Effect of Metformin Treatment on some Blood Biomarkers in Women with Endometriosis.

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Abstract

Endometriosis is a common women health disorder that occurs when Endometrial-like tissue grows outside the uterus. This may lead to irregular bleeding , pelvic pain, infertility and other complications. Metformin, because of its activity to improve insulin sensitivity, it is used for the treatment of diabetes; it also has a modulatory effect on ovarian steroid production and has anti-inflammatory properties, all may suggest its possible effect in treatment of endometriosis. This study was planned to determine the effect of metformin on serum levels of interleukin- eight(IL-8), Tumor necrosis factor-alpha (TNF –α) and estradiol (E2) production , and related symptomatic changes that accompany with endometriosis (pelvic pain, dysmenorrhea and menorrheaga) after three months of study. Blood samples were obtained from those taking metformin and measure the serum levels of (IL-8) , TNF –α and (E2) were measured before and after three months of taking a metformin .Metformin therapy resulted in a significant reduction in the clinical symptoms of endometriosis (pelvic pain and dysmenorrhea) and insignificant changes in menorrhagia. Metformin therapy resulted in a significant reduction in the serum levels of IL-8, TNF- α while insignificant reduction in estradiol E 2 in the study group after 3 months of treatment .In conclusion the results of this study, demonstrated that metformin may be a well-tolerated treatment for endometriosis that relieved pain and reduces menstrual disorders and serum levels of the inflammatory markers (IL-8 and TNF-α) are decreased in study group treated with metformin after 3 months due to its anti-inflammatory effects.

Keywords: Endometriosis, Metformin, TNF – a ,IL-8, E 2.

فعاليه دواء الميتفورمين في بعض المؤشرات الحيويه في الدم على النساء اللاتي يعانين من داء بطانه الرحم الهاجره

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الخلاصـــة

بطانة الرحم الهاجره هو من اكثر الامراض شيوعا لدى النساء و الذي يحدث نتيجه أنسجة تشبه بطانة الرحم تنمو خارج الرحم و هذا قد يؤدي إلى اضطراب الدوره الشهريه ,آلام الحوض والعقم ومضاعفات اخرى.

الميتفورمين يستخدم لعلاج مرض السكري الذي يحسن الحساسية للانسولين و لديه أيضا خصائص مضادة للالتهابات وتأثير على الإنتاج الستيرويد المبيض، لهذا االحتمال ان يكون له دور فعال في علاج البطانة الرحم الهاجره .

لدراسه ما إذا كان استخدام الميتفورمين فعالا على بطانة الرحم الهاجره من خلال تقييم استخدام الميتفورمين ١٥٠٠ملغ يوميا لمده ثلاث اشهر على مستويات الانترلوكين– ٨ و عامل نخر الورم ألفا وانتاج الستيرويد في الدم والتغييرات في الأعراض التي تحدث مع بطانة الرحم (ألم الحوض، عسر و غزاره الطمث)بعد ثلاثة أشهر من اخذ العلاج .

تشمل جمع عينات من نساء من مختلف الاعمار وعددهم ٣٠ يعانين من داء بطانه الرحم الهاجره و وينقسمون الى:

١-نساء مصابين بالمرض ويستخدمن العلاج التقليدي(حبوب منع الحمل, هرمون البروجيسترون, عقار الدانازولDanazol , مثيل محرر هرمونات التناسلGn-RH-Agonists) .

٢- نساء مصابين بالمرض يستخدمن العلاج التقليدي اضافه الى ١٥٠٠ ملغ من الميتفورمين.

تشمل اخذ عينات من الدم قبل وبعد ثلاثه أشهر من اخذ الميتفورمين واجراء بعض التحاليل المختبريه على العينات التي تشمل مستوى الانترلوكين ٨(*interleukin-8*), عامل نخر الورم (*tumor necrosis factor alpha*) و الاستروجين E2 ومقارنة النتائج .

