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ABSTRACT

of the dissertation for the degree of Doctor of Sciences

MODELLING OF THE INTERSTITIAL CYSTITIS/PAINFUL BLADDER SYNDROME, INVESTIGATION OF THE MULTIFACTORIAL FACTORS IN ETIOPATHOGENESIS AND STUDY THE ROLE OF DIAGNOSTIC BIOMARKERS

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GENERAL CHARACTERISTICS OF THE RESEARCH

Actuality of the theme and the degree of research. Interstitial Cystitis /Painful Bladder Syndrome (İC/PBS) refers to diseases, which is diagnosed and treated with difficulty. According to RICE (RAND Interstitial Cystitis Epidemiology studies from 2,7% up to 6,5% of the women in the United States suffer from the symptoms of the urinary bladder which is an indication of the wide spread of the disease[1]. It is supposed that IC/PBS is observed much before being diagnosed[2].

Recently due to better comprehension of pathophysiological mechanisms of diagnostic opportunities the discovery of this disease among the women has risen at least up to 2 %. Along with it, it is difficult to diagnose IC/PBS, because its symptoms are very identical to a number of urological and gynecological diseases[3].

IC/PBS is known in medicine over 50 years, but the development of pathological mechanisms of this disease has not yet been clarified, its etiology has not been studied up to the end and its diagnostics is based on the exclusion of other diseases. It is supposed that the potential pathophysiological reasons consist of the defect in the glicozaminoglycan layer of the apical surface of the bottom of urinary bladder, well as of autoimmune, inflamed and neurogen mechanizm, infections and others.

IS/ASKS ethiology may have numerous reasons. For better clarification of their reasons and comprehension of this issue

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[1] Berry, S.H. Prevalence of symptoms of bladder pain syndrome/interstitial cystitis among adult females in the United States / S.H.Berry, M.N.Elliott, M.Suttorp [et al.] // J Urol, – 2011. 186(2), – p. 540-544; Konkle, K.S. Comparison of an Interstitial Cystitis/Bladder Pain Syndrome Clinical Cohort With Symptomatic Community Women From the RAND Interstitial Cystitis Epidemiology Study / K.S.Konkle, S.H.Berry, M.N.Elliott [et al.] // J Urol, – 2012. 187(2), – p. 508-512

[2] Fang, Z., Xu, K. Interstitial Cystitis/Bladder Pain Syndrome: a Review and an Update // Current Bladder Dysfunction Reports, – 2016. 11(4), – p. 391–398.

[3] Davis, N.F., Gnanappiragasam, S., Thornhill, J.A. Interstitial cystitis/painful bladder syndrome: the influence of modern diagnostic criteria on epidemiology and on Internet search activity by the public // Transl Androl Urol, – 2015. 4(5), – p. 506-511; Rosen, J.M., Klumpp, D.J. Mechanisms of pain from urinary tract infection // Int J Urol, – 2014. 21 (Suppl 1), – p. 26-32.

experimental models are created. As a rule, to analyze the functions of the urinary bladder models are based on the intrabladder or systemic harmful stimuls[4]. The investigations carried out on animals displayed that the mast cells are responsible for the urinary bladder inflammation and pain[5]. But despite these studies the role of the mast cells has not been defined.

IC/PBS is looked upon as a chronical pain syndrome connected with urinary bladder. But to discover this violation there is not yet an objective marker or a test has not yet been conducted. It has been determined the diagnostics of this disease is distinguished with the lack of characteristic symptoms and is carried out with the methodology of "removing"[6].

One of the reasons in the birth of difficulties in the diagnostics and treatment of IC/PBS is the absence of biochemical markers in the tissues and liquids (blood and urine) that are considered a sign of the disorder. One of the approaches aimed at determining biomarkers is the study of the activity of methabolits. The investigators study the patients having one or several IS/PBS with matters, which distinguish the patients, who do not have this pathology, which take part in growing, propagation, development and other related processes[7].

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[4] Birder, L., Andersson, K-E. Animal Modelling of Interstitial Cystitis/Bladder Pain Syndrome // Int Neurourol J, – 2018. 22(Suppl 1), – p. S3-9.

[5] Bicer, F. Chronic pelvic allodynia is mediated by CCL2 through mast cells in an experimental autoimmune cystitis model / F.Bicer, C.Z.Altuntas, K.Izgi [et al.] // Am J Physiol Renal Physiol, – 2015. 308(2), – p. F103-113.

[6] Аль-Шукри, С.Х. Симптоматика и цистоскопическая картина у женщин с синдромом болезненного мочевого пузыря / С.Х.Аль-Шукри, И.В.Кузьмин, М.Н.Слесаревская [и др.] // Ученые записки СПбГМУ им. акад. И.П.Павлова, – 2017. 24 (4), – с. 50–54; Зайцев, А.В. Синдром болезненного мочевого пузыря/интерстициальный цистит: современные подходы к диагностике и лечению / А.В.Зайцев, М.Н.Шаров, Р.А.Ибрагимов [и др.] // Врач скорой помощи, – 2018. №8, – с.16-26.

[7] Parker, K.S. Urinary Metabolomics Identifies a Molecular Correlate of Interstitial Cystitis/Bladder Pain Syndrome in a Multidisciplinary Approach to the Study of Chronic Pelvic Pain (MAPP) Research Network Cohort / K.S.Parker, J.R.Crowley, A.J.Stephens-Shields [et al.] // EBioMedicine, – 2016. 7, – p. 167-174.

In the recent years much attention is paid to the biomarkers of IC/PBS, one of which is the nerve growth factor (NGF). It has been discovered that because of the block of receptor tropomyosin kinase or tyrosine kinase in NGF afferent nerve edges the transfer and strengthing of nociceptive signals takes place[8]. Clinical and experimental information shows that there is a direct relation between the rise of the level of NGF in the tissue of the urinary bladder and in urine and intensification of clinical appearances during IC/PBS[9]. It is supposed that NGF may potentially contribute to spread and keep chronical inflammation the observed during IS/PBS[10]. This hypothesis is supported by the achieved by our previous information on the relation between the leukocytes on the walls of the urine bladder and on the composition of the mast cells in IC/PBS experiment[11].

