Functional endometrial polyps in infertile asymptomatic patients: a possible evolution of vascular changes secondary to endometritis

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Objective: Functional polyps and chronic endometritis are among the most common abnormalities seen in the endometrium of patients with implantation failures and recurrent miscarriages. In this study we describe morphological vascular changes in endometrial samples from asymptomatic infertile patients and their association with chronic endometritis and polyp.

Study design: We selected 435 asymptomatic infertility patients submitted to office-based diagnostic hysteroscopy and endometrial biopsy. We described vascular changes and searched for histologic signs of endometritis and functional polyps in the endometrial samples. We explored the associations between these conditions.

Results: Signs of endometritis, vascular changes and polyps were identified in 176 (40.5%), 168 (38.6%) and 102 (23.4%) cases, respectively. There was a significant association between endometritis and vascular changes. The more frequent vascular alteration (70%) was the hyaline thickening of vessels, a morphological pattern very similar to the thick-walled vessels of polyps. Polyps were associated with endometritis in 28 (27.4%) cases and with other vascular changes besides the vascular stalk in 14 (13.7%). All the polyps with vascular changes had histologic evidence of endometritis. There was a significant association between inflammatory phenomena and vascular changes, even among cases of polyps.

Conclusions: Endometrial samples from infertile patients present a broad spectrum of vascular changes, most of them associated with endometritis. This association is also identified in functional polyps. Our results suggest that these alterations may be etiologically related. It is possible that the vessel axis of functional polyps actually may originate from the evolution of the vascular changes associated with endometritis. This would place functional polyps among the spectrum of inflammatory endometrial diseases.

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1. Introduction

Functional polyps and endometritis are among the most common abnormalities seen in the endometrium of patients with implantation failures and recurrent miscarriages. Although research findings in this area have not been unanimous, most researchers agree that the identification and correction of such abnormalities can improve rates of successful pregnancy [1–5].

Endometrial polyps correspond to localized overgrowth of the mucosa, which is characterized by a vascular axis of arterialized spiral vessels that involves variable fibrous stroma and/or irregular glands. They can be multiple and present with variable sizes, from a few millimeters to more than 2 cm [5]. Polyps localized in the functional layer, hormonally responsive, are classified as functional polyps.

The etiopathogenesis of polyps is not well described. It has long been supposed that they were the result of hormonal dysfunction [5], but an inflammatory component has been considered, at least in a subset of polyps [6–8]. An association between polyps and endometriosis has also been demonstrated [9,10]. Eutopic endometrium of patients with endometriosis presents significant disturbances in the populations of cells involved with the inflammatory response [11]. The elegant demonstration of reduced HOXA10 and HOXA11 expression, which is indicative of endometrial receptivity...
failure in uteri with polyps, has given an important insight into the eutopic endometrium [12].

The inflammatory phenomena in the endometrium are challenging to investigate. Cyclical endometrial inflammation is a physiological event that is present at the time of implantation as well as during menstruation, and it is a consequence of the interactions between the endocrine and immune systems [13]. Deregulation of this inflammatory response can lead to infertility and/or recurrent spontaneous abortions, as it is found in endometriosis and certain autoimmune conditions, such as systemic lupus erythematosus (SLE) and antiphospholipid syndrome (APS) [13,14]. Many eutopic endometrial changes in patients with endometriosis have been described, including disturbances in populations of inflammatory cells and cytokines [11,15,16].

Chronic endometritis is often identified in patients with unexplained infertility, and it is assumed to be caused by a variety of microbiological agents, such as bacteria, viruses, and parasites. In most cases, however, no causal pathogen can be isolated; regardless, an antibiotic regimen is generally prescribed [2]. Blood vessels are important players in the inflammatory process, and are the principal morphological element of functional polyps, where large caliber arterialized vessels are seen in the functional layer. In this study, we describe the vascular changes in endometrial samples from infertile patients and their association with endometritis and functional polyps, in order to delineate potential pathways to be investigated.

