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ASSESSMENT OF OXIDATIVE PROTEINS MODIFICATION LEVEL IN INFERTILE MALES EJACULATE SECONDARY TO TOXOCARA INVASION

Оцінка рівня окисної модифікації білків в еякуляті інфертильних чоловіків на тлі токсокарозної інвазії

Abstract

Our research was aimed at revealing level of proteins oxidative proteins modification in ejaculate homogenate for patients with reproductive damages secondary to toxocara invasion.

Materials and methods. *The article contains the data of researching proteins oxidative modification in ejaculate and values for congenital immunity cells factors in 89 men being divided into five groups depending on spermatozoa DNA values and presence or lack of toxocara invasion.*

The results. *According to the data obtained it has been established that increasing OPM values with different intensity in ejaculate depending on peculiarities of damaging factor were being observed. Incomplete phagocytosis of the neutrophil link was determined, but only in the 3rd and 4th groups was noted a simultaneous decrease in HSTsp and HSTst, which indicated a deep damage to the bactericidal system of phagocytes, which also, apparently, occurs due to inhibition of the metabolism of immunocompetent cells and their bioenergetic resources by toxic products of life activity of helminths. It was in the groups with the most pronounced oxidative stress that DNA fragmentation of spermatozoa was also observed, which, in turn, was combined with the more severe forms of pathozoospermia in the 4th and 5th groups we discovered earlier.*

Conclusions. *Thus, insignificant OPM increasing in patients of the third and fourth groups (with antibodies against toxocara) is connected with defense formed by helminths against damaging factors, such as OPM and phagocytosis, that is*

Реферат

Мета роботи. *Виявлення рівня окисної модифікації білків в еякуляті у пацієнтів із порушеннями репродуктивної функції на тлі токсокарозної інвазії.*

Матеріали та методи. *У статті висвітлено дані дослідження окисної модифікації білків в еякуляті та показників клітинних факторів вродженого імунітету 89 чоловіків, які були розділені на п'ять груп, залежно від рівня ДНК сперматозоїдів та наявності/відсутності токсокарозної інвазії.*

Результати. *Згідно з отриманими даними, встановлено, що у пацієнтів усіх досліджуваних груп спостерігалося збільшення рівня ОМБ в еякуляті різної інтенсивності, залежно від особливостей фактора, що ушкоджує. Визначався незавершений фагоцитоз нейтрофільної ланки, проте тільки в 3-й і 4-й групах відмічено одночасне зниження НСТсп і НСТст, що вказувало на глибоке ураження бактерицидної системи фагоцитів, що так само, мабуть, відбувається через пригнічення метаболізму імунокомпетентних клітин та їх біоенергічних ресурсів токсичними продуктами життєдіяльності гельмінтів. Саме в групах, з найбільш вираженим окислювальним стресом спостерігалася також і фрагментація ДНК сперматозоїдів, що, у свою чергу, поєднувалося з виявленими нами раніше більш важкими формами патозооспермії у 4-й та 5-й групах.*

Висновок. *У пацієнтів 3-ї та 4-ї груп (з наявністю антитіл до токсокарів) незначне збільшення ОМБ пов'язане з формуванням*

confirmed by incomplete phagocytosis secondary to functional and metabolic reserve deficiency. High OPM values in patients of second and fifth groups indicate only influence of environment (industrial pollutants in air), many of them can be themselves booster to develop and maintain OPM high values.

Keywords: *oxidative proteins modification, cells factors of congenital immunity, toxocariasis, male infertility.*

гельмінтами захисту проти ушкоджуючих їх факторів, таких як ОМБ та фагоцитоз, що підтверджується незавершеністю фагоцитозу на тлі дефіциту функціонально-метаболічного резерву. У пацієнтів 2-ї та 5-ї групи (з відсутністю антитіл до токсокарів) високі значення ОМБ вказують лише на вплив факторів навколишнього середовища (промислові полютанти в атмосферному повітрі), багато з яких можуть самі бути пусковим механізмом для розвитку та підтримки високого рівня ОМБ.

