development Index countries: a secondary analysis of the World Health Organization Multicountry Survey on Maternal and Newborn Health. BJOG. 2014;121(Suppl 1):101–109. doi: 10.1111/1471-0528.12631

6 Souza RT, Cecatti JG, Passini R Jr, Tedesco RP, Lajos GJ, Nomura ML, et al; Brazilian Multicenter Study on Preterm Birth study group. The burden of provider-initiated preterm birth and associated factors: Evidence from the Brazilian Multicenter Study on Preterm Birth (EMIP). PLoS One. 2016;11(2):e0148244. doi: 10.1371/journal.pone.0148244

7 Passini R Jr, Cecatti JG, Lajos GJ, Tedesco RP, Nomura ML, Dias TZ, et al; Brazilian Multicentre Study on Preterm Birth study group. Brazilian multicentre study on preterm birth (EMIP): prevalence and factors associated with spontaneous preterm birth. PLoS One. 2014;9(10):e109069. doi: 10.1371/journal.pone.0109069

8 Kramer MS, Papageorghiou A, Culhane J, Bhutta Z, Goldenberg RL, Gravett M, et al. Challenges in defining and classifying the preterm birth syndrome. Am J Obstet Gynecol. 2012;206(2): 108–112. doi: 10.1016/j.ajog.2011.10.864

9 Fuchs F, Senat MV. Multiple gestations and preterm birth. Semin Fetal Neonatal Med. 2016;21(2):113–120. doi: 10.1016/J.SINY.2015.12.010

10 Santana DS, Souza RT, Surita FG, Argenton JL, Silva CM, Cecatti JG. Twin pregnancy in Brazil: a profile analysis exploring population information from the National Birth E-Registry on Live Births. BioMed Res Int. 2018;2018:9189648. doi: 10.1155/2018/9189648

11 Santana DS, Silveira C, Costa ML, Souza RT, Surita FG, Souza JP, et al; WHO Multi-Country Survey on Maternal and Newborn Health Research Network. Perinatal outcomes in twin pregnancies complicated by maternal morbidity: evidence from the WHO Multicountry Survey on Maternal and Newborn Health. BMC Pregnancy Childbirth. 2018;18(1):449. doi: 10.1186/s12884-018-2082-9

12 Vogel JP, Torloni MR, Seuc A, Betrán AP, Widmer M, Souza JP, et al; Maternal and perinatal outcomes of twin pregnancy in 23 low- and middle-income countries. PLoS One. 2013;8(8):e70549. doi: 10.1371/journal.pone.0070549

13 Chawanpaiboon S, Vogel JP, Moller AB, Lumbiganon P, Petzold M, Hogan D, et al. Global, regional, and national estimates of levels of preterm birth in 2014: a systematic review and modelling analysis. Lancet Glob Health. 2019;7(1):e37–e46. doi: 10.1016/S2214-109X(18)30451-0

14 Harrison MS, Goldenberg RL. Global burden of prematurity. Semin Fetal Neonatal Med. 2016;21(2):74–79. doi: 10.1016/J.SINY.2015.12.007

15 Frey HA, Klebanoff MA. The epidemiology, etiology, and costs of preterm birth. Semin Fetal Neonatal Med. 2016;21(2):68–73. doi: 10.1016/J.SINY.2015.12.011

16 Smith V, Devane D, Begley CM, Clarke M, Higgins S. A systematic review and quality assessment of systematic reviews of randomised trials of interventions for preventing and treating preterm birth. Eur J Obstet Gynecol Reprod Biol. 2009;142(1):3–11. doi: 10.1016/j.ejogrb.2008.09.008

17 Roberts D, Brown J, Medley N, Dalziel SR. Antenatal corticosteroids for accelerating fetal lung maturation for women at risk of preterm birth. Cochrane Database Syst Rev. 2017;3: CD004454. doi: 10.1002/14651858.CD004454.pub3

18 Mackeen AD, Seibel-Seamon J, Muhammad J, Baxter JK, Berghella V. Tocolytics for preterm premature rupture of membranes. Cochrane Database Syst Rev. 2014;(2):CD007062. doi: 10.1002/14651858.CD007062.pub3

19 Kenyon S, Boulvain M, Neilson JP. Antibiotics for preterm rupture of membranes. Cochrane Database Syst Rev. 2013;(12): CD001058. doi: 10.1002/14651858.CD001058.pub3

20 Shepherd E, Salam RA, Middleton P, Makrides M, McIntyre S, Badawi N, et al. Antenatal and intrapartum interventions for preventing cerebral palsy: an overview of Cochrane systematic reviews. Cochrane Database Syst Rev. 2017;8:CD012077. doi: 10.1002/14651858.CD012077.pub2

21 Lawn JE, Kinney MV, Belizan JM, Mason EM, McDougall L, Larson J, et al. Born Too Soon Preterm Birth Action Group. Born too soon: accelerating actions for prevention and care of 15 million newborns born too soon. Reprod Health. 2013;10(Suppl 1):S6. doi: 10.1186/1742-4755-10-S1-S6

22 Georgiou HM, Di Quinzio MKW, Permezel M, Brennecke SP. Predicting preterm labour: current status and future prospects. Dis Markers. 2015;2015:435014. doi: 10.1155/2015/435014

23 Glass HC, Costarino AT, Stayer SA, Brett CM, Cladis F, Davis PJ. Outcomes for extremely premature infants. Anesth Analg. 2015; 120(6):1337–1351. doi: 10.1213/ANE.0000000000000705

24 Locatelli A, Consonni S, Ghidini A. Preterm labor: approach to decreasing complications of prematurity. Obstet Gynecol Clin North Am. 2015;42(2):255–274. doi: 10.1016/j.ogc.2015.01.004

25 França EB, Passos VMA, Malta DC, Duncan BB, Ribeiro ALP, Guimarães MDC, et al. Cause-specific mortality for 249 causes in Brazil and states during 1990-2015: a systematic analysis for the global burden of disease study 2015. Popul Health Metr. 2017; 15(1):39. doi: 10.1186/s12963-017-0156-y