2. Materials and methods

In this study we included consecutive endometrial samples obtained during 2009 and 2010 from infertile patients with regular menstrual cycles. Individuals were recruited at two private reproduction and infertility centers, one in São Paulo and another in Porto Alegre, Brazil. The study was approved by the Department of Pathology Scientific Committee of the Faculdade de Medicina da Universidade de São Paulo and by the Ethical Committee for Research Projects of the Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo (Comissão de Ética para Análise de Pesquisa – CAPesq) (process number 021/12). The patients underwent office-based diagnostic hysteroscopy followed by blind endometrial aspiration biopsy using a silicone urethral catheter number 8. Four hundred and thirty-five patients were included in the study, aged between 23 and 49 years (35.8 ± 4.9 y). Endometriosis was investigated in 157 patients, either by laparoscopic confirmation, or by a strong clinical presentation (significant pelvic pain, associated with menstrual period, that has increased over time). It was identified in 111 patients and was excluded in 46. All the biopsies were performed after the 10th day of the menstrual cycle until the 5th postovulatory day. All cycles were spontaneous without the use of drugs.

The tissues were fixed in 10% buffered formalin and routinely processed for paraffin embedding. Four-micron sections were stained with hematoxylin and eosin, and all slides were examined independently by two pathologists (FMC and FNA). For cases with discrepant interpretation, a consensus was determined by simultaneous examination under a dual-head microscope. The microscopic study was performed on the functional layer of the mucosa. The criteria for endometritis were the presence of plasma cells in the stroma and/or a significant number (moderate to intense) of other inflammatory cells (lymphocytes, neutrophils, eosinophils and histiocytes) associated with signs of epithelium and/or vessel aggression. We considered whether each biopsy showed these alterations and so fulfilled the diagnostic criteria. Spindle-cell alteration of the stroma associated with a lesser degree of inflammatory cells was considered to be suspicious for endometritis [17], as well as cases with a significant inflammatory cellular infiltrate but without plasma cells or epithelial and/or vessel aggression.

For the purpose of statistical analysis, we grouped cases that fulfilled the criteria for endometritis with those that had highly suggestive findings under the designation “signs of endometritis”. The vascular changes that were qualitatively investigated were: high vascular density with endothelial proliferation and swelling, hyaline thickening of the vessel wall with luminal occlusion, fibrinoid degeneration of the vessel wall and small vessel thrombosis (Fig. 1). The criteria for the diagnosis of polyps were the identification of at least two of the following three features: thick-walled vessels in the functional layer (Fig. 2), fibrous or collagenous stroma, and irregular gland architecture [18]. The associations between the categorical variables were investigated by the Pearson’s chi-square test. Statistical analyses were performed using MedCalc for Windows (version 11.5.0.0; MedCalc Software, Mariakerke, Belgium), and a p-value less than 0.05 was considered significant.

3. Results

Cases of endometritis that fulfilled the established histological criteria were found in 107/435 (24.6%) samples, while 69/435

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Fig. 1. Endometrial vessel showing segmental fibrinoid degeneration, near a proliferative gland (hematoxylin–eosin stain – original magnification ×400).

Fig. 2. Vascular axis of a functional polyp (hematoxylin–eosin stain – original magnification ×400).
Table 1: Vascular changes and association with endometritis in 435 endometrial biopsies from asymptomatic infertile patients.

<table>
<thead>
<tr>
<th>Type of vascular change</th>
<th>N (%)</th>
<th>Endometritis*</th>
</tr>
</thead>
<tbody>
<tr>
<td>High vascular density with endothelial proliferation and swelling</td>
<td>30 (17.8)</td>
<td>28 (93.3%)</td>
</tr>
<tr>
<td>Hyaline thickening of the vascular wall</td>
<td>44 (26.2)</td>
<td>35 (79.5%)</td>
</tr>
<tr>
<td>High vascular density with endothelial proliferation and swelling with luminal occlusion</td>
<td>1 (0.6)</td>
<td>1 (100%)</td>
</tr>
<tr>
<td>High vascular density with endothelial proliferation and swelling associated with hyaline vascular wall thickening with luminal occlusion and segmented fibrinoid degeneration</td>
<td>2 (1.2)</td>
<td>2 (100%)</td>
</tr>
<tr>
<td>High vascular density with endothelial proliferation and swelling associated with small vessel thrombosis</td>
<td>13 (7.7)</td>
<td>10 (76.9%)</td>
</tr>
<tr>
<td>Small vessel thrombosis</td>
<td>1 (0.6)</td>
<td>1 (100%)</td>
</tr>
<tr>
<td>Hyaline vascular wall thickening with luminal occlusion and small vessel thrombosis</td>
<td>6 (3.6)</td>
<td>5 (83.3%)</td>
</tr>
<tr>
<td>Total</td>
<td>168</td>
<td>144 (85.7%)</td>
</tr>
</tbody>
</table>