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أدى العلاج الميتفورمين في المرضى الذين يعانون من التهاب بطانة الرحم في انخفاض كبير في عسر الطمث وآلام الحوض أسفرت أيضا عن انخفاض كبير في ل IL-8 و TNF-a لمستويات مصل - لمجموعة الدراسة بعد ٣ أشهر من العلاج ,.مما يشير إلى أنها قد يكون لها الإمكانات العلاجية للميتفورمين كما عقار للالتهاب البطاني الرحمي.

الكلمات المفتاحية : داء بطانه الرحم الهاجره، الميتفورمين،TNF-a، IL-8 ، E2.

Introduction

Endometriosis is a common health problem in women .It got its name from the word endometrium, which is the tissue lining the uterus [(1)] .During a woman's menstrual period, this tissue thickens in preparation for a fertilized egg (pregnancy). If there is no fertilization, the tissue breaks down and bleeds with each menstrual period, allowing it to exit the body.

In endometriosis, the tissue that normally lines the uterus grows outside the uterus. Most commonly, it is found on the ovaries, fallopian tubes, tissue that holds the uterus in place, outer surface of the uterus, or the lining of the pelvic cavity. Other sites for growths can include the bladder, bowel, cervix, rectum and vagina, or vulva [(2)].

The different theories involved in the pathogenesis of endometriosis indicate that the etiology of endometriosis is complex and multifactorial, involving hormonal,genetic immune and environmental components [(3)]Although the etiology of disease is undetermined, four main hypotheses have been circulated as understandable causes [(4)] :

A. Sampson's theory of retrograde menstruation.

B. Ceolomic metaplasia and induction theories (an extension of the ceolomic metaplasia theory).

C. The embryonic rest theory.

D. Lymphatic and vascular metastasis theories. Several theories considering the pathogenesis of endometriosis are shown in Table-1 , retrograde menstruation may be one of the initiating steps in the pathogenesis of superficial endometriosis, genetic and micro environmental factors that prevent clearance of ectopic lesions and allow remodeling of peritoneum are essential for the propagation of endometriotic lesions [(3,5)].

Table (1): Mechanism of the different theories in the pathogenesis of endometriosis [(6)]

Theory	**Mechanism**
Retrograde menstruation	Flow of endometrial content into pelvis, allowing implantatic endometrial lesions
Metaplasia	Transformation of peritoneal tissue or cells into endometrial tissue through hormonal and/ or immunological factors
Hormones	Estrogen-driven proliferation of endometrial lesions. Resistar to progesterone-mediated control of endometrial proliferatior
Oxidative stress and Inflammation	Recruitment of immune cells and their production of cytokin that promote endometrial Growth
Immune Dysfunction	Prevention of eliminating menstrual debris andpromotion of implantation and growth of endometrial lesions
Apoptosis suppression	Promoting survival of endometrial cells and Down regulation apoptotic pathways
Genetic	Alteration of cellular function that increases attachment of endometrial cells and evasion of these cells from immune clearance
Stem cells	Initiation of endometriotic deposits by undifferentiated cells natural ability to regenerate

Tumor necrosis factor-alpha TNF-α is the main pro-inflammatory cytokine known to impair glutathione (GSH) production by several ways, making an environment conducive to the development of oxidative stress(OS). This pathogenic cycle of GSH disturbances and enhanced TNF-α production may be active in the female reproductive tract in endometriosis. *An in vitro* study investigating endometriosis-associated infertility showed that the quality of spermatozoa may be decreased following incubation with TNF-α in a dose- and time-dependent manner[7].

