http://dx.doi.org/10.35630/2199-885X/2021/11/6.7

EFFECT OF SOME BIOCHEMICAL FACTORS ON FEMALE REPRODUCTIVE FUNCTION

Received 15 September 2021;
Received in revised form 17 October 2021;
Accepted 21 October 2021

***Natalia Gudinskaya*[1], *Oksana Boiko*[1]✉, *Rushaniya Mukhamedzyanova*[1], *Yurii Dotsenko*[1], *Shakhrydin Tokhtarov*[2], *Anzhela Ramaeva*[3], *Galiya Khairulaeva*[4], *Polina Gudinskaya*[1]**

[1] *Astrakhan State Medical University, Astrakhan;*
[2] *Grozny Republican Clinical Hospital named after Sh.Sh. Ependiev, Grozny;*
[3] *Alexandro-Mariinsky Regional Clinical Hospital, Astrakhan Region;*
[4] *Tinaki Rehabilitation Center, Astrakhan Region, Russia*

✉ *oboyko08@mail.ru*

ABSTRACT — The study aimed to determine the effect of certain biochemical factors on the female reproductive function. Analysis showed that two biochemical factors classified as inflammatory markers have a significant difference in their concentrations in women with impaired fertility and those without fertility disorders. These are C-reactive protein (CRP) and lactoferrin (LF). It is reasonable to assume that these inflammatory markers are associated to some extent with the development of reproductive disorders due to possible formation of inhibin A/LF complexes, which may reduce the biological activity of inhibin A, in patients' serum. The effect of CRP on inhibin A activity is most likely to be systemic and associated with the effect of CRP high concentrations on inhibin A function on the body level. In particular, it is possible that CRP has an effect on IL-1βsynthesis in ovarian follicles.

KEYWORDS — C-reactive protein, lactoferrin, interleukins, fibrinogen, α2 macroglobulin reproduction.

INTRODUCTION

According to a number of researchers, an inflammatory process in patients, accompanied by a number of biochemical factors in the serum, may reduce the relevance of sufficient levels of inhibin A, belonging to the group of Smads [2]. Inhibin A is a protein secreted by ovarian granular cells, among others, and it has an effect on the secretion of follicle-stimulating hormone (FSH). Inhibin A is present in blood plasma and in follicular fluid in large quantities. Its main function is to inhibit follitropinsecretion by the pituitary gland by affecting the hypothalamus [7, 11].

Factors that cause a decrease in the biological activity of inhibin A primarily include cytokines and acute-phase proteins. Finding out the causes of a decrease in the level and activity of inhibin A in women with inflammatory diseases of the reproductive system, including infectious diseases, will both improve the quality of diagnosis and provide new opportunities for the development of fertility treatment methods [1, 3, 8, 10].

The study aimed to determine the effect of certain biochemical factors on the female reproductive function.

MATERIALS AND METHODS

The study is focused on the blood serum of women with chronic inflammatory processes, in particular chronic trichomoniasis. Blood serum of almost healthy fertile women having no trichomoniasis, including in past medical history, was used as a control.

160 women with chronic trichomoniasis were examined. They were from 22 to 29 years old, with the average age of 24.1+3.45.

The level of inhibin A in the blood serum of all the examined women was determined with the method of enzyme immunoassay on days 3–5 of the menstrual cycle, as recommended by WHO. 35 healthy fertile donor women without trichomoniasis and with no history of STDs (the average age of 24.3+1.41), were examined as a control.

The phenotype of lymphocytes was determined using a fluorescent microscope with monoclonal antibody straining (produced by State Research Center Institute of Immunology, Moscow, Russia).

Immunoglobulin levels in the serum were measured with the standard method of radial immunodiffusion (Mancini method), for which monospecific antisera were used (National Research Center for Epidemiology and Microbiology named after Academician N.F. Gamaleya, Moscow, Russia). The content of immunoglobulins was expressed in mg/%.

Cytokines were produced through spontaneous synthesis of IL-1β, IL-8, IL-10 and IFNγ. Lymphoid cells were preincubated for 24 hours. The concentration of the above cytokines was determined with immunoenzyme method. Concentrations of IL-1β, IL-8 were determined using test kits produced by Protein Contour (St. Petersburg), the ones of IL10 andIFNγ – with Immunotech test kits (France).

Lactoferrin was determined with immunoenzyme method using test kits produced by Cytokine company (St. Petersburg).

Concentrations of α2-macroglobulin and C-reactive protein were determined with nephelometric method.

Fibrinogen concentration was determined with coagulological method.

RESULTS

Levels of IFNγ, IL-8 and IL-1β were determined to describe the functional state of Th1 and Th2 in the patients. These cytokines are universal regulators of both inflammatory responses and the whole immune system, with a wide range of biological effects including antibody formation, proliferation of T and B lymphocytes and induction of synthesis of other cytokines [4, 5, 9].