* Samples fulfilled the diagnostic criteria or were highly suggestive.

(15.9%) cases showed histological changes that were highly suspicious for endometritis. Therefore, 176/435 (40.5%) samples in total were designated as having signs of endometritis. Vascular changes were identified in 168/435 (38.6%) cases. The distribution of the types of vascular involvement and their association with signs of endometritis are shown in Table 1. Polyps were identified in 102/435 (23.4%) cases. The associations of polyps with endometritis and vascular changes are shown in Table 2.

One-hundred and forty-four (85.7%) of the 168 cases with vascular changes were associated with signs of endometritis, while endometritis without vascular changes was found in only 32/435 (7.3%) cases (p < 0.0001). Twenty-three of the cases with vascular changes had thrombi and/or fibrinoid degeneration of vessel wall, which was suggestive of vasculitis (Fig. 1). Nineteen (82.6%) of these cases were associated with endometritis. Among the cases of endometritis without vascular changes, 14/32 (43.7%) had thick-walled vessels that were characteristic of polyps (Fig. 2). Forty-two patients with a diagnosis of endometritis without polyps underwent a second biopsy after antibiotic treatment with vibramycin, 100 mg every 12 h, administered for 14 days. Of these patients, five (11.9%) were diagnosed with polyps on the second biopsy. As the vascular change characterized by hyaline thickening of the vessel wall with luminal occlusion was very similar to the thick-walled vessels of polyps (Figs. 2 and 3), we considered the diagnosis of polyp only when the vascular alteration was seen in the functional layer and associated with altered stroma and/or irregular gland architecture. Hyaline thickening of the vessels was observed in 124 (70.4%) of the 176 cases with vascular changes, either isolated or together with other alterations. In eight (6.4%) of these cases, it was associated with thrombi. In 13/124 (10.5%), there were also vessels of polyps fragments. A summary of the frequencies of endometritis in different groups of patients can be seen in Table 3.

4. Comments

This is a descriptive morphological study that intentionally worked with a high-risk population for the diagnosis of endometritis and polyps. The patients were recruited from two infertility centers that receive the more complex cases of our country. Forty-six percent of our population (data not shown) presented at least one implantation failure or miscarriage before the biopsy. In this study we intentionally omitted the hysteroscopy findings because we desired to explore just the morphological alterations. We included in the study only the biopsies performed between the end of the second week of the proliferative phase and the first post-ovulatory week, because breakdown and secretory endometrium can harbor physiologic inflammatory phenomena, including vascular alterations.

We identified a large spectrum of vascular changes in the functional layer of the endometrium of 38.6% of asymptomatic infertile women, and we demonstrated a close association between these alterations and endometritis. More than 70% of the vascular alterations corresponded with vessel wall hyaline thickening, which is very similar in morphology to the thick-walled vessels along the vascular axis of polyps. This alteration in some cases was...
associated with thrombi and/or fibrinoid degeneration of the vessel wall, suggesting a vasculopathy, perhaps related to inflammation. We hypothesized that the vessel axis of polyps might represent an evolving stage of vasculopathy that was related to endometritis. This hypothesis needs confirmation with further studies. Examining the cases of polyps, we observed that all the cases with vascular changes had signs of active endometritis. Half of the cases of polyps with endometritis had no vascular changes, only the presence of a vascular axis, but even so, the frequency of endometritis among polyps was higher than among the cases without vascular changes (27.5% vs. 10.5%, p = 0.0001). Moreover, we could clearly identify polyps in the second biopsy of a number of patients who were treated with vibramycin. These facts support our hypothesis that polyps can be on the spectrum of stages that lead to chronic endometritis, probably a consequence of the whole process.

