RESEARCH ARTICLE

Antiproliferative Effect of Metformin on the Endometrium - a Clinical Trial

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Abstract

Background: Unopposed estrogen has a central role in development of endometrial benign, premalignant and malignant lesions. The aim of this study was to evaluate the anti-estrogenic effect of metformin on endometrial histology in comparison with progesterone. Materials and Methods: A total of 43 patients who were referred to our center for abnormal uterine bleeding and had a histologic diagnosis were disordered proliferative endometrium or simple endometrial hyperplasia were included and randomly distributed in two groups treated with metformin (500mg Bid) or megestrol (40mg daily), respectively, for three months. After this period the patients were evaluated by another endometrial biopsy to assess the impact of the two drugs in restoring normal endometrial histology. Results: Our findings revealed that metformin could induce endometrial atrophy in 21 out of 22 patients (95.5%) while this positive response was achieved in only 13 out of 21 patients (61.9%) in the megstrol group. In addition two low grade endometrial carcinomas in the metformin group responded very well. Conclusions: We conclude that metformin could be used as an effective antiestrogenic agent in control of abnormal endometrial proliferative disorders.

Keywords: Endometrial hyperplasia - metformin - mestrol - anti-estrogenic influence - endometrial atrophy

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Introduction

Based on numerous epidemiologic and experimental studies it has been speculated that unopposed estrogen has a central role in development of endometrial benign, premalignant and malignant lesions (Fryer et al., 2002; Eftekhar et al., 2009; Chao et al., 2013; Campagnoli et al., 2013; Acmaz et al., 2014). Anovulatory cycles are common at menarche and menopause and usually induce benign endometrial proliferation including disordered proliferative endometrium and simple endometrial hyperplasia without nuclear atypia. Prolonged anovulatory cycles due to PCO or other hyperestrogenic states such as estrogen secreting tumors often lead to increased endometrial proliferation and cause complex hyperplasia with or without atypia, endometrial polyps or type I endometrial carcinoma (Huang et al., 2008). Although there is no doubt regard to role of estrogenic agents in developing of abnormal endometrial proliferation, recent understanding of genetic and molecular basis of endometrial carcinoma lead to a new terminology for benign and true premalignant endometrial lesion proposed by international group of pathologist in 2000 (Hardie et al., 2003; Jarboe et al., 2010). Based on this new classification, those proliferations that represent hormonal field effect e.g. disordered proloferative endometrium, endometrial hyperplasia (simple or complex) without nuclear atypia and endometrial polyp can be included in benign category whereas those that showing genetically altered crowded glands with clonal expansion (endometrial intraepithelial neoplasia-EIN) categorized as true premalignant group (Mutter et al., 2007; Libby et al., 2009).

Clinically, due to well-known histological changes induced by steroid hormones on the endometrium, exogenous hormone therapy has been used as an effective therapeutic tool in various situations. It is well recognized that high dose progesterone can be used for treatment of patients with atypical endometrial hyperplasia and well differentiated endometrial carcinoma who desire preserve fertility or those who are poor candidate for hysterectomy (Mutter et al., 2002a; 2002b; Mutter et al., 2007; Nevadunsky et al., 2013) .Treatment of the hyperplastic or neoplastic endometrium with high dose progesterone causes inactive or atrophic glands in a decidualized stroma in association with reverse of architecheral abnormality and nuclear atypia. In a cohort study conducted by Wheeler et al. in 44 patients who had atypical hyperplasia or low grade endometrial carcinoma, they concluded that persisting of architectural abnormalities and nuclear atypia more than 6 months of therapy strongly indicates for treatment failure (Wheeler et al., 2007).

In recent years numerous studies have indicated that

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metformin could be effective in endometrial cancer risk reduction in PCOS patients in association with effective antiproliferative activity in endometrial hyperplasia, low grade endometrial carcinoma and even in endometrial serous carcinoma cell line (Perino et al., 1987a; 1997b; Shen et al., 2008; Shen et al., 2008; Prat et al., 2009; Sarfstein et al., 2013; Shafiee et al., 2013). However clinicopathologic studies regard to metformin in restoring normal endometrial morphology or change to atrophic endometrium in compare with high dose progesterone are limited. Therefore this clinical trial conducted to examine the effect of metformin on disordered proliferative endometrium and simple endometrial hyperplasia in comparison with progesterone to assess metformin clinical usefulness in these situation.