It is supposed that IC/PBS consists of the changes on the walls of the urinary bladder[12]. Recently the heparine-binding epidermal growth factor (HB-EGF), which is in the centre of attention of the majority of researches, and epidermal growth factor (EGF) levels are important factors for the proliferation of epithelial cells[13]. Besides, in

[8] Prato, V., Taberner, F.J., Hockley, J.R.F. Functional and Molecular Characterization of Mechanoinsensitive "Silent" Nociceptors // Cell Rep, – 2017. 21(11), – p. 3102-3115.

[9] Liu, H-T. Urinary nerve growth factor level is increased in patients with interstitial cystitis/bladder pain syndrome and decreased in responders to treatment / H-T.Liu, P.Tyagi, M.B.Chancellor [et al.] // BJU Int, – 2009. 104(10), – p. 1476-1481.

[10] Tyagi, P. Recent advances in imaging and understanding interstitial cystitis [version 1; peer review: 2 approved] / P.Tyagi, C.H.Moon, J.Janicki [et al.] // F1000Research, 2018. 7(F1000 Faculty Rev), – p. 1771.

[11] Шолан, Р.Ф. Уровень фактора роста нервов и его связь с содержанием лейкоцитов и тучных клеток при экспериментальном интерстициальном цистите / синдроме болезненного мочевого пузыря // Урологические ведомости, – 2019. 9(3), – с. 5–11.

[12] Слесаревская, М.Н., Игнашов, Ю.А., Кузьмин, И.В. Современные подходы к диагностике синдрома болезненного мочевого пузыря // Урологические ведомости, – 2017. 7(2), – с. 25-30.

[13] Keay, S.K., Birder, L.A., Chai, T.C. Evidence for Bladder Urothelial Pathophysiology in Functional Bladder Disorders // Biomed Res Int, – 2014. – p. 865463; Kim, S.R. NGF and HB-EGF: potential biomarkers that reflect the effects of fesoterodine in patients with overactive bladder syndrome / S.R.Kim, Y.J.Moon, S.K.Kim [et al.] // Yonsei Med J, – 2015. 56 (1), – p. 204-211

the pathogenesis of IC/PBS the role of inflamation protein is not excluded. The EGF was identified as one of the biomarkers in the urine of the patients who had IC/PBS, but nowadays the importance of the EGF levels from a clinical point of view is unclear. There was very little information about the concentration of HB-EGF and cytokines of the patients who had IC/PBS.

Then the study of the urine or serium biomarkers during IC/PBS seems to be hopeful for the raising the effect of the early diagnostics, because the diagnostics based only on the clinical symptoms and cystoscopic image is not enough.

Many factors of reason of IC/PBS have been described, nevertheless, the present understanding of the cause and consequence relations shows that as a result of trauma with poisonous matters, allergens or bacterias from the urinary bladder may be the reason of the process of development of inflammation[14].

The suggested pathophysiological reasons include the defect in the glicozaminoglican layer in the appical surface, inflammatory process, which causes the disfunction of urothelium, autoimmune and neurogen mechanisms, infectious diseases and others. In this case the chronical inflammation is regarded the main pathology approved in a number of studies IC/PBS. The histopathological researches concerning the urine bladder attaches importance to the role of immune mechanisms of IC/PBS with relation through cell , this does not exclude the fact that autoimmun inflammation which subgroup of patient with this disease is probably integral part of pathophysiology[15].

Thus, the topicality of the study of IC/PBS is explained by various reasons, particularly with its development mechanisms, absence of unanimity concerning the potential biomarkers of this

[14] Игнашов, Ю.А., Кузьмин, И.В., Слесаревская, М.Н. Синдром болезненного мочевого пузыря: исторические аспекты // Урологические ведомости, – 2016. 6(3), – с. 5-10; Birder, LA. Pathophysiology of interstitial cystitis // International Journal of Urology, – 2019. 26(Is1), – p. 12-15

[15] Wang, X. Evidence for the role of mast cells in cystitis-associated lower urinary tract dysfunction: a multidisciplinary approach to the study of chronic pelvic pain research network animal model study / X.Wang, W.Liu, M.O'Donnell [et al.] // PLoS One, – 2016. 11(12), – p. e0168772.

disease and with demands connected with perspective studies concerning the perspective studies able to illuminate the clinical and cystoscoptic scene. Though it has been studied for many years there is no a unanimous opinion concerning the etiopathogenesis of this disease. Along with it, an optimal experimental model has not been developed up to now connected with the study of this disease.

The object and subject of the research. The object of the study has been five experimental IC/PBS models and 129 patients with IC/PBS. The subject of the investigation is the specific peculiarities of biomarkers, morphological and infrastructural changes in the mucous membrane, interrelation of specific biomarkers with cytokins.

Aims and objectives of the research work. The goal of the research is the study of multifactoral factors in IC/PBS pathogenesis and specific biomarkers for their early diagnostics.

The objectives of the research:

1. Creation of a model, which characterizes the IC/PBS model, and the comparison of its analogues.

2. Estimation of the morphological changes in different experimental models of the uroepithelium in the urinary bladder.

3. The study of the ultrastructural changes in the mucous membrane, which have taken place in the interstitial cystitis of modeled experimented animals.

4. The study of specific biomarkers in the blood and urine in interstitial cystitis modeled experimented animals and determination of the degree of their reliability, the study of relations changes taken place in their structure of the mucous membrane.

5. The study of clinical manifestations and consequences of hydrodistensia in patients with IC/PBS.

6. Determination of the existence of specific biomarkers in the blood and urine of patients with interstitial cystitis and the degree of truthfulness for the early diagnostics, the degree of disease with them, correlation between them and seriousness of the disease.

7. Specification of immune-pathogenesis and neuro-immune inter cellular relations in IC/PBS urinary bladder.

8. Substantiation of suggestions concerning the optimisation of algorithm for the patients with IC/PBS.

The research methods. During the research five experimental IC/PBS models were created on the basis of 53 white New Zealand female rabbits. The IC/PBS models were achieved by means of irritating matters injected into their bags. The clinical examination covered 129 persons with IC/PBS and 20 patients without this disease. To fulfill the goals and objectives of the research the histological light and electron-microscopic examination, anamnesis collection, questionnaire, instrumental, biopsy, biochemical and statistical methods have been used.