TNF-α secretion is stimulated by IL-1 and bacterial endotoxin. When mediated by IL-8, TNF-α has been known to promote the growth of endometriotic cells [8] .The mechanisms connecting endometriosis and infertility involved:

- Distorted pelvic anatomy, including adhesions resulting from endometriosis, which can impair oocyte release or prevent ovum pickup and transport, [9]as well as damaged or plugged fallopian tubes or acquired or congenital uterine defects.[10].
- Altered peritoneal function, including increases in fluid volume; concentration of activated microphages; prostaglandins; IL-1, IL-6, TNF-alpha, IgG, and IgA antibodies; lymphocytes; an ovum capture inhibitor preventing cumulus -fimbria interaction;[11].
- Endocrine and an ovulatory disorders, including luteinized unruptured follicle syndrome (LUF), luteal phase defect, abnormal follicular growth, and premature as well as multiple luteinized hormone surges. It has been hypothesized that LUF may not be a consequence of endometriosis, but, in fact, may be a cause or cofactor in the development of the disease[9].
- Impaired implantation, with evidence suggesting that endometriosis may be responsible for reduced expression of the (alpha(v)beta(3) integrin)αvβ3 cell adhesion molecule during the time of implantation [11].

The diagnosis of endometriosis can be substantiated only by direct visualization during laparoscopy or laparotomy confirmed by tissue biopsy [12] Larger lesions may be seen within the ovaries as ovarian endometriomas or "chocolate cysts" as they contain a thick brownish fluid, mostly old blood. However, smaller endometriosis implants cannot be visualized with ultrasound technique [12]. Surgically, endometriosis can be staged I–IV according to the (Revised Classification of the American Society of Reproductive Medicine)[13]. In principle the various stages show these findings:

Stage I (Minimal): Endometriosis restricted to only superficial lesions and possibly a few filmy adhesions, there are isolated incidents of endometrial tissue growth outside the uterus [14].

Stage II (Mild): This diagnosis occurs when there are several small implants and a few small areas of scar tissue or adhesions and some deep lesions are present in the cul-de-sac.[15].

Stage III (Moderate): As in stage II, plus presence of endometriomas on the ovary and more adhesions. The implants in stage three must be superficial and deep.

Stage IV (Severe): This is the most severe stage of endometriosis. Patients with stage IV endometriosis will have many superficial and deep implants as well as large adhesions found.

The purpose of medical management is to minimize proliferation/reduce pain, inhibit inflammation, minimize menstrual volume, frequency and oppose E2 action[16] .

Medical treatment included :Initial therapy should include a non-steroidal anti-inflammatory drugs (NSAIDs) such as :

a- Naproxen 500 mg at first then followed by 250 mg orally three times daily, or

b-Ibuprofen 800 mg as single dose, then 400 mg orally every 6 to 8 hours or

c- Mefenamic acid 500 mg orally then followed by 250 mg every 6 hours.[17].

Surgical treatment is highly effective for the alleviation of symptom, pain reduction and can increase fertility in sub-fertile women [18].Medical management is based on hormonal suppression of endometriotic lesions and is particularly effective when amenorrhea occurs via down -regulation of the hypothalamic -pituitary-ovarian axis [19].As shown in table-2.

Table (2): Hormonal Treatment for Endometriosis [20].

Medication	Indication	Dosing	Comment
Depot MDPA (Depo- Provera)[R]	Pain relief	150 mg intramuscularly every three months	Common usage in primary care
MDPA (Provera)[R]	Pain relief	30 to 100 mg daily, given orally	Common usage in primary care
Oral contraceptives	Pain relief	0.02 to 0.03 mg ethinyl estradiol and 0.15 mg desogestrel daily (cyclically for six months	Common usage in primary care
Levonorgestrel intrauterine system (Mirena)[R]	Pain relief	Intrauterine system Can be placed easily	Common usage in primary care
Gonadotropin-releas hormone analogues (e.g. goserelin [Zoladex],	Pain relief	3.75 mg of leuprolide injected every four weeks or 3.6 mg of goserelin implanted subcutaneously for six months	Expensive; significant side effects (hypoestrogenic symptoms)
Nafarelin (Synarel)[R]	Pain relief	200 mcg intranasally twice daily for s months	Expensive; significant side effects
Danazol	Pain relief	200 mg given orally three times Daily 400 mg given orally twice daily for six months	Significant androgenic side effects
Gestrinone	Pain relief	2.5 mg given orally twice weekly for six months	Hot flashes

This study was conducted to:-

1- Investigate the effect of metformin on symptoms of endometriosis and biochemical markers.