In this study, we carried on a qualitative evaluation of endometrial blood vessels. Makhija et al. conducted a morphometric analysis of vessels and found an average blood vessel concentration that was significantly higher in 13 cases of endometrial polyps compared to controls. They also analyzed 17 cases of infertility and observed no statistically significant difference in the mean value for blood vessel numbers, congestion or vascularity compared with the control group. The authors concluded that inadequate vascularization of the endometrium is not a feature that is involved in unexplained infertility, as proposed in older studies [19]. Another study evaluated microvessel density (MVD) in the endometrium, counting endothelial cells highlighted by the immunohistochemical determination of CD34. The MVD in endometrial polyps was higher than in the normal endometrium in the secretory phase, and the authors demonstrated a statistically significant difference between the surrounding endometrium and polyps within either of the two phases [20].

Although our study is purely descriptive, it raises the possibility of blood vessels as the primary target of an inflammatory process, as observed in lupus erythematosus and antiphospholipid syndrome. These conditions have been linked with infertility and spontaneous miscarriages [13,14]. A microbiological etiology of endometritis cannot be excluded, but it would seem more reasonable to consider an immunological etiology for inflammatory disturbances of the endometrium, particularly as the pathogen is not identified in most cases [2]. In further support of this hypothesis, the demonstrable inflammatory endometrial alterations in eutopic endometrium, and the higher frequency of polyps in endometriosis, also indicate a close relation between endometritis, polyps and infertility [2,9,10]. Importantly, endometriosis is a disease that is known to have a strong immune component [15].

Eutopic endometrium from patients with endometriosis shows characteristics that are potentially implicated with the pathogenesis of endometriosis secondary to the retrograde passage of endometrial cells into the peritoneal cavity [21]. The overexpression of metalloproteinases (MMP), important enzymes in the degradation and reconstruction of the extracellular matrix, is an important alteration in eutopic endometrium [20,21]. One family member, MMP-2, has been shown to be overexpressed in endometrial polyps from patients with endometriosis [20].

Higher levels of cytokines, particularly interferon–gamma, in polyps of infertile women led to the suggestion of an inflammatory etiology for polyps identified by hysteroscopy [8]. These authors suggested that the localized overgrowth of the endometrium that characterizes the polyps might be secondary to the inflammatory reaction. Previously, others authors linked endometritis with polyps [6,7], but the diagnosis of polyps was performed by hysteroscopic evaluation. If one considers an inflammatory etiopathogenesis for polyp development, a more diffuse or multifocal mucosal involvement would be expected. Actually, Cicinelli et al. described polyposis rather than poly in their cases of proven endometritis [6,7]. All these data indicate a possible intrinsic alteration of the endometrium that favors the development of polyps. The reduced receptivity of polyps demonstrated by the reduced expression of HOXA10 and HOXA11 further supports this hypothesis [12]. In this context, it is possible that polypectomy, which is proposed by various groups as the ideal treatment for infertile patients [4,5,22], is not sufficient to treat the diffuse, or at least multifocal, involvement of the mucosa. In the cases of polyps in our cohort, 14/102 (13.7%) showed other vascular alterations besides the vascular axis, meaning a probable high risk of development of new vascular axes and novel polyps. In this scenario, polyps themselves are not the cause of the infertility, but they might be a consequence of a vasculopathy, which is probably inflammatory or autoimmune. This can explain why pregnancy rates after polypectomy, although higher than in cases without this procedure, still do not reach very high numbers [23]. It is important to emphasize that there are different types of polyps and that the aspects we discussed here refer to those that originated and presented in the functional layer of the endometrium, and are more common in young patients. Surely the etiopathogenesis of the different subsets of polyps might differ from each other.

Although our results are preliminary, they present histological evidence of endometrial vessels as the target of an inflammatory process, and encourage future studies to investigate the molecular basis of the vascular changes. The knowledge of etiological factors involved in the endometrial vasculopathies can be applied in the management of infertile patients.

Conflict of interest

The authors have no conflicts of interest.

References


