RESEARCH ARTICLE

Cytokine Profile and Intracellular Matrix Component Degradation Indicators in Male Ejaculate with Chronic Urethritis Before and Following the Treatment

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Abstract

Aim: The present study was aimed to identify the local level alterations in pro-inflammatory, anti-inflammatory cytokines, and matrix metalloproteinase indicators as well as their tissue inhibitors in male ejaculate with chronic urethritis. **Materials and Methods:** Antiviral therapy was given as preparation of valacyclovir, administered per os in dosage 500 mg twice a day during 10 days, then 500 mg once a day during 20 days; antibacterial therapy was implemented with doxycycline in dosage 100 mg twice a day during 10 days. Immunomodulator was used as recombinant human interleukin-2 in dosage 500 thousand IU 1.0 ml subcutaneously in the shoulder area every day. Laboratory researches included cytokine status and acute-phase protein analyses initially and after 28 days it has been evaluated with specific reagents of "R&D Diagnostics Inc." (USA) by means of sandwich-variant solid-phase immunoassay analysis. The results were processed with immunoassay analyzer "Multiscan" (Finland). Cytokine number and metalloproteinase system indicators were evaluated with the help of calibration curve by means of the computer program. The number was evaluated in PN/mlилиNG/ml. The data were statistically processed with Statistica 10 and R. **Conclusion:** The study presents the local level cytokine response phenotypes in men with chronic herpetic, chlamydial, and mixed urethritides depending on the etiologic factor based on immunologic indicators, defined by means of specific and highly sensitive statistic methods. Additional differential markers of chronic urethritides in men have been proposed. Immunologic evaluation of the efficiency of the etiotropic therapy as well as schemes coupled with cytokine therapy, defining effects of the latter per se, has been carried.

Key words: Chlamydiosis, cytokines, herpes, immunomodulators, matrixmetalloproteinase

INTRODUCTION

Nowadays, there are no doubt concerning the fact that HSV infecting can alter human bodily immune response to different pathogens.[1] In 85% of cases, genital herpes (GH) is associated with the other pathogens.[2] Structural analysis of the sexually transmitted infections evidenced that GH is mostly associated with chlamydial infection,

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being due to the common epidemiological patterns attributed to these agents.[1] Data were supported by the cluster analysis, which revealed the greatest likelihood of HSV-1,2 with *Chlamydia trachomatis*.[3] Immunological mechanisms, in particular, the balance of the pro- and anti-inflammatory cytokines, and acute-phase protein play an important role in the onset of chronic urethritis. Cytokines, as molecules of short distance action, practically do not enter blood flow during immune response, and consequently, they are found in the blood serum in low concentrations.[4] There is a high likelihood of their detection on the local level that has been undertaken in our investigation.

MATERIALS AND METHODS

A total of 132 males have been screened for sexually transmitted infections according to generally accepted standards. Males of the main group have been subdivided into two subgroups (MG): 42 males with chronic viral urethritis (genital herpes [GH]), 24 males with chronic chlamydial urethritis (chlamydiosis), and 36 males with viral-chlamydial urethritis (GH associated with chlamydiosis). MG mean age was 33.0 ± 4.2 years. Comparison group (control group) was apparently healthy 30 males, mean age was 31.1 ± 1.1 years. Antiviral therapy was implemented with the preparation valacyclovir, administered per os in dosage 500 mg twice a day during 10 days, then 500 mg once a day during 20 days; antibacterial therapy was implemented with doxycycline in dosage 100 mg twice a day during 10 days. Immunomodulator was used as recombinant human interleukin-2 in dosage 500 thousand IU 1.0 ml subcutaneously in the shoulder area every day. Laboratory researches included cytokine status and acute-phase protein analyses initially and after 28 days it has been evaluated with specific reagents of "R&D Diagnostics Inc." (USA) by means of sandwich-variant solid-phase immunoassay analysis. All patients were monitored during 28 days (laboratory investigations included cytokine status and acute-phase protein analyses initially and after 28 days). Levels of tumor necrosis factor (TNFα), TNFβ, IL-1β, IL-2, IL-2sR, IL-13, IL-17, IL-22, IL-10, IL-13, $TGFβ_1$, IFNα and IFNγ, matrix metalloproteinase 8 and 9 types (MMP-8 and MMP-9), and their tissue inhibitors 1 and 2 types (TIMP-1, TIMP-2) in the ejaculate have been evaluated with specific reagents of "R&D Diagnostics Inc." (USA) by means of sandwich-variant solid-phase immunoassay analysis according to the provided instructions. The results were processed with immunoassay analyzer "Multiscan" (Finland). Cytokine number and metalloproteinase system indicators were evaluated with the help of calibration curve by means of the computer program. Number was evaluated in PN/mlилиNG/ml. To assess the immunomodulatory own effect, Zemskov A.M. formula was applied (2003):[5]

$$(P_{starting}-P_t)/P_{starting} – (P_{starting}-P_m)/P_{starting}×100\%$$

Where

$P_{startig}$ - absolute level of the parameter value before the treatment,

P_t - the same, following the traditional therapy,

P_m - the same, following the traditional therapy with the immunomodulator.