26 Offord DR, Kraemer HC. Risk factors and prevention. Evid Based Ment Health. 2000;3(3):70–71. doi: 10.1136/ebmh.3.3.70

27 Fletcher RH, Fletcher SW, Fletcher GS. Risco: princípios básicos. In: Fletcher RH, Fletcher SW, Fletcher GS. Epidemiologia clínica: elementos essenciais. 5. ed. Porto Alegre: Artmed; 2014. Cap. 4

28 Rose G. Sick individuals and sick populations. Int J Epidemiol. 2001;30(3):427–432, discussion 433–434. doi: 10.1093/ije/30.3.427

29 Institute of Medicine. National Research Council. Committee to Reexamine IOM Pregnancy Weight Guidelines. Weight gain during pregnancy: reexamining the guidelines. Washington, DC: The National Academies Press; 2009

30 Masho SW, Bishop DL, Munn M. Pre-pregnancy BMI and weight gain: where is the tipping point for preterm birth? BMC Pregnancy Childbirth. 2013;13(1):120. doi: 10.1186/1471-2393-13-120

31 Torloni MR, Betrán AP, Daher S, Widmer M, Dolan SM, Menon R, et al. Maternal BMI and preterm birth: a systematic review of the literature with meta-analysis. J Matern Fetal Neonatal Med. 2009; 22(11):957–970. doi: 10.3109/14767050903042561

32 Honest H, Hyde CJ, Khan KS. Prediction of spontaneous preterm birth: no good test for predicting a spontaneous preterm birth. Curr Opin Obstet Gynecol. 2012;24(6):422–433. doi: 10.1097/GCO.0b013e328359823a

33 Koullali B, Oudijk MA, Nijman TAJ, Mol BWJ, Pajkrt E. Risk assessment and management to prevent preterm birth. Semin Fetal Neonatal Med. 2016;21(2):80–88. doi: 10.1016/j.siny.2016.01.005

34 Phillips C, Velji Z, Hanly C, Metcalfe A. Risk of recurrent spontaneous preterm birth: a systematic review and meta-analysis. BMJ Open. 2017;7(6):e015402. doi: 10.1136/bmjopen-2016-015402

35 Baer RJ, Yang J, Berghella V, Chambers CD, Coker TR, Kuppermann M, et al. Risk of preterm birth by maternal age at first and second pregnancy and race/ethnicity. J Perinat Med. 2018;46(5): 539–546. doi: 10.1515/jpm-2017-0014

36 McManemy J, Cooke E, Amon E, Leet T. Recurrence risk for preterm delivery. Am J Obstet Gynecol. 2007;196(6):576.e1–576.e6, discussion 576.e6–576.e7. doi: 10.1016/j.ajog.2007.01.039

37 Shah NR, Bracken MB. A systematic review and meta-analysis of prospective studies on the association between maternal cigarette smoking and preterm delivery. Am J Obstet Gynecol. 2000; 182(2):465–472. doi: 10.1016/s0002-9378(00)70240-7

38 Florek E, Piekoszewski W, Hubert A, Kornacka MK. Influence of active and passive smoking during pregnancy on newborn health. In: Koskinen CJ, (ed.) Handbook of smoking and health. Hauppauge: Nova Science; 2011:264–307.

39 Huang SH, Weng KP, Huang SM, Liou HH, Wang CC, Ou SF, et al. The effects of maternal smoking exposure during pregnancy on postnatal outcomes: a cross sectional study. J Chin Med Assoc. 2017;80(12):796–802. doi: 10.1016/j.jcma.2017.01.007

40 Behrman R, Butler A, editors. Institute of Medicine; Committee on Understanding Premature Birth and Assuring Healthy Outcomes. Preterm birth: causes, consequences, and prevention. Washington, DC: National Academies Press; 2007

41 Menon R. Oxidative stress damage as a detrimental factor in preterm birth pathology. Front Immunol. 2014;5:567. doi: 10.3389/fimmu.2014.00567

42 Conde-Agudelo A, Romero R. Cervical phosphorylated insulin-like growth factor binding protein-1 test for the prediction of preterm birth: a systematic review and metaanalysis. Am J Obstet Gynecol. 2016;214(1):57–73. doi: 10.1016/j.ajog.2015.06.060

43 Hee L. Likelihood ratios for the prediction of preterm delivery with biomarkers. Acta Obstet Gynecol Scand. 2011;90(11): 1189–1199. doi: 10.1111/j.1600-0412.2011.01187.x

44 Goldenberg RL, Iams JD, Mercer BM, Meis PJ, Moawad A, Das A, et al. Maternal-Fetal Medicine Units Network. The Preterm Prediction Study: toward a multiple-marker test for spontaneous preterm birth. Am J Obstet Gynecol. 2001;185(3):643–651. doi: 10.1067/mob.2001.116752

45 van der Ven J, van Os MA, Kazemier BM, Kleinrouweler E, Verhoeven CJ, de Miranda E, et al. The capacity of mid-pregnancy cervical length to predict preterm birth in low-risk women: a national cohort study. Acta Obstet Gynecol Scand. 2015;94(11): 1223–1234. doi: 10.1111/aogs.12721

46 Abbott DS, Hezelgrave NL, Seed PT, Norman JE, David AL, Bennett PR, et al. Quantitative fetal fibronectin to predict preterm birth in asymptomatic women at high risk. Obstet Gynecol. 2015;125(5): 1168–1176. doi: 10.1097/AOG.0000000000000754

47 Villar J, Papageorghiou AT, Knight HE, Gravett MG, Iams J, Waller SA, et al. The preterm birth syndrome: a prototype phenotypic classification. Am J Obstet Gynecol. 2012;206(2):119–123. doi: 10.1016/j.ajog.2011.10.866

48 Barros FC, Papageorghiou AT, Victora CG, Noble JA, Pang R, Iams J, et al. The distribution of clinical phenotypes of preterm birth syndrome: implications for prevention. JAMA Pediatr. 2015;169 (3):220–229. doi: 10.1001/jamapediatrics.2014.3040

