Original Article

Asymmetric dimethylarginine (ADMA), nitric oxide metabolite, and estradiol levels in serum and peritoneal fluid in women with endometriosis

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Abstract

Background: Increase in nitric oxide (NO) concentration accompanied by alteration in peritoneal immune defense reactions is involved in the pathogenesis of endometriosis. Asymmetric dimethylarginine is an endogenous competitive inhibitor of NO synthase. This study was designed to compare NO metabolite (nitrite), asymmetric dimethylarginine, and estradiol concentrations in serum and peritoneal fluid (PF) of patients with and without endometriosis.

Materials and Methods: Subjects were assigned to two groups based on their laparoscopic results. The groups consisted of women with and without endometriosis (90 and 89 participants, respectively). The serum and peritoneal levels of nitrite (stable NO metabolite), asymmetric dimethylarginine, and estradiol were measured using enzyme-linked immunosorbent assay (ELISA) kits. These parameters were analyzed and compared between the groups statistically using SPSS software version 16.

Results: Both nitrite and asymmetric dimethylarginine levels were significantly higher in the serum of the participants from both groups than those in the PF group ($P < 0.05$). However, no significant difference in the asymmetric dimethylarginine level was detected between the two groups. In addition, the PF level of nitrite increased significantly in patients with endometriosis when compared with non-endometriosis subjects ($P < 0.05$). The PF levels of estradiol in both groups were significantly higher than the serum levels of estradiol ($P < 0.05$).

Conclusions: The NO metabolite level of PF implies the possible role of NO in the pathogenesis of endometriosis.

Key words: Asymmetric dimethylarginine, endometriosis, infertility, nitric oxide, peritoneal fluid

Introduction

Endometriosis is a complex disease. It is defined as the presence of functional endometrial glands and stroma outside the uterine cavity.[1] The disease is a common gynecological problem that is often associated with pelvic pain and infertility.[2] Many women suffering from pelvic pain and dysmenorrhea have endometriosis. Furthermore, according to laparoscopic studies, endometriosis is present in more than 50% of women with unexplained fertility.[3-5] A prevalence of 2-22% of endometriosis is observed among asymptomatic women, while this incidence is 40-60% in women with dysmenorrhea.[6] In spite of extensive studies conducted and the wide acceptance of John Sampson's theory of retrograde menstruation, the causes of endometriosis have remained ambiguous.[1,7] Moreover, endometriosis is broadly known as a pelvic inflammatory problem, which is related to distorted function of immune-related cells in the peritoneal environment. Many studies comply with this notion, proposing that the peritoneal fluid (PF) of women with endometriosis has a larger number of activated macrophages that secrete different local products, such as growth factors and cytokines,[8-11] as well as additional immune mediators such as nitric oxide (NO).[12-15]

NO is a known free radical that is involved in various physiological and pathophysiological processes in

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different organs, including the human female reproductive tract.[16-19] It can directly stimulate the production of vascular endothelial growth factor (VEGF); consequently, it is involved in angiogenesis of the endometrium.[20]

Asymmetric dimethylarginine (ADMA) is an endogenous competitive inhibitor of NO synthase (NOS).[21] Elevated level of ADMA is related to reduced systemic NO production.[22,23] Furthermore, ADMA inhibits NO production in cultured human macrophages in a concentration-dependent way.[24] Laparoscopy is a gold standard for diagnosis of endometriosis.[25] Yet, it is an invasive procedure, requiring surgical skill and general anesthesia, also having its own risks. In addition, significant limitations exist in the visual inspection of the pelvis, particularly in the diagnosis of retroperitoneal and deep infiltrative lesions.[26] Thus, diagnosis of endometriosis by a simple blood test would help in overcoming these problems, while significantly protecting women's health.[27] In this study, we attempted to compare NO and ADMA concentrations in the PF and serum of patients with and without endometriosis.

Materials And Methods

This study was approved by the ethics committee of Isfahan University of Medical Sciences (grant code 187086). Accordingly, all patients were informed about the research protocol and all necessary information was given, and they were included in the protocol after their consent was obtained. The samples were drawn from women who were subjected to laparoscopy for evaluation of infertility or pelvic pain at the Isfahan Fertility and Infertility Center. The patients with hypertension, coronary arterial diseases, diabetes, renal diseases, active pelvic inflammatory diseases, or polycystic ovarian syndrome were excluded. As the laparoscopy was done, the patients were divided into two groups, namely, women with and without endometriosis (group I, $n = 90$ and group II, $n = 89$, respectively). Written informed consent was obtained from all the participants.

Before induction of anesthesia, venous blood samples were obtained from all the participants. The samples were centrifuged, and then the serum samples were stored at a temperature of −20°C until measurements were carried out. Before any manipulations, the PF samples were collected from pelvis. Then, the bloody fluids were discarded and the PF samples were also centrifuged. The supernatants were stored at a temperature of −20°C for analysis.