The obtained data were statistically processed with the software package program Statistica 10 and R. Data were presented as the median and two quartiles (Me, Q_{25}, and Q_{75}). Intra- and inter-group differences were assessed with Mann–Whitney test within the applied program. To decide which cytokines in patients with chronic ureteritis ejaculate reliably distribute patients' groups depending on the etiological factor, the discriminant analysis (DA) was used. To check the relationship or in dependence between values, the criterion χ^2 was established. To reveal sensitivity and specificity of selected parameters' changes, the linear regression technique with receiver operating characteristic (ROC) curves creating in the program MedCalc was applied. Confidence level was selected 95%, i.e. null hypotheses were rejected in the event that reached level of meaning P of the statistical parameter used was less 5%. Study implementation rate abled to estimate the results with 95–99% certainly using the relevant statistic methods.

RESULTS

In analyzing anti-inflammatory cytokines in the ejaculate in men with chronic herpetic urethritis depending on the etiological factor, it has been found that levels of TNFβ (1.2 times, $P < 0.05$), IL-2sR (6 times, $P < 0.001$), IL-17 ($P < 0.05$), IFNα (2.8 times, $p<0.001$) grew, IL-1β (was changeable only in this group; 6.3 times, $P < 0.001$), and IL-2 (1.3 times, $P < 0.05$) decreased against the background of low IL-10 (1.5 times, $P < 0.001$) and $TGFβ_1$ (1.3 times, $P < 0.05$) and high IL-13 (1.4 times, $P < 0.05$) and IL-22 (2.3 times, $P < 0.001$, Table 1); TNFα and IFNγ concentration were unchangeable. Viral-chlamydial urethritis group demonstrated elevated levels of TNFα (3.6 times, $P < 0.001$), TNFβ (1.3 times, $P < 0.001$), as well as significant increase of IFNα (2.5 times, $P < 0.01$), IL-13 (2.5 times, $P < 0.01$) and IL-22 (2.3 times, $P < 0.001$), while levels of IL-2 (1.1 times, $P < 0.05$) and IL-10 (2.3 times, $P < 0.001$) were lower than benchmarks. IL-1β, IL-2sR, IL-17, IFNγ, and $TGFβ_1$ content in patients of this group were not different from the reference values.

Male group with chlamydial infection evidenced the following multidirectional changes of pro-and anti- inflammatory cytokines: TNFα levels (3.5 times, $P < 0.001$), IL-2sR (18 times, $P < 0.001$), IFNα (3.1 times, $P < 0.01$), IL-22 (1.8 times, $P < 0.01$), and $TGFβ_1$ (1.3 times, $P < 0.05$) were higher than control values, but TNFβ content (1.1 times, $P < 0.05$), IL-2 (1.2 times, $P < 0.05$), IL-10 (4 times,

$P < 0.001$), and IL-13 (1.2 times, $P < 0.05$) decreased. IL-1β, IL-17, and IFNγ levels were unchangeable [Table 1].

While assessing condition of intercellular matrix indicators in the ejaculate, elevation of MMP-8 in all male groups has been ascertained: In male group with chlamydial urethritis - 5.5 times ($P < 0.001$, Table 2), in group with mixed urethritis - 4.5 times ($P < 0.001$), and in group with viral urethritis - 3.5 times ($P < 0.001$). MMP-8 values in group with viral urethritis were significantly higher than those of chlamydial infection group ($P < 0.05$).

A significant decrease of MMP-9 level has been noted: In viral urethritis, values were the lowest (9 times, $P < 0.01$), in mixed urethritisMMP-9 level - 8 times less, and in chlamydial urethritis - 7 times less ($P < 0.05$). In all groups, TIMP-1 and TIMP-2 values were not beyond the control values.

