Review Article

Role of iron in the oxidative stress in the pathophysiology of endometriosis: A new concept to know the potential therapeutic benefit

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ABSTRACT

Endometriosis is a multifactorial disease characterized by inflammatory changes in the pelvic cavity and symptoms of pain, infertility, and menstrual irregularities. Several theories have been proposed since Samson's theory of retrograde menstrual flow. Iron has been found to be one of the major factors involved in the above-mentioned theory. Abundant retrograde flow of blood into the pelvic peritoneum, followed by the destruction and consumption of the free red cells by the macrophages results in an iron overload both intra-and extracellular. Being a strong catalyst to the formation of free radicals, iron contributes significantly to the rise in the levels of reactive oxygen species, which cause the oxidative stress (OS). Besides stimulating the formation of free radicals and contributing to OS, increased iron in the macrophages also activates a sustained and overstimulated inflammatory response that is responsible for much of the symptoms seen in endometriosis; in particular infertility, due to extensive intraperitoneal adhesions.

While genetic factors play a role in determining a woman's response to OS, lifestyle also plays an important part. Dietary deficiency of vitamin C, vitamin E, and micronutrients such as selenium and manganese results in an acquired deficiency of antioxidants and an exaggerated response to the OS. Consumption of fresh fruits, green vegetables, etc. that are rich in antioxidants has been shown to be beneficial in alleviating the symptoms of endometriosis as well as in improving pregnancy rates. Theoretical advantages seem to be present in localized iron chelation as a method of medical management of endometriosis; however, further studies need to be conducted to confirm that the benefits outweigh the risks.

Keywords: Endometriosis, iron, oxidative stress

INTRODUCTION

Endometriosis is defined as the presence or development of functional endometrial glands and stroma outside the uterine cavity. Common sites include the pelvic peritoneum, ovaries, and recto-vaginal septum. It is a benign disease usually characterized by symptoms of severe dysmenorrhea, dyspareunia, pelvic pain, and infertility.[1,2]

Despite being a disease with a large number of complications, and being a subject of many obstetric and gynecological studies, the etiology is yet to be clearly defined. However, it is known that endometriosis is a disease of multifactorial origin. A combination of genetic, anatomical, hormonal, environmental, and immunological factors is said to be responsible for the pathogenesis of this disease.[3-5]

The most widely accepted theory in the pathophysiology of endometriosis is Samson's theory of Retrograde Menstruation, described in 1927. According to this theory, the backward flow of menstrual bleeding is responsible for the implantation and invasion of endometrial tissue in the pelvic peritoneal cavity.[1,6] The deposits may then metastasize to different sites within the body, for example the lungs or the brain. However, ectopic endometrial implants develop in only 10-15% of patients, while

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in a majority of women, most of the endometrial cells get resorbed by the peritoneal cavity. This reflux of menstrual endometrial tissue through the fallopian tubes into the peritoneal cavity results in the entry and presence of free red blood cells. In addition, the endometrial implants undergo cyclical growth and shedding with bleeding which further increases the quantity of red cells within the cavity.

Activation of the immune system results in an increase in the concentration of macrophages in the peritoneal fluid. As in other tissues in the body, pelvic and peritoneal macrophages also have primarily two functions; first, in initiating an inflammatory response.[7,8] and second, in iron homeostasis. Lysis of the red cells in the peritoneal cavity by the immune system results in an iron overload in the peritoneal fluid, the ectopic endometrial tissues, and the peritoneum adjacent to the lesions.[9] Phagocytosis of these red cells, or endocytosis of the hemoglobin-haptoglobin complex, results in the presence of iron in the macrophages. The iron is either sequestered in the macrophages as ferritin or returned to the peritoneal transferrin. Iron is a potent catalyst that generates free radicals, which are the main contributors to the oxidative stress (OS) in a patient with endometriosis. A study done by Jean-Christophe Lousse *et al.,* in 2009 shed some light on this fact and proved that iron storage is statistically significantly increased in the peritoneal macrophages of women with endometriosis in comparison to those without the disease; a fact that correlates with iron overload in the peritoneal fluid.[10]

Iron transport is also increased in patients with the disease, as the expression of transferrin receptors by the peritoneal macrophages is higher and transferrin concentrations in the peritoneal fluid are increased. Studies have been conducted which involved staining the endometriotic implants with Prussian blue, where both intracellular and extracellular iron clusters have been visualized.[10]

WHAT ARE FREE RADICALS?