49 Requejo J, Merialdi M, Althabe F, Keller M, Katz J, Menon R. Born too soon: care during pregnancy and childbirth to reduce preterm deliveries and improve health outcomes of the preterm baby. Reprod Health. 2013;10(Suppl 1):S4. doi: 10.1186/1742-4755-10-S1-S4

50 Lumley J, Chamberlain C, Dowswell T, Oliver S, Oakley L, Watson L. Interventions for promoting smoking cessation during pregnancy. Cochrane Database Syst Rev. 2009;(3):CD001055. doi: 10.1002/14651858.CD001055.pub3

51 Scherman A, Tolosa JE, McEvoy C. Smoking cessation in pregnancy: a continuing challenge in the United States. Ther Adv Drug Saf. 2018;9(8):457–474. doi: 10.1177/2042098618775366

52 Honest H, Forbes CA, Durée KH, Norman G, Duffy SB, Tsourapas A, et al. Screening to prevent spontaneous preterm birth: systematic reviews of accuracy and effectiveness literature with economic modelling. Health Technol Assess. 2009;13(43):1–627. doi: 10.3310/hta13430

53 To MS, Skentou C, Chan C, Zagaliki A, Nicolaides KH. Cervical assessment at the routine 23-week scan: standardizing techniques. Ultrasound Obstet Gynecol. 2001;17(3):217–219. doi: 10.1046/j.1469-0705.2001.00369.x

54 Iams JD, Goldenberg RL, Meis PJ, Mercer BM, Moawad A, Das A, et al. The length of the cervix and the risk of spontaneous premature delivery. National Institute of Child Health and Human Development Maternal Fetal Medicine Unit Network. N Engl J Med. 1996;334(9):567–572. doi: 10.1056/NEJM199602293340904

55 Berghella V, Roman A, Daskalakis C, Ness A, Baxter JK. Gestational age at cervical length measurement and incidence of preterm birth. Obstet Gynecol. 2007;110(2 Pt 1):311–317. doi: 10.1097/01.AOG.0000270112.05025.1d

56 Romero R, Conde-Agudelo A, Da Fonseca E, O'Brien JM, Cetingoz E, Creasy GW, et al. Vaginal progesterone for preventing preterm birth and adverse perinatal outcomes in singleton gestations with a short cervix: a meta-analysis of individual patient data. Am J Obstet Gynecol. 2018;218(2):161–180. doi: 10.1016/j.ajog.2017.11.576

57 Dodd JM, Jones L, Flenady V, Cincotta R, Crowther CA. Prenatal administration of progesterone for preventing preterm birth in women considered to be at risk of preterm birth. Cochrane Database Syst Rev. 2013;(7):CD004947. doi: 10.1002/14651858.CD004947.pub3

58 Norman JE, Marlow N, Messow CM, Shennan A, Bennett PR, Thornton S, et al. Vaginal progesterone prophylaxis for preterm birth (the OPPTIMUM study): a multicentre, randomised, double-blind trial. Lancet. 2016;387(10033):2106–2116. doi: 10.1016/S0140-6736(16)00350-0

59 Shirodkar VN. Some observations on non-malignant conditions of the cervix. J Obstet Gynaecol India. 1953;3:287–289

60 McDonald IA. Suture of the cervix for inevitable miscarriage. J Obstet Gynaecol Br Emp. 1957;64(3):346–350. doi: 10.1111/j.1471-0528.1957.tb02650.x

61 Eleje GU, Ikechebelu JI, Eke AC, Okam PC, Ezebialu IU, Ilika CP. Cervical cerclage in combination with other treatments for

preventing preterm birth in singleton pregnancies. Cochrane Database Syst Rev. 2017;(11):CD012871. doi: 10.1002/14651858.CD012871

62 American College of Obstetricians and Gynecologists. ACOG Practice Bulletin N. 142: Cerclage for the management of cervical insufficiency. Obstet Gynecol. 2014;123(2 Pt 1):372–379. doi: 10.1097/01.AOG.0000443276.68274.cc

63 Goya M, Pratcorona L, Merced C, Rodó C, Valle L, Romero A, et al. Cervical pessary in pregnant women with a short cervix (PECEP): an open-label randomised controlled trial. Lancet. 2012;379(9828):1800–1806. doi: 10.1016/S0140-6736(12)60030-0

64 Cruz-Melguizo S, San-Frutos L, Martínez-Payo C, Ruiz-Antorán B, Adiego-Burgos B, Campillos-Maza JM, et al. Cervical pessary compared with vaginal progesterone for preventing early preterm birth: a randomied controlled trial. Obstet Gynecol. 2018;132(4):907–915. doi: 10.1097/AOG.0000000000002884

65 Saccone G, Maruotti GM, Giudicepietro A, Martinelli P; Italian Preterm Birth Prevention (IPP) Working Group. Effect of cervical pessary on spontaneous preterm birth in women with singleton pregnancies and short cervical length: a randomized clinical trial. JAMA. 2017;318(23):2317–2324. doi: 10.1001/jama.2017.18956

66 Nicolaides KH, Syngelaki A, Poon LC, Picciarelli G, Tul N, Zamprakou A, et al. A randomized trial of a cervical pessary to prevent preterm singleton birth. N Engl J Med. 2016;374(11):1044–1052. doi: 10.1056/NEJMoa1511014

67 Dugoff L, Berghella V, Sehdev H, Mackeen AD, Goetzl L, Ludmir J. Prevention of preterm birth with pessary in singletons (PoPPS): randomized controlled trial. Ultrasound Obstet Gynecol. 2018;51(5):573–579. doi: 10.1002/uog.18908

68 Kyvernitakis I, Maul H, Bahlmann F. Controversies about the secondary prevention of spontaneous preterm birth. Geburtshilfe Frauenheilkd. 2018;78(6):585–595. doi: 10.1055/a-0611-5337

69 Conde-Agudelo A, Romero R. Predictive accuracy of changes in transvaginal sonographic cervical length over time for preterm birth: a systematic review and metaanalysis. Am J Obstet Gynecol. 2015;213(6):789–801. doi: 10.1016/j.ajog.2015.06.015