In vitro production of IL-1β by mononuclear cells in the healthy women averaged 102.6±8.4 pg/ml and ranged from 96.1 to 111.0 pg/ml. The rate of spontaneous IL-1β production in the patients with chronic trichomoniasis was significantly higher and equaled to 292.7±12.1 pg/ml ($p<0.001$). Therefore, it becomes evident that there is definite correlation between the degree of IL-1β production and trichomoniasis infection.

IL-8 is known as a classic pro-inflammatory cytokine that induces an acute inflammatory response, increasing the adhesive capacity of neutrophils. While its concentration in the healthy women was 19.7±2.3 pg/ml, the rate of spontaneous production of IL-8 in the patient group was lower ($p<0.001$)than the reference values, being only 10.9±2.1 pg/ml, which suggests a decrease in the phagocytic activity of neutrophils, associated with it.

The level of IFNγ is equally important for normal functioning of the immune system; as known, it regulates immune response intensity and intensifies the expression of antigens belonging to classes I and II of the major histocompatibility complex. IFNγ stimulates bactericidal activity of macrophages and neutrophils by activating oxygen-dependent and oxygen-independent mechanisms. Besides, this mediator also influences the functional state of NK cells, the first line of protection against viral and bacterial infections. It was determined that the level of IFNγ in the healthy women was 25.10±1.60 pg/ml, while spontaneous production of IFNγ in the patients with trichomoniasis was almost three times lower and equaled to 8.3±1.1 pg/ml ($p<0.01$).

Another important immunoregulatory cytokine is IL-10, an anti-inflammatory agent produced by Th2. It is considered that its biological function is mainly to inhibit IFNγ production. Spontaneous production of IL-10 in the group of healthy women was from 114.0 to 250.0 pg/ml (178.3±41.5 pg/ml on average). Its concentration in the patients was evaluated — up to 1564.0±123.0 pg/ml, i.e. more than 8-fold ($p < 0.001$). It is obvious that the lack of IFNγ production, on the one hand, reflects the impaired functional activity of CD4+ cells in the patients, and, on the other hand, may lead to infection tolerance.

The content of immunoglobulins IgA and IgM in the patients was within normal limits, though there were changes in their ratio (as a rule, an increase in the level of IgA and/or decrease in the level of IgM), which was identified in two thirds of the patients.

Therefore, examination of the cytokine profile in the women with chronic trichomoniasis clearly demonstrates prevailing development of the Th-2 type immune response.

It is known that cytokines initiate synthesis of secondary mediators of inflammation — acute-phase proteins — by hepatocytes. These include, among others, plasma glycoproteins produced by the liver in response to tissue damage. Plasma proteins are divided into several classes depending on the increase in their concentrations in case of acute-phase states. A decision was made to examine concentrations of lactoferrin (LF), C-reactive protein (CRP), α-2-macroglobulin and fibrinogen. According to the authors' data, the level of CRP in the group of healthy women was 0.3±0.01μg/ml. Its concentration in the patients showed more than 65-fold increase — up to 19.5±0.8 μg/ml ($p < 0.001$), but this is much lower than in most bacterial infections and more typical of chronic viral diseases.

The level of lactoferrin in the group of healthy women under examination was 445.0±104.0 ng/ml. Its concentration in the patient group was elevated almost 8-fold – up to 3,475.0±178.0 ng/ml ($p < 0.001$). Such values are more often seen in acute infections (including sexually transmitted diseases) and are not typical of most chronic bacterial infections at all, although bacteria are capable of synthesizing protein that is immunochemically similar to human lactoferrin [6, 10].

Then the authors performed a retrospective analysis of variation series, with the results obtained being grouped regardless of the concentration of inhibin A and the inflammatory factors under study, in their relationship with the state of the reproductive function of the women having trichomoniasis. As mentioned above, infertility was observed in 30 women in the patientgroup with normal levels of inhibin A, while the rest kept fertile or had no reliable data on fertility disorders. The conducted analysis showed that only two inflammatory markers in the blood of the women having a chronic inflammatory process have a significant difference in their concentrations in the women with impaired fertility and those without fertility disorders. These are C-reactive protein and lactoferrin.

It is natural to assume that these inflammatory markers are associated to some extent with the devel-

opment of reproductive impairment in the women suffering from trichomoniasis. The authors admit possible formation of inhibin A/LF complexes in the patients' serum which may reduce the biological activity of inhibin A. This is quite possible in at least two ways. Firstly, it is electrostatic interaction of several molecules of inhibin A, having a sufficiently high negative charge, with LF polycation. Secondly, there may be a mechanism of mostly hydrophobic interaction between LF molecules and inhibin A.