OS generally develops when there is an imbalance between the radical-generating and the radical-scavenging systems, i. e., antioxidants. By definition, free radicals are species with one or more unpaired electrons in their outer orbit. The two major types of free radicals are Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS).[5,11,12] The most important ROS are the superoxide radical, hydrogen peroxide, hydroxyl radical, and the singlet oxygen radicals. Hydroxyl radicals are the most reactive free radical species and have the ability to react with a wide range of cellular constituents including amino acid residues, purine, and pyrimidine bases of deoxyribonucleic acid (DNA) and membrane lipids (lipid peroxidation). Therefore, it is imperative that the ROS be continuously inactivated and maintained at the minimal amounts that are necessary for cellular function.

The radical-scavenging systems can be both non-enzymatic and enzymatic antioxidants. The non-enzymatic antioxidants are obtained from dietary intake in the form of beta-carotenes, vitamin C, vitamin E, selenium, manganese, taurine, and hypotaurine. On the other hand, the body produces several antioxidant enzymes such as catalase, superoxide dismutase, glutathione reductase, and molecules such as glutathione and nicotinamide adenine dinucleotide (NADH). NADH has the highest reduction power among the endogenous antioxidants due to its high reactivity with free radicals besides its high intracellular concentration. A disruption in this balance between ROS production and antioxidant defence results in the OS and its related harmful effects.

OXIDATIVE STRESS AND ENDOMETRIOSIS

Iron overload in the peritoneal cavity and macrophages contributes to the formation of ROS. It has been hypothesized that the abundance of menstrual reflux might overwhelm the peritoneal protective mechanisms, leading to an accumulation of red cells and subsequently, free iron due to red cell lysis.[13] A study by Foyouzi *et al.,* in 2004 suggested that the OS has a biphasic dose-response, i. e., only moderate doses of ROS induce endometrial growth and proliferation, whereas higher doses do not, due to the direct cytotoxic effects and higher rates of apoptosis.

ROS are predominantly generated in the peritoneal activated macrophages, triggered by the accumulated iron. Iron, thereby, compromises the function of the macrophages, while also enhancing the activation of nuclear factor (NF)-κB, a proinflammatory transcriptional factor. The enhanced inflammatory response results in the increased production of cytokines and interferons.[6,11,12,14] These inflammatory mediators are the factors implicated in the symptoms of pain and infertility associated with the condition. The increase in the OS may also be furthered by the depletion in the antioxidant reserve. Some women have a genetically decreased expression of certain enzymes involved in the defence against OS, while in the other set of patients, the extent of OS results in the depletion of the antioxidant levels. It has been demonstrated that the vitamin E levels are significantly lower in the peritoneal fluid of women with endometriosis, as a result of the increased OS.[15] Another study demonstrated that the levels of superoxide dismutase and glutathione peroxidase are also decreased in the peritoneal fluid of women with the disease.

OS increases vascular endothelial growth factor (VEGF) production, which promotes the growth of the endometrial implants and stimulates angiogenesis. This effect is partly mediated through glycodelin. Glycodelin is a glycoprotein whose expression is stimulated by OS, and it acts as an autocrine factor within the ectopic endometrial tissue by augmenting the expression of VEGF.[1]

OS is also known to be the contributing factor to the formation of intraperitoneal adhesions; one of the key factors implicated in the infertility associated with endometriosis.[16] The enhanced inflammatory response furthers the formation of these adhesions. This occurs due to the mechanical hindrance of the sperm-egg encounter. The endometrial implants and the adhesions distort the normal anatomy of the female reproductive system, i. e., the fallopian tubes and the ovaries. Blockage of the tubes impairs the normal movement of the eggs from the ovaries to the uterus. Destruction of the fimbriae results in failure in the oocyte pick-up during ovulation. Structural destruction of the ovaries as a result of the ectopic tissue results in a decrease in the ovarian volume and the number of viable eggs; and the toxic effects of the free

radicals reduce the viability of those eggs that remain.[17] A study done by Mansour *et al.,* in 2007 demonstrated that oocytes incubated in the peritoneal fluid of women with endometriosis exhibited increased DNA damage.

Besides the above-mentioned effects, the OS also has detrimental effects on the sperms that enter the female reproductive tract, usually destroying them before effective fertilization can take place. The levels of ROS in women with endometriosis are toxic to sperm plasma and the acrosomal membranes, thus resulting in loss of motility and decreased ability in penetrating the oocyte. A concurrent study by Mansour *et al.,* in 2009 showed that even spermatozoa incubated in the peritoneal fluid of women with endometriosis exhibited increased DNA fragmentation.[18,19]

On those occasions that fertilization does occur, the presence of endometriosis results in poor quality embryos and by extension, poor implantation rates. Besides the negative effect on sperm motility, the increased levels of iron and the resulting OS also arrest the division of the fertilized egg leading to embryo death. This is the reason why success rates of *in vitro* fertilization (IVF) are unpredictable and the procedure prone to failure. Excessive levels of ROS can interfere with the IVF by lowering the quality of the eggs, inducing early embryonic apoptosis after intracytoplasmic sperm injection (ICSI) and by hampering the intrauterine growth of the blastocysts.