70 Esplin MS, Elovitz MA, Iams JD, Wapner RJ, Grobman WA, et al. Predictive accuracy of serial transvaginal cervical lengths and quantitative vaginal fetal fibronectin levels for spontaneous preterm birth among nulliparous women. JAMA. 2017;317(10):1047–1056. doi: 10.1001/jama.2017.1373

71 Conde-Agudelo A, Papageorghiou AT, Kennedy SH, Villar J. Novel biomarkers for the prediction of the spontaneous preterm birth phenotype: a systematic review and meta-analysis. BJOG. 2011;118(9):1042–1054. doi: 10.1111/j.1471-0528.2011.02923.x

72 Li S, Dunlop AL, Jones DP, Corwin EJ. High-resolution metabolomics: review of the field and implications for nursing science and the study of preterm birth. Biol Res Nurs. 2016;18(1):12–22. doi: 10.1177/1099800415595463

73 Dettmer K, Hammock BD. Metabolomics - a new exciting field within the “omics” sciences. Environ Health Perspect. 2004;112(7):A396–A397. doi: 10.1289/ehp.112-1241997

74 Romero R, Espinoza J, Gotsch F, Kusanovic JP, Friel LA, Erez O, et al. The use of high-dimensional biology (genomics, transcriptomics, proteomics, and metabolomics) to understand the preterm parturition syndrome. BJOG. 2006;113(Suppl 3):118–135. doi: 10.1111/j.1471-0528.2006.01150.x

75 Patti GJ, Yanes O, Siuzdak G. Innovation. metabolomics: the apogee of the omics trilogy. Nat Rev Mol Cell Biol. 2012;13(4):263–269. doi: 10.1038/nrm3314

76 Dettmer K, Aronov PA, Hammock BD. Mass spectrometry-based metabolomics. Mass Spectrom Rev. 2007;26(1):51–78. doi: 10.1002/mas.20108

77 Kell DB. Metabolomics and systems biology: making sense of the soup. Curr Opin Microbiol. 2004;7(3):296–307. doi: 10.1016/j.mib.2004.04.012

78 Horgan RP, Clancy OH, Myers JE, Baker PN. An overview of proteomic and metabolomic technologies and their application to pregnancy research. BJOG. 2009;116(2):173–181. doi: 10.1111/j.1471-0528.2008.01997.x

79 Putri SP, Nakayama Y, Matsuda F, Uchikata T, Kobayashi S, Matsubara A, et al. Current metabolomics: practical applications. J Biosci Bioeng. 2013;115(6):579–589. doi: 10.1016/j.jbiosc.2012.12.007

80 Dessì A, Marincola FC, Fanos V. Metabolomics and the great obstetrical syndromes – GDM, PET, and IUGR. Best Pract Res Clin Obstet Gynaecol. 2015;29(2):156–164. doi: 10.1016/j.bpobgyn.2014.04.023

81 Kenny LC, Black MA, Poston L, Taylor R, Myers JE, Baker PN, et al. Early pregnancy prediction of preeclampsia in nulliparous women, combining clinical risk and biomarkers: the Screening for Pregnancy Endpoints (SCOPE) international cohort study. Hypertension. 2014;64(3):644–652. doi: 10.1161/HYPERTENSIONAHA.114.03578

82 Odibo AO, Goetzinger KR, Odibo L, Cahill AG, Macones GA, Nelson DM, et al. First-trimester prediction of preeclampsia using metabolomic biomarkers: a discovery phase study. Prenat Diagn. 2011;31(10):990–994. doi: 10.1002/pd.2822

83 Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. J R Stat Soc. 1995;57(1):289–300. doi: 10.2307/2346101

Delayed-Interval Delivery in Dichorionic Twin Pregnancies: A Case Report of 154 Latency Days

Parto diferido em gravidez gemelar bicoriônica: Um caso com 154 dias de latência

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Abstract

Premature delivery often complicates multifetal pregnancies, placing neonates at risk of serious morbidity and mortality. In select cases, preterm birth of one sibling may not require delivery of the remaining fetus(es), which may remain *in utero* for a delayed-interval delivery, consequently improving neonatal morbidity and mortality. Currently, there is no consensus on the best protocol for the optimal management of these cases. We report one case of delayed-interval delivery of a dichorionic pregnancy assisted in our center. In this case, prophylactic cerclage, tocolytic therapy and administration of broad-spectrum prophylactic antibiotics enabled delivery at 37 weeks, corresponding to 154 days of latency, which is, to our knowledge, the longest interval described in the literature. The attempt to defer the delivery of the second fetus in peri-viability is an option that should be offered to parents after counseling, providing that the clinical criteria of eligibility are fulfilled. The correct selection of candidates, combined with the correct performance of procedures, as well as fetal and maternal monitoring and early identification of complications increase the probability of success of this type of delivery.

Keywords

- delayed-interval delivery
- twin pregnancy
- preterm birth
- maternal morbidity
- neonatal morbidity

Resumo

O parto pré-termo espontâneo complica frequentemente as gestações multifetais, condicionando elevada morbimortalidade perinatal. Em determinados casos, o nascimento prematuro do primeiro feto pode não requerer o nascimento do(s) feto(s) restante(s), que podem permanecer *in utero*, com o objetivo de diminuir a morbidade e mortalidade neonatal. Atualmente, não existe consenso quanto à melhor atitude clínica nas situações de parto diferido. Descrevemos um caso de parto diferido de gravidez bicoriônica vigiado no nosso centro. Neste caso, a realização de cerclagem, a terapêutica tocolítica e a administração de antibioticoterapia de largo espectro permitiu o parto às 37 semanas do segundo gêmeo, o que corresponde a 154 dias de latência, que, segundo o nosso conhecimento, é o intervalo de diferimento mais

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Palavras-chave
- Parto diferido
- Gravidez gemelar
- Parto pré-termo
- Morbidade materna
- Morbidade neonatal

longo descrito na literatura. A tentativa de diferir o parto do segundo feto na periviabilidade é uma opção que deve ser oferecida aos progenitores, após aconselhamento e desde que se cumpram os critérios clínicos de elegibilidade. A seleção correta das candidatas, em conjunto com a realização de corretos procedimentos, monitorização fetal e materna e identificação precoce de complicações aumentam a possibilidade de sucesso deste tipo de parto.