The main provisions for defense:

– experimental IC/PBS models have been created and the most effective translation model has been determined;

– morphological changes in the urinary bladder ultrastructure changes have been determined in the special layer of mucous membrane;

– the quantity of NGF and EGF was determined in the blood and urine of the animals under experiment after the first and fourteenth day of the experiment and correlation analysis has been conducted between these markers;

– a questionnaire was conducted and the tension of symptom was determined, changes were discovered in the mucous membrane of the urinary bladder by means of cystoscopy and hydrodistention;

– the level of biomarkers and cytokins was determined in the blood of the patients, in whose bloods and urines there are IC/PBS.

Scientific novelty of the research:

– An effective experimental model has been created for the study of IC/PBS etiopathogenesis in animals under experiment.

– The main factors, which play an important role in the creation of the experimental model of interstitial cystitis disease, have been studied.

– The electron-microscopic character of the mucous membrance of urinary bladder has been given and the results of the ultra-structure analysis of the mucous membrance of urinary bladder

have been introduced.

– The specific biomarkers in the diagnostics of disease in the blood plasma and in the urine of the patients have been studied and the degree of correctness have been appreciated.

– Intracellulare relations have been discovered in the immunopathogenesis of the urinary bladder.

Theoretical and practical significance of the research. The preclinical model of IC/PBS has been created, it repeats the main features of the disease, it may be used in for the study of the pathogenesis of IC/PBS in practice. Implementation of cystoscopy and hydro-distance during the examination of patients having IC/PBS helps the efficiency of the differential diagnostics with other situation which expressed by pain syndrome.

Discovery of NGF, HB-EGF and EGF of biomarkers in the blood and urine of the patients is useful both for diagnostic goals and for learning the etiology of the disease.

Approbation and application. The main provisions of the dissertation have been reported in research-practical conferences: ESSIC 2019 (European Society for the Study of Interstitial Cystitis, annual meeting – 2019), 5-7 December, Amsterdam, Netherlands; SUFU 2020 (Society of Urodynamics, Female Pelvic Medicine and Urogenital Reconstruction, annual meeting – 2020), February 25-29, Scootsdale, Arisona, USA; TJOD – 2020 (Türk Jinekoloji ve Obstetrik Derneği online sympozyumu – 2020), 4-6 aralık, Türkiye. Scientific-Practic Conference Dedicated to the Birthday of A.Aliev Baku; 4th Health, Sciences and Innovation Congress, – Baku, Azerbaijan, – july 5-6, – 2021; 2nd International Congress On Applied Scences, – Azerbaijan, – November 8-10, – 2021.

The first discussion of the dissertation was held in the joint sitting of the staff of the Departments of General Surgery, Urology, Pathological Anatomy, Gynecology, Department of Endocrinology, Toxicology, Biochemistry of the Research Centre of Azerbaijan Medical University, Research Institute of Pulmonary Diseases, Department of Radiology and Hemostasiology of the Scientific Research Institute of Gynecology, Hemodialysis Laboratory, Chair of Urology of the Institute of Qualification of Physicians , Institute of

Physiology named after A.Garayev of the Academy of Sciences (July 8. 2021, Records No 1). The Scientific seminar of the dissertation was held in the sitting of the BED 2.06/3 One-Time Dissertation Council at AMU (February 3, 2022, Record No 6).

Twenty-one articles, 6 abstracts and one chapter dedicated to the topic of the dissertation of the author have been published.

Name of the organization where the dissertation is performed. The dissertation was performed at the Republican Treatment-Diagnostic Center of the Ministry of Health of the Republic of Azerbaijan, as well as in the Research Centre, Department of the Pathological Anatomy, Department of Histology, Embryology and Sitology, Immunology laboratory of the Azerbaijan Medical University, Laboratory of the Central Hospital of Oilworkers.

The total volume of the dissertation with a sign including a separate volume of the structural units of the dissertation. The dissertation consists of an introduction, five chapter each consisting of several sections, conclusion and abbreviations, practical recommendations, list of the literature used in the dissertation and the list of the abbreviations.

There are 38 tables, 25 graphics, 28 photos. The introduction consists of 7 pages (11656 symbols), the first chapter consists of 47 pages (93358 symbols), the second chapter consists of 20 pages (25668 symbols), the third chapter consists of 37 pages (48688 symbols), the fourth chapter consists of 37 pages (46874 symbols), the fifth chapter consists of 51 pages (97883 symbols), conclusion – 7 pages (12879 symbols), summary – 2 pages (3781 symbols), practical recommendations – 1 page (972 symbols), the total volume of dissertation excluding the list of literature –341759 symbols.

DESIGN AND RESEARCH METHODOLOGIES OF THE RESEARCH

The researches submitted in the dissertation are of experimental-clinical nature.

The experimental part of the dissertation has been carried out on 53 white New Zealand female rabbits weighing 1500-2000 g. To

create IC/PBS models in those animals different chemical and toxic matters have been injected into their urine bladders and an inflammation of the urine bladder has been created. An IS/PBS model has been created in the rabbits by several means, particularly through the injection of intrabladdery IC/PBS. For this purpose the animals under experiment have been divided into six groups and a different origins IC /PBS model has been created in 1-5 groups, and in the 6th group there were intact animals (Table 1).

Table 1.

ASKS/IS modelled rabbit groups

№ of the studied group	Number of animals	Modelling of IS/PBS
I group	8	70 % spirit solution has been injected into the urinary bladder
II group	7	Injection of protamine sulfate into the borrom of the urinary bladder
III group	8	Injection of 0,5% HCl solution has been injected into the urinary bladder
IV group	15	Injection of urine into the wall of the animal taken from the urinary bladder of the animal
V group	7	Injection of 0,9% NaCl solution into the wall of the urinary bladder
VI group (intact)	8	Not any solution has been injected

The studies have been conducted in one and 14 days after the IS/PBS modelling. After the lapse of 14 days the rabbits were injected 200mg/kg doze of pentobarbital, then transabdominal cystectomy was made. The extracted urinary bladder was kept in the buffer of 10 per cent neutral formalin solution. On the next day it was studied microscopically. Then parallel sections were made, their thicknesses being 0,3-0,4 cm, it allowed to view the walls of the urinary bladder simultaneously. Then with the purpose of fixation the obtained specimen were kept for 24 hours in 10% formalin solution

buffer. On the following days with the purpose of dehydratisation they were kept in 75%, 85%, 95% and 99,9% spirit solutions.