2-Investigate the effects of metforminon serum interleukin- 8 (IL-8), tumor necrosis factor alpha (TNF-α)and estradiol (E2)levels at baseline and after 3 months of treatment**,** compared to baseline values.

Patients and methods

Thirty women were included in this study from AL-Elwiyah Maternity Teaching Hospital and Al-Yarmok Teaching Hospital for the period from January 2014 to June-2014. Verbal consent was obtained from all women prior to enrollment in the study.

Inclusion criteria

1- Women undergo diagnostic laparoscopy for pelvic pain or treated for some causes of infertility.

2- Women undergo diagnostic laprotomy for ovarian cyst or acute abdominal pain who were found to have endometriosis.

3-Women came for follow up for endometriosis.

Grouping of patients

A-Control group

Fifteen patients diagnosed by laparoscopy to have different stages of endometriosis. These patients were complaining of one or more symptoms such as dysmenorrhea, pelvic pain or menorrhagia as seen in table -3.

They received classical drugs such as danazol capsules (isoxazolic derivative of a synthetic steroids,17 α- ethinyl testosterone) , some women treated with zoladex (goserelin acetate implant) ,and some women treated with oral contraceptive pills as seen in table - 4.

Table (3): Patient's characteristics of women with endometriosis.

Patient's characteristics	Control group	Study group	P value (control versus study group)
Age in years (mean ± S.D)	34.69±7.476	32.93±7.488	0.5404
Duration of infertility in years (mean ± S.D).	4.045±1.387	4.100±1.955	0.9415
Dysmenorrhea	6/15(40.00%)	12/15(80.00%)	0.02
Pelvic pain	5/15(33.33%)	9/15(60%)	0.14
Menorrhagia	5/15(33.33%)	2/15(13.33%)	0.19

Table (4): Number of patients treated with Danazol , goserelin acetate (zoledax)[R], and oral contraceptive for control groups.

Drugs	No. of Patients
Danazol	(4)26.66%
(goserelin acetate) Zoledax[R]	(10)66.66%
oral contraceptive pill	(1)6.66%

B. Study Group

Fifteen patients with stages (I-IV) endometriosis diagnosed by laparoscopy or laprotomy and were complaining of one or more symptoms such as pelvic pain or menorrhagia and received metformin at 500 mg every 8 hour in addition to classical drugs as shown in table -5.

Table (5): Numbers of patients treated with Danazol, zoledax [R], and oral contraceptive pills plus metformin(study group).

Drugs	No. of Patients
Danazol and metformin [R]	(6)40%
Zoledax[R] and metformin [R]	(5)33.33%
oral contraceptive and metformin [R]	(4)26.66%

Methods

Sampling

Blood samples were obtained for the estimation of the cytokines as (IL-8[21] and TNF-α [22])in addition to estradiol E2 [23]levels at the start of the study from the fasting patients in morning and also at the follow up visits after three months.Estimation of the cytokines IL-8, TNF-α level was performed using a commercially available enzyme -linked immunosorbent assay (ELISA) kits.

Estimation of the estradiol E2 was performed using Radioimmunoassay.

Five ml of venous blood was withdrawn aseptically into dry plastic tubes. Then the collected blood samples were centrifuged at 3000g for 15 min and the separated serum were stored at −20 °C until time of assay.

Statistical analysis

The collected data were tabulated, compared and proper statistical analyses were performed.

