The association between polycystic ovaries and endometrial cancer

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BACKGROUND: Women with polycystic ovary syndrome (PCOS) are assumed to be at increased risk of endometrial cancer (EC), albeit of a more differentiated type with better prognosis than in normal women. This study was designed to test these assumptions, as evidence for them is lacking. METHODS: The prevalence of polycystic ovaries (PCO), as a marker of PCOS, was investigated in ovarian sections from 128 women with EC and 83 with benign gynaecological conditions. The expression of the prognostic markers p53, Ki67, Bcl2 and cyclin D1 was also investigated by immunohistochemistry in endometrial tumours from 11 women with PCO and 16 with normal ovaries. RESULTS: Overall, PCO were similarly prevalent in women with EC (8.6%) and benign controls (8.4%); however, in women aged <50 years, PCO were more prevalent in women with EC (62.5 versus 27.3%, \( P = 0.033 \)). Cyclin D1-expressing endometrial tumours tended to be more prevalent in women with PCO compared to normal ovaries (36.4 versus 6.25%, respectively, \( P = 0.071 \)). Bcl2-, p53- and Ki67-expressing tumours were similarly prevalent. CONCLUSIONS: The association between PCOS and EC appears confined to premenopausal women. The tendency for cyclin D1-expressing endometrial tumours to be more prevalent in women with PCO challenges the assumption that EC prognosis is improved in women with PCOS.

Key words: cancer/endometrium/polycystic ovaries/prevalence/prognosis

Introduction

Polycystic ovary syndrome (PCOS) is one of the most common endocrinopathies in the humans, affecting 5–10% of women of reproductive age (Futterweit and Millauer, 1988). Since its original description by Stein and Leventhal in 1935 (Stein and Leventhal, 1935), studies using ultrasound have shown that at least 23% of young women exhibit polycystic ovary (PCO) morphology (Polson et al., 1988; Clayton et al., 1992). On detailed questioning, it was found that many of these women (up to 70%) had had other symptoms of PCOS (Polson et al., 1988; Clayton et al., 1992). Indeed, it has been suggested that the PCO is part of the essential phenotype of PCOS (Jacobs, 2001).

An association between PCOS and endometrial cancer (EC) was first suggested in 1949 by Speert (Speert, 1949), who noted an increased incidence of cystic ovaries in young women with EC. This association was investigated by Jackson and Docherty (1957) who recruited 16 patients with EC from ‘several thousands’ with Stein–Leventhal syndrome and a further 27 patients with Stein–Leventhal syndrome and PCO on ovarian biopsy. The authors erroneously concluded that the prevalence of EC in women with PCO was 37% (i.e., 16 of 43). Despite its methodological problems, this publication is commonly cited in support of an association between PCOS and EC. Indeed, strategies to reduce the risk of EC in women with PCOS are advised by the Royal College of Obstetrics and Gynaecology (Royal College of Obstetricians and Gynaecologists, 2002), the Health information website of the National Library of Medicine of the USA and standard textbooks of obstetrics and gynaecology (DiSaia and Creasman, 2002). There is only limited evidence to support an association between EC and PCOS; indeed, it has been suggested that the ovaries of women with EC are morphologically more similar to those of normal women than those with PCOS (Ramzy and Nisker, 1979). Furthermore, in a longitudinal study of 750 women with PCOS, mortality from EC was not increased (Pierpoint et al., 1998), and it has been suggested that the prognosis for EC is better when it occurs in women with PCOS, since their endometrial tumours tend to exhibit a greater degree of differentiation, and thus have a better prognosis, than in women without PCOS (Jafari et al., 1978).

We have reviewed the evidence supporting an association between EC and PCOS and found it to be inconclusive (Hardiman et al., 1998).
Methods

These proteins are thus considered good markers of prognosis cell proliferation, the increased expression of which in cancer-cell cycle arrest, apoptosis induction and DNA repair, and its overexpression in EC is associated with a worse prognosis (Weidner et al., 1994). These proteins are thus considered good markers of prognosis in EC, hence their investigation in this study.

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No other selection criteria were applied, and patients with gynaecological cancers other than EC were excluded. All laboratory reagents were obtained from Sigma-Aldrich (Poole, Dorset, UK), unless otherwise noted.