Then these specimen were kept in xylol solution and was put into paraffin and histological blocks were created. With the aid of microtomes sections were created, their thickness being 3-5 microns (Leica RM 2125 RTS, Germany). In order to discover the incisions with standard staining of hematoxylin-eosin and fat cells was stained by May-Grünwald Giemsa (Merck, Germany)[16].

The prepared micropreparations were enlarged by means of an microscope (Leica DM 750, Germany). During the microscopic examination all the observed changes were registered with the help of a camera attached to the microscope. Successively 10 view fields of micropreparat were greatly magnified and viewed, the number of lymphocytes, neutrophils, eosinophils and mast cells were counted. In each of these fields the cell infiltration was appreciated by means of the following scale: 0 – there are no extravascular leukocytes and mast cells; 1 – number of mast cells is less than 20; 2 – there are 20-45 leukocytes and mast cells; 3 – the number of leukocytes and mast cells is over 45; The scores of all the sections are counted and divided into 30 (the possible highest result) and multiplied to 100. The scores of leukocytes and mast cells for each urinary bladder were average in all three sections. The number of leukocytes and corpulent cells were counted with × 200 optic magnifier[17].

The materials obtained for electron-microscopic examination were being fixed within 15 minutes through an immersion of 2,5% glutaraldehyde and 2,5% paraformaldehyde solution and phosphate buffer (pH=7,4) with 0,1% picric acid solution. Then within a night they were placed in the share of new fixator. The next postfixation was realized within two hours in the mixture in one per cent 4-

[16] Mutsaddi, S. Comparison of histochemical staining techniques for detecting mast cells in oral lesions / S.Mutsaddi, V.S.Kotrashetti, R.S.Nayak [et al.] // Biotech Histochem, – 2019. 15, – p. 1-10.

[17] Bayrak, O. Chemical cystitis developed in experimental animals model: Topical effect of intravesical ozone application to bladder / O.Bayrak, S.Erturhan, I.Seckiner [et al.] // Urol Ann, – 2014. V 6, – p. 122-126; Bjorling, D.E. Mast cells mediate the severity of experimental cystitis in mice / D.E.Bjorling, T.J.Jerde, M.J.Zine [et al.] // J Urol, – 1999. 162(1), – p. 231-236.

osmium oxide and in 0,1 M phosphate buffer (pH=7,4) in 1,5 % potassium ferrocyanide solution. After the process of dehydration procedure araldite-epon blocks were prepared. Ultra-thin sections (35-70 mm) were dyed with 2% saturated solution of uranyl acetate, then in 0,1 M NaOH solution in with 0,6 per cent pure lead citrate solution (Serva, Germany). Examination and registration of the painted and unpainted ultra-thin sections were carried out in 80-100 kilowatt accelerating tension in JEM-1400 electron microscope (Japan). The photographing of the wall of the urinary bladder have been realized with a digital Veleta camera (Olympus, Japan) and iTEM program (Germany).

The nerve growth factor (NGF) was determined by using the Emax® set with the strong phase immunoferment methodology (ELISA). The concentration of NGF in blood and urine was determined by means of Medicspec 6000M apparatus (Israel). Epidermal growth factor was determined by using the set of epidermal growth factor (Cusabio Biotech Co., Ltd., China) with a strong phase of immunoferment methodology (ELISA).

The IS/PBS of clinically diagnosed 129 patients has been examined. The criteria of inclusion of the patients into the study:

• Grown up people over 20 years of age;

• Patients having inconvenience symptoms connected with urinary bladder (pain, pressure, uneasiness) (in cases when there are not her infection and other identificated reasons going on over six months);

• Patients subjected to cystoscopy and patients without other injuries of the urinary bladder;

• Absence of active phase infections in the urinary channel;

• Absence of active phase infections in the exit of the urinary bladder;

• Absence of obvious neurogen dysfunction in the urinary bladder.

Exclusive criteria:

• The patients have got intraurinary IS treatment within the last month;

• Existence of diseases or pelvis pains, which may be reason of

urine excretion in the process urination, including neurological diseases, urine bladder stones, obvious prolapse of pelvis, intravesical obstructions;

• Patients with heavy cordium-lung stagnant heart insufficiency, arrhythmia, weak and not regularly traced hypertonia;

• Patients with uncontrolled diagnosis of severe urinary infection;

• Patients with coagulopathic diseases;

• Women with tumours in the pelvis organs in their anamnesis;

• Pregnant and feeding mothers.

Twenty women were included into the group under control, (Table 2).

Table 2.

Characteristics of the groups under research

Indicator	Patients with ASKS/IS n=129)	Controlling group (n=20)
Men Women, n/%	3/2,3 126/97,7	1/5,0 19/95,0
Age, year	46,4±13,9 [21; 76]	35,3±9,7 [20; 53]
Duration of the disease, year	6,0 ±2,8 [1; 8,0]	-
Term of clinical manifestations of ASKS, month	32,6±21,8 [6; 122,0]	-

ASKS/IS diagnose was chosen by taking into account the criteria of the National Institute of Diabetes and Digestive and Kidney Disease[18].

To determine the degree of clinical manifestation patients filled special questionnaires: symptoms of Pelvic Pain and Urgency/ Frequency Patient Symptom Scale[19]; VAS – visual analog scale;

[18] Gillenwater, J.Y., Wein, A.J. Summary of the National Institute of Arthritis, Diabetes and Kidney Diseases. Workshop on Interstitial Cystitis. National Institutes of Health, Bethesda, Maryland, August 28-29, 1987 // J.Urology, – 1988. V 140, – p. 203-206.

[19] Parsons, C.L. Increased prevalence of interstitial cystitis: previously unrecognized urologic and gynecologic cases identified using a new symptom

ICSI – O'Leary Sant Interstitial Cystitis Symptom Index[20].