To make a decision what variables (cytokines in the ejaculate) in men with chronic urethritis differ the totalities under the investigation (patient groups, subdivided according to the etiological factor), we have applied the DA. Step-by-step analysis of the discriminant function defined those cytokines and acute-phase proteins (variables), contributing strongly to the difference between the patient groups with chronic urethritis (totalities). Thus, the model has been constructed, enabling to forecast the best affiliation to patient groups (totalities) some or other ejaculate samples. While looking into the canonical discriminant axis (separation degree of groups under consideration), we revealed the following [Figure 1]: Indicators of controls were significantly different from those of the groups under the investigation; splitting of high-stress indicators between the groups depending on the etiological factor has been established.

Majority of variables came into the model in the ejaculate after totalities and function defining: TNFα, TNFβ, IL-1β, I-2, IL-2sR, IFNα, IL-10, IL-13, IL-22, $TGFβ_1$, MMP-8, MMP-9, and TIMP-1 ($\lambda = 0.0048$, F-statistics <0.00001). The exceptions were IL-17, IFNγ, and TIMP-2.

Quality of the constructed model was 98.48% in the test sample. To testify the interrelation or the independence (χ^2) among the proposed indicators in the ejaculate of male groups with chronic urethritis in reducing IL-1β level <1.0 pg/ml ($\chi^2 = 6.11$, $P < 0.01$), cytokine profile phenotype typical for the viral etiology of chronic urethritis should be proposed; TNFα elevating more than 10.0 pg/ml ($\chi^2 = 4.82$, $P < 0.01$) and IL-13 more than 80 pg/ml ($\chi^2 = 2.13$, $P < 0.05$) indicate viral-chlamydial origin of chronic urethritis; in IL-2s relevating more than 3.0 pg/ml and IL-10 decreasing <3.0 pg/ml chlamydial etiology of chronic urethritis in men should be proposed.

The subsequent estimation of the studied indicators values has been conducted with a linear regression method - ROC-analysis, enabling to narrow the set of chronic urethritis immunologic markers. The linear regression method is designed to determine sensitivity and specificity of chosen inflammatory mediators (identified according to the results of the DA) in the ejaculate. Their concentration has been estimated by means of the square limited by ROC-curve

Table 1: Anti-inflammatory cytokines levels in the ejaculate in men with chronic urethritis depending on the etiological factor

Indicators (Me; Q_{25};Q_{75})	Controls (n=30)	Patients with chronic urethritis depending on the etiology (Me; Q_{25};Q_{75})		
		Viral urethritis (*n*=42)	Mixed urethritis (*n*=36)	Chlamydial urethritis (*n*=24)
TNFα, pg/ml	2.6 (1.56;3.05)	3.78 (3.02;4.51)$^{\#\#}{}_{1\text{-}2}$,$^{\#\#}{}_{1\text{-}3}$	10.37*** (3.62;10.82)$^{\#}{}_{2\text{-}3}$	7.41*** (4.74;10.12)
TNFβ, pg/ml	9.69 (5.49;13.24)	10.82* (7.91;11.78)$^{\#}{}_{1\text{-}2}$,$^{\#\#}{}_{1\text{-}3}$	12.91* (5.89;22.11)$^{\#\#}{}_{2\text{-}3}$	8.26* (4.69;9.26)
IL-1β, pg/ml	3.32 (2.01;4.65)	0.5*** (0.32;0.61)$^{\#\#}{}_{1\text{-}2}$,$^{\#\#}{}_{1\text{-}3}$	3.83 (0.87;6.45)	3.35 (1.87;4.12)
IL-2, pg/ml	113.34 (89.65;135.21)	97.78* (81.11;106.31)	102.19* (95.0;110.21)	100.2* (87.67;100.86)
IL-2sR, pg/ml	0.28 (0.04;0.06)	1.82*** (0.09;3.15)$^{\#\#}{}_{1\text{-}2}$,$^{\#}{}_{1\text{-}3}$	0.08 (0.03;0.09)$^{\#\#}{}_{2\text{-}3}$	4.1*** (3.48;4.9)
IL-17, pg/ml	11.31 (8.44;13.28)	14.11* (8.69;22.88)$^{\#}{}_{1\text{-}2}$	11.65 (10.32;13.45)	13.34 (9.33;14.01)
IFNγ, pg/ml	18.82 (14.23;22.13) (13,0-25,96)	14.79 (14.45;15.97)	15.05 (14.7;16.96)	15.11 (14.25;17.42)
IFNα, pg/ml	4.82 (3.21;4.98) (1.2-5.13)	14.92*** (6.25;18.5)$^{\#}{}_{1\text{-}2}$	10.81** (4.81;16.45)$^{\#}{}_{2\text{-}3}$	13.11** (8.92;16.23)
IL-10, pg/ml	10.01 (5.28;10.61)	6.21*** (0.99;11.56)$^{\#}{}_{1\text{-}2}$,$^{\#\#}{}_{1\text{-}3}$	4.37*** (0.63;1.86)#2-3	2.26*** (1.36;2.89)
IL-13, pg/ml	34.88 (22.54;41.31)	51.11* (44.12;67.89)$^{\#\#}{}_{1\text{-}2}$,$^{\#}{}_{1\text{-}3}$	99.37** (90.16;104.77)$^{\#\#}{}_{2\text{-}3}$	28.07* (11.23;38.88)
IL-22, pg/ml	74.57 (48.91;104.32)	163.21*** (134.53;174.81) $^{\#}{}_{1\text{-}3}$	161.69*** (85.47;236.11)$^{\#}{}_{2\text{-}3}$	123.11** (84.78;136.71)
$TGFβ_1$, pg/ml	191.16 (105.54;248.52)	148.22* (80.66;158.91)$^{\#}{}_{1\text{-}2}$,$^{\#\#}{}_{1\text{-}3}$	193.98 (139.56;265.19) $^{\#}{}_{2\text{-}3}$	255.24* (200.13;401.78)