Introduction

In the last decades, multifetal pregnancies have been increasing as a result of a higher mean maternal age and increased use of assisted reproductive technologies.[1–4] As a result, there is a high risk of preterm premature rupture of membranes and preterm delivery, placing neonates at risk of serious morbidity and mortality.[1,2] In twin pregnancies, delivery of the second twin generally follows the birth of the first fetus shortly thereafter. However, in selected cases, preterm birth of one sibling may not require delivery of the remaining fetus(es), who may remain *in utero* for an extended period. This event is defined as a delayed delivery of the second twin, and has been reported as a management strategy to decrease morbidity and optimize the survival of the remaining fetuses after the spontaneous preterm birth of one fetus during a multifetal gestation.[1,5,6] Despite the interest in this subject, in the literature there is still a lack of an universally-accepted protocol for the optimal management of these cases.[1–3,7,8] Here, we report the results of one dichorionic pregnancy with a delayed delivery of 154 days assisted at our center.

Case Report

A 33 year-old healthy woman, with 2 previous late abortions, was followed in our institution due to a dichorionic twin pregnancy. She had two embryo transfers after a successful assisted reproductive technology (ART) cycle. She was admitted to our emergency department at 15 weeks due to regular contractions. During the pelvic examination, bulging membranes and a complete cervical dilatation was observed, with subsequent en caul delivery of the presenting fetus. After the delivery of the first twin, the uterine contractions ceased. There were no signs of chorioamnionitis. The amniotic membrane of the second twin remained intact, and ultrasonography showed a healthy remaining fetus. The parents were informed about the option of deferring delivery of the remaining fetus along with its benefits and possible complications for the mother and remaining fetus. After the parents decided to defer the delivery of the remaining fetus, cervical cultures were taken, and a McDonald cerclage was performed under general anesthesia. Tocolysis with 25 mg of indomethacin was administered 4 times a day for 2 days, and broad-spectrum antibiotics (ampicillin and gentamycin) were administered for 7 days. The mother was continuously monitored through clinical assessment and laboratory tests. No signs of infection were listed, and serial ultrasonography confirmed fetal growth and wellbeing. The cervical length was monitored weekly with transperineal ultrasonography. Due to maternal and fetal stability, the patient was discharged at the 17th week of pregnancy. A transperineal ultrasound revealed a stable cervix, with 21 mm in length. The remaining pregnancy was as expected. At the 37th week, the cervical cerclage suture was removed. And at 37 weeks and 1 day, 154 days after the delivery of the first fetus, the remaining fetus was delivered vaginally. The second baby weighed 2,980 g, and the Apgar score was 9 at the 1st minute, and 10 at the 5th minute. No maternal morbidity occurred after the delivery, and the baby girl had an uneventful neonatal course.

Discussion

Delayed-interval delivery was first reported in the 1960s as a means of prolonging pregnancy for multifetal gestations after spontaneous delivery of the first fetus.[6,9] Since then, several case reports of asynchronous delivery have been published. To our knowledge, the published case with the longest interval between the births of both twins was also 154 days.[10] This prolongation of the gestational period enables the reduction of premature and neonatal morbidity, and increases the survival rate of the remaining fetus.[1,7,10,11] Consistent with this, Van der Straeten et al[12] reported a decrease of 13.4% in mortality with a delayed delivery of the second fetus.[2,12] It is essential that a number of conditions for deferred delivery of the second fetus are present: multifetal gestation with delivery of the first fetus before the 30th week, diamniotic pregnancy, intact membranes in the remaining gestational sac, and absence of fetal or maternal indication for delivery.[2,7,11,13] These inclusion criteria were all fulfilled in our case. The optimal management for a delayed-interval delivery has not yet been defined. Cerclage, tocolysis, hospitalization, and antibiotic therapy are all still controversial procedures.[1,2,7–9,13]

The use of prophylactic cerclage is the most controversial issue among the recommended procedures.[7,8,11,13,14] For some authors, it is a routine procedure, while for others it is recommended only if the etiology of the spontaneous delivery is cervical insuffiency.[7,8,11] Zhang et al[15] concluded that in cases of delayed-interval delivery, immediate cervical cerclage after the first delivery is associated with a significantly longer delivery interval between twins without increasing the rate of intrauterine infection.[2,10,15] The median interdelivery interval was 8 and 25 days in patients without and with cervical cerclage respectively.[10] Cerclage can minimize the exposure

of the fetal membranes to vaginal bacteria, and may provide stability to the cervix.[2] In our case, we performed a cervical cerclage and no intrauterine infection was detected. The use and duration of the tocolysis in cases of asynchronous delivery are not well established.[11] Some authors recommend routine tocolysis after the birth of the first twin, until the contractions cease.[7,13] Others use tocolytic therapy even if there is no contractility, in cases of the performance of cerclage. In these cases, a course of 48 hours of tocolytic therapy may curb the uterine contractions precipitated by the cervical manipulation.[16] Furthermore, studies demonstrate that the perinatal results in situations of threatened labor are not better when tocolysis are used as a maintenance therapy, or with repeated courses of tocolysis.[11] Because of that, tocolysis with indomethacin was performed 48 hours after the prophylactic cerclage. Some authors advocate strict bed rest in the hospital until the delayed delivery. Others believe that a prolonged hospitalization is not necessary. Up to now, no management has proved to be superior to the other.[7,13,17,18] In the case herein reported, given the maternal and fetal stability, the patient was discharged.