The prevalence of PCO morphology in subjects operated on for EC or for benign gynaecological conditions was assessed by examination of archived haematoxylin–eosin-stained 5 μm ovarian sections from each patient. In clinical histopathological practice, PCO morphology is often diagnosed using a qualitative assessment (Russell and Farnsworth, 1999; Robboy et al., 2000). To enhance reproducibility and reduce ambiguity, semiquantitative criteria for the diagnosis of PCO morphology were devised in this study, these being the presence of more than eight peripherally arranged follicular cysts per section and less than five corpora albicantia per section, the latter as evidence of reduced ovulation rate. In borderline cases (six to eight follicular cysts and five to seven corpora albicantia), a diagnosis of PCO morphology was made if capsular thickening and stromal hyperplasia/hyperthecosis were also apparent. Sections that failed to achieve these criteria were judged as normal. All sections were independently microscopically examined and scored for ovarian morphology by OCP, JC and EB according to these criteria.

Cyclin D1, Bcl2, p53 and Ki67 expression was immunohistochemically assessed in paraffin-embedded 5 μm endometrial tumour sections. Sections were deparaffinized in xylene, rehydrated in alcohol and incubated for 10 min in 3% (v/v) hydrogen peroxide. Antigen retrieval was performed by microwaving sections in 10 mM citrate buffer (pH 6.0) for 10 min, leaving them in hot buffer for 5 min, then cooling for 30 min in distilled water at 25°C. Sections were washed and blocked for 15 min in 10% rabbit (cyclin D1, p53, Bcl2) or 10% goat (Ki67) serum, then incubated with mouse monoclonal antibodies against cyclin D1 (clone DCS-6, Novocastra, Newcastle, UK), Bcl2 (clone 124, Dako, Ely, UK) or p53 (clone DO-7, Dako), each diluted 1:100, or a goat polyclonal against Ki67 (MIB-1, Immunotech, Marseille, France) diluted x25, for 1 h (Bcl2, p53 and Ki67) or overnight (cyclin D1). After washing, sections were incubated with appropriate biotinylated secondary antibodies, and immunocomplex detection was performed using the streptavidin–biotin horseradish peroxidase complex method (StreptABC Complex Kit, Dako) with 3,3′-diaminobenzidine development. Sections were then counterstained with Mayer’s hemalum (Merck, Lutterworth, UK) and mounted. Fresh paraffin-embedded ovarian sections (from blocks 1- to 6-month-old) were used as a positive control tissue. Negative controls were incubated with phosphate-buffered saline (PBS).

Immunostaining was assessed by light microscopy and scored independently by two observers (OCP, LTWF), each section being scored on two separate occasions to ensure reproducibility. Sections were scored positive for cyclin D1, Bcl2 and p53 if more than 10% of tumour cell nuclei were positively stained. This value was chosen as a threshold, as it was just above the PBS-negative control background levels. The Ki67-staining index for each section was calculated as the percentage of tumour cell nuclei stained positive in five randomly selected fields of views (Rolfe et al., 2001, 2002; Ellis et al., 2002).

Statistical analysis was performed using SPSS 11 for Windows XP and post hoc power analysis using G-power for Macintosh. The difference in PCO prevalence between the EC and control group was tested using Chi-square analysis, with a Mantel–Haenszel correction to adjust for differences in age distribution, and using Fisher’s exact test in age-restricted groups. Differences in immunostaining were tested by Fisher’s exact test (cyclin D1, Bcl2 and p53) or Student’s t-test (Ki67). P < 0.05 was taken as the threshold level of significance.

Funding sources had no involvement in any aspect of the study design, execution, interpretation or publication.

et al., 2003). Most studies have investigated the association between EC and anovulatory infertility, rather than PCOS, while others have shown an association between PCOS and endometrial hyperplasia (Chamlilian and Taylor, 1970) and assumed that the relationship extends to EC. Many studies contain no description of the type of hyperplasia, although one stated that 14% of women with endometrial hyperplasia developed EC within 14 years (Chamlilian and Taylor, 1970). Despite this lack of evidence, clinicians often advise patients with PCOS that they are at risk of developing EC and recommend the contraceptive pill or progestogens to induce withdrawal bleeds. This treatment is provided despite there having been no randomized control trials that support its utility, or to assess the most effective treatment options to manage the potential risk of EC in women with PCOS.