This increased DNA damage to the sperms, oocytes, and embryos is proposed to be responsible for the increased miscarriages and fertilization and implantation failures seen in women with endometriosis. The excessive ROS levels implicated in the pathogenesis of endometriosis-induced infertility is therefore the potential and most promising target for medical management of this disease.

EVIDENCE-BASED MEDICAL MANAGEMENT

As mentioned above, the peritoneal fluid of women with endometriosis-induced infertility has been found to have elevated ROS and/or insufficient anti-oxidant defence mechanisms.[11-13] Lifestyle factors such as inadequate dietary intake of antioxidants may also contribute to the OS seen in this condition. It has been demonstrated in numerous studies that a significant reduction in the risk of endometriosis is seen in women who consumed a larger proportion of green vegetables and fresh fruits as part of their daily diet. Mier-Cabrera *et al.,* conducted a study in 2008 which reported that women with endometriosis had lower intake of vitamin A, C, E, zinc, and copper in their diet compared to those without the disease. The introduction of an antioxidant-rich diet in the patients increased the peripheral concentrations of vitamins A, C, and E within 3 months in comparison to the control group with normal diet.[15] The high antioxidant diet also resulted in an increase in the activity of enzymes like SOD and glutathione peroxidase within the same time period.[20] A study done by Westphal *et al.,* in 2004 demonstrated a 33% increase in pregnancy rates in women placed on a nutritional supplementation diet rich in antioxidants.

Vitamin E is an important lipid-soluble antioxidant molecule that enhances the activity of the free-radical scavenging system in the body. It also interrupts the lipid peroxidation which is an important step in the free-radical induced OS. It is a well-known fact that vitamin E works synergistically with Selenium, a micronutrient. An increase in the dietary intake of nuts, sunflower seeds, and extra-virgin olive oil can be advised as they are known to be rich in vitamin E.

Vitamin C on the other hand is a water soluble ROS scavenger. It protects against oxidative damage by neutralizing free radicals like the hydroxyl and superoxide anions, and hydrogen peroxide. Vitamin C and E are often prescribed in combination due to their synergism because of their actions on the aqueous and hydrophobic phases of cellular damage, respectively. Vitamin C also helps regenerate the tocopheroxyl free radical which is the active component of vitamin E. Iron overload in patients with endometriosis sheds some light on iron chelation as a potential therapeutic measure in patients with the disease. Patients with endometriosis very often have symptoms of menorrhagia with prolonged bleeding. Therefore, the iron overload in the pelvic/peritoneal cavity would usually be associated with an overall decrease in the body iron content due to menstruation. For this reason, any therapy with iron chelators must be localized to the peritoneal cavity, for example, with intrapelvic implants that release desferrioxamine gradually over several months.

CONCLUSION

Endometriosis is a multifactorial disease characterized by inflammatory changes in the pelvic cavity and symptoms of pain, infertility, and menstrual irregularities. Despite high prevalence and extensive studies, the pathophysiology of this disease still remains enigmatic. Several theories have been proposed since Samson's theory of retrograde menstrual flow. But the mechanism behind the development of the disease is still a debatable topic. Recent studies have brought to light OS as a key factor in the presentation and progression of the disease. OS is a phenomenon that is triggered by free radicals, which in turn are present in high quantities in patients with the disease owing to a multitude of factors.

Iron has been found to be one of the major factors involved in the above-mentioned theory. Abundant retrograde flow of blood into the pelvic peritoneum, followed by the destruction and consumption of the free red cells by the macrophages results in an iron overload both intra- and extracellular. Being a strong catalyst to the formation of free radicals, iron contributes significantly to the rise in the levels of ROS, which cause the OS. Studies have also shown that it is the imbalance between this stress and the innate antioxidant defence mechanism that is more relevant to the pathophysiology of the disease, which would explain the difference in the severity of the disease among different women.

Besides stimulating the formation of free radicals and contributing to OS, increased iron in the macrophages also activates a sustained and overstimulated inflammatory response that is responsible for much of the symptoms seen in endometriosis; in particular infertility, due to extensive intraperitoneal adhesions.

While genetic factors play a role in determining a woman's response to OS, lifestyle also plays an important part. Dietary

deficiency of vitamin C, vitamin E, and micronutrients such as selenium and manganese results in an acquired deficiency of antioxidants and an exaggerated response to the OS. Consumption of fresh fruits, green vegetables, etc. that are rich in antioxidants has been shown to be beneficial in alleviating the symptoms of endometriosis as well as in improving pregnancy rates.

Theoretical advantages seem to be present in localized iron chelation as a method of medical management of endometriosis; however, further studies need to be conducted to confirm that the benefits outweigh the risks.

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