The possible role of zinc in the etiopathogenesis of endometriosis

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Summary

Purpose of investigation: Aim of the study was to evaluate the possible involvement of zinc in the complex pathogenic process behind the onset and perpetuation of endometriotic lesions. To study the level of zinc serum between a group of patients affected by endometriosis and a group of healthy patients. *Materials and Methods:* The study included 86 women: 42 patients whose histodiagnosis had revealed pelvic endometriosis and 44 healthy patients. The authors measured the serum zinc concentration for all patients. *Results:* The group of patients with endometriosis presented serum zinc concentration of 1,010 ± 59.24 μg/l. The observation group presented a serum zinc concentration of 1,294 ± 62.22 μg/l. *Conclusion:* The results showed that serum zinc levels in women with endometriosis are decreased and this seems to actually confirm that this micro-element can possibly affect the multifactorial pathogenesis of the disease. As a matter of fact, zinc interferes with many biological processes, among which inflammation and immunity, which seem to be the base of the development of the lesions. Therefore, the authors believe that this hypothesis requires more attention and further investigation to determine its reasonableness. If the results are confirmed, this study opens up future prospects as for the treatment of endometriosis, taking into account also the role of zinc in the onset of male sterility and the development of testicles. Zinc could in fact be used as marker to detect women at high risk of endometriosis and for the elaboration of a new treatment for sterility, from which these women often suffer.

Key words: Serum zinc levels; Endometriosis; Role of zinc.

Introduction

Due to its wide and underestimated spread, endometriosis is considered a very interesting subject in gynaecological practice and research. Nonetheless, the etiopathogenesis of this affliction is still to be fully defined. As a matter of fact, despite the many studies carried out, to this day the greatest difficulty is obtaining scientific evidence that may justify its pathogenesis with special reference to both genetic and environmental predisposing factors. In general, the most recognized etiopathogenic hypotheses are three: possible retrograde menstrual flow causing the dispersion of endometrial cells through the tubes and into the peritoneal cavity; possible metaplastic process of the coelomic epithelium [1]; or possible lymphatic or haematogenous spread of endometrial cells [2]. In spite of these hypotheses, it is widely known that the illness onset depends on a complex series of factors which constitute the ground needed for many molecular events to spark off and lead to the disease [3]. This study is part of this debate and aims at assessing the possible involvement of zinc in the complex pathogenic process behind the onset and perpetuation of endometriotic lesions. In fact, this micro-element seems to be involved in many pro-apoptotic and antioxidant processes, as well as in the remodelling of the extracellular matrix whose alteration could transmit to the endometrial cells a potential invasive endometriotic phenotype. Concerning this, the authors assessed the difference in quantity of zinc serum between a group of patients affected by endometriosis and a group of healthy patients to establish whether a change in the quantity of such metal in the two groups could be statistically associated to endometriosis in a significant way.

Materials and Methods

After obtaining their informed consent, the authors selected 42 patients whose histodiagnosis had revealed pelvic endometriosis and gathered them at the general practice for gynaecology and obstetrics and/or sterility of the Department of Gynaecologic Obstetric and Reproduction Sciences, Second University of Naples, Naples (Italy). They were compared to an observation group of 44 young women presenting negative anamnesis with regards to dysmenorrhoea, dyspareunia, pelvic pain, negative CA-125 levels, and negative pelvic ultrasound with regard to ovarian cysts. The latter group was therefore considered supposedly not affected by endometriosis and examined in the same place and period. The protocol for the research project was approved by the Ethics Committee of our University Department.

The average age was 34 years (range: 19-45) in the group with endometriosis and 29 years (range 22-45) in the observation group (Table 1). The authors excluded from the study all the patients with amenorrhoea, declared menopause, related pathologies, and possible cases of endometriosis without histological evidence.

To detect the serum zinc concentration the authors took a sample of blood from all the patients and kept it at -20°C until the dosage, which was performed at the department for health, preventive medicine and medical statistics of Second University of Naples.

Revised manuscript accepted for publication April 17, 2013

Table 1. — *Features of the two groups*

	Patients with endometriosis	Observation group
Number	42	44
Average age	34.21	29.38
Age range	19-45	22-45
SD	5.8	7.21
Median	35	26

Table 2. — *Serum zinc levels*

	Patients with endometriosis	Observation group
Zn (average ± SD) µg/L	1,010 ± 59.24*	1,294 ± 62.22*

* $p < 0.05$.

