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Original article

Augmented oxidative stress in infertile women with persistent chlamydial infection

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ABSTRACT

There is established association between oxidative stress, infections of genital tract and fertility. Genital tract infections may provoke increased production of free radicals and generate oxidative stress that can be involved in pathophysiology of a number of reproductive diseases and complications during pregnancy. The aim of this study was to determine connection between oxidative stress and infertility associated with persistent chlamydial infection. Serum samples of infertile women with tubal factor infertility (TFI), women with multiple spontaneous abortions (MSA) and fertile women was screened for *C. trachomatis* MOMP specific IgG and IgA antibodies and cHSP60 specific igG antibodies using ELISA. The levels of superoxide anion radical, nitric oxide and reduced glutathione were determined spectrophotometricaly. Serum levels of testosterone, luteinizing hormone and follicle stimulating hormone were determined by enzyme-linked fluorescent immunoassay method. Our results showed that persistent infection was more prevalent in TFI than in MSA group, whereas seropositivity was higher in MSA than in TFI group of patients. We also found that superoxide anion was significantly lower, while LH was markedly higher in TFI and MSA group of patients. However, when our results were analyzed according to the serological status of chlamydial infection, we found that parameters of oxidative stress, superoxide anion and index of oxidative stress, defined as relative ratio between superoxide anion and nitrites sum and glutathione $((O_2^{\bullet-} + NO_2^-)/GSH)$ were significantly elevated in infertile patients with persistent chlamydial infection compared to seropositive and seronegative patients. Our findings point to the possible impact of *Chlamydia trachomatis* infection on prooxidative-antioxidative balance that can influence fertility potential in women with persistent chlamydial infection.

1. Introduction

Oxidative stress (OS) is a condition characterized by an imbalance between prooxidants and antioxidants. This relationship can be disrupted by increased levels of reactive nitrogen species (RNS) and/or reactive oxygen species (ROS), or by reduction of antioxidant defense mechanisms [1]. A certain amount of ROS is required for maintenance of normal cellular functions. On the other hand, overproduction of ROS can overpower antioxidant defense mechanisms, thereby creating an environment that is unsuitable for normal physiological reactions. In women, this imbalance has been involved in the pathophysiology of a number of reproductive diseases including tubal factor infertility, polycystic ovary syndrome, endometriosis and unexplained infertility. In addition, this condition is associated with pregnancy complications such as miscarriage, recurrent pregnancy losses, preeclampsia, as well as intrauterine growth restriction [2]. Nitric oxide (NO) is a signal molecule with vasodilatory properties involved in several physiological and pathological processes [2]. Although the vasodilatory effect of NO may be therapeutic, excessive production

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can influence the structure and function of proteins, thereby causing changes in the catalytic activities of enzymes, changes in the organization of the cytoskeleton, and disturbances in cell signal transduction. Moreover, NO is a key factor that leads to the endothelial dysfunction associated with infertility states in both men and women [3].

Among others, *C. trachomatis* genital tract infection is widely associated with the failure of human reproduction. Spread of chlamydial infection to the upper part of the female genital tract can cause pelvic inflammatory disease with serious scarring in the fallopian tubes leading to tubal factor infertility [4]. Inflammatory responses to genital chlamydial infection lead to activation of polymorphonuclear leukocytes and macrophages, resulting in an increased production of ROS and oxidative stress. In that way, OS may possibly participate in multiple pathological changes that affect reproductive function of both men and women. Pathological mechanisms include lipid peroxidation, oxidative DNA damage, modulation of gene expression and inhibition of protein synthesis [5]. In several cell lines, chlamydial infection has proved to be the cause of the release of ROS and the products of lipid peroxidation [6]. Peroxidation of surrounding cells may induce cell lysis and consequently can facilitate spreading of chlamydial elementary bodies. This may partially explain the inflammation and cell damage occurring during chlamydial infections [7]. Moreover, infection of epithelial cells of fallopian tubes can cause oxidative damage to DNA, which may result in elevated levels of 8-OHdG (8-hydroxy-2-deoxyguanosine), as observed in women with chlamydial infection and tubal infertility [8]. A biomarker of endogenous oxidative DNA damage, 8-OHdG is also associated with a lower rate of fertilization and low quality oocytes [9].

It has been reported, in both human and animal studies, that female sex hormones might influence host susceptibility, innate and adaptive immune response and outcome of chlamydial infection. [10–13]. Other findings suggest that oxidative stress genes are, at least in part, under the control of sex hormones which may exert either antioxidative [14–16] or prooxidative effects [17,18]. Finally, hormonal imbalance is considered as a common cause of female infertility [19–21].