This study was designed to test the hypotheses that women with PCOS are at increased risk of EC, and that they have a better prognosis when EC is diagnosed than do women without PCOS. To accomplish this, differences in the prevalence of PCO morphology, as a marker of PCOS, were investigated in ovarian sections from women who underwent hysterectomy for EC or for benign conditions. To investigate prognosis, p53, Ki67, Bcl2 and cyclin D1 protein expression was investigated by immunohistochemistry in endometrial carcinomas removed from women with PCO or normal ovaries. Cyclin D1 is a cell-cycle protein whose expression level in endometrial glands is correlated with carcinogenic progression (Quddus et al., 2002). Bcl2 is an antiapoptotic protein (Adams and Cory, 1998), the decreased cytoplasmic and increased nuclear expression of which appear to correlate with a worse prognosis in EC (Sakurai et al., 1998). In many solid tumours, decreases in Bcl2 expression increase the rate of apoptosis which, in turn, stimulates cell proliferation giving a worst prognosis (Nakopoulos et al., 1999; Ohkouchi et al., 2002). p53 has an important role in cell-cycle arrest, apoptosis induction and DNA repair, and its overexpression in EC is associated with a worse prognosis (Ohkouchi et al., 2002). Ki67 is a marker of mitotic index and cell proliferation, the increased expression of which in cancerous tissues confers a worse prognosis (Weidner et al., 1994). These proteins are thus considered good markers of prognosis in EC, hence their investigation in this study.

This was a cross-sectional retrospective study carried out between December 2002 and December 2003 at the Royal Free and University College Medical School, London, with consent from the local ethics committee. The experimental group (n = 128) comprised all patients who had been diagnosed with EC, for whom archived ovaries and endometrial tissue were available, following total hysterectomy and bilateral salpingo-oophorectomy or bilateral oophorectomy. This was a cross-sectional retrospective study carried out between December 2002 and December 2003 at the Royal Free and University College Medical School, London, with consent from the local ethics committee. The experimental group (n = 128) comprised all patients who had been diagnosed with EC, for whom archived ovaries and endometrial tissue were available, following total hysterectomy and bilateral salpingo-oophorectomy or bilateral oophorectomy. The control subjects (n = 83) were age matched (±5 years) to the experimental subjects and were derived from a larger group of patients with benign gynaecological conditions operated on in the hospitals above over the same time periods, for whom archived ovaries and, in most cases, endometrium were available. Controls had also undergone total hysterectomy and bilateral salpingo-oophorectomy or bilateral oophorectomy.

Statistical analysis was performed using SPSS 11 for Windows XP and post hoc power analysis using G-power for Macintosh. The difference in PCO prevalence between the EC and control group was tested using Chi-square analysis, with a Mantel–Haenszel correction to adjust for differences in age distribution, and using Fisher’s exact test in age-restricted groups. Differences in immunostaining were tested by Fisher’s exact test (cyclin D1, Bcl2 and p53) or Student’s t-test (Ki67). P < 0.05 was taken as the threshold level of significance.

Funding sources had no involvement in any aspect of the study design, execution, interpretation or publication.
Results

Investigation of patient records revealed that there was no significant difference in the ethnicity profile between subjects with EC and benign gynaecological conditions (Table I). It also showed that patient records could not be used to determine consistently and without bias whether patients had PCOS. Archived ovarian sections were therefore investigated from each subject to determine the prevalence of features consistent with PCO morphology as a surrogate for PCOS.

Overall, the prevalence of PCO was comparable in women with EC or benign gynaecological conditions [11 of 128 (8.6%) versus 7 of 83 (8.4%), respectively, $P = 1.00$]. Post hoc power analysis indicated the experiment had a power of 0.834 and 0.999 to detect a two- or threefold increase, respectively, in PCO prevalence in the EC set at $P < 0.05$; no significant difference was however noted. Ethnicity appeared not to influence PCO prevalence as in the EC set; 10 women with PCO were Caucasian and 1 was of unknown ethnicity, while in the benign controls with PCO, 6 were Caucasian and 1 was of Indian-Asian origin. When subjects were subdivided by age (Figure 1), there were again no differences in the prevalence of PCO between the EC patients and benign controls, albeit non-significant effects were noted in women aged 20–39 years and 40–49 years. When subjects were divided into two groups aged <50 years and ≥50 years (50 years being the average age of menopause), the prevalence of PCO in patients aged <50 years was greater in those with EC than in controls [10 of 16 (62.5%) versus 6 of 22 (27.3%), respectively, $P = 0.033; \beta = 0.153$ by post hoc power analysis]. No difference was noted in PCO prevalence in patients aged ≥50 years.