The CRP effect on inhibin A activity can hardly be explained with formation of complexes. They are most likely to be systemic and associated with the effect of CRP high concentrations on inhibin A function on the body level. In particular, it is possible that CRP has an effect on IL-1β synthesis in ovarian follicles. It is known from literature that this cytokine is synthesized by the granulosa cells and that high concentrations of IL-1β in the follicular fluid correlate with the degree of maturity and with the frequency of oocyte fertilization [1, 3, 11]. On the other hand, IL-1β is a pro-inflammatory cytokine that plays an essential role in inflammatory response unfolding. It can induce most of its local and general manifestations. It is also known that high concentrations of CRP (10 μg/mL and more) can inhibit IL 1β synthesis along with prostaglandin E2 and glucocorticoids.

CONCLUSION

Therefore, the study results can be summed up as follows. The group of patients with a chronic inflammatory process demonstrate significant variability in the concentration of inhibin A (4.3 — 29.4 ng/l). Secondary infertility was diagnosed in 63.2% of cases in the group of patients with reduced levels of inhibin A, while the group of patients with normal levels of inhibin A demonstrated only 12.9% of cases of secondary infertility development. It was found out that C-reactive protein and lactoferrin were the only acute-phase proteins that had a prognostic value and differed significantly in the groups of women with subsequent infertility and those with a chronic inflammatory process that was not complicated with infertility.

The authors declare no conflict of interest in the manuscript being submitted.

REFERENCES

1. **Askari SG, Khatbasreh M, Ehrampoush MH, Sheikhha MH, Eslami H, Taghavi M, Shahrokhi S, Andishmand S.** The relationship between environmental exposures and hormonal abnormalities in pregnant women: An epidemiological study in Yazd, Iran. Women Birth. 2018;31(3): e204–e209. DOI: 10.1016/j.
2. **Ahmad I, Jagtap DD, Selvaa Kumar C, Balasinor NH, Babitha Rani AM, Agarwal D, Saharan N.** Molecular characterization of inhibin A: Structure and expression analysis in Clariasbatrachus. Gen Comp Endocrinol. 2018;261:104–114. DOI: 10.1016/j.
3. **Baerwald A, Vanden Brink H, Hunter C, Beuker D, Lim H, Lee CH, Chizen D.** Age-related changes in luteal dynamics: preliminary associations with antral follicular dynamics and hormone production during the human menstrual cycle. Menopause. 2018;25(4):399–407. DOI: 10.1097/GME.
4. **Boiko O.V., AkhmineevaA.Kh., Gudinskaya N.I., Boiko V.I., Kozak D.M.** Age-related changes in immune, morphological and biochemical parameters of the male reproductive system. Advances in Gerontology. 2014. Vol. 27, No. 1, p. 50–53.
5. **Boiko V.I., DotsenkoYu.I., Boiko O.V.** Acute-phase proteins in saliva of workers at a sour gas condensate processing plant. Russian Clinical Laboratory Diagnostics. 2011. No. 6, p. 18–20.
6. **BoikoO.V., NikolaevA.A., KozakD.M., GudinskayaN.I., Sakharov M., Gudinskaya P., DotsenkoYu.I.** Isolation and purificationof protein thatis immunologicallysimilar to human lactoferrin ArchivEuroMedica. 2021. Vol. 11,No. 2,p. 10–12.
7. **Gallastegui N, Coe RM, Torres A, Harrington TJ.** Thrombocytopenia associated with high levels of inhibin. A case study. Blood Cells Mol Dis.;76:22–24. DOI: 10.1016/j.bcmd.2019.01.001.
8. **Gaccioli F, Aye ILMH, Sovio U, Charnock-Jones DS, Smith GCS.** Screening for fetal growth restriction using fetal biometry combined with maternal biomarkers. Am J Obstet Gynecol. 2018;218(2S):S725-S737. DOI: 10.1016/j.
9. **Kristensen SG, Mamsen LS, Jeppesen JV, Bøtkjær JA, Pors SE, Borgbo T, Ernst E, Macklon KT, Andersen CY.** Hallmarks of Human Small Antral Follicle Development: Implications for Regulation of Ovarian Steroidogenesis and Selection of the Dominant Follicle. Front Endocrinol (Lausanne).2018 ;8:376. DOI: 10.3389/fendo.
10. **Korzun I.A., Ploskonos M.V., Syatkin S.P., Blagonravov M.L., GushchinaYu.Sh., Eremina I.Z., Kaitova Z.S., Navid M.N., Aissa A.A.** Components of seminal plasma as factors regulating apoptosis of male gametes. «FEBS Open Bio», Vol. 9, Suppl. 1, 2019, P. 370.
11. **Singnoi W, Wanapirak C, Sekararithi R, Tongsong T.** A cohort study of the association between maternal serum Inhibin A and adverse pregnancy outcomes: a population-based study. BMC Pregnancy Childbirth. 2019;19(1):124. DOI: 10.1186/s12884-019-2266-y.