Materials and Methods

After institutional review board approval, a prospective clinical trial was conducted. All patients who referred in three months period between May to August 2013 for abnormal uterine bleeding and underwent endometrial office biopsy or D&C in our center and their tissue diagnosis were disordered proliferative endometrium (DPE) or simple hyperplasia (SH) included in this study. Past medical history gathered from patient interview records and patients with history of metformin sensitivity, renal failure, anorexia, anemia, skin rashes, diabetes mellitus, gynecologic neoplastic disorderes and patients on estrogen or progestrone were excluded. 43 patients who fitted with including criteria categorized in two groups in randomized fashion. The first group treated with metformin (500 mg in the first week to 1000 mg in the forth week) and the second group was administrated medroxyprogesterone acetate -Megestrole (40 mg daily) for three months. After 3 months all patients in both groups underwent secondary endometrial biopsy for evaluation of treatment response.

Results

Amongst 43 patients, 22 (51.16%) case were treated with Metformin and 21(48.83%) patients with Megestrol acetate for the period of three months. The mean number of gravida, parity and abortus in obstetric history of patients for Metformin and Megestrol groups are shown respectively in Table 1.

In the Metformin group, 2 (9.1%) patients had blood glucose level of 126-200 mg/dL, which got normalizes during the period of 3 month treatment. From the studied group of Megerstrol, 2 (9.5%) patients had blood glucose level of 126-200 mg/dL and 1 (4.8%) patient had blood glucose level of over 200 mg/dL, which there were no changes after the period of 3 month treatment (Table 3, 4). Due to small number of patients with abnormal level of blood glucose level, evaluation of P value was not possible. The frequency of patients with high blood glucose level of 125 mg/dL among the Metformin and Megestrol groups are illustrated in Figure 1 and 2, before and after treatment.

The histology of the endometrial biopsy in two groups before and after treatment of Metformin and Megestrol, was described as below: *i*) Simple Hyperplasia (S.H); *ii*) Disordered proliferative Endometrium (D.P.E); *iii*) Atrophic Endometrium (A.E); *iv*) Endometrioid Endometrial Carcinoma (E.E.C); *v*) Complex Hyperplasia (C.H)

The histology of atrophic endometrium (A.E) indicates positive response to the medical treatment. Referring to table 4, before intervention the most common pathology in Metformin (40.9%) and Megestrol (71.4) groups was disordered proliferative endometrium (D.P.E). The frequency of different types of histologic findings before and after treatment in both groups is illustrated in Figure 3. Due to small sample size evaluation of P value was not possible. The histology of endometrium after treatment was A.E with frequency of 21 (95.45%) in Metformin and 13 (61.9%) in Megestrol group. According to table 5 it was apparent that the number of cases with positive response to Metformin (21-95.5%) were much higher than the Megesrtol group (14-63.6%) (Figure 4). In addition there was only 1 (4.5%) case with no response

Table 1. Obstetric History of the Patients for Metformin and Megestrol Groups

	Group	N	Mean±Std. E	Std. Deviation	Std. Error Mean	p value
Gravida	Metformin	22	2.82±0.53	2.5	0.533	0.75
	Megestrol	21	3.00±0.39	1.789	0.39	
Para	Metformin	22	2.27±0.39	1.856	0.396	0.94
	Megestrol	21	2.24±0.27	1.261	0.275	
Abortus	Metformin	22	0.50±0.25	1.185	0.253	0.44
	Megestrol	21	0.76±0.22	1.044	0.228	

Table 2. Blood Sugar before Treatment

	BS before treatment			Total
	<126	126-200	>200	
Metformin	20(90.9%)	38(88.4%)	0	22(100%)
Megestrol	18(85.7%)	2(9.5%)	1(4.8%)	21(100%)
Total	38(88.4%)	4(9.3%)	1(2.3%)	43(100%)