Cyclin D1, Bcl2, p53 and Ki67 expression was immunohistochemically assessed in archived paraffin-embedded endometrial tumour biopsies from the 11 EC subjects with PCO morphology identified above, and from a further 16 EC subjects who had normal ovaries, age matched to those with PCO (controls). Endometrial tumours which stained positively for cyclin D1 tended to be more prevalent in women with PCO than in those with normal ovaries [4 of 11 (36.4%) versus 1 of 16 (6.25%), respectively, $P = 0.071; \beta = 0.02$ by post hoc power analysis; Figure 2]. No differences were noted in the prevalence of Bcl2-, p53- or Ki67-staining tumours. In all cases, endometrial tumours from women with PCO or normal ovaries expressed p53 at a moderate-to-strong level. The prevalence of Bcl2-positive endometrial tumours was also comparable in women with PCO or normal ovaries (approximately 75%) as was the Ki67-staining index (approximately 35%; Figure 2).

Discussion

These results support an association between PCO morphology and EC in women aged <50 years. Post hoc analysis indicated that this test had relatively low statistical power however; thus, this interpretation is made with caution. No association between PCO and EC was noted in the total subject set or in women aged >50 years. Since there was no difference in ethnicity between the subjects with EC and those with benign gynaecological conditions, this should not have biased the results.
association between PCOS and EC is consistent with the hypothesis that the stimulatory effect of estrogen on the endometrium, if unopposed by progesterone, can induce endometrial carcinogenesis (Genazzani et al., 2001). In anovulatory women, agonist-bound progesterone receptor-A inhibition of the transcriptional effects of estrogen receptor in the human reproductive system is limited (Giangrande and McDonnell, 1999). In keeping with this, the incidence of ‘fibromyoma’, myohypertrophy and endometrial hyperplasia is increased in women with PCOS (Jackson and Docherty, 1957; Chamlian and Taylor, 1970). PCOS is associated with hyperinsulinaemia and insulin resistance (Futterweit and Millauer, 1988), worsening with increasing age (Dunaif and Finegood, 1996). Aside from exacerbating the hyperandrogenism by enhancing aromatase activity (Randolph et al., 1987), these endocrine factors may underlie the association between PCO and EC, since insulin and IGF-I stimulate EC cells in vitro (Nagamani and Stuart, 1998).

Hyperandrogenaemia has been associated with EC in post-menopausal women on the basis that those with testosterone levels in the upper quartile have an approximate threefold increased risk of EC (Lukanova et al., 2004). The lack of association between PCO and EC in post-menopausal women in the present study may reflect the hypoestrogenic environment in post-menopausal women. Alternatively, the absence of an increased prevalence of PCO morphology in women aged ≥50 years with EC may be because it is difficult to identify such morphology in this age group. PCO can, however, be reliably identified in post-menopausal women by ultrasonography (Birdsall and Farquhar, 1996), and it is unlikely that the histological analysis used in this study would be less sensitive. Thus, it is probable that elements of PCO morphology remain after the menopause and, if present, would have been detected in this study. Indeed, hyperandrogenism commonly persists in post-menopausal women with PCOS (Birdsall and Farquhar, 1996; Winters et al., 2000).

A limitation of this study is that subjects were characterized as having PCO rather than PCOS. It was impossible to identify women with PCOS from patient records, consistently and without bias, because the clinical and endocrine details required were infrequently recorded for these subjects who were being treated either for EC (many of whom were post-menopausal) or for other gynaecological disorders unrelated to PCOS. Subject follow-up could also not be performed, as many were untraceable or deceased. Up to 70% of women with PCO morphology exhibit other symptoms supportive of a diagnosis of PCOS (Polson et al., 1988; Clayton et al., 1992). In the present study, the concordance between PCO morphology and PCOS was likely to be higher, since the definition of PCO morphology used included the criterion that a reduced number of corpora albicantia were present, suggestive of impaired ovulation. Such a criterion has not been previously used and, if satisfied in association with the presence of more than eight cystic follicles, is supportive of a retrospective diagnosis of PCOS. It is possible that a few subjects with normal ovaries may have had symptoms of hyperandrogenaemia and ovulatory dysfunction supportive of a diagnosis of PCOS (The Rotterdam ESHRE/ARSM-Sponsored PCOS and Consensus Workshop Group, 2004). Indeed, an ovary was classed as normal if less than eight follicles were present, even if ovulatory dysfunction was indicated by the absence of corpora albicantia. This situation seldom occurred; however, few ovaries classed as normal had a limited prevalence of corpora albicantia. It is also possible that some of the controls operated on for benign gynaecological conditions may have had occult endometrial tumours. The level of contamination of the control group was probably insignificant, however, since the subjects had been gynaecologically assessed, and endometrial histopathological assessment was made in most cases as hysterectomy was performed. Finally, no ECs were noted in post-operative records when hysterectomy was not performed, albeit these records were sometimes limited in the length of follow-up.

