Revista Española de Ozonoterapia vol. 7, nº 1. pp. 77-81, 2017
Editado por AEPROMO (Asociación Española de Profesionales Médicos en Ozonoterapia)

ISSN: 2174-3215

artículo original

The use of Ozonized Physiologic Saline in Gynecologic patients with Uterine Myoma and Endometrial Cancer in the Postsurgical Period

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Keywords

Abstract

The complex therapy of the patients with malignant and nonmalignant tumors of uterine includes radical surgery with hormonotherapy, polychemotherapy and radiation therapy. These methods have a whole number of side effects on a patient organism such as toxic and immuno- suppressive effects as well as balance of pro- and antioxidant systems disorders. In this connection it is important to include in complex postoperative therapy auxiliary methods of treatment, which reduce intoxication, modify antioxidant protection level, normalize lipid peroxidation intensity and immunity. One of such methods is ozone-therapy. Treatment of a whole number of diseases including cancers demonstrates positive effects.

Immune system realizes elimination of tumor cells and restore the homeostasis of the organism. Reasonability of immunocorrecting therapy is identified if it changes immune cell repertoire and serum level of soluble immune cell membrane antigens or not. Also it is interesting to investigate lipid peroxidation process and antioxidant protection state. Some physicochemical forces can increase reactive oxygen species production and destroy tumor cells. So these forces can be used in anticancer therapy.

In this connection the aim of our research was to study the immunologic status before and after treatment with ozonized physiological saline in gynecologic patients with malignant and nonmalignant tumors for pathogenetic justification of ozone usage in persons suffering from hysteromyoma and endometrial cancer in the post-surgical period..

Suggestion on how to quote this paper:
Olga S. Yanchenko et al. (2017). The use of Ozonized Physiologic Saline in Gynecologic patients with Uterine Myoma and Endometrial Cancer in the Postsurgical Period.. *Revista Española de Ozonoterapia*. Vol. 7, nº 1, pp 77-81

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INTRODUCTION

The complex therapy of the patients with malignant and nonmalignant tumors of uterine includes radical surgery with hormonotherapy, polychemotherapy and radiation therapy. These methods have a whole number of side effects on a patient organism such as toxic and immuno-suppressive effects as well as balance of pro- and antioxidant systems disorders. In this connection it is important to include in complex postoperative therapy auxiliary methods of treatment, which reduce intoxication, modify antioxidant protection level, normalize lipid peroxidation intensity and immunity. One of such methods is ozone-therapy. Treatment of a whole number of diseases including cancers demonstrates positive effects.

Immune system realizes elimination of tumor cells and restore the homeostasis of the organism. Reasonability of immunocorrecting therapy is identified if it changes immune cell repertoire and serum level of soluble immune cell membrane antigens or not. Also it is interesting to investigate lipid peroxidation process and antioxidant protection state. Some physicochemical forces can increase reactive oxygen species production and destroy tumor cells. So these forces can be used in anticancer therapy.

In this connection the aim of our research was to study the immunologic status before and after treatment with ozonized physiological saline in gynecologic patients with malignant and nonmalignant tumors for pathogenetic justification of ozone usage in persons suffering from hysteromyoma and endometrial cancer in the post-surgical period.