The serum level of estradiol was measured using an enzyme-linked immunosorbent assay (ELISA) kit (Diagnostics Biochem Canada Inc., Ontario, Canada). The serum and PF levels of nitrite (stable NO metabolite) were measured using an assay (Promega Corporation, Madison, WI, USA) that involves the Griess reaction. Briefly, after adding sulfanilamide solution and incubating the mixture, *N*-(1- naphtyl) ethylenediamine dihydrochloride solution was added. Then, the absorbance was measured by a microreader at a wavelength of 540 nm. The nitrite (NO metabolite) concentration of the samples was determined by comparing to the nitrite standard reference curve. The serum and PF levels of ADMA were measured using ELISA kit (DLD Diagnostika GmbH, Hamburg, Germany). Briefly, ADMA in the samples competes with solid phase-bound ADMA for a fixed number of rabbit anti-ADMA. The anti-rabbit/peroxidase was used to detect the antibody bound to the solid phase ADMA, which is inversely proportional to the ADMA concentration of serum or PF.

Data are expressed as mean ± SEM. Data were analyzed using SPSS version 16. Unpaired *t*-test was applied to compare the parameters between the groups. Paired *t*-test was used to compare the serum and PF levels of ADMA and nitrite in each group. $P < 0.05$ were considered statistically significant.

Results

The data for estradiol, ADMA, and NO levels in serum and PF from patients with endometriosis (case) and without endometriosis (control) are given in Figure 1. No significant difference was observed in estradiol levels between the groups. However, the estradiol concentration in PF was significantly higher than that in the serum ($P < 0.05$). Both nitrite and ADMA levels in the serum of the patients from both groups were significantly higher than those in PF ($P < 0.05$). However, the PF level of nitrite increased significantly in endometriosis patients when compared with non-endometriosis subjects ($P < 0.05$). In addition, the serum levels of ADMA and nitrite and the level of ADMA in PF were not significantly different between the two groups.

Discussion

In this study, the levels of estradiol, ADMA, and nitrite in serum and PF were compared between women with and without endometriosis. We found that both ADMA and nitrite levels in PF were lower than in serum in endometriosis and non-endometriosis subjects, and endometriosis caused an increase in the nitrite level in PF but not in serum. No significant difference was observed in estradiol levels between the groups, but the estradiol concentration was higher in PF than in serum ($P < 0.05$).

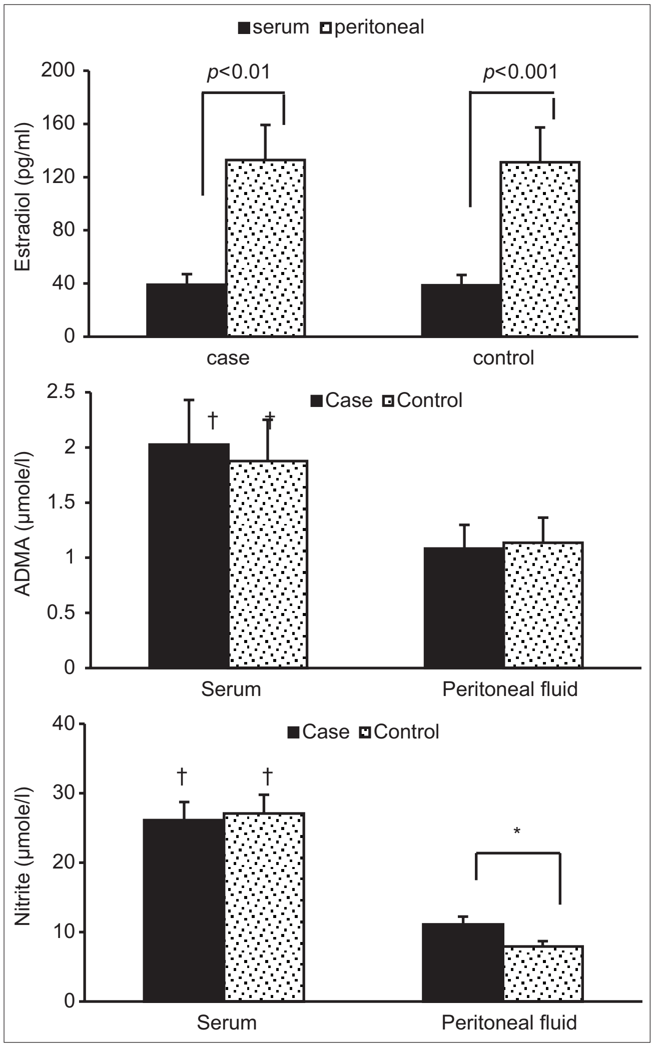

Figure 1: ADMA, NO metabolite (nitrite), and estradiol levels in serum and PF from the patients with endometriosis (case) and — without endometriosis (control). †indicates significant difference from PF ($P < 0.05$) and *indicates significant difference from the other group ($P < 0.05$)