Statistical significance of differences between groups: With controls: P<0.05*, P<0.01**, P<0.001***, between groups with chronic urethritis depending on the etiology: P<0.05$^{\#}$, P<0.01$^{\#\#}$, P<0.001$^{\#\#\#}$, where 1, 2, and 3 - groups under investigation

and the axis of false-positive classification ratio (AUC). It is known that the more AUC, the higher likelihood of suggested diagnostic sigh [Table 3].

For an objective assessment of chronic urethritis clinical symptoms manifestation gravity, we modified G.I. Marchuk method scoring (innovatory proposal No. 2858 from 10 April 2017. Quantitative method of the manifestation gravity of chronic urethritis patients' condition based on clinical-immunological indicators), where score 0 corresponded to the absence of the clinical sign, score 1 corresponded to the minor symptom manifestation, score 2 - moderate, and score 3 - major manifestation. This has enabled the objective assessment of the indicators manifestation gravity in the dynamics. In males with chronic viral urethritis before treatment, there has been estimated clinical symptoms' major manifestation, corresponding 24 scores. Following the treatment, all patients with chronic urethritis had the PCR and DIF negation.

As a result of the monovariant anti-viral treatment in herpetic urethritis male groups, there has been noted a decrease of clinical laboratory disorders manifestation (18 scores). Like this, in male groups having received standard anti-viral therapy, there has been palliation due to eruptions elimination and reducing lymphadenopathy syndrome 1.3 times ($P < 0.05$). There have been no changes from the part of the pain syndrome, dysuria, and sexual disorders that require, in our view, anti-viral therapy continuation or

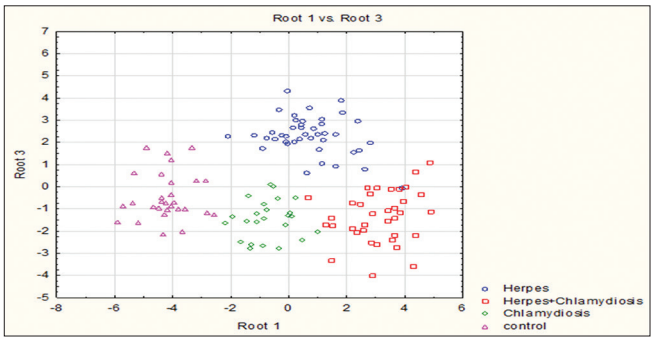

Figure 1: Projections of the available observations on the two-dimensional plane formed by the canonical discriminant axes 1 and 3