MATERIALS AND METHODS

The study population consisted of 100 gynecological patients treated in the Gynecological Clinic of the Regional Clinical Hospital of Nizhny Novgorod. Out of the 100 women, 43 had uterine myoma and 58 had endometrial cancer (adenocarcinoma of endometrium). According to the FIGO criteria all cases of cancer were stage I. the diagnosis of uterine myoma and endometrial cancer were con- firmed by the examination of biopsy and cytologic specimens. The prevailing concomitant diseases were hypertension (70% of patients), coronary heart disease (30%), obesity (20%) and insular diabetes (15%). All patients were submitted to the radical surgery including supravaginal uteroctomy. The study population was subdivided into two groups. The control group of patients, including 22 patients with uterine myoma and 26 endometrial cancer patients (the median age was 58.7 years, range 28 - 79), received a standard postsurgical therapy for the treatment. The treatment of the main group of patients, 20 with hysteromyoma and 32 with endometrium cancer (the median age 60.3 years, range 28 - 79), combined the standard ther- apy and intravenous infusion of ozonized physiologic saline (0,9% NaCl). Physiologic saline were bubbled with gas mixture, containing ozone oxygen in ozonizer. Concentration of ozone in physiological saline 400 µg/L. A course of 10 sessions of ozone-therapy included ozonized saline infusion, 200 mL a day each day starting from the second day after surgery. Ozone concentration and the procedure frequency were worked out and confirmed in earlier gynecological, oncological and therapeutic researches.

Immunophenotype assay. Cell expression of CD3, CD4, CD8, CD16, CD20, CD95 and HLA-DR antigens of periferal blood mononuclear cells (PBMC) was quantified by flow cytometry. The PBMC was in staining buffer (PBS, pH 7.4 – 7.6) with corresponding antihuman monoclonal antibody for 30 minutes at 4ºC. The treated cells underwent the second antibody staining of FITC-goat antimouse immunoglobulin for labeling cells. Cells were washed twice with staining buffer, suspended and fixed with fixation buffer (4% paraformaldehyde). Samples were analyzed using a FACScan (Becton Dickinson, San Jose, CA).

Soluble form of CD-antigens determination. Soluble CD38, CD50, HLA-I and HLA-DR were measured by a sandwich ELISA using corresponding capture antibodies coated at 100 μg/mL in saline buffer (pH 7.0) into 96-well ELISA plates at room temperature overnight. After 4 washes in PBS supplemented with 0.1% Tween-80 (PBS-T), serum was added at 1:1 dilution in PBS and incubated at room overnight. After 4 washes in PBS, captured soluble form of CD-antigens were detected with corresponding antibody at 100 μg/mL followed by color detection using HRP. All analyses were carried out in duplicate wells, and results were expressed in conditional units as a mean of soluble CD-antigens (CU/ml).

Lipid peroxidation and antioxidant system activity analys*is*. To determine lipid peroxidation and antioxidant system activity Fe^{2+}- and H_2O_2-induced biochemiluminescence assay was used. The essence of the method is that ions of metal with variable valence catalyzing decay of hydrogen peroxide lead to generation of free radicals (R', OH', RO, O'_2, RO'_2). Radicals cause free-radical oxidation. Recombination of RO'_2 results in forming of unstable tetroxide which decay and emit photon. Photon emission can be registered. To register biochemiluminescence serum of patient venous blood was used. Following chemiluminescence parameters were taken in account: Imax (mv) – maximum intensity of luminescence, it characterizes lipid peroxidation capacity of biological object; S (mv/30sec) – total luminescence during 30 sec describes discontinuity of the free-radical chain reaction; tg (-2α) – describes speed of free-radical process attenuation in serum of blood.

The serum levels of lipid peroxidation products such as dien (DC) and triene conjugates (TC) were measured by reading of absorbance of lipid solution in methanol- hexane mixture at 233 and 275 nm. The level of the Shiff bases as the final products of peroxidation was measured by reading of absorbance at 420 nm (Fletcher).

Statistical Analysis: Data are expressed as means ± SD. The differences between the means of variables was analyzed by the Mann-Whitney U test.

Results and Discussion. Before surgery and any therapy the blood of the patients suffering from endometrial can- cer contained significant higher levels of CD16+, CD20+ and HLA-DR+ PBMC as well as lower levels of CD3+ and CD95+ cells relative to healthy controls ($p<0.05$) (Table 1).