Ключові слова: *окислювальна модифікація білків, клітинні фактори вродженого імунітету, токсокароз, чоловіче безпліддя.*

INTRODUCTION

Globally, 48,5 million couples are suffering from infertility. One of six couples in United Kingdom is categorized as infertile. In developing countries, infertility affects one of four couples. Male infertility constitutes about 40–50% of the incidence. A minimum of 30 million men worldwide are infertile. Mortality rate is higher in men with impaired semen quality than those who have normal semen quality. The initial evaluation of a male partner of an infertile couple should be done if there is a delay in the pregnancy in the female partner for one year or more from unprotected sexual intercourse. It can be done earlier if there is a predisposing factor for infertility. Tendency to increasing male factor in childless couples requires thorough study on this problem [1–3]. Deterioration of ejaculate in its qualitative parameters is not pathology but it's a result of harmful influence of environment (ecological, social, economical, infectional). These factors affect organism on cellular and molecular levels and result in pathozoospermia [4, 5].

Toxocariasis is one of the factors able to cause teratogenic modifications in males. Toxocariasis is helminthiasis which is one of the most frequently found over Ukraine [6, 7].

It is known, that oxidative stress is the main factor in destabilization of host genome in helminthiasis. Oxidative stress is cell damage after oxidation through formation of free radicals, which being in abundance are able to initiate disturbances in spermatozoa through induction of oxidative damage of lipids, proteins and DNA [8].

Oxidized proteins modifications (OPM) are precursors markers of oxidative cell damage. Recent studies have shown that activation of free radicals oxidation in particular with oxidized proteins is great danger in damage of genetic material such as somatic and generative host cells [9–10].

Recent reports concerning results of clinical and experimental studies have allowed the authors to emphasize in this field the aspects studied insufficiently. For instance, OPM was always being assessed in the blood, but similar studies in ejaculate have not been performed though they would be more important.

Hence the aim of our studies is to reveal peculiarities for oxidative proteins modification in ejaculate of patients with disturbances of reproductive function secondary to toxacara invasion.

MATERIAL AND METHODS

89 males aged between 20 and 45 have been examined within our research. All men have presented an agreement in written form for participation in the studies. The agreement has been approved by Institutional Committee on Bioethics and corresponded to the bases of Helsinki Declaration, as well as approved by the Committee on Bioethics at State Institution «Zaporozhye Medical Academy of Postgraduate Education of Ministry of Health of Ukraine», and corresponded to ethic, moral and legal requirements of the Order № 281 of Ministry of Health of Ukraine from 01.11.2000.

All patients have been divided into 5 groups. The first group (control one) included 12 fertile healthy men who had 1–2 children aged between 1 and 5 years. The second group (comparison) included 27 infertile patients with standard condition of spermatozoa DNA fragmentation and deprived of antibodies to toxocara.

The third group included 20 infertile men with standard condition of spermatozoa DNA fragmentation and antibodies to toxocara.

The fourth and fifth groups included 15 infertile men each with high values in spermatozoa DNA fragmentation, and antibodies to toxocara (4th group) and their lack (5th group) respectively.

Complex examination has been carried out including studying level in spermatozoa DNA fragmentation, presence or lack of toxocara invasion, cellular activity of congenital immunity factors and oxidative proteins modification in ejaculate homogenate extraction.

Oxidative proteins modification has been assessed in ejaculate homogenate extraction (patent RU№ 2525437C1). Ejaculate homogenate extraction has been obtained by process «freezing – thawing» with preliminary congelation of ejaculate at – 40 C and just at the time of performance it has been defrosted and subsequent centrifugation has been done at 3000 G for precipitation of spermatozoa membranes flaps. Liquid over-sedimentary was homogenate extraction, that is spermatoplasm with internal spermatozoa content. To obtain the whole numbers the recount of optical density obtained has been done in the samples studied on spermatozoa amount in 1 ml of ejaculate (units of optical density 10000 mln spermatozoa in 1 ml of ejaculate). (patent UA№ 122056).

Spermatozoa DNA fragmentation has been performed by technique Sperm Chromatin Dispersion (patent RF № 2373288). Standard values for this index were considered those below 30% from 500 spermatozoa calculated.

Toxocara invasion has been diagnosed by revealing in blood serum the amount of antibodies immunoglobulins G (Ig G) to toxocara using technique IFA with set of reagents «Vitrotest», Ukraine. Positive value as compared to optical density in specimen studied to utmost value of negative control has been calculated in every experimental sample. The result was considered as positive at values > 1,1.

Assessment for phagocytic activity of neutrophils in the blood based on the method for determination of absorptive and digestive capacity to microbe test-culture after joint preincubation has been carried out [11].