A t-test is any statistical hypothesis test in which the test statistic follows a Student's t distribution if the null hypothesis is supported. It can be used to determine if two sets of data are significantly different from each other. .When the scaling term is unknown and is replaced by an estimate based on the data, the test statistic (under certain conditions) follows a Student's t distribution [24] .Frequency, mean and standard deviation (SD) were used to describe data .P value was considered positive and significant if less than 0.05.

Results and Discussion

The study sample included (30) patients comprising of 15 female as control groups and 15 female as study groups; endometriosis was diagnosed by laparotomy or laparoscopy as shown in table - 6.

Table(6): Endometriosis diagnosis by laparotomy or laparoscopy

Diagnostic routes	Causes	No. of patients
Laparoscopy	pelvic pain	**3**
Laparoscopy	Treated some causes of infertility	**7**
Laparotomy	ovarian cyst	**8**
Laparotomy	acute abdominal pain	**6**
follow up	Endometriosis	**6**

Endometriosis classified into stages according to the revised criteria of the American Society of Reproductive Medicine (ASRM) [13] Table -7 represents the % of stages in endomtrosis were I, II, III and IV in 3(10%), 14(46%), 8(27%) and 5(17%) patients.

Endometriosis can occur in teenagers and adult women of all ages, but most typically it occurs in women ages 23 – 45 for control groups 34.69±7.476.and for study groups are 32.93±7.488 as shown in Table-3.

Table(7): Stages of endometriosis.

Stages of endometriosis	Frequency
Minimal (I)	3/30 (10%)
Mild (II)	14/30(46%)
Moderate (III)	8/30(27%)
Sever (IV)	5/30(17%)

Dysmenorrhoea is the most common reported symptom in endometriosis, it occurs in six women in control groups (34.69±7.476.and twelve women in study groups are 32.93±7.488 as shown in table -3. Endometriosis is estimated to occur in 5/15(33.33%) of women presenting with pelvic pain for control groups , 9/15(60%) in study groups also showed that 5/15(33.33%) of women presenting menorrhagia in control groups and 2/15(13.33%) in study groups as shown table-3.

The serum levels of TNF- α , IL-8 and E2 in the control and study groups levels in the endometriosis before treatment and after 3 months of treatment. As shown in Table -8.

Table (8): Tumor necrosis factor-alpha (TNF- α), Interlukin-8(IL-8) and Estradiol (E_2) levels in the control and study groups:

	Control group(mean ± S.D)		Study group (mean ± S.D)		P value
	Before(1st visit)	After(2nd visit)	Before(1st visit)	After(2nd visit)	
TNF-α (pg/ml)	83.90±9.23	78.05±8.88	89.90±11.83	68.39±10.91	
P	0.15		0.004		
P1					0.23
P2					0.05
IL-8 (pg/ml)	41.31±6.376	39.86±6.239	52.70±8.646	44.54 ± 13.94	
P	0.09		0.04		
P1					0.16
P2					0.01
E_2 (pg/ml)	133.2±32.33	114.8±30.43	89.90±11.83	68.39±10.91	
P	0.37		0.25		
P1					0.92
P2					0.34

P< 0.05 is significant &P> 0.05 is not significant.

P: levels at start of the study versus after 3 months.

P1: levels at 1start of the study for Control versus study group.

P2: levels after 3 months of the study for Control versus study group.

in the present study there was a significant reduction in the number of cases with dysmenorrhea and pelvic pain (P< 0.05) after three months of metformin therapy. as seen in table -9.

Metformin significantly decreased pain and the level of C-reactive protein after 6 months of treatment in patient with endometriosis associated chronic pelvic pain [25] Rannou F *et al* 2007 , study demonstrated that IL-6, may be considered as a major regulator of C-reactive protein gene[25] tumor necrosis factor alpha [26] , nuclear factor κB [27] and transforming growth factor beta- one (TGFβ-1) [28] .The inflammation associated with endometriosis, through increased levels of peritoneal fluid Vascular Endometriosis growth factor (VEGF), may promote angiogenesis for progressive growth of endometriosis[29] .

