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Comparison of the hemostatic effects of a levonorgestrel-releasing intrauterine system and leuprolide acetate in women with endometriosis: A randomized clinical trial

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ABSTRACT

Introduction: The hemostatic and inflammatory systems may activate each other. Endometriosis is a chronic inflammatory disease affecting 10% of women. The objective of this study was to compare the hemostatic effects of two treatments widely prescribed to women with endometriosis: the levonorgestrel intrauterine system (LNG-IUS) and the gonadotropin-releasing hormone analog (GnRHa) leuprolide acetate.
Materials and Methods: In this randomized open-label controlled trial, 44 women with endometriosis were randomly allocated to one of two groups: 22 women were assigned to use LNG-IUS and 22 to use GnRHa. The assessed variables were D-dimers, fibrinogen, prothrombin time, activated partial thromboplastin time, coagulation factors (F) II, V, VII, VIII, IX, X, and XI, antithrombin (AT), protein C, free protein S, tissue plasminogen activator (t-PA), α2-antiplasmin, thrombin-antithrombin complex, and prothrombin fragment 1 + 2. All variables were assessed before treatment and six months after treatment onset.
Results: In the LNG-IUS group, FVIII decreased 10% after six months of use. In the GnRHa group, there was a 6% increase in AT, 29% reduction in D-dimers, and 19% increase in t-PA. The LNG-IUS users exhibited a significantly greater reduction of FVIII than the GnRHa users (LNG-IUS: −6.4 ± 14.3% vs. GnRHa: 4.2 ± 12.3%, p = 0.02). The women in the GnRHa group exhibited a greater increase of AT than the LNG-IUS users (LNG-IUS: −0.7 ± 9.5% vs. GnRHa: 6.5 ± 10.1%, p = 0.02).
Conclusion: Both hormonal treatments for endometriosis exhibited no association with a procoagulant profile.

Introduction

Essentially, all situations leading to a systemic inflammatory response can be associated with some degree of activation of coagulation [1,2]. This process may range from a mild activation of coagulation that can only be detected by coagulation markers to a more robust coagulation activation that may be clinically relevant [2]. Inflammatory mediators activate coagulation mainly through the production/release of cytokines [mainly interleukin (IL)-6, but also tumor necrosis factor-α (TNF-α), IL-1 and IL-2]. Other mechanisms include the induction of tissue factor (TF) expression, inhibition of the anticoagulant pathways (protein C system, tissue factor pathway inhibitor, and antithrombin) and fibrinolysis [2–4]. During the inflammatory process, platelets are activated as a direct effect of the action of endotoxins, proinflammatory mediators, and thrombin [4]. Likewise, coagulation can also modulate inflammation through the action of the components of the thrombin-fibrin formation pathway. Fibrin increases macrophage adherence. Thrombin has important implications for inflammation due to its effects on endothelial cells [increase of cell adhesion molecule expression and stimulation of platelet-activating factor (PAF), IL-6, and IL-8 production] as well as on monocytes and macrophages (production of IL-8) [4]. Conditions such as sepsis with disseminated intravascular coagulation, acute myocardial infarction, and adult respiratory distress syndrome are some examples of the close relationship between an exacerbated inflammatory response and disorders of the hemostatic system [4–6].

Endometriosis is an inflammatory chronic disease that affects 10% of women of reproductive age [7,8] and is characterized by the presence of

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endometrial tissue outside the uterus. Currently, endometriosis is considered a proliferative, estrogen-dependent condition [9] caused by retrograde menstruation, coelomic metaplasia, and lymphatic dissemination in women who exhibit immune and genetic susceptibility [10,11]. The presence of the plasminogen activator inhibitor (PAI) and inflammatory factors in the peritoneal fluid of women with endometriosis indicates activation of the hemostatic and inflammatory systems [12]. The concentrations of urokinase plasminogen activator (u-PA) and PAI-1 are higher in the normal and ectopic endometrial tissue of women with endometriosis than in women without endometriosis [12].

The therapeutic alternatives available for women with endometriosis are surgical or pharmacological. The pharmacological agents used to control the disease symptoms include anti-inflammatory drugs (e.g., non-steroidal anti-inflammatory agents) and hormonal therapies, such as progestogens, a levonorgestrel-releasing intrauterine system (LNG-IUS), gonadotropin-releasing hormone analogs (GnRHa), and combined oral contraceptives [11].

Due to the proinflammatory state characteristic of endometriosis and to the cross-talk between the inflammatory and hemostatic systems, knowledge of the effects of the treatments for endometriosis on the hemostatic system is relevant, especially because changes in the hemostatic system may modulate the inflammatory system. The objective of the present study was to compare the effects on hemostasis of two treatments widely prescribed to women with endometriosis, LNG-IUS and GnRHa leuprolide acetate.

Material and Methods

A randomized, prospective, open-label, controlled clinical trial (www.clnicaltrial.gov: NCT02158845) was conducted to evaluate 44 patients with endometriosis, aged 18 to 40 years, who were consecutively selected at the pelvic pain and endoscopy outpatient clinic of the University Hospital of Ribeirao Preto School of Medicine, Brazil, between February 2010 and September 2012. The diagnosis of endometriosis was confirmed by laparoscopy and histology performed 3 to 24 months before inclusion in the study. All patients had chronic pelvic pain, and none of them had been treated with either hormone contraceptives for at least 3 months or with depot medroxyprogesterone acetate or GnRHa for at least 6 months at the time of randomization.