immunomodulating preparation administering. In the group of patients treated with the combined therapy of recombinant IFN-γ preparation, minor severity of disorders has been noted (9 scores). It is connected with their solution of cutaneous manifestations in all patients and lymphadenopathy inguinal lymph nodes decreasing in 25% men, as well as pain syndrome reduction and urination recovery in 45% of these patient categories. Sexual complaints in 60% of men were poorly identified or were absent. Men with chronic viral-chlamydial urethritis pro treatment scored 25 on the gravity degree of clinical signs and laboratory indicator levels. Following monovariant antiviral and antibacterial therapy, there has been gravity reducing of clinical laboratory disorders (19 scores against 25). Manifestations of mucous discharge from the urethra ($P < 0.05$) became statistically soundless as well as a number of men with complaints of ejaculation delay decreased by 8.3%. Male group treated with recombinant IFN-γ noted reducing the number of bubble rush, edema, and erythema intensity twice; lymphadenopathy syndrome became twice less manifested ($P < 0.05$). Patients with pain syndrome, complaints of dysuria, and sexual disorders reduced by 2.3 times, and intensity of symptoms also decreased 1.5 times ($P < 0.05$). Men treated with recombinant IL-2 noticed herpetic rash fully disappearing on the smooth skin and mucous membrane as well as relief of pain syndrome practically in 90% (11 patients) of this group. Among sexual complaints, it was only erectile distress, and its intensity reduced 1.8 times. Following monovariant antibacterial therapy, patients with chlamydial infection had reduced manifestation of clinical laboratory estimation of the chronic urethritis from 23 to 17 scores in 25% (23 patients). It is interrelated with pain syndromes severity reducing 1.5 times ($P < 0.05$), as well as dysuria complaints (difficulty urinating was noticed throughout all treatment period). From the sexual disorders, there was noticed reducing of discomfort severity while erecting 1.3 times ($P < 0.05$). Patients treated with recombinant IFN-γ noted pain syndrome relief ($P < 0.05$), although.

Dysuria remained in patient seven after combination therapies with immunomodulator. It should be stressed that this patient group following treatment reported a decrease of sexual disorders complaint expression reducing of erectile dysfunction and orgasm absence by 2 times in 80% (6 men) and reducing manifestation of clinical laboratory signs

Table 2: Content of mediators influencing intercellular matrix condition and their complexes in the ejaculate in men with chronic urethritis depending on the etiological factor

Indicators (Me; Q_{25}:Q_{75}) ng/ml	Patients with chronic urethritis (Me; Q_{25};Q_{75}) (n=102)	Patients with chronic urethritis depending on the etiology (Me; Q_{25};Q_{75})		
		Viral urethritis (n=42)	Viral-chlamydial urethritis (n=36)	Chlamydial urethritis (n=24)
MMP-8	3.98 (1.56;3.05)	14.23*** (2.45;26.16)$^{\#1\text{-}3}$	18.55*** (1.78;40.08)	22.28*** (11.32;36.12)
MMP-9	0.11 (0.002;0.238)	0.013** (0.004;0.013)$^{\#1\text{-}3}$	0.026** (0.008;0.039)	0.038* (0.037;0.05)
TIMP-1	3.57 (3.06;3.59)	3.45 (3.07;4.09)	3.39 (3.09;4.1)	4.2 (4.01;4.36)
TIMP-2	73.65 (72.65;74.85)	74.01 (73.66;74.11)	73.02 (69.12;75.31)	71.52 (71.32;71.71)

Statistical significance of differences between groups: With controls: *P<0.05, **P<0.01, ***P<0.001, between groups with chronic urethritis depending on the etiology: $^{\#}P$<0.05, $^{\#\#}P$<0.01, $^{\#\#\#}P$<0.001, where 1, 2, and 3 - groups under investigation

Table 3: Indicators diagnostic validity - square values under the ROC-curve

Indicator	GH	GH+chlamydiosis	Chlamydiosis
TNFα	0.68	0.94	0.93
IL-1β	0.99	0.51	0.51
IL-2sR	0.87	0.85	1
IFNα	0.98	0.92	0.97
IL-10	0.89	0.97	0.98
IL-13	0.68	0.99	0.65
IL-22	0.99	0.97	0.95
MMP-8	0.86	0.71	0.99
MMP-9	0.92	0.87	0.81

from 23 to 10 score. Male group treated with recombinant IL-2 reported reducing lymphadenopathy expression in all patients of this subgroup by 2.3 times. Only one-fourth (25%) retained sexual disorders.