We opted for broad-spectrum prophylactic antibiotics, which are routinely administered by most authors to prevent the onset of an infection.[7,9,11] There is no consensus about the antibiotics of choice, the duration of the treatment, and the route of administration. However, the therapeutic scheme used in situations of preterm premature rupture of membranes could be extrapolated to cases of asynchronous delivery, because the infectious agents are similar.[11] In addition to protecting against infections, antibiotics often have tocolytic properties.[7,9] Given the cervical stability, no steroid therapy was administered at 24 weeks. Most studies demonstrate that maternal morbidity associated with asynchronous delivery is rare.[9] However, some authors describe a considerable incidence of serious maternal morbidity due to intrauterine sepsis and septicemia.[6,7] Careful monitoring can prevent the more serious maternal risks. We do not report any maternal complication. Neonatal survival and morbidity are primarily dependent on gestational age at birth.[6,7] Different clinical centers describe different survival rates, which range from 29% to 82%.[10] The long-term outcome and neurological development seems to be comparable to those of children with the same gestational age.[9,19] In the case reported here, the fetal short-term outcomes were optimal, with no neonatal morbidity.

In conclusion, delayed-interval delivery is a useful and possible therapeutic option for the management of the remaining fetus, enabling the improvement of neonatal survival and decreasing morbidity.[1,5,6] Selecting optimal candidates for delayed-interval delivery is fundamental, and parents should always be counseled about the potential risks and benefits of the procedure.[6,11,20,21] Further research in this field is needed to generate standardized management guidelines for the deferred delivery.[1–3,7,8] In the case herein reported, the performance of prophylactic cerclage, a short course of tocolytic therapy, and the administration of broad-spectrum prophylactic antibiotics enabled the delivery of the second fetus at 37 weeks, corresponding to 154 days of latency.

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

References

1 Galvão A, Gonçalves D, Rocha A, Rodrigues A, Buchner IG, Cunha A, Braga J. Delayed interval delivery of a second twin resulting in septic shock in the mother. Nascer Crescer. 2016;25(4):241–243
2 Aydin Y, Celiloglu M. Delayed interval delivery of a second twin after the preterm labor of the first one in twin pregnancies: delayed delivery in twin pregnancies. Case Rep Obstet Gynecol. 2012;2012:573824. doi: 10.1155/2012/573824
3 Arabin B, van Eyck J. Delayed-interval delivery in twin and triplet pregnancies: 17 years of experience in 1 perinatal center. Am J Obstet Gynecol. 2009;200(2):154.e1–154.e8. doi: 10.1016/j.ajog.2008.08.046
4 Văduva CC, Constantinescu C, Țenovici M, Văduva AR, Niculescu M, DiȚescu D, et al. Delayed interval delivery in twin pregnancy - case reports. Rom J Morphol Embryol. 2016;57(3):1089–1098
5 The Royal Women's Hospital. Multiple pregnancy - interval birth delivery: clinical guideline. Melbourne: The Royal Women's Hospital; 2019
6 Roman AS, Fishman S, Fox N, Klauser C, Saltzman D, Rebarber A. Maternal and neonatal outcomes after delayed-interval delivery of multifetal pregnancies. Am J Perinatol. 2011;28(2):91–96. doi: 10.1055/s-0030-1262513
7 Raposo MI, Cardoso M, Ormonde M, Stokreef S, Correia L, Pereira A. Obstetric management of delayed-interval delivery. Case Rep Womens Health. 2017;16:11–13. doi: 10.1016/j.crwh.2017.09.002
8 Zorilă GL, Marinaș MC, Florea M, Pătru CL, Drăguşin RC, Căpităescu RG, et al. Stillbirth in dichorionic twins discordant for major and minor anomaly, followed by asynchronous delivery - a rare occurrence. Case presentation. Rom J Morphol Embryol. 2016;57(3):1117–1123.
9 Yodoshi T, Tipton E, Rouse CA. A case of delayed interval delivery with a successful hospital move. Case Rep Pediatr. 2015;2015:802097. doi: 10.1155/2015/802097
10 Benito Vielba M, De Bonrostro Torralba C, Pallares Arnal V, Herrero Serrano R, Tejero Cabrejas EL, Campillos Maza JM. Delayed-interval delivery in twin pregnancies: report of three cases and literature review. J Matern Fetal Neonatal Med. 2019;32(2):351–355. doi: 10.1080/14767058.2017.1378336
11 Rodrigues F, Pereira J, Rodrigues T, Montenegro N. [The clinical challenge of delayed interval delivery in multiple pregnancies]. Acta Obstet Ginecol Port. 2015;9(2):148–153. Portuguese
12 Van der Straeten FMA, De Ketelaere K, Temmerman M. Delayed interval delivery in multiple pregnancies. Eur J Obstet Gynecol Reprod Biol. 2001;99(1):85–89. doi: 10.1016/S0301-2115(01)00376-1
13 Udealor PC, Ezeome IV, Emegoakor FC, Okeke DO, Okere PC. Delayed interval delivery following early loss of the leading twin, case report. Case Rep Obstet Gynecol. 2015;2015:213852. doi: 10.1155/2015/213852
14 Centeno M, Clode N, Tuna M, Graça LM. [Delayed delivery – perinatal and maternal outcomes]. Acta Obstet Ginecol Port. 2009;3(3):128–133. Portuguese
15 Zhang J, Johnson CD, Hoffman M. Cervical cerclage in delayed interval delivery in a multifetal pregnancy: a review of seven case series. Eur J Obstet Gynecol Reprod Biol. 2003;108(2):126–130. doi: 10.1016/S0301-2115(02)00479-7
16 Eleje GU, Ikechebelu JI, Eke AC, Okam PC, Ezebialu IU, Ilika CP. Cervical cerclage in combination with other treatments for preventing preterm birth in singleton pregnancies. Cochrane Database Syst Rev. 2017; (11):CD012871. doi: 10.1002/14651858.CD012871
17 Cozzolino M, Seravalli V, Masini G, Pasquini L, Di Tommaso M. Delayed-interval delivery in dichorionic twin pregnancies: a single-center experience. Ochsner J. 2015;15(3):248–250.