Assessment for oxygen-dependent metabolism of neutrophils (NBT-test) and functional cell reserve (NBT-test induced) has been performed [12].

Activity for myeloperoxidase (MPO) in neutrophils has been determined by cytochemical method [13].

Method with bromphenol blue has been used for assessment of cation proteins (CP) in neutrophils [14].

Statistical analysis of data obtained was performed using computer programs set STATISTICA (StatSoft Statistic v.7.0.). Statistical significance of compared values with distribution different from standard, assessed by Kolmogorov-Smirnov test, has been established using Wald-Wolfowitz runs test at the significance level of 0,05. The data under consideration are presented as median (Me) and interquartile scope (RQ), presenting difference between meanings of 75 and 25 percentiles (RQ = 75% UQ – 25% LQ), where UQ is upper quartile and LQ is lower quartile.

RESULTS

In assessment of OPM level value reduced in 25% was revealed in second group compared to control group (Table 1).

Table 1

OPM values in ejaculate of infertile men with regard for presence or lack of toxocara invasion Me (75% Q – 25% Q = RQ)

Value, units	Group 1 (n = 12)	Group 2 (n = 27)	Group 3 (n = 20)	Group 4 (n = 15)	Group 5 (n = 15)
OPM units optical density x10000/mln sperm in 1 ml	11,45 (16,45–11,45 = 5,20)	18,60* (11,10–5,10 = 6,00)	14,40*,** (21,90–6,90 = 15,00)	16,90*,** (26,30–14,80 = 11,50)	26,05*,** (57,05–18,10 = 38,95)

Notes: ** – statistically significant difference compared to control group (p < 0,01), ** – statistically significant difference compared to 2 group (p < 0,01)*

When studying values of functional and metabolic status for neutrophils in patients of second group it has been revealed reduced absorptive (NPN) and digestive (NPI) capacities of neutrophils at 30 and 120 minutes by 25% and 29% and by 32% and 52% as for values in control group respectively (Table 2).

Values of NBTsp-test have been decreased by 19% while the values of NBTst-test corresponded to the values in control group.

Concerning values of microbicidal system it may be said that CP level was decreased by 20%, while MPO activity was increased by 9%, that is not statistically reliable, but clinically this is very important with regard to control group.

Thus, in patients of second group functional failure was noted for neutrophils link in phagocytic system secondary to preservation of functional and metabolic neutrophils reserve proving incomplete phagocytosis.

While determining OPM level in the third group the value increased by 26% and 32% was revealed as compared to both control and comparison groups respectively (Table 1).

After studying values for functional and metabolic neutrophils status in patients of third group it must be noted lowering both neutrophils absorptive capacity at 30 min and 120 min by 43% and 33% and their digestive function by 32% and 54% comparatively control group.

Values of NCT test (spontaneous and stimulated) have been decreased by 29% and 10% comparatively control group respectively.

Various modifications were being observed in

microbicidal induction: CP level was decreased by 22%, MPO activity was increased by 27% compared to control group (Table 2).

Thus, in the third group functional failure was observed in neutrophilic link of phagocytic system secondary to exhaustion in functional and metabolic reserve proving incomplete phagocytosis.

During OPM assessment in the fourth group values increased by 47% and 96% compared to control group has been registered (Table 1).

Lowering neutrophils absorptive and digestive capacities was observed at 30 min and at 120 min by 43% and 32%, and 32% and 50% compared to control group respectively.

Values of NBT-test (spontaneous and stimulated) have been decreased by 19% and 15% comparatively control group respectively

CP level was decreased by 13% compared to control group, while MPO activity corresponded with them (Table 2).

Thus, functional failure in neutrophils link of phagocytic system secondary to exhaustion of functional and metabolic reserve was observed in the fourth group proving incomplete phagocytosis.

Values increased by 127% and by 203% compared to both control and comparison groups were observed during OPM level assessment in the fifth group.

While studying values of neutrophils functional and metabolic status lowering absorptive and digestive capacity at 30 min and at 120 min by 33%, 25% and 36%, 57% compared to control group was observed.

The values of spontaneous NBT-test were lower by 9% than control ones, while the values of NBT stimulated – test correlated to values in control group.

The level of CP was lowered by 35% while MPO activity corresponded to control values.

Thus, failure in neutrophils link of phagocytic system secondary to functional and metabolic cells reserve preserved has been observed in patients of fifth group, that showed incomplete phagocytosis.