Ejaculate of men with chronic viral urethritis treated with Valacyclovir revealed IL-10 and IL-13 levels normalization. IL-2sR level decreased by 2 times ($P < 0.01$). IL-1β and MMP-9 values were unchanged able following the antiviral therapy and were beyond the control values. IFNα, IL-22, and MMP-8 changes concentration dynamics following the therapy were absent, their meanings stayed elevated during the treatment. TNFα as prior the therapy, as during it was unchangeable and in control values. Analyses of changes in cytokine levels and acute-phase proteins in men with herpetic urethritis treated with valacyclovir and recombinant IFNγ determined IL-2sR and IL-10 normalization. IL-1β production has increased 3 times and MMP-9 - 2 times in providing the therapy, and IFNα and MMP-8 concentration lessened 1.5 times, though indicators have not reached control values. IL-13 and IL-22 levels were unchanged in providing the therapy and stayed high during the therapy. Minimal dynamics to value stabilization had IL-13, IL-22, MMP-8, and MMP-9 levels. TNFα meanings were unchanged in providing the therapy. Men with viral urethritis treated with valacyclovir combined with the recombinant IL-2 demonstrated all indicators normalization (IL-1β, IFNα, IL-10, IL-13, IL-22, and MMP-9), except: IL-2sR, which level was 2 times more than control meanings, and MMP- 8, which meanings 2.5 times decreased during the treatment, but did not reach control ($P < 0.05$). TNFα meanings were unchanged able providing the treatment and were in the reference limits.

Analysis of the mediators local content in men with mixed infection treated with the standard therapy (valacyclovir + doxycyclin) has noted the following changes: TNFα, IFNα, IL-13, IL-22, and MMP-8 on 28th day of therapy were unchangeable and stayed high and were consistent with protreatment data, there were no differences between the groups pro and following the treatment. IL-10 providing the therapy increased twice, and MMP-9 meanings elevated trice ($P < 0.05$) but did not reach control ones. IL-1β and IL-2sR values were in the reference limits as pro, as following the treatment. Combine standard therapy and recombinant IFNγ provided stabilization of the following indicators: IL-10, IL-13, and MMP-9. TNFα values decreased 40%; IL-22 dropped 35%, not enabling to reach controls providing combined therapy. IFNα value remained steady high in dynamics and was twice higher controls. MMP-8 fell 3.5 times providing the therapy ($P < 0.05$), although its level has not reached reference levels. IL-1β and IL-2s remained stable during the treatment and match control values. IFNα, IL-10, and MMP-9 levels in male group treated with recombinant IL-2 reached the normal ones. TNFα values declined trice, IL-13 - 2.5 times, IL-22 - 1.5 times, and MMP-8 - twice, though having not reached the normal ones. IL-1β remained the same during the treatment.

IL-2sR, IL-10, and IL-13 values in ejaculate of men treated with single antibacterial therapy have normalized and matched controls. Although TNFα, IFNα, IL-22, and MMP-8 content following the therapy leveled off and matched group levels prior treatment. There was no dynamic of MMP-9 levels: They remained steady following 28 days standard antiviral and antibacterial treatment. IL-1β remained the same as prior, as following the treatment. IL-2sR, IFNα, IL-10, IL-13, and MMP-9 levels of men treated in conjunction with recombinant IFNγ have normalized and matched those of controls. TNFα, IL-22, and MMP-8 levels following the treatment considerably reduced: TNFα and IL-22 1.5 times, and MMP-8 trice, having not reached control ones. IL-1β remained the same as prior, as following therapy. Discharge from the urethra in men treated with recombinant IL-2 had IL-2s R and IL-10 normal levels. TNFα, IFNα, IL-13, IL-22, MMP-8, and MMP-9 value dynamics have not been noted. Their concentration following the treatment leveled off and has not reach control levels. IL-1β remained the same during therapy.

Viral urethritis treatment effect study based on standard schemes as well as mixed ones with immunomodulators would be appropriate with the complex therapeutic schemes, embracing antiviral and immunomodulating preparations, owing to the positive change dynamics of cytokines and acute-phase proteins levels in the ejaculate. At the same time, studied inflammatory mediator values following the recombinant IL-2 therapy in 91% of patients returned to control ones and following the recombinant IFNγ indicators in 80% of men comply with controls. Mixed urethritis treatment effect study based on recombinant IL-2 therapy has shown that 52% of patients' examined inflammatory mediator values returning to control ones and indicators of the 86% of male subgroup treated with recombinant IFNγ have been consistent with the controls. Complex therapeutic scheme would be appropriate in chronic chlamydial urethritis including antibacterial and

immunomodulating preparations: 76% of patients' examined inflammatory mediator values returning to control ones, following the recombinant IFN- 2; 89% of male indicators met the controls following the recombinant IFNγ.