Table(9): Changes in clinical symptoms in endometriosis control and study group before and after three months of treatment .

	Control group [N(%)]		Study group [N(%)]		P value	
	Before1st visit	After2nd visit	Before1st visit	After2nd visit	Before1st visit	After2nd visit
Dysmenorrhea	7/13 53.8%	6/15 40.00%	6/15 40 %	12/15 80.00%	0.02	0.02
P		0.46		0.02		
Menorrhagia	4/13 30.76%	5/15 33.33%	6/15 40 %	2/15 13.33%	0.19	0.61
P		0.88		0.09		
Pelvic pain	4/13 30.76%	5/15 33.33%	3/15 20%	9/15 60%	0.14	0.51
P		0.88		0.02		

N: number of positive cases/total number.

Thus , endometriosis had a direct effect on adhesion formation[30] . Moravek *et al* (2009) ,provided a preliminary data about the effectiveness of rosiglitazone, an insulin sensitizer, in treating endometriosis -related pain in six patients and concluded that it was effective and promising for usage in endometriosis patients desiring the chance to conceive [31] .

The result of this study showed that the level of IL-8 was significantly decreased after 3 months of metformin therapy (P< 0.05). IL-8 level was significantly higher in the endometriosis groups than in the control groups (p=0.01). Table 8.

Arici *et al* 1998. reported" that IL-8 is produced in the human endometrium *in vivo*, especially in glandular cells"[32] .IL-8 raise the proliferation of endometrial stromal cells as a potential autocrine growth factor [36] .

A previous study Y. Takemura *et al* [33] has shown that metformin in non decidualized ovarian endometriotic stromal cells could reduce IL-1b-induced IL-8 production, aromatase activation and proliferation. So far only a reduction of aromatase activation and a reduction of proliferation

has been demonstrated in *vitro* in ovarian endometriotic stromal cells after treatment with metformin (Y. Takemura *et al* 2007) [33] .The results of Iwabe *et al* [8] clearly demonstrated that "IL-8 is a growth-promoting factor in normal endometrium as well as in endometriotic cells". The study of Baracz *et al* 2012. [34]excluded the primary role of IL-8 in the formation of endometriosis -associated peritoneal adhesions and confirmed the role of cytokines in the pathogenesis and progression of endometriosis.The result of the present study showed that there was significant reduction in TNF-α levels in women with endometriosis after 3 months of metformin treatment.The result of Iwabe *et al 2001* [8] ,proved that it through induction of IL-8 gene and protein expression in endometriotic stromal cells ,TNF-α stimulated proliferation of endometriotic stromal cells in a dose - dependent fashion. The addition of anti-TNF-α antibody or anti-IL-8 antibody, lead to neutralized the proliferated effect of the endometriotic stromal cells, and the stimulatory effects of TNF-α [34] Bedaiwy *et al* (2002)[35] demonstrated that serum TNF-α had a high degree of sensitivity and specificity so it could be surprisingly used to differentiate between patients with and without endometriosis.

The result of the current study showed that there was no significant reduction in level

of estradiol E2 in women with endomterosis treated with metformin for periods of 3 months and this disagree with the study of Mansfield *et al* 2003 [36] . Also table 8 showed that the significant reduction in estradiol E2 ,FSH ,insulin -stimulated progesterone production in granulosa cells Thus, metformin may be effective in treatment endometriosis through suppression both ovarian and local production of estrogen [36] . So the result from this study about serum estradiol E2 may be attributed to the small sample size and short time of study or the dose of metformin was not enough to lower of estradiol E2 level.

Conclusion

From the results of this study, it was found that metformin may be well tolerated treatment for endometriosis that relieved pain and reduced menstrual disorders.Serum levels of the inflammatory markers (IL-8 and TNF-α) are decreased in study groups with metformin after 3 months indicating that metformin may be have anti-inflammatory effect.

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