Potassium test was conducted in two stages: In the first stage 40 ml of sterillized water was injected into the urinary bladder, then the patients appreciated the birth of pains and the immediate desire of urination within five minutes. In the second stage within five minutes 40 ml 0,4-M KCL solution was injected slowly into the urinary bladder[21]. If the patients do not feel pain in the urinary bladder, but they feel urgency during potassium test, they are asked to evaluate the degree of USS-urgency severity scale[26].

The USS questionnaire list is appreciated by four scores: 0 score – lack of the felling of urgency; 1-2 scores – mild, average urgency; 3 scores – mild, heavy; 4 scores – unable to resist urination.

During cystoscopy the vacuum of urine canals, the cystoscopic capacity of the urine bladder, peculiarities of the optic environment (transparency, hematuria, optic environment) are taken into consideration, the state of the mucous membrane of the urinary bladder is evaluated (ordinary, hyperemic, totally hyperemic), existence of pathological derivatives (Hunner injury, submucose bleedings, diffuse submucosal bleedings the leukoplakia of the urine bladder) was kept under attention. The hydrodistensy of the urinary bladder was performed with the following methodology: the urinary bladder was filled with physiological solution till the intra urinary bladder pressure rose 80-100 cm water column. The urinary bladder remained in the enlarged state for two minutes. Then a repeated cystoscopy was conducted, the urinary bladder was emptied, the amount and colour of the leaking liquid were registered[22].

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questionnaire and intravesical potassium sensitivity / C.L.Parsons, J.Dell, E.J.Stanford [et al.] // Urology, – 2002. 60(4), – p. 573-578.

[20] Chung, S.D. Urgency severity scale could predict urodynamic detrusor overactivity in patients with overactive bladder syndrome / S.D.Chung, C.H.Liao, Y.C.Chen [et al.] // Neurourol Urodyn, – 2011. 30, – p. 1300-1304.

[21] Parsons, C.L. The role of urinary potassium in the pathogenesis and diagnosis of interstitial cystitis / C.L.Parsons, M.Greenberger, L.Gabal [et al.] // J Urol, – 1998. 159, – p. 1862–1866.

[22] Rigaud, J. Hydrodistension in the therapeutic management of painful bladder syndrome / J.Rigaud, D.Delavierre, L.Sibert [et al.] // Prog Urol, – 2010. 20(12), – p. 1054-1059.

The number of plasmatic and mast cells was valued in bioptats obtained during the cystoscopy of the wall of the urinary bladder: for this purpose in the last stage deep biopsy was realized in the mascular layer of the urinary bladder wall under a short period anesthesia. The specimen was placed in 10 % buffer of neutral xylol solution, then were placed in 75%, 85%, 95% and 99,9% concentrations in spirit solutions. Then they were kept in xylol solution and placed in paraffin blocks, a series of sections, the thickness of which were 3-5 microns, were prepared and they painted with hematoxilin eosine and May-Grunwald Giemsa for the discovery of mast cells. To look through the micropreparations the Olympus PM10SP camera systems have been used. In each 10 exemplary field plazmatic and corpulent cells have been counted like the followings: 0 - no extravascular leucocytes and corpulent cells; 1 - less than 20 plasmacyte and mast cells; 2 – 20-45 cells: 3 – over 45 cells. The balls of 10 sections are counted and divided into 30 (the most highest result) and multiplied to 100. The number of leucocytes and corpulent cells were counted by ×200 optic by magnifier.

Cystoscopy in men was implemented into life mainly with the help of elastic fibrocystoscopic parameters.

Five indicators have been used in the analysis of cystoscopic parameters: 0 – absence of changes in the mucous membrane, I – there are rare, at least in its two squares there are glomeruliations, II – there are diffuse submembrane bleedings, III – diffuse bleeding in mucous membrane, IV – Hunner damages.

The following biomarkers have been determined: NGF – nerve growth factor, HB-EGF – Heparin-binding EGF-like growth factor and EGF-epidermal growth factor and C – reactive protein. The growth factor of nerves was determined by using the Emax® set with the strong phase immunoferment methodology (ELISA) in Medispec 6000M apparatus (Israel). HB-EGF concentration of Human HB-EGF ELISA (Germany) have been measured with test sets. EGF concentration was measured with Human EGF R Quantikine ELISA Kit (Germany) test sets.

The serum COBAS C311 was measured using autoanalyser (ROCHE Diagnostics GmbH, Germany) through immunotur-

bidimetric method. The concentration of Interleukins - IL-1β, IL-6, IL-8, Tumor Necrosis Factor-α (TNF-α) was determined by the relevant test systems based on morning blood and urine of Vector-Best company (Novosibirsk, Rossia) and it bases on solid phase immunoenzyme analysis (IFA is one of the two most commonly used tests for ANAs. Typically, HEp-2 cells are used as a substrate to detect the antibodies in human serum). This analysis (IFA) was based on appropriate test systems.

Statistic analysis of the obtained results has been fulfilled with the application of Windows (version 12.0, SPSS Inc., Chicago, İllinois, USA) by using SPSS program. The indicators have been expressed by means of mean quantity ± standart diversion (SD), as well as by means of figures per cents.

All the main indicators among the groups were compared with the help of Manna-Uitni and Kruskal-Uollis criteria. For the comparison of average indicators t-criterium of Student has been calculated. The degree of correlation relations among different indicators have been counted the degree of Spirmen correlation factor (R2). By calculating the determination factor (R2) correlation-regression analysis has been conducted. The statistical indicators on the level of $p<0.05$ were considered to be important.

RESULTS OF THE PERSONAL RESEARCHES OF THE AUTHOR

Results of experimental researches. We have distinguished and appreciated five models of animals of ASKS/IS, classified them as inflammation of urinary bladder, which has emerged as a result of injection of chemical agent into the wall of the urinary bladder. For this purpose we used our own versions of experimental model of ASKS/IS obtained by means shown below: I) by means of injection of 70% alcoholic solution into the urinary bladder; II) injection of protamine sulfate in the urinary bladder; III) injecting 0,5% HCl solution into the urinary bladder; IV) injecting the urine taken from the urinary bladder in the wall of the urinary bladder; V) injecting. 0,9% NACl solution into the wall of the urinary bladder.