DISCUSSION

Naslednikova *et al*. reported that mononuclear capacity to secrete TNFα in patients with chronic herpetic infection is practically normal. In herpes simplex virus, long-term persistency IL-2-induced production has been significantly lower than in healthy donors,[6] confirming the results obtained during our investigation. TNFα and IL-2 deficit might bear evidence to the insufficient antiviral activity of the cellular immunity, enabling viral long-term persistency. TNFβ is not only merely bound with the receptors of TNF (TNFRI, TNFRII and tumor necrosis factor receptor superfamily member 14 [TNFRSF14]) but also is a mediator of herpesvirus invasion (herpesvirus early mediator). TNFRSF14 jointly with its ligands-homologs TNF, (LT)-α and LT-β lymphotoxin, immunoglobulins, and $CD160^{+}$ forms a complex multisignal system of the immune system inflammation and homeostasis regulation,[7,24] having been demonstrated during the study with TNF β level in viral and mixed urethritis groups being higher controls. TNF is long known to suppress chlamydial infection and to play an important role in chlamydial elimination not bounded with IFNγ.[7] Hakimi *et al*. conflicting data (2014) display that found by the authors IL-1, IL-2, IL-6, IL-17 elevated levels, and TFNα in plasma and sperm might indicate male infertility and influence negatively on spermatogenesis and steroidogenesis.[8] However,Lu *et al*. have shown that defense from genital infection by means of live Chlamydia immunization correlates with T-cell response and is characterized by IFNγ high level and IL-17 low level.[9] Andrew *et al*. in their investigation have illustrated that, in IL-17 absence, clinical gravity in chlamydial genital lesions is considerably lessened;[10] like this, in our study, IL-17 level in the chlamydial infection group was not different from the control values.

One of the viral persistence mechanisms is a synthesis stimulation of ineffective IgG-antibodies which suppresses immunity and NK-cells and stimulates MMP-9 productions.[11,12] Hayshi *et al*. experimental work (2009) illustrates that HSV with IgG immune complexes stimulates macrophages by Fc receptors, TLR 2 and 3 types, and inducing MMP-9 production.[11]

Apart from properly neutralizing effect, IgG-HSV immune complexes stimulate pro-inflammatory mediators (IL- 8, MMP-9, and leukotriene B4) production[11] that might strengthen inflammation and clinical severity manifestation. Other pro-inflammatory cytokines (IL-1β, IL-6, and TNFα) also strengthen MMP-9 production,[5] and MMP-9 excess contributes to the viral transmission and progression of the disease, not in keeping with the results of our investigation. Shurshalina *et al*. demonstrated that on the background of the viral infection female shave reliable elevation of the MMP-1 local activity and simultaneous level declining of the tissue inhibitor of the matrixmetallo proteinase (TIMP)-1 in the mucous of the cervical channel, being a significant indicator of the intensity of the inflammatory process and reiterating viral destructive potential of various gravity.[13]

According to Spiridonova *et al*., it is high relapse rate that is linked to the synthesis reduction rate of the lymphocytes IFNγ and NK cytotoxicity, although the duration of the acute-phase of inflammation was directly dependent on IFNα secretion.[14] However, as IFNα production defect, as IFNγ is thought to exist.[15] In view of Shperling *et al*., elevated IL-10 level might be a marker of herpetic process chronization; however, we have reveal edits level decrease in all groups under examination.[16]

In chlamydiosis, there was monotony of credits of the specific antibodies to *C. trachomatis,* as well as a reliable increase of IL-1β and IL-6, being a marker of the inflammatory process, which does not compile with the results of this study. Squith *et al*.[17] showed, in their study, that high IL-13 level could promote sensitivity to the chlamydial infection and inflammation increase. Majority of the investigators consider IFN, TFN, and IL-1 to be the most significant anti-chlamydial immunity factors.[2] The special role plays IL-1, secreting the first by intact epithelial cells, and its lymphocyte-activating function is necessary for the following development of the immunity response-specific face in urogenital chlamydia (UGC).[18] Balance between the production levels of IFNγ and IL-10 is thought to influence on the outcome of the chlamydial infection, resulting in full recovery or complications. IL-10 secretion in the long-term infection increases significantly. IL-10 inhibits in phagocytes and APC transcription and production of cytokines, responsible for the inflammatory response (TNF and IL-12,).[19] Asymptomatic UGC infection in some men results in autoimmune response in hsp60 and infertility.[18] Elevation of hsp60 content in pathology produces inflammatory cytokines and induces metalloproteinase synthesis and lipoproteins oxidizing.[18] MMP activity depends on their gene expression level and activator and inhibitor presence. MMP not only could take part in the infection elimination but also these enzymes expression strengthening could degrade intercellular matrix and cicatrization.[20] IL-17 stimulates MMP-9 production, which, for its part, increases neutrophil in filtration and stimulates sun favorable complications by means of chemokines enzyme modification.