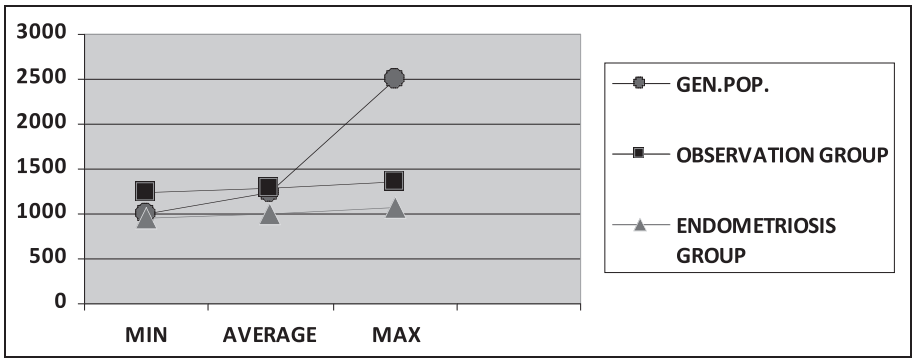

Table 3. — *Comparison in zinc distribution*

The zinc analysis on the serum was carried out with a Perkin Elmer 5,000 atomic absorption spectrophotometer using an air-acetylene flame at the following operative conditions: zinc (wavelength 213.9 nm, slit 0.7 nm, air-acetylene flame); calibration performed using solutions at concentrations of 0.00 - 0.05 - 0.10 - 0.20 - 0.50 -0.80 -1.00 -1.50 - 2.00 mg/l).

Results

The authors achieved the following results: the group of patients with endometriosis (42 women, average age of 34.21 years, age range between 19 and 45 years, standard deviation -SD-value 5.8, median value 35) presented serum zinc concentration of 1,010 ± 59.24 µg/L; the observation group (44 women, average age of 29.38 years, age range between 22 and 45 years, SD value 7.21, median value 26) presented a serum zinc concentration of 1,294 ± 62.22 µg/L (Table 2).

The results were analysed comparing the average values (± standard deviation) in the two groups and a statistical analysis using the Student's *t*-test for unpaired data was carried out. The data obtained were considered statistically significant for one $p < 0.05$.

In Table 3, a comparison between the levels of zinc in the general population, those of the group with endometriosis, and those of the observation group is shown.

Discussion

From the study the authors gather that the serum zinc levels in the group of patients with histodiagnosis of endometriosis are lower than those in the observation group where patients are healthy and do not present any sign or symptom of the disease. The difference between the two groups is statistically significant. This micro-element could be involved in the etiopathogenesis and/or evolution of endometriosis (Figure 1).

Zinc is a basically intracellular micro-element that can be found in the whole body: in organs, tissues, intracellular and extracellular fluids, with a serum concentration ranging from 1,000 to 2,500 µg/L. 70% of it can be found in the erythrocytes, where it is integrated in enzymes and transcription factors which allow the hematopoietic cells to have a regular and physiological genic activity. Zinc also influences the regulation of homeostasis between cell-mediated immunity and humoral immunity with effects the immune system's functionality. As a mater of fact, it seems that zinc can stimulate the production of lymphocytes, while its deficiency can reduce the efficiency of cell-mediated immunity [4].

In general, the highest level of zinc is detected in the brain and pancreas, probably due to its antioxidant functions and to the fact that it serves as cofactor for a large quantity of enzymes. Zinc is included in about 300 enzymes where it has a catalytic, cocatalytic or structural function. By the 3-10% it is a cofactor of proteins and responsible for proteins assembly and possible changes in conformation.

Thanks to its massive presence in the enzymes, this trace element is employed at every stage of the cell cycle, from replication to repair, as it is cofactor of enzymes such as DNA and RNA polymerase, reverse transcriptase, and thymidine kinase.

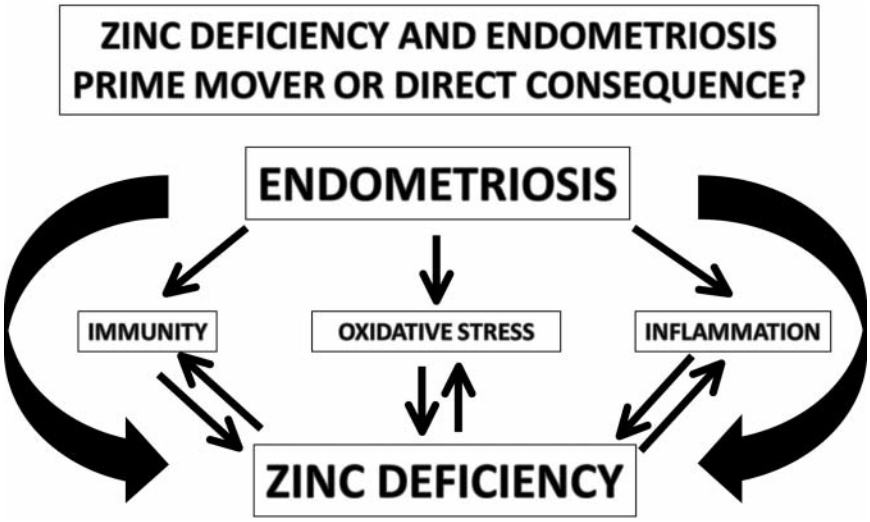

Figure 1. — Zinc and etiopathogenesis and/or evolution of endometriosis.