We think that the experimental model created by the injection of the urine taken from the urinary bladder of the animal into the wall of the same animal is particularly praiseworthy. One of the leading theories of pathogenesis is the existence of venomous matters in the urine of the İC/PBS patients. This, unequivocally, tells that there may be a normal component in the existing anomal matter or component in the urine of IC patients. Thus, by injecting the drops of chloride accid, protamine sulfate as physiological solution we create an inflammatory situation in the urinary bladder. The majority of the intrabladder agents may inflict nonselective damages on the mucous membrane and on the glicozaminoglycan layer. By means of the created experimental models the neurogen inflammation of the urinary bladder was studied.

On the first day of the study the analysis of the concentration of the growing factor revealed a notable increase in the level of biomarkers in comparison with the groups under control. So that in the first group the level of NGF has been higher 46,0% ($p<0,05$), in the second group – 41,0% ($p<0,05$), in the third group – 60,4% ($p<0,01$), in the fourth and fifth groups were 71,3% ($p<0,01$) and 44,0% ($p<0?05$) respectively. The highest increase on the level of NGF was observed in the forth group of animals. In the same period of examination the level of NGF in the urine was high only in the fourth group in comparison with the group under control (68,2%, $p<0,01$), in other groups the level of increase was low. After 14 days the increase of NGF level in comparison with the group under control has not changed ($p<0,05$), but particularly in the fourth group a great difference (90,1% ($p<0,001$) has been noted. Concentration of NGF in urine was notably higher in all the groups under control, but in the fourth group differences of statistic importance were not observed. On the first day, the wide variability of NGF levels in the blood of rabbits of groups III, IV and V, and in the urine of group IV attracted attention. After 14 days notable changes in the blood of the first and fourth groups of animals were observed only in the urine of the fourth group of animals.

In different periods of the study when the NGF indicators were compared, it became evident that in the first group within 14 days in

comparison with the first day these indicators in the urine and blood have grown 35,1% ($p<0,05$) and 8,7%. In the second group of rabbits, on the contrary, it has been observed that after 14 days the level of NGF in blood 4, 4%, in urine 21,6% has reduced. As a result of injecting 0,5% HCl solution into the urinary bladder of the of rabbits with İC/PBS (III group) and in animal group with 0,9% NaCl solution (group V) and in both of the two biological solution reduction of NGF was registered. In the third group the composition of NGF in blood has reduced 29,3%, in urine 14,3 per cent in comparison with the first day of the 14^{th} days experiment. In the fifth group the NGF concentration has reduced in blood 30,8%, in urine 30,5 per cent in the 14^{th} day. In the fourth experimental model on the 14^{th} day the volume of NGF in blood has increased 65,5% ($p<0,01$) in comparison with the first day, in urine -- 52,7% ($p<0,05$).

Thus, in each of experimental groups the intra-group analysis revealed the tendency shown below:

– in the first observation group in different periods the tendency of increase was observed both in blood and in urine on NGF level. After fourteen days the creation of the model in comparison with the initial indicator the volume of NGF increased 35,1% ($p<0,05$) in blood, 8,7% in urine.

– in the second group on the 14th day of the study on the level of growth of nerves a very small reduction was found.

– in the third group the NGF concentration has reduced 29,3% in blood, has increased 14,3% in urine.

– A significant increase was identified when in group number IV after urination when it is compared with the first days after 14 days in the level of NGF some 65,5% ($p<0,01$) and 52,7% ($p<0,05$) was observed in the blood and urine.

– A decrease was observed in the group number V in the concentration of NGF in the blood and urine. It is necessary to highlight that compared to the initial observation period, the difference in blood was 30.8%, in urine - 30.5%.

On the 14^{th} day of a strong positive, statistically significant correlation was observed in the blood ($r = +0,715$, $p=0,05$). High direct correlation was observed in the V group ($p<0,01$) as well. A

very weak negative relationship between NGF indicators was recorded in the control group.

It means, that more pronounced changes in NGF levels in blood and urine might be observed in the he ASK model (a bare bones approach to the critique of nursing research for use in practice) pattern by urine injection into the urinary bladder wall compared with the control group. Levels of NGF in the blood of Group II animals were correlated with levels of NGF in the urine. In comparison of the indicators of the control group with groups I, II, III and V, statistically significant changes in the blood and their absence in the urine were found. In this case, NGF levels in animals with the ASK model on the basis of urine injection into the urinary bladder wall were statistically significantly different from those of other intact group options as well. Dynamic research revealed statistically significant changes in NGF concentration in animals belonging to models of urinary bladder wall.

The day after modeling, the level of EGF in the blood in the experimental groups did not differ significantly compared to the Intact Group. Also, no significant difference in this factor in the blood between the ASK model has been recorded. Epidermal growth factor (EGF) (It is a protein that stimulates cell growth and differentiation by binding to its receptor, EGFR). Relatively high level of EGF in the urine was revealed in the group II, III and IV rabbits. It exceeded the rate of intact animals 33, 3% ($p<0,05$), 48,2% ($p<0,05$) and 34,6% ($p<0,05$).

After 14 days, the concentration of EGF in the blood and urine was 17.3% and 15.8% higher in group I than in group VI, respectively. In group II, a decrease in EGF levels in the blood (17.4%) and urine (3.3%) was observed during the same observation period compared to the group of intact animals. The volume of epidermal growth factor in the blood of III group rabbits is observed to be increased rather than the indicators in group VI exceeded 18.3% and 17.1% in urine. In Group IV, EGF levels in blood and urine were higher by 63.1% ($p<0.01$) and 49.2% ($p<0.05$) in urine compared with intact animals. In group V, 68.8% ($p <0.01$) and 17.2% decrease in blood and urine EGF concentrations was observed

in comparison to the intact group.

Observing the EGF dynamics during the observation period, its increase was recorded in Group I on Day 14 compared to day 1 only in the blood and the difference was on average 31.4% ($p<0.05$). In the II group of the research, the EGF level in the blood and urine decreased by 14% and 19.4% ($p<55.0$), respectively, compared with the first day after 0.05 days. In the V group, EGF in blood and urine after 14 days decreased by 85.8% ($p<0.001$) and 12.3%, respectively as well. In Group III, the concentration of this marker in the blood increased slightly after two weeks, and in the urine decreased by 59.9% ($p<0.05$). In this case, after 14 days, the maximum increase in EGF in the blood was observed in animals of the IV experimental group; the difference with the initial indicator was 61.1% ($p<0.01$). During the same period of the research, a 22.3% increase in the level of EGF in urine was recorded.