Table 3. Blood Sugar after Treatment

	BS after treatment		Total
	<126	126-200	
Metformin	22 (100.0%)	0	22 (100%)
Megestrol	18 (85.7%)	3 (14.3%)	21 (100%)
Total	40 (93.0%)	3 (7.0%)	43 (100%)

Table 4. Pathology of Metformin and Megestrol Groups Before and After Treatment

Group		Pathology before treatment D.P.E	Pathology after treatment A.E	Total
Metformin	S.H=8	1(12.5%)	7(87.5%)	8(100%)
	D.P.E=9	0	9(100%)	9(100%)
	E.E.C=2	0	2(100%)	2(100%)
	C.H=3	0	3(100%)	3(100%)
Total	22	1(4.5%)	21(95.5%)	22(100%)
Megestrol	S.H =6	2(33.3)	4(66.7%)	6(100%)
	D.P.E=15	6(40 %)	9(60%)	15(100%)
Total	21	8(38.1%)	13(61.9%)	21(100%)

*E.E.C=0

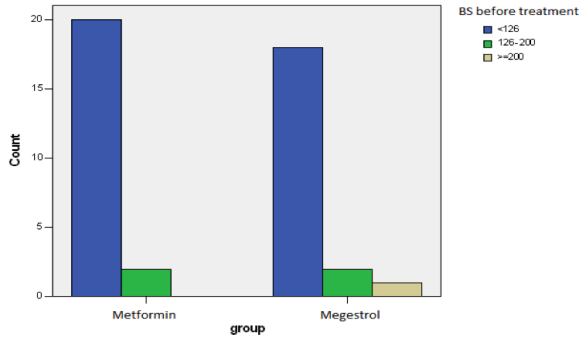

Figure 1. BS before Treatment in Metformin and Megestrol Groups

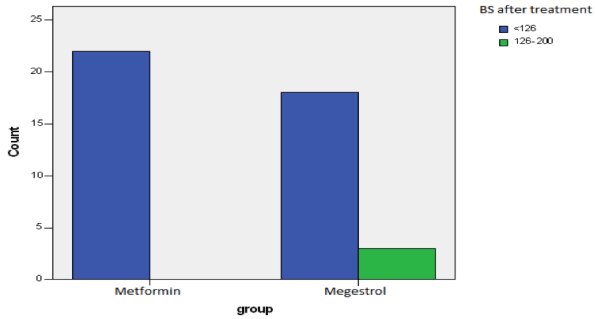

Figure 2. BS after Treatment in Metformin and Megestrol Groups

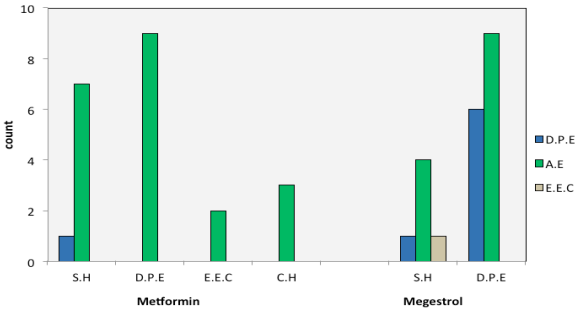

Figure 3. Pathology of Metformin and Megestrol Groups Before and After Treatment

Table 5. Response to Medication in Metformin and Megestrol Groups

Response to medication		Negative	Positive	Total
Group	Metformin	1(4.5%)	21(95.5%)	22(100%)
Group	Megestrol	8(38.1)	13(61.9%)	21(100%)
Total		9(20.5%)	34(79.5%)	43(100%)

to Metformin in comparison with 8 (38.1%) cases in Megestrol administered group. Among the Metformin administered group, before treatment, there were 2 (9.1%) cases of endometrioid endometrial carcinoma (E.E.C), and 3 (13.6%) of complex hyperplasia (C.H) also. After 3 months treatment with Metformin all of these lesions responded to the medication and were converted to atrophic endometrium. (Table 4). Due to small number of patients evaluation of P value was not possible.