Additionally, zinc serves as cofactor for the superoxide dismutase (SOD) enzyme (involved in the control of oxidative stress in cells) and for this reason it plays a leading role in the apoptotic process of programmed cell death [5].

Finally, zinc is responsible for the appropriate neurode generative equilibrium of the extracellular matrix, matrix metalloproteinases (MMPs) functionality also depends on it.

Having analysed and summarized all the functions carried out by zinc in the human body and by virtue of the results obtained through the present study, which show a quantitative reduction of zinc in patients with endometriosis, the authors assume a possible involvement of zinc in the pathogenesis and perpetuation of the aforementioned pathology.

The present authors cannot be certain that the deficiency of this metal is actually one of the factors that spark off processes which subsequently lead to the formation of endometriotic lesions. Nonetheless, they can surely identify a reasonable cause and effect connection between zinc deficiency and the ability of endometriotic cells to develop invasive, phlogistic, and anti-apoptotic features.

In fact, it is known that the endometrial cells that colonise ectopic sites acquire a special phenotype which makes them develop the ability to take root on a different tissue, elude the immune response, provoke neo-angiogenesis processes, stimulate the production of cytokines responsible for chronic phlogosis, and prevent the apoptotic process [6]. In literature it is extensively documented that zinc is involved in all the aforementioned processes and therefore the deficiency the authors detected in patients with endometriosis could be the single or joint cause of all the alterations entailed in this pathology.

Firstly, the authors will analyse the modifications in the immune system. In addition to the numerous functions carried out in the body, it is also well known that zinc assures the correct functioning of the immune system as it seems to stimulates the production of lymphocytes. Zinc deficiency causes a decrease in the ability of natural-killer cells to lyse, as well as in the percentage of precursors of cytotoxic T cells. The final result is the alteration of cell-mediated immunity [7, 8]. This statement is confirmed by the fact that zinc is proved to be necessary for the biological activity of thymulin (TT), an hormone produced by the thymus and essential for the differentiation and growth of CD4+ cells. The hormone presents itself in two isoforms: one is active and zinc-bound (ZnFTS), while the other is inactive and zinc-unbound (FTS). The ratio between total TT and active thymulin (AT) represents a marker to detect deficiency in the micro-element (TT/AT>2 = deficiency; TT/AT<2 = mild deficiency). Therefore, zinc acts directly on the functional homeostasis of the specific immune system defence processes through TT, for which it serves as cofactor. Thanks to all this it can be presumed that the defect in the intrinsic immune system - widely proved in women with endometriosis - can be both the result of an idiopathic alteration of the body, and the consequence of a constitutional or dietary zinc deficiency. The deficiency has negative effects on macrophages' functionality and alters their intracellular death and phagocytosis processes and stimulate the production of cytokines. Such modifications increase the possibility for endometrial cells not only to survive, but also to take root. All this leads to a state of immune under-functioning, which has been proved also by a study carried out by Prasad *et al.* that showed that the reduction of IL-2, IL-1β and TNF-α gene expression occurs in conjunction with the decrease in zinc concentration available [9]. This effect is probably due to the ion action on the zinc-dependant transcription factors involved in the genes expression of these cytokines [10, 11].

This ion is also responsible for the inactivation of other enzymes (MMPs) which play a role in immune and phlogistic mechanisms. In their catalytic site, or active site, MMPs contain a zinc ion that interacts with a cysteine residue and prevents the binding and cleavage of the substrate keeping the enzyme in an inactive form. The concentration of this ion is therefore essential for the production and activation of MMPs [12].

In endometriosis, endometrial fibroblastic cells were also proven to increment the production of MMPs as a consequence of the increase in pro-inflammatory cytokines [13, 14]. The latter, in turn, further influence zinc metabolism [15]. As a matter of fact, many studies documented higher ion consumption when cytokines such as IL-6 and TNF-α were present [16]. Consequently, the ion was proven to be less disposed to bind to and then inactivate MMPs. The result is a massive activation of these enzymes which ease the advance of endometriotic lesions as they metabolize the extracellular matrix and facilitate the penetration of endometrial cells [17]. Furthermore, it seems that chronic phlogosis affects the homoeostasis of metallothionein, a protein which binds zinc after dietary absorption [18]. Metallothionein increases with age and in case of chronic inflammation and leads to a continuous zinc sequestration at intracellular level and to the subsequent decrease of zinc available to fulfil the enzymatic functions in which it serves as cofactor [19, 20].