So, in each of the experimental groups the analysis of the EGF level covers the following dynamics:

– After 14 days, in the I observation group, a statistically significant increase in the level of EGF in the blood (31.4%, $p<0.05$) and a slight decrease in urine were noted.

– On the 14th day of the research in the II group the epidermal growth factor in the blood is 19,4% and it is 55,0% ($p<0,05$) in the urine and it is observed to be decreasing.

– The tendency of increasing EFG concentration in the blood in the III group is observed, and its increasing has also been observed in the urine 59,9% ($p<0,05$).

– After 14 days in the group IV, the level of EGF in the blood and in the urine increased significantly compared to the first day after urination. The increase is observed like the following: 61,1% ($p<0,01$) and 22,3%.

– The decrease of EGF concentration was observed in the blood and urine in the fifth (V) group. It is 85,8% ($p<0,001$) in the blood, and it is 12,3% in the urine compared to the initial observation period.

The research that is carried out in the dynamics, in the highest direct correlation that is observed in after modeling in a day in the I

group (r=+0,976, p<0,001) is mentioned to be decreased after 14 days and this decrease is seen like the following (r=+0,500, p<0,01). The correlation relationship is lower than the highest direct relationship (r=+0,847, p<0,001) in the II group after 14 days it is very low like this (r=+0,076, p=0,581). The contact changed from high retardation (r=-0,810, p<0,001) to weak (r=-0,810, p<0,001), in the first days of the experiment in the III group and in this case the correlation direction of the relationship was preserved. The weak correlation in the IV group, which tends to weaken further during the entire observation period was noted. The mutual relationship was noted to strengthen of the interaction from weak (r=0,359, p<0,05) to the middle (r=0,635, p<0,01) in the V group. The EGF indicators in the blood and in the urine of the intact animal groups were correlated with the average back and it is like this (r=-0,664, p<0,01). Statistically significant strong relationships between NGF and EGF were determined both in early days and after 14 days. After a day from the experiment very high correlation in the blood and in the urine was determined; it is (r=-0,942, p<0,001) in the II group and it is (r=+0,956, p<0,001) in the IV group; after 14 days it is (r=+0,936, p<0,001) in the I group. The determination coefficient indicating the degree of dependence R^2=0,888, it means 89% which was formed depending on as a result of injection of protamine sulfate (group II) in the ASK position the changing in the NGF leads the changing in EGF.

The coefficient of determination indicating the degree of dependence is R^2= 0,913, it means 91% which is demonstrated between NGF and EGF among the animals that are not ASK; the NGF changing lead to the changing in EGF. The I group rabbits that were created after injecting a 70% alcoholic solution to the urinary bladder of the ASK/IS experimental animals, the determination coefficient is R^2=0,877 between NGF nad EGF; it means that in the case of the changing of the 88%, the NGF changing lead to the EGF changing. The average correlation relationship between the markers was noted after 14 days in the blood (r = -0,565, p<0,01) in the I group and in the IV group it is (r =0,561, p<0,01) in the blood. In the IV group experimental İC/PBS rabbits that were created by by

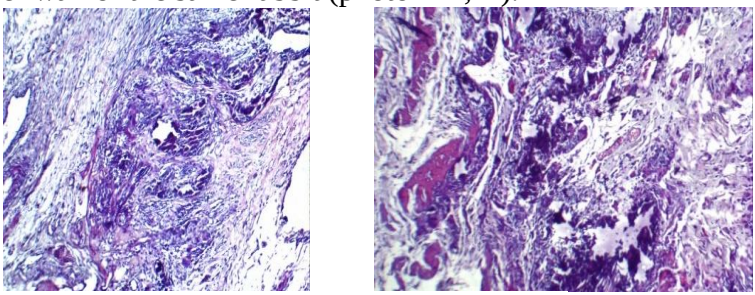
injecting urine from the bladder into the urinary bladder wall, the determination coefficient is R^2=0,315 between NGF and EGF; it means that in the case of 31% the changing in NGF lead to the changing in EGF.

So, in the presence of IC / PBS, the changing in nerve growth factor and epidermal growth factor are noted in different variants of the experimental model, but more pronounced changing is observed in experimental IC / PBS caused by toxins in the urine.

After 14 days of the experiment, the macroscopic examination of the urinary bladder wall of the experimental group of animals revealed a thickening of the subcellular layer.

The leukocyte infiltration of tissue and edema (dropsy) was noted in the microscopic examination in the I group. After the injection of protamine sulfate (group II) infiltration of lymphocytes and neutrophils in the vascular region of the urinary bladder wall of animals were noted during the histological examination.

The inflammatory components were observed in the histological analysis of the urinary bladder wall of rabbits in the III group. The abundant infiltration of lymphocytes and neutrophils were determined in the histograms. Though we do not observe total necrosis in the experimental model of IC/PBS unlike the first two groups. Necrosis, mixed inflammatory infiltration was detected in the micropreparations of group IV animals of the IC / PBS model obtained by injecting urine from the urinary bladder of the experimental rabbit into the urinary bladder wall of the same rabbit (photo 1 A, B).



A B

Photo 1. IV group. Necrosis and mixed inflammatory infiltration due to urinary injection in the urinary bladder wall in A and B (growth x100, size HE)

Hemosiderin accumulation was also detected in histograms of experimental group IV animals against the background of mixed inflammatory infiltration (photo 2).

After creating the toxic model, cell necrosis developed in the urinary bladder wall of the animals, and great multinucleated histiocytes were found against the background of inflammatory infiltration.