The complex interplay between oxidative stress response, sex hormones and persistent chlamydial infection in the etiology of female infertility is poorly understood. Understanding the mechanisms of their mutual relationships may provide useful for treatment and/or prognosis of infertility patients. Therefore, serum levels of oxidative stress biomarkers and female reproductive hormones were estimated in Chlamydia positive and negative women with tubal factor infertility, multiple spontaneous abortions and in fertile women with the aim to determine their possible association with persistent chlamydial infection and tubal factor infertility.

2. Materials and methods

2.1. Study population and design

This prospective case control study involved female patients presenting to the Department of Gynecology and Obstetrics, at the Clinical Center in Kragujevac between July 2011 and December 2012. The Ethical Committee at the Public Health Institute Kragujevac approved the study and written informed consent was obtained from all subjects as stated by the Declaration of Helsinki.

In this study, infertility was defined according to WHO definition as failure to achieve a clinical pregnancy after 12 months or more of regular unprotected sexual intercourse either due to the inability to conceive or carry a pregnancy to a live birth. In agreement with this definition, inclusion criteria for study entry were infertility due to tubal factor or multiple spontaneous abortions for at least 12 months duration. Fertile controls were women who had given birth within the last 18 months. Exclusion criteria were the use of contraceptive therapy, known uterine anomalies, history of uterine surgery, current vaginal or cervical infection, chronic or systemic illness and male factor infertility.

A total of 104 age matched women, 33 with tubal factor infertility (TFI), 54 with multiple spontaneous abortions (MSA) and 17 fertile controls were consecutively recruited in the study. Infertility assessment was carried out by standard procedures. Tubal factor infertility was verified by hysterosalpingography and multiple spontaneous abortions were identified by means of patients' medical records. Out of 104 enrolled patients, 94 patients (10 patients were excluded from this analysis due to indeterminate serological data, see 2.3. antibody analysis) were additionally subdivided in 3 groups based on their Chlamydia antibody positivity into: seropositive patients with serological evidence of persistent chlamydial infection (n = 23), seropositive patients with serological evidence of previous/recent chlamydial infection (n = 29) and seronegative patients (n = 49).

2.2. Microbiology screening

After clinical examination, vaginal and cervical samples were collected from all subjects. Sterile dacron-tipped swabs were taken from the vaginal sidewall and cervical canal, inserted in sterile tube containing transport medium and transported to the laboratory within 2 h. All subjects were tested for the presence of *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Trichomonas vaginalis*, *Candida albicans* and *Treponema pallidum* using standard microbiology methods.

2.3. Antibody analysis

Serum samples were obtained from peripheral blood, centrifuged and stored at −20 °C until further analysis. All samples were screened for *C. trachomatis* MOMP specific IgG and IgA and C. *trachomatis* HSP60 (heat shock protein 60) specific IgG using commercial ELISA kits (Dia.Pro, Milano, Italy and Medac, Wedel, Germany, respectively). Serum samples were diluted 1:101 (MOMP) or 1:50 (cHSP60) with sample buffer and tested in microplate wells, according to the manufacturer's instructions. The absorbance was measured at 450 nm using RT-2100C microplate reader (Rayto, Shenzhen, PR China). Results were evaluated semiquantitatively by calculating ratio of optical density of sample value (S) and cut-off value (Co). The result was interpreted as negative when the S/Co ratio was less than 0.9, undefined when S/Co ratio was greater than or equal to 0.9 and less than 1.1, and positive when the ratio was greater than or equal to 1.1. Anti-MOMP IgG and/or IgA seropositive patients with concurrent anti-cHSP60 seropositivity are defined as patients with serological evidence of persistent chlamydial infection (cHSP60 seropositive). Patients who were anti-MOMP IgG and/or IgA seropositive, but lacking anti-cHSP60 IgG antibody, are defined as seropositive patients with serological evidence of previous/recent chlamydial infection (MOMP seropositive). Finally, patients lacking anti-MOMP and anti-cHSP60 antibodies are defined as seronegative patients without serological verification of chlamydial infection. Six patients seropositive for anti-cHSP60 IgG antibody, but seronegative for IgA/IgG against *C. trachomatis* MOMP and four patients with anti-cHSP60S/Co values in the equivocal zone were excluded from serological analysis.

2.4. Determination of superoxide anion radical (NBT assay)

The concentration of superoxide anion radical ($O_2^{\bullet -}$) was determined by spectrophotometric method based on the reduction of nitroblue tetrazolium (NBT) to nitroblue-formazan in the

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