Finally, it must be remembered that zinc has an antioxidant function especially crucial to prevent the starting of endometriosis. As a matter of fact, many studies showed how an increase in oxidative stress (or rather reactive oxygen species) can provoke pro-inflammatory reactions which serve as perfect substratum for the activation and taking root of endometrial cells. The proof that peritoneal fluid in women suffering from endometriosis contains a higher quantity of reactive oxygen species was already provided by Ota *et al.* in 2000 and confirmed by Shigetomi *et al.* in 2012 [21, 22]. The latter in particular proved that the increase in copper-zinc superoxide dismutase and glutathione peroxidase in women with endometriosis is persistent and constant. Superoxide dismutases are a class of tightly correlated enzymes that catalyze the dismutation of superoxide anion into oxygen and hydrogen peroxide. As zinc is one of the constituents of these enzymes, its deficiency could play a role in endometriosis [23]. This theory is supported by a study carried out in France on 20 people (aged between 59 and 85) which showed that zinc supplementation reduces the production of oxidized proteins generated by oxidative stress due to chronic inflammation [24, 25].

Ultimately, the present authors can affirm that this microelement directly or indirectly falls within all the factors that, at the moment, are supposed to concur in the etiopathogenesis of endometriosis. For this reason it is reasonable to conjecture a connection between this pathology and zinc deficiency.

In general, the influence of nutrition on health as been regarded with attention since the dawn of medicine. Concerning this, it must be noted that many studies show a cause-effect connection between zinc supplementation and the resolution of pathologies affecting the reproductive system, while others directly or indirectly associate zinc deficiency with endometriosis and the symptomatology related to it. In 1961 and 1966, Prasad *et al.* noticed that a daily zinc supplementation of 75 mg in Iranian patients about 20 years old, suffering from dwarfism, scarce development of genital organs, and limited brain liveliness determined the normalization of sexual functions and the increase in height within a period of about two months [26-30].

Another study carried out by Darwish *et al.* and conduced in the gynaecology and obstetrics department of the University of Assiut and Al-Azhar (Egypt) showed that the 18.8% out of 2,493 patients examined was affected by endometriosis. This signifies that the disease is common in Egypt where zinc deficiency was actually noticed in Egyptian and Iranian peoples. The present authors can moreover suppose a pathogenetic connection between zinc deficiency in these populations and the increase in the incidence of endometriosis [31].

Finally, in 2009 Mier-Cabrera *et al.* proved that women affected by endometriosis present a lower quantity of zinc, copper, vitamin A, C, and E than healthy women. The study also revealed a greater increase in the activity of superoxide dismutase and glutathione peroxidase in patients with endometriosis than in healthy patients after the administration of a diet with a high percentage of antioxidants for about three months [32].

These data are confirmed by the study carried out by Parazzini *et al.* which highlights the cause-effect connection between the type of diet and the probability to suffer from endometriosis. In particular, patients with endometriosis declared their diet was rich in red meat and ham, while healthy patients had a diet mainly based on vegetables and food rich in antioxidant elements and vitamins [33].

Other studies proved that the administration of antioxidants in patients with endometriosis can reduce symptomatology related to it, especially dysmenorrhoea. Such data are certainly important, above all in order to manage patients whose symptomatology can not be cured through surgery [34, 35].

Conclusion

In conclusion, the present data are consistent with the claims of prior studies but, as the sample examined was exiguous, the authors cannot describe with certainty the actual role of zinc in the endometriotic pathology, nor establish whether its deficiency is the cause or consequence of a series of molecular mechanisms which cross and intersect with one another. Nevertheless, all the reactions explained

above in which zinc takes part are surely the cause of the reduction of zinc concentration. For this reason, even if it was proved that this micro-element is not a joint cause of pathogenesis, but rather one of the progression factors of the pathology, it would all the same have significant implications for its therapeutic employment. In conclusion, nowadays, endometriosis must be certainly considered a social disease and we must aim at further broaden our knowledge to try and understand the pathogenetic mechanisms and hence develop new treatments that may contribute to the fight against this disease. In this scenario, zinc could play a leading role.

The present results showed that serum zinc levels in women with endometriosis are decreased and this seems to actually confirm that this micro-element can possibly affect the multifactorial pathogenesis of the disease. As a matter of fact, zinc interferes with many biological processes, among which inflammation and immunity, which seem to be the base of the development of the lesions. Therefore, the authors believe that this hypothesis requires more attention and further investigation to determine its reasonableness.

If the results are confirmed, this study opens up future prospects as for the treatment of endometriosis, taking into account also the role of zinc in the onset of male sterility and the development of testicles. Zinc could in fact be used as marker to detect women at high risk of endometriosis and for the elaboration of a new treatment for sterility, from which these women often suffer.

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