Hence, on the basis of all reports presented it should be necessary to conclude that increasing OPM level with different intensity was observed in all patients irrespectively of group.

Probably, atmospheric air pollution serves as a background for development of oxidative stress, in particular, for OPM. It is precisely the chemical pollutants exert unspecific influence on free radicals production, that is particularly evident in the fifth group, while the lower OMP value in the third and fourth groups is explained by available toxocara invasion (as display of competition for efficient forms O_2) [15, 16].

Incomplete phagocytosis of neutrophils link was observed in all groups under research, although simultaneous lowering NBTsp. and NBTst. was observed only in the third and fourth groups, indicating deep damage in phagocytic microbicidal system, that, probably, occurs because of suppression to metabolism in immunocompetent cells and their bioenergetic resources with toxic products resulted from vital functions of helminths.

Besides, spermatozoa DNA fragmentation was observed just in the groups with the most pronounced oxidative stress, that was combined with more severe forms of pathozoospermia revealed earlier in the fourth and fifth groups [17, 18].

Table 2

Characteristic of functional and metabolic status of neutrophils in infertile males with regard for presence or lack of toxocara invasion

Me (75% Q – 25% Q = RQ)

Values, units	1 group (n = 12)	2 group (n = 27)	3 group (n = 20)	4 group (n = 15)	5 group (n = 15)
NPI at 30 min, %	66,5 (68,0–66,0 = 2,0	50,0* (54,0–38,0 = 16,0)	38,0* (53,0–35,0 = 18,0)	38,0* (48,0–38,0 = 10,0)	45,0* (58,0–38,0 = 20,0)
NPN at 30 min, c.u.	2,2 (2,3–2,1 = 0,2)	1.5* (1,8–1,3 = 0,5)	1,5* (1,7–1,4 = 0,3)	1,5* (1,7–1,4 = 0,3)	1,4* (1,5–1,3 = 0,2)
NPI at 120 min, %	56,0 (57,0–55,0 = 2,0)	40,0* (50,0–33,0 = 17,0)	38,0* (50,0–25,0 = 25,0)	39,0* (50,0–30,0 = 12,0)	42,0* (52,0–30,0 = 22,0)
NPN at 120 min, c.u.	3,0 (3,1–2,9 = 0,2)	1,4* (1,7–1,2 = 0,5)	1,4* (1,8–1,2 = 0,6)	1,5* (1,7–1,4 = 0,3)	1,3* (1,5–1,0 = 0,5
NBTsp., c.u.	2,1 (2,2–1,9 = 0,3)	1,7* (1,8–1,3 = 0,5)	1,5 (2,0–1,5 = 0,5)	1,7* (1,7–1,4 = 0,3)	1,9 (2,0–1,7 = 0,3)
NBTst., c.u.	2,0 (2,2–1,9 = 0,3)	2,0 (2,0–1,8 = 0,2)	1,8 (2,0–1,3 = 0,7)	1,7** (2,0–1,5 = 0,5)	2,0 (2,3–1,9 = 0,4)
CP, c.u.	2,3 (2,3 – 2,2 = 0,1)	1,6* (1,8–1,5 = 0,3)	1,8* (1,9–1,6 = 0,3)	2,0* (2,0–1,5 = 1,6)	1,5* (1,9–1,2 = 0,7)
MPO, c.u.	2,2 (2,3–2,1 = 0,2)	2,4 (2,7–2,1 = 0,6)	2,8* (2,8–1,6 = 1,2)	2,3 (2,6–2,1 = 0,5)	2,3 (2,8–2,0 = 0,8)

Notes: ** – statistically significant difference compared to control group ($p < 0,05$), ** – statistically significant difference compared to 2 group ($p < 0,05$)*

CONCLUSION

1. The data obtained allowed to establish, that increasing OPM values with different intensity in ejaculate were observed in patients of all groups studied depending on damaging factor peculiarities.

2. Insignificant OPM increasing in patients of third and fourth groups, probably, is connected with forming by helminths (toxocara) defense against their damaging agents, in particular, OPM and phagocytosis, that is confirmed by incomplete phagocytosis secondary to deficiency of functional and metabolic reserve.

3. The high OPM values in patients of second and fifth groups, probably indicate only influence of environment (industrial pollutants in air), many of them may be booster for development and maintenance of high OPM values.

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