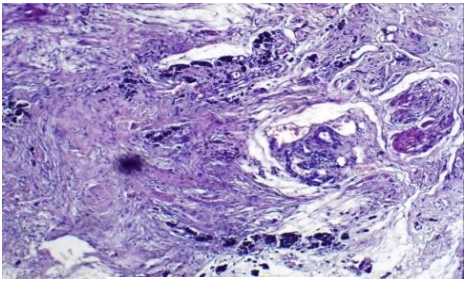
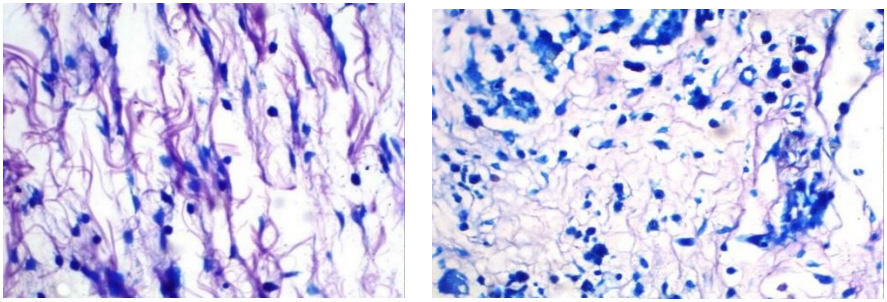


Photo 2. Group IV
Hemosiderin accumulations and mixed inflammatory infiltration due to urinary injection in the urinary bladder wall (growth x100, size HE)

We observed the areas of infiltration of fat cells in the animals on the histogram (photo 3 A, B) after insertion into the urinary bladder wall and obtaining a toxic model.



A B

Photo 3. Group IV. Obstructive cell infiltration in the urinary bladder wall in a test animal injected with urine into the urinary bladder wall (growth x400, size Mey-Grunvald Gimza - MGG)

Sparse lymphocytes and cell edema (dropsy) were determined in the micro-preparations of IC / PBS model that was formed by injection of 0.9% NaCl (group V). Morphological studies of the urinary bladder wall of controlled animals proved that no changing happens in the urine bladder of the animals.

So, inflammatory infiltration of lymphocytes and neutrophils was observed in animals with experimental IC / PBS models generated by chemical agents. Necrosis, inflammatory infiltration of lymphocytes and neutrophils, accumulation of hemosiderin, great multinucleated histiocytes and obstructed cells were observed in the histograms of the urinary bladder wall of animals with a toxic model (group IV). Edema (dropsy) of group V histology revealed edema, presumably in response to injection, but no inflammatory infiltration was observed, only rare inflammatory cells were found.

In the experimental models of IC / PBS, we studied the properties of the epithelial lining of the bladder mucosa at the ultrastructural level. In the IV and V groups in the experimental models, it means from 15 rabbits injected with urine taken from the animal's bladder into the urinary bladder wall (group IV) and from 7 animals (group V) injected 0.9% NaCl solution into the urinary bladder wall have been used; in this case the latter has been considered to be controlled one. In the conrol group (V experimental group) no gaps were found between the superficial urotelial (umbrella) cells at low growth of the electron microscope in the epithelial lining of the bladder mucosa. In the IV experimental group edematous fluid, located mainly in the peripheral cells of the endothelial cells of the capillaries of the mucous membranes and postcapillary venules of the posterior capillaries, entering mainly from the fenestra, spreads between the basal and interstitial cell layers of the epithelial lining of the bladder, disrupting the integrity of individual parts of the bladder. In this case, in this group, in the preparations between the individual superficial urotelial cells, there are cavities, such as a narrow slit, which is traced to the bladder cavity.

In the IV experimental group, besides the above mentioned instructions, even at 20,000 growth of the electron microscope in

ultranasic sections no hemispherical gaps between plasmolemmas of adjacent surfaces of urothelial cells. Both the apical parts of the lateral surfaces of superficial urotheliocytes as well as in the most lateral parts in the highest growth of the electron microscope (from 80.000 to 100.00) close contact of the adhesion points of the outer layers of plasmolemmas, which is the only morphological feature of the hemato-urinary barrier, is not detected. In the IV group expreimental animals ultrastructural features characteristic of the asymmetric single membrane of the bladder are not found in the apical parts of superficial urotelial cells.

When urine is injected under the mucous base of the bladder of the white New Zealand rabbits, in the structural elements of both the plaque and the epithelial lining of the bladder mucosa the complex of pathomorphological changes were observed. Perivascular infiltration with inflammatory cells and clearly expressed edema (dropsy) of the private plaque of the mucous membrane of the bladder were noted. The presence of neutrophils, lymphocytes, and a large number of activated platelets in the venous space at a single point of view can be considered a sign of an ongoing inflammatory process (Photo 4).

At the ultrastructural level in the controlled drugs, the special plate of the rabbit's mucous membrane was represented by a dense, unstructured connective cells with sets of collagen fibers of different orientations.

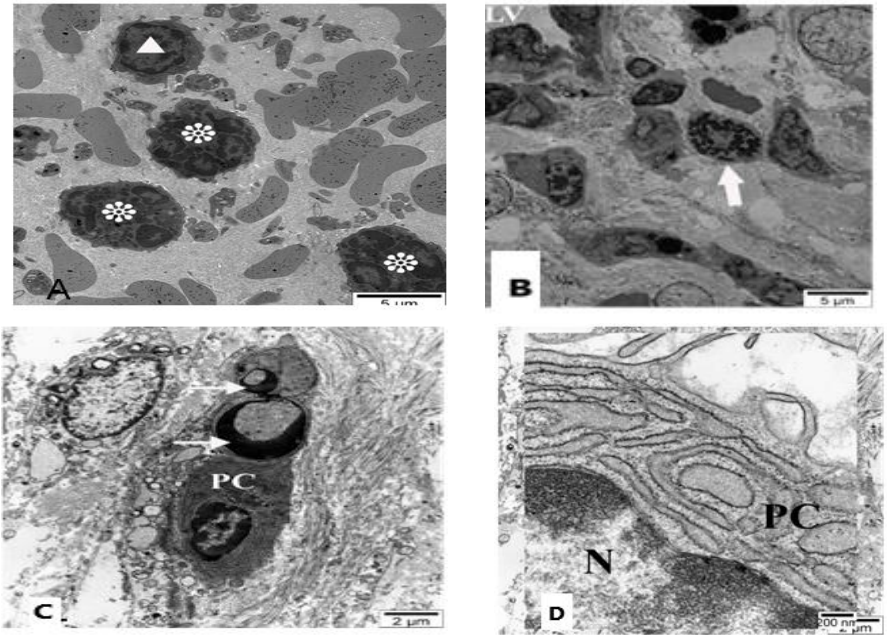