Table 1 - Relative quantity of PBMC in endometrial cancer patients before and after treatment

Cell population %	Healthy controls n=40	Control group n=26		Main group n=32	
		Before treatment	After treatment	Before treatment	After treatment
$CD3^+$	72,0±7,0	55,4±2,9*	51,7±2,6*	50,4±2,2*	53,5±2,9*
$CD4^+$	39,0±5,0	45,5±3,8	44,2±3,7	43,5±3,1	43,6±3,4
CD8+	23,0±4,0	22,1±2,4	19,6±2,1	4,6±2,3	22,8±1,9
$CD16^+$	12,0±6,0	28,2±1,9*	24,3±2,2*	27,1±1,6*	20,0±1,5*▼
$CD20^+$	12,0±3,0	23,5±2,9*	27,5±2,6*	22,3±2,5*	27,8±2,8*
$CD95^+$	52,0±11,0	14,2±1,8*	19,8±1,7*	22,2±2,4*	25,6±3,1*
HLA-DR^+	14,0±7,0	30,4±3,1*	29,7±2,1*	30,7±3,1*	28,8±2,7*

** - significant differences in comparison with healthy controls ($p<0.05$)*
▼ - significant differences in comparison with pretreatment values ($p<0.05$)
n – number of patients

Before the treatment persons with hysteromyoma had significant higher levels of $CD8^+$, $CD20^+$ and HLA-DR^+ PBMC and lower $CD3^+$ and $CD95^+$-cells relative quantity in comparison with healthy controls ($p<0.05$) (Table 2). The differences in CD4+ and CD8+-lymphocytes concentrations were not established in cancer patients as well as in patients with hysteromyoma.

We found that persons with malignant and nonmalig- nant pathology had significant increased preoperative serum levels of sCD38, sCD95, sHLA-I and sHLA-DR molecules then in healthy controls. But the concentrations of sCD50 and sCD95 antigens were higher in patients with hysteromyoma relative to endometrial cancer patients ($p<0.05$) (Table 3, 4).

Standard therapy in endometrial cancer patients and uterine myoma patients was not attended significant changes in PBMC repertoire. In patients with endometrial cancer relative quantity of CD16+ lymphocytes as well as serum levels of sCD38, sCD95, sHLA-I and sHLA-DR molecules went down to normal ($p<0.05$) only after ozone-therapy but not after standard treatment. We did not find significant differences in sCD50 concentration before and after any treatment.

Table 2 - Relative quantity of PBMC in hysteromyoma patients before and after treatment

Cell population %	Healthy controls n=40	Control group n=22		Main group n=20	
		Before treatment	After treatment	Before treatment	After treatment
CD3+	72,0±7,0	48,2±2,8*	48,6±2,4	50,2±2,6*	50,6±2,7
CD4+	39,0±5,0	40,4±3,1	40,3±3,1	41,5±3,4	40,8±3,2
CD8+	23,0±4,0	28,3±2,1*	27,6±2,3	30,2±2,4*	23,2±1,9*▼
CD16+	12,0±6,0	20,6±1,8*	25,0±2,1	20,8±1,7*	21,8±2,7
CD20+	12,0±3,0	28,2±2,6*	25,4±2,4	30,2±2,2*	20,3±2,4*▼
CD95+	52,0±11,0	21,8±1,8*	29,8±1,7*▼	30,2±2,4*	24,4±2,8*▼
HLA-DR+	14,0±7,1	28,0±2,1*	31,6±2,9	32,8±2,7*	29,6±2,8

** - significant differences in comparison with healthy controls (p<0.05)*
▼ - significant differences in comparison with pretreatment values (p<0.05)
n – number of patients

After ozonized saline infusion in hysteromyoma patients we found that the number of CD8+, CD20+ and CD95+ PBMC went down to standard as well as the serum levels of sCD38, sCD50 and sHLA-DR molecules (p<0.05). The concentration of sHLA I also changed after ozone-therapy, but it went up to normal. Treatment with ozone led to further decreasing of the sCD95 level.