ELSEVIER

Contraception xx (2016) xxx – xxx

Contraception

Original research article

Immunologic evaluation of the endometrium with a levonorgestrel intrauterine device in solid organ transplant women and healthy controls

Caron R. Kim*,[1], Otoniel Martinez-Maza, Larry Magpantay, Clara Magyar, Jeffrey Gornbein, Radhika Rible, Peggy Sullivan

University of California, Los Angeles, Department of Obstetrics and Gynecology, 10833 Le Conte Ave., Los Angeles, CA 90095

Received 20 December 2015; revised 23 June 2016; accepted 27 June 2016

Abstract

Objective: The objective was to describe the endometrial milieu of stable transplant patients and healthy women before and after levonorgestrel intrauterine system (LNG-IUS) insertion.

Study design: Women between 18 and 45 years of age desiring LNG-IUS insertion were enrolled with a 2:1 ratio of healthy to stable solid organ transplant patients. The first visit entailed a blood draw, uterine lavage and endometrial biopsy followed by LNG-IUS insertion. Follow-up visit involved a repeat serum draw, uterine lavage and endometrial biopsy. Cytokine levels were measured in the uterine lavage and serum by quantifying inflammatory biomarkers. Immunohistochemistry staining was performed on the endometrial tissue to measure macrophage levels. Statistical analysis included a nonparametric analysis that compared medians of the marker levels before and after intrauterine device (IUD) insertion within the group and between the two groups.

Results: Sixteen participants completed the study: 5 solid organ transplant patients and 11 healthy patients. For the serum, there were no marked changes in the cytokines or soluble receptor levels in either group after IUD insertion. However, in the uterine lavage, there was an increase in cytokine levels post-IUD insertion for both healthy and transplant women. For the endometrial tissue, there was evidence of macrophage activity in both groups after device insertion.

Conclusions: This pilot study investigated the uterine environment of the transplant patient population. Findings have pointed to the strong local inflammatory response following LNG-IUS insertion for the transplant recipients. In addition, these preliminary findings will help power a larger study that can investigate the safety and effectiveness of the IUD in this patient population.

Implications: Findings from this pilot study suggest that the IUD is inducing a local inflammatory reaction in the uterus of the transplant patient as in the healthy control. A larger study can build on these preliminary results to pursue the efficacy and safety of IUD use among solid organ transplant patients.

Keywords: Intrauterine device; Immunology; Transplant; Endometrium

1. Introduction

Limited data exist on the use of intrauterine contraception in women who require chronic immunosuppression, such as solid organ transplant (SOT) recipients. Pregnancy during an immunosuppressed state has the potential for serious morbidity, secondary to worsening of maternal health conditions, which directly affects fetal and maternal health [1]. Providing reliable, long-term contraception for these women is important for the morbidity associated with unplanned pregnancy. However, practitioners are reticent to provide intrauterine devices (IUDs) for SOT recipients in the absence of studies revealing not only the safety and effectiveness of IUDs but their mechanism of action and effects on immune/inflammatory biomarkers in this select group.

Investigations on the IUD's mechanism of action have centered on the healthy, immunocompetent endometrium. These studies revolve around the local inflammatory reaction with the IUD. Macrophages, neutrophils and plasma cells

* Corresponding author.

E-mail addresses: kimcr316@gmail.com, caronkim@mednet.ucla.edu (C.R. Kim).

[1] Present address: 20 Avenue Appia, Geneva 1211, Switzerland.

http://dx.doi.org/10.1016/j.contraception.2016.06.021

have played the main roles in this inflammatory process [2–6]. In turn, the macrophages produce various cytokines that further trigger the inflammatory response. Therefore, macrophages and cytokines are our players of interest in this study.

Macrophages are present in the endometrium with an active IUD in place [4,7]. The levonorgestrel (LNG)-IUD remodels the endometrium with stromal cell decidualization and has been associated with the marked presence of neutrophils and macrophages. [8] An earlier study has also highlighted the endometrial changes with elevated leucocytes and tissue IL-8 levels 1 month after LNG-IUD insertion [7].