Exclusion criteria were as follows: obese patients with a body mass index (BMI) $\geq$30 kg/m^2, smokers, diabetics, alcohol or drug users, patients currently wishing to conceive, patients with chronic diseases (except endometriosis), patients with infectious processes, patients with a personal and/or family history of thromboembolic events, and patients taking medications known to interfere with inflammation markers (such as hormonal and nonhormonal anti-inflammatory agents) within the 15 days before the study.

The sample size was calculated with the STATA® program (StataCorp LP, Texas, USA). Considering D-dimers as a global marker of activated coagulation [13] and their average value (mean and standard deviation - SD) in women not using hormonal contraception as a reference (0.2 $\pm$ 0.1 μg/mL) [14], 16 participants per group were necessary to induce changes equivalent to 1 SD six months after the onset of treatment, with an alpha of 5% and a test power of 80%. To compensate for potential losses, a larger number of participants were included in each group.

The study was approved by the institutional review board of the hospital, and all patients gave written informed consent to participate.

Patients were randomized by a computer program (http://www.randomizer.org/) at a 1:1 ratio into the LNG-IUS (Mirena®, Bayer Health Care, AG, Berlin) group (n = 22) and the GnRHa (Lupron® Depot 3.75 mg, Abbott, Illinois, USA) group (n = 22). Randomization was performed in blocks (with 4 patients in each block), and the codes were placed in sealed envelopes. The investigator in charge of randomization (RAF) was not the same as the one (JCRS) who informed each participant of the group to which she was allocated. The LNG-IUS group underwent placement of the device according to the manufacturer's instructions, always by the same examiner, while the GnRHa group was treated with 3.75 mg leuprolide injected intramuscularly on a monthly basis for 6 months. Insertion of LNG-IUS was performed within the first five days after menstruation, and GnRHa was first applied within the first five days after menstruation and then every 28 days.

Assessment was performed at two time points: before and six months after the onset of treatment. The reason for choosing a six-month interval was that the use of GnRHa for longer periods of time is associated with significant side effects, such as bone mass loss [15], and some studies showed that the most beneficial effects of GnRHa for endometriosis occur within six months [16]. The same examiner measured the participants' systolic (SBP) and diastolic blood pressure (DBP), body weight, and height at both assessments. The participants were asked to come to the hospital for assessment after a 12-h fasting period, and the blood samples were collected after measurement of the clinical variables mentioned above. A total of 20 mL of blood was collected with minimal stasis and stored in non-vacuum conical plastic tubes containing 3.2% sodium citrate as an anticoagulant at room temperature. Blood samples were processed within 2 h of collection. Whole blood was centrifuged at 120 $\times g$ (700 rpm) in a Sorvall RC 3 centrifuge (Sorvall Kendro Laboratory Products GmbH, Langenselbold, Germany) at room temperature (mean, 22 °C; range, 18–24 °C) for 15 min. Platelet-poor plasma was obtained by centrifuging the remainder of the sample at 1600 $\times g$ (2500 rpm) for 30 min at 4 °C using a Universal 32 R centrifuge (Hettich Zentrifugen, Tuttlingen, Germany). All samples were stored at −80 °C. Prior to the analyses, the samples were rapidly thawed to 37 °C in a water bath, and all tests were performed in duplicate simultaneously to eliminate interassay variability. All tests were performed in the Haemostasis Laboratory of the Ribeirão Preto School of Medicine. The technician was blind to the study groups.

The following coagulation tests were performed.

(i) Coagulation times: Thrombin time (TT), activated partial thromboplastin time (APTT), and prothrombin time (PT) were assessed using an automated coagulation analyzer (STA compact, Diagnostica Stago, Asnieres-Sur-Seine, France). The following reagents were used: for PT, calcium thromboplastin; for APTT, cephalin with silica as a contact activator (STA-PTT automated 5); and for TT, human calcium thrombin (STA Thrombin 10). The results are reported as patient TT/control TT ratio, patient APTT/control APTT ratio, and International Normalized Ratio (INR = patient PT/control PT raised to the ISI power).

(ii) Procoagulatory variables: Fibrinogen was quantified using the Clauss coagulation method using kits from Diagnostica Stago (Asnières-sur-Seine, France) and the STA automated coagulation analyzer, which detects clots by photometry. The concentrations of coagulation factors II, V, VII, VIII, IX, X, and XI were determined by coagulometry using a Behring coagulation system (BCS, Dade Behring, Marburg, Germany).

(iii) Activation of the coagulation cascade: The concentrations of the thrombin-antithrombin complex (TAT) and prothrombin fragment (PF) 1 + 2 were determined by ELISA using a kit from Dade Behring (Marburg, Germany) and the EL 808 Reader (Bio Tek Instruments, Winooski, VT).

(iv) Fibrin turnover: D-dimers were measured using an ELISA kit (bioMérieux SA, Marcy-l'Etoile, France) in a Mini-Vidas analyzer (Vitek Systems; bioMérieux, France).

(v) Natural anticoagulants: Protein C and antithrombin were measured using the automated chromogenic method with BCS and a kit from Dade Behring (Marburg, Germany). Free protein S was determined by ELISA using a kit from Diagnostica Stago (Asnières-sur-Seine, France) and an EL 808 Reader (Bio Tek Instruments, Winooski, VT).