The 128 EC subjects and 83 controls were obtained by searching the RFH and UCH histopathology sample databases for archived ovaries from subjects who underwent surgery as described in the methods. No other selection criteria were applied except that they should have EC (experimental group) or benign gynaecological pathology (controls); other gynaecological cancers were excluded. Archived ovaries were available for all subjects and endometrium for all EC subjects and most controls. The number of EC cases at the RFH (1987–2000) can be estimated from the annual UK EC incidence at this time (approximately 5000 cases/year) divided by the number of UK treatment centres (approximately 300 medium-large hospitals before 2000). Thus, the RFH dealt with 16.6 cases/year before 2000, an estimate supported by a senior gynaecologist operating in the RFH in the 1990s. Most gynaecological cancer treatment transferred from the RFH and four other London hospitals to UCH in 2000, i.e. 100 EC cases/year (6*16.6), albeit the RFH retained approximately five cases/year. From these case loads and the sample collection periods, an estimated 532 cases of ECs were available to the study, 232 from the RFH and 300 from UCH. 128 EC cases (24%) were found by our database searches. It is possible that some subjects with EC may not have been found due to sample loss or miscoding. It is also possible that ovaries were not always available, as some of the 532 cases would have been treated with radiotherapy alone, due to tumour stage and/or comorbidity which precluded surgery. The study was inherently unbiased though, as it investigated all subjects with EC found on the databases. Even though our estimates suggest that not all EC cases treated in the hospitals were studied, there are no reasons to believe that sample selection was systematically biased.

Our results challenge the assumption that endometrial tumours in patients with PCOS are less aggressive and have a better prognosis. The expression of the cell-cycle and apoptotic proteins investigated is related to tumour prognosis; thus, it was expected that their expression would differ between endometrial tumours from patients with normal or polycystic ovaries. The expression of Bcl2, p53 and Ki67 in endometrial tumours was, however, unaffected by ovarian morphology. Furthermore, cyclin D1-expressing endometrial tumours tended to be more prevalent in women with PCO rather than normal ovaries, albeit this difference was of limited significance (P = 0.071), and post hoc analysis showed that the statistical
power of the test was low ($\beta = 0.02$); this observation must therefore be viewed with caution. It may though suggest that cyclin D1 overexpression is involved in the pathogenesis of endometrial tumours in women with PCO. Certainly, these results provide no support for the contention that the prognosis of EC is better in women with PCOS.

Recent studies suggest that endometrial carcinogenesis involves perturbation of multiple processes such as ras proto-oncogene expression, microsatellite instability, apoptosis, tumour immortalization (via telomerase activation), tumour-suppressor gene expression, proteolysis, adhesion, angiogenesis and clonal expansion (Inoue, 2005). The results of this study suggest that some patients with PCOS may develop EC via a pathway that involves cyclin D1, a member of the G1 cyclin family which regulates the G1/S transition in the cell cycle. Cyclin D1 overexpression is associated with gene amplification and transcriptional dysregulation in cancers (Sherr, 1999); indeed, cyclin D1 expression increases progressively in endometrial glands during endometrial carcinogenesis (Quddus et al., 2002). Progesterone inhibits endometrial proliferation, in part by inhibiting c-Jun recruitment to the cyclin D1 promoter and, consequently, cyclin D1 transcription and its pro-proliferative effects (Dai et al., 2003). Cyclin D1 overexpression in endometrial tumours in women with PCOS may occur due to genetic lesions or because luteal phase progesterone synthesis is reduced or absent.

More than 50 years after an association between PCOS and EC was first suggested, the nature of this association remains unclear. The results of this study suggest the association may be confined to premenopausal women. Furthermore, protein expression studies do not support the assumption that endometrial tumours in women with PCOS have a better prognosis.

Acknowledgements

This study was partly funded by a Research Bursary from The North London Nuffield Hospital (OCP) and the Annie McCall Trust (AJL).

Conflicts of interest

The authors state that no conflicts of interest were present in this publication.

References


Submitted on June 2, 2005; resubmitted on October 31, 2005; accepted on November 9, 2005