18 Doger E, Cakiroglu Y, Ceylan Y, Kole E, Ozkan S, Caliskan E. Obstetric and neonatal outcomes of delayed interval delivery in cerclage and non-cerclage cases: an analysis of 20 multiple pregnancies. J Obstet Gynaecol Res. 2014;40(7):1853–1861. doi: 10.1111/jog.12435
19 Rosbergen M, Vogt HP, Baerts W, et al. Long-term and short-term outcome after delayed-interval delivery in multi-fetal pregnancies. Eur J Obstet Gynecol Reprod Biol. 2005;122(1):66–72. doi: 10.1016/j.ejogrb.2004.11.036
20 Graça LM. Medicina materno-fetal. 5. ed. Lisboa: Lidel; 2017
21 Tran PL, Desveaux C, Barau G, Iacobelli S, Boukerrou M. Delayed-interval delivery in multifetal pregnancy: a review and guidelines for management. Gynecol Obstet (Sunnyvale). 2015;5:333. doi: 10.4172/2161-0932.1000333

Instructions to Authors

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Original Articles, complete prospective, experimental or retrospective studies. Manuscripts containing original clinical or experimental research results have priority for publication.

Case Reports, of great interest and well documented from the clinical and laboratorial point of view. In the letter of referral, authors should indicate new or unexpected aspects in relation to already published cases. The text of Introduction and Discussion sections should be based on an updated bibliographic review.

Review Articles, including comprehensive reviews, meta-analysis or systematic reviews. Spontaneous contributions are accepted. The methods and procedures adopted for obtaining the text should be described, and based on recent references, including the current year. As this subject is still subject to controversy, the review should discuss the trends and lines of research under way. In addition to the text of the review, there should be an abstract and conclusions. See the 'Instructions to Authors' section for information on the text body and title page;

Letters to the Editor, dealing with editorial matters or not, but presenting relevant information to readers. Letters can be summarized by the editor, but maintaining the main points. In case of criticism to published works, the letter is sent to the authors so their reply can be published simultaneously;

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Title

When writing a scientific article, the researcher should focus on the manuscript title, which is the business card of any publication. It should be elaborated very carefully, and preferably written only after the article finalization. A good title adequately describes the manuscript content. Generally it is not a phrase, because it does not contain the subject, only verbs and arranged objects. Titles rarely contain abbreviations, chemical formulas, adjectives, names of cities, among others. The title of manuscripts submitted to RBGO must contain a maximum of 18 words.

Abstract

The abstract should provide the context or basis for the study, establish the objectives, basic procedures, main outcomes and key findings. It should emphasize new and important aspects of the study or observations. Since the abstract is the only substantive part of the article indexed in many electronic databases, authors should ensure it reflects the article content in an accurate and highlighted manner. Do not use abbreviations, symbols and references in the abstract. In case of original articles from clinical trials, authors must inform the registration number at the end of the text.

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

Objective: What was done; the question posed by the investigator.

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

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

Conclusion: The conclusions; the answer to the question asked.

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Among the included items are the review objective to the question asked, data source, procedures for selecting the studies and data collection, the results and conclusions. The abstracts of systematic review articles submitted to RBGO must be structured in six sections and contain a maximum of 250 words:

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

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

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

Data synthesis: State the main results of the review and the methods used to obtain them.

Conclusions: Indicate the main conclusions and their clinical usefulness.

Informational abstract of unstructured type of review articles, except systematic reviews and case studies

It shall contain the substance of the article, covering the purpose, method, results and conclusions or recommendations. It exposes enough details so readers can decide on the convenience of reading the full text (Limit of words: 150).

Keywords

The keywords of a scientific paper indicate the thematic content of the text they represent. The main objectives of the aforementioned terms are the thematic content identification, indexing of the work in databases, and rapid location and retrieval of contents. The keyword systems used by RBGO are DeCS (Health Sciences Descriptors - Lilacs Indexer) and MeSH (Medical Subject Headings - MEDLINE-PubMed Indexer). Please choose five descriptors that represent your work on these platforms.

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Introduction

The **Introduction** section of a scientific article has the purpose of informing what was researched and the reason for the investigation. This part of the article prepares the reader to understand the investigation and justification of its realization. The content informed in this section should provide context or basis for the study (i.e. the nature of the problem and its importance); state the specific purpose, research objective, or hypothesis tested in the study or observation. The study objective usually has a more precise focus when formulated as a question. Both the primary and secondary objectives should be clear, and any analyzes in a pre-specified subgroup should be described; provide strictly relevant references only and do not include data or conclusions of the work being reported.

Methods

According to the Houaiss dictionary, **Methods** "is an organized, logical and systematic process of research". The method comprises the material and procedures adopted in the research in order to respond to the central research question. Structure the Methods section of RBGO starting with the study design; research scenario (place and period in which it was performed); sample of participants; data collection; intervention to be evaluated (if any) and the alternative intervention; statistical methods used and the ethical aspects of the study. When thinking about the writing of the study design, reflect if it is appropriate to achieve the research objective, if the data analysis reflects the design, and if what was expected with use of the design was achieved to research the theme. Following, the guidelines used in clinical or epidemiological research that should be included in the section Methods of manuscripts sent to RBGO:

Types of study (adapted from Pereira, 2014*):

Case Report (Case study): In-depth investigation of a situation in which one or a few people are included (usually up to ten);

Case series: A set of patients (for example, more than ten people) with the same diagnosis or undergoing the same intervention. In general, these are consecutive series of patients seen in a hospital or other health institution for a certain period. There is no internal control group formed simultaneously. The comparison is made with external controls. The name of external or historical control is given to the group used to compare the results, but that was not constituted at the same time within the study: for example, the case series is compared with patients from previous years.

Transversal (or Cross-sectional) study: Investigation to determine prevalence; examine the relationship between events (exposure, disease, and other variables of interest) at any given time. Cause and effect data are collected simultaneously: for example, the case series is compared with patients from previous years.

Case-control study: Particular form of etiological investigation of retrospective approach in which the search of causes starts from the effects. Groups of individuals, respectively with and without a particular health problem are compared in relation to past exposures in order to test the hypothesis that exposure to certain risk factors is the contributing cause of the disease. For example, individuals afflicted with low back pain are compared with an equal number of individuals (control group) of the same sex and age, but without low back pain.