Another approach to studying the endometrial environment is investigating the infiltrates surrounding and attaching to the IUD. One study demonstrated attachment of leucocytes to the IUD, and these cells produced prostaglandins, contributing to the inflammatory response [9]. Although this study focused on the inert IUD, there have been subsequent studies following the same idea by performing a uterine lavage to examine cells on the device [10]. Given these findings, we quantified biomarker levels through uterine lavage and endometrial sampling.

There were several studies that informed the selection of cytokines in this study. One study reported the endometrial tissue and fluid in postmenopausal women with an IUD and found that there was an increase in IL-6 and TNF-α in the endometrium with the IUD in place [10]. Another noted IL-1α, IL-1β and IL-6 as products of the macrophages and T lymphocytes in their evaluation of endometrium [3]. We were also curious to investigate the macrophage subtypes 1 and 2 (M1 and M2) due to their distinct properties. M1s are proinflammatory cells that produce proinflammatory cytokines (IL-6, TNF-α, IL-23), whereas the M2s have anti-inflammatory properties, producing IL-10 [11].

Before investigating the safety and effectiveness of the IUD in the SOT group, there has to be an understanding of the IUD's effects on the endometrium in this population. The main objective of this study was to investigate the uterine environment of both the transplant patient and healthy controls by examining the histology and inflammatory markers, focusing on the macrophage with its subtypes. Our hypotheses were the following: (a) Transplant patients would display a smaller difference in endometrial inflammatory markers after IUD insertion compared to the healthy controls, and (2) proinflammatory macrophages (type 1 macrophages) would predominate in the healthy women, while anti-inflammatory macrophages (type 2 macrophages) would predominate in the transplant patient.

2. Materials and methods

2.1. Study participants

We recruited women between the ages of 18 to 45 years interested in the LNG-IUD as a birth control method. Inclusion criteria for the healthy controls included no immunosuppressive drug use in the past 3 months and no contraindications to IUD insertion. Exclusion criteria were current pregnancy, current pelvic infection and known to be HIV positive. For the SOT patient, we applied similar inclusion/exclusion criteria in addition to the criterion of being stable on her immunosuppressant regimen. We aimed for a case: control ratio of 1:2. After establishing eligibility, we reviewed and signed appropriate consents with the participants. We obtained the Institutional Review Board approval prior to study initiation.

2.2. Study visits

The study consisted of two visits. Visit 1 for both the SOT and healthy patients started with a blood draw by using a BD Vacutainer Serum collector. To exclude those with any local infections, we performed gonorrhea and chlamydia (GC/CT) cultures on all patients prior to IUD insertion per clinic protocol. The SOT patients had a prescreening visit that entailed contraceptive counseling and STD screening including HIV and rapid plasma reagin testing, GC/CT cultures and wet mounts. Prior to IUD insertion, we performed a uterine lavage followed by an endometrial biopsy (EMB). For the uterine lavage, we inserted a saline sonohysterography catheter through the cervix. We then pushed 3 ml of sterile normal saline through the catheter and withdrew the wash contents using a syringe. We performed the lavage under ultrasound guidance to ensure proper placement of the catheter in the uterine cavity. We avoided placement in the cervical canal to minimize withdrawal of cervicovaginal secretions. In addition, the reason to perform the lavage prior to the EMB was to minimize blood contamination of the wash that an EMB could induce if performed first. For the EMB, we used a Pipelle to withdraw endometrial tissue with a maximum of three passes.

The study participant returned 4–6 weeks later for visit 2. The participants completed a five-question exit survey at this visit. We took a repeat blood draw. Then we performed a uterine lavage and endometrial biopsy while keeping the IUD in place.

2.3. Immunoassay of cytokines and soluble receptors in serum and lavage fluids

We transported serum and lavage samples to the lab within 2 h of collection. We centrifuged the fluid sample and then subaliquoted the supernatant and serum into microtubes.

The assays used in this study were two multiplexed immunometric assay panels (Luminex platform) for cytokines (IL-1β, IL-6, IL-8, IL-10, TNF-α and IFN-γ) and for soluble receptors (sIL-2R, sIL-6R, sTNFR2 and sCD14) (R&D Systems). The Luminex xMAP system uses spectrally addressed bead sets, each of which is conjugated with a different capture monoclonal antibody specific for a given target molecule. The antibody-conjugated beads react with the sample and a secondary detection antibody to form a