There are several points to discuss here. The first one is NO metabolite. It was reported that PF of endometriosis patients contains higher concentrations of NO[17,28] and that the endothelial NOS located in the endometrium produces NO.[29,30] The endometrium of women with endometriosis contains a larger amount of NO[31,32] and NOS.[30,32] In addition, the number of peritoneal macrophages (PMs) in the peritoneal cavity is higher than other cells. In fact, 82-99% of the PF cell population belongs to the macrophage cells.[32-34] Several other studies have reported an increase in the number of total PF cells, macrophages, and also cell concentration in patients with endometriosis in comparison with the control group.[8,35-37] Furthermore, NOS activity and expression of inducible NOS (iNOS) of the PMs have been reported to be higher in these patients,[38] and their iNOS isoform has been reported to increase in the tissues.[32] Endothelial NOS and iNOS[39,40] are also stimulated by the cytokines that are secreted from endometrial cells, immune cells, or macrophages to release NO at higher concentrations.[29,30,40-43] Genetic and environmental factors, as well as immunologic alterations play a role in the development of endometriosis.[44] The irregular immune response may cause the macrophages and/or endometrial cells to inhibit implantation and also produce elevated concentration of NO.[45] The elevated number and activity of macrophages in endometriosis was initially considered in the concept of low-grade inflammation.[17] This has been accompanied by liberation of some immune mediators like NO[17,38,46] and extra cytokines.[38,46] Since NO can directly induce VEGF production, it is involved in endometrial angiogenesis.[20,40] In the same way, VEGF can stimulates the release of NO from endothelial cells.[47] Therefore, the nitrite level in PF in endometriosis patients could be increased by several pathways, and possibly this biomarker could be considered for diagnostic purposes.

The second point to discuss is estradiol levels in serum and PF. Endometriosis is known as an estrogen-dependent inflammatory disease.[48] Estrogen stimulates the growth of endometriotic issue in primates, including humans.[49] Estrogens can locally originate from ovary, or can be formed from the circulating estrone sulfate, or from the inactive precursor of adrenal dehydroepiandrosterone sulfate (DHEA-S), dehydroepiandrosterone (DHEA), and androstenedione.[50]

In women with endometriosis, estrogen is produced from three main sources in the body. First, the conversion of circulating androstenedione to estrone is catalyzed by aromatase in adipose tissue and skin, and is subsequently converted to estradiol. This estradiol and estrone enter the circulation and approach the endometriosis sites. Second, the estrogen secreted from the ovary reaches the endometriotic tissue via circulation. Also, during each ovulation, large amounts of estradiol are added to pelvic implants because of follicular rupture. The last source of estradiol is cholesterol, which is changed to estradiol in endometriosis. This conversion is due to the complete set of steroidogenic genes expressed by endometriosis tissue.[51] Therefore, the above mechanism may involve increase in the estradiol level in PF.[52]

Angiogenesis of the endometrium is promoted by estrogen, controlling the expression of some factors like VEGF.[53] Inflammatory and immune responses, angiogenesis, and apoptosis in women with endometriosis are altered in favor of survival and replacement of endometriotic issue.[54-58] It is also reported that the increased activity or production of numerous compounds like NO causes estrogen to have direct effects on endothelial function and vascular reactivity.[59] Thus, it seems that in patients with endometriosis, the level of NO metabolite will be increased by different immune and inflammatory responses and estradiol increase in PF is a key factor in endometriosis that elevates the NO metabolite, as estrogen promotes the persistence and survival of endometriotic tissue.[49] Thus, because of the enhanced levels of estrogen in endometriosis, increased level of NO metabolite is observed.

The last point to be considered with regard to ADMA is endometriosis. It is reported that by stimulation of dimethylarginine dimethyl aminohydrolase (DDAH) activity, estrogen decreases plasma ADMA,[59] which is an endogenous competitive inhibitor of NOS. Methylation of arginine residues in intracellular proteins produces ADMA via methyltransferases.[60-62] Furthermore, the ADMA metabolism is catalyzed by DDAH[63] to dimethylamine and citrulline.[64] The study of Holden *et al*. illustrated three main issues. The first issue is that circulating ADMA is decreased in women after estrogen replacement therapy. Secondly, the endothelial cells reduce the release of ADMA *in vitro*, a nd the final issue is that the endothelial cell DDAH enzyme activity is stimulated by estrogen.[59] At mid cycle of premenopausal women, estrogen reaches its maximum circulating concentration and plasma NO peaks.[65] The population in our study was in reproductive age, and aging seems to be a factor affecting the ADMA levels of plasma. Moreover, the participants were in the proliferation phase. Thus, it is probable that reduction in peritoneal levels of ADMA and increase in PF levels of NO are caused by the effects of estrogen.

Conclusion

According to the data available, a hypothesis can be proposed regarding the estrogen function and the crosstalk between inflammation and proliferation in endometriosis. The high PF levels of NO (not serum levels of NO) in endometriosis patients may signify the importance of narrow increase in macrophage activating factors. As increased levels of NO and ADMA were not observed in both groups, the disease seems not to be associated with a significant modulation in the levels of circulating NO in this age range.

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