Most authors consider herpetic and chlamydial infection treatment to be complex: Etiological and pathogenic, applying combinations of various medications with a different mechanism of action, since in sufficiency of the different links of the immune system, its failure to eliminate virus from the body and antiviral monotherapy cannot produce the desired

results.[21] There are very few works devoted to the mixed viral-chlamydial infection treatment of the men urogenital tract, whereas these patient therapies generate recurrent difficulties. For instance, doxycycline immunotherapy is known to penetrate through cell membranes relatively easy and lessens intravascular and systemic MMP secretion and activity, predominantly 1, 2, and 9th types, suppresses IL-6 anti-inflammatory cytokine secretion, acute-phase C-reactive protein, nitrogen oxide, and oxygen forms, enabling HSV foci healing, thereby improving patient state.[22] It should be mentioned that doxycycline, being the most potential MMP inhibitor, suppresses MMP activity *in vivo* in less elevated medication concentration in blood compared to those necessary for its antimicrobial effect.[18] Skulason *et al*. have conducted a study, where they tested antiviral and wound-healing effects of hydrogels containing monocarpine only and monocarpinein combination with a small dose of doxycycline *in vivo* against herpes-viral labialis infection.[23] Monocarpine has been reported to show high antimicrobial activity *in vivo* and to suppress HSV in 1 min, preventing virus penetrating in cell membranes of mucous body parts. While doxycycline combination is active against labial herpes on each disease stage, monocarpine ceases viral replication and doxycycline enable rapid erosion epithelization due to MMP-9 suppression. Notwithstanding, doxycycline has been noted to obtain high toxify to male generative cells. Considered all, macrolides and tetracyclines yield immunomodulating effect embracing direct dose-dependent lymphocyte mitosis and phagocytosis suppression, inflammatory cytokine secretion reducing, such as TNFα, IL-1, IL-6, and IL-8, and secretion rising of anti- inflammatory cytokines IL-2, Il-4, and IL-10.[24]

CONCLUSION

In general, male group with chronic urethritis on the systemic level TNFα, IL-2sR, IFNα, MMP-8 high levels, and MMP-9 deficit, against the background of the disregulatory interrelation of the anti-inflammatory cytokines IL-13 and IL-22 (IL-10 level decline at high IL-13 and IL-22 levels are noted) and normal levels of their tissue inhibitors (TIMP-1 and TIMP-2), is detected.

Patients with chronic urethritis on the local level demonstrate IL-1β deficit, IL-2sR, and IFNα high levels (the highest one among all groups under the investigation), against the background of the lowest $TФPβ_1$ levels. MMP-8 high levels and the lowest MMP-9 levels are detected. In male group with mixed urethritis TNFα, TNFβ and IL-13 the most vivid elevation, high MMP-8 level and low MMP-9 level have been defined. Men with chronic chlamydial urethritis had the least TNFβ, high IL-2sR, IL-10 and IL-13 deficit, and $TGFβ_1$ overproduction. MMP-8 hyperproduction and MMP-9 deficit have been defined. It should be noted that the investigation of the intercellular matrix indicators in patients with chronic urethritis has been conducted for the first time, there were no similar investigations noted in Russian and foreign databases.

DA, χ^2 method (Chi-square), and ROC-analysis results allowed to narrow the differentiating indicators diagnostic search and to detect the additional diagnostic criteria of chronic urethritides in men on the local levels, defining which at the beginning of the therapy and in dynamics enable to personalize therapeutic tactics of each person in this patient category.

1. In IL-1β level decline below 1.0 pg/ml, viral origin of chronic urethritis in men should be proposed;
2. TNFα elevation higher than 10.0 pg/ml and IL-13 higher than 80 pg/ml points to the mixed origin of chronic urethritis;
3. In IL-2sR elevation higher than 3.0 pg/ml and IL-10 reducing lower than 3.0 pg/ml, chlamydial etiology of chronic urethritis in men should be proposed.

Investigated indicator level study in venous blood serum and the ejaculate of men with chronic urethritis was informative and revealed their concentration changes by which a particular infectious agent can be supposed to participate.

Standard therapy did not provide cytokine profile and acute- phase protein level rehabilitation. When immunomodulators were added, cytokine imbalance and extracellular matrix indicator disorder reduced expression was evidenced: The own effect of recombinant IL-2 immunomodulator in chronic viral urethritis was 15%, in chlamydial urethritis - 7%, and in mixed urethritis - 8%, and recombinant IFNγ own effect in chronic viral urethritis was 8%, in chlamydial infection - 13%, and in mixed urethritis - 7%.

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