Discussion

Our findings in this study revealed that metformin

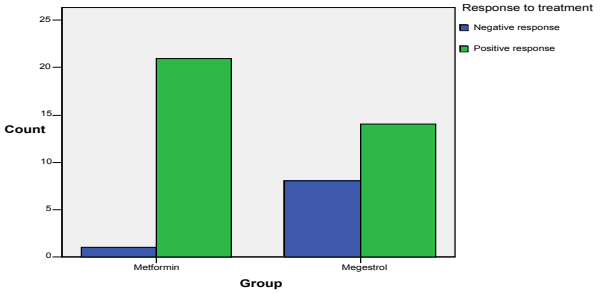

Figure 4. Response to Treatment in Metformin and Megestrol Groups

could be effective as well as megestrol in resolving of benign enodometrial proliferative lesions. In the new scheme for endometrial proliferative disorders and precancerous lesions, DPE and EH without atypia were included in benign category with no malignant potential and endometrial intrepithelial neoplasia (EIN) considered as a true precancerous lesion with significant association of co-existance or subseqent endometrial endometriod carcinoma (Shafiee et al., 1999; Wheeler et al., 2006).

In general the benign lesions usually induced by hormonal imbalance(unopposed estrogen) whereas the premalignant proliferation caused by monoclonal growth and mutation of tumor-suppressor genes in the affected glands. Management depends on the type of underlying disease, histologic diagnosis, reproductive status of the woman, whether the patient is on hormone replacement therapy or not and her general health. Benign endometrial hyperplasia responds well to medroxyprogesterone acetate (MPA), 10 mg orally, or micronized progesterone, 300 mg orally, once a day for 14 days per month for 3 months. Such cyclic regimens lead to withdrawal bleeding; a biopsy specimen is obtained at the end of the progestin therapy at 3-4 months. Complete responders should be maintained on cyclic progesterone therapy or, if appropriate, combined cyclic or continuous HRT. If a partial response is obtained, another 3-month trial with MPA, 10 mg orally four times per day, or megestrol acetate, 80 mg, for 3 months may be carried out. Non- responders and patients with intractable breakthrough bleeding may have transabdominal hysterectomy.

In a cohort study conducted by Libby et al. they found that cancer incidence in metformin user diabetic patients were significantly lower than the diabetic patients who were never on metformin after adjusting for age, sex, A1c hemoglobin, deprivation, smoking and other drug use (Huang et al., 2009). The plausible mechanism of antiproliferative effect of metformine lies in activating of AMPK pathway and enhance activation of AMPK by LBK1 which lead to lowering of cellular energy level for tumoral proliferation. Recent laboratory evidences showing that three distinct drugs (AMPK-activator) delayed tumorigenesis in tumor-prone mice. This findings suggest that AMPK activators could have therapeutic benefit for the treatment of cancer in humans (Huang et al., 2008). In another study the investigators showed that metformin acts as an antagonist to testosterone on endometrial glandular cell line and concluded that metformin could be effective in resolving of insulin

resistance effect of high androgen level in PCO patients (Zhang et al., 2011).

Our results were in line with previous preclinical studies regard to anti proliferative role of metformin in control of endometrial cell growth (Zhou et al 2001; Fryer et al., 2002; Hardie et al 2003; Zakikhani et al., 2006; Huang et al., 2008). All of 22 patients except one in metformin group respond very well and histology of the endometrium convert to atrophic endometrium. Although the current study has been focused on the antiproliferative effect of metformin in benign endometrial lesions, presence of 2 and three patients with EEC and CH in metformin group (probably for fertility desire reason) show that this medication could be effective in restoring inactive endometrium in malignant or premalignant conditions. This limited finding were in line with findings of a recent study regarding to anti-carcinogenic effect of metformin (Ko et al., 2014). Whether it might exert its effects through influence on miRNAs (Avci et al., 2013) is a question which requires attention.

In summary, the current study showed that treatment of the patients with abnormal endometrial proliferation (DPE and SH) with metformin in three months induced endometrial atrophy and prevents abnormal cell growth. Other large scale clinical trials should be conducted to establish the anti-proliferative effects of insulin sensitizing agents in patients with more serous conditions such as endometrial intraepithelial neoplasia and endometrial carcinoma.

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