Cohort study: Particular form of investigation of etiological factors in which the search of effects starts from the cause; therefore, the opposite of case-control studies. A group of people is identified, and pertinent information on the exposure of interest is collected, so the group can be monitored over time, checking those who do not develop the disease in focus, and if the prior exposure is related to occurrence of disease. For example, smokers are compared to nonsmoker controls; the incidence of bladder cancer is determined for each group.

Randomized study: This has the connotation of an experimental study to evaluate an intervention hence the synonym of *intervention study*. Can be performed in a clinical setting; sometimes referred to simply as clinical trial or clinical study. It is also conducted at the community level. In clinical trials, participants are randomly assigned to form groups called study (experimental) and control (or testimony), whether submitted or not to an intervention (for example, a drug or vaccine). Participants are monitored to verify the occurrence of outcome of interest. This way, the relationship between intervention and effect is examined under controlled observation conditions, usually with double-blind evaluation. In the case of a **randomized study**, inform the number of the Brazilian Registry of Clinical Trials (REBEC) and/or the number of the International Clinical Trials Registration Platform (ICTRP/OMS) on the title page.

Ecological study: Research performed with statistics: the unit of observation and analysis is not constituted of individuals, but of groups of individuals hence the synonyms: study of groups, aggregates, clusters, statistics or community. For example, research on the variation of mortality coefficients for diseases of the vascular system and per capita consumption of wine among European countries.

Systematic Review and Meta-analysis: Type of review in which there is a clearly formulated question, explicit methods are used to critically identify, select and evaluate relevant research, and also to collect and analyze data from the studies included in the review. There is use of strategies to

limit bias in the localization, selection, critical evaluation and synthesis of relevant studies on a given topic. Meta-analysis may or may not be part of the systematic review. Meta-analysis is the review of two or more studies to obtain a global, quantitative estimate of the question or hypothesis investigated; and employs statistical methods to combine the results of the studies used in the review.

Source: *Pereira MG. Artigos Científicos – Como redigir, publicar e avaliar. Rio de Janeiro: Guanabara-Koogan; 2014.

Script for statistical review of original scientific papers

Study objective: Is the study objective sufficiently described, including pre-established hypotheses?

Design: Is the design appropriate to achieve the proposed objective?

Characteristics of the sample: Is there a satisfactory report on the selection of people for inclusion in the study? Has a satisfactory rate of responses (valid cases) been achieved? If participants were followed up, was it long and complete enough? If there was a pairing (eg. of cases and controls), is it appropriate? How did you deal with missing data?

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Sample size: Has adequate information on sample size calculation been provided? Is the logic used to determine the study size described, including practical and statistical considerations?

Statistical Methods: Was the statistical test used for each comparison informed? Indicate if the assumptions for use of the test were followed. Was there information about the methods used for any other analysis? For example, subgroup analysis and sensitivity analysis. Are the main results accompanied by accuracy of the estimate? Inform the p value and confidence interval. Was the alpha level informed? Indicate the alpha level below which the results are statistically significant. Was the beta error informed? Or indicate the statistical power of the sample. Has the adjustment been made to the main confounding factors? Were the reasons that explained the inclusion of some and the exclusion of others described? Is the difference found statistically significant? Make sure there are sufficient analyzes to show the statistically significant difference is not due to any bias (eg. lack of comparability between groups or distortion in data collection). If the difference found is significant, is it also relevant? Specify the clinically important minimal difference. Make clear the distinction between statistically relevant difference and relevant clinical difference. Is it a one- or two-tailed test? Provide this information if appropriate. What statistical program is used? Inform the reference where to find it, and the version used.

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The purpose of the Results section is to show the study findings. It is the original data obtained and synthesized by the author with the aim to answer the question that motivated the investigation. For the writing of the section, present the results in logical sequence in the text, tables and illustrations, first mentioning the most important findings. Do not repeat all information of the tables or illustrations in the text. Emphasize or summarize only important observations. Additional or supplementary materials and technical details may be placed in an appendix where they will be accessible without interrupting the flow of the text. Alternatively, this information may be published only in the electronic version of the Journal. When data are summarized in the results section, provide numerical results not only in derived values (eg. percentages), but also in absolute values from which the derivatives were calculated, and specify the statistical methods used for their analysis. Use only the tables and figures necessary to explain the argument of the work and evaluate its foundation. When scientifically appropriate, include data analysis with variables such as age and sex. Do not exceed the maximum limit of five tables, five charts or five figures. Tables, charts and/or figures should be included in the body of the manuscript and do not count the requested limit of 4000 words.

ATTENTION!

In Case Studies, the Methods and Results sections should be replaced by the term Case Description.

Discussion

In the **Discussion** section, emphasize the new and important aspects of the study and the conclusions derived therefrom. Do not repeat details of data or other information presented in the introduction or results sections. For experimental studies, it is useful to begin the discussion by briefly summarizing the main findings, comparing and contrasting the results with other relevant studies, stating the limitations of the study, and exploring the implications of the findings for future research and clinical practice. Avoid claiming precedence and referring to incomplete studies. Do not discuss data not directly related to the results of the presented study. Propose new hypotheses when justifiable, but qualify them clearly as such. In the last paragraph of the Discussion section, cite which information of your work contributes relatively to advancement of knowledge.

Conclusion

The **Conclusion** section has the function of relating the conclusions to the objectives of the study, but authors should avoid unfounded statements and conclusions not adequately supported by data. In particular, authors should avoid making statements about economic benefits and costs unless their original includes economic analysis and appropriate data.

References

A study is based on the results of other research that preceded it. Once published, it becomes support for future work on the subject. In the report of their research, authors state the references of prior works consulted that they deem pertinent to inform readers, hence the importance of choosing good References. Properly chosen references lend credibility to the report. They are a source for convincing readers of the validity of facts and arguments presented.

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Please check the American Medical Association (AMA) Citation Style to format your references.

*The Instructions to Authors of this journal were elaborated based in the literary work ***Artigos Científicos: Como redigir, publicar e avaliar de Maurício Gomes Pereira, Editora Guanabara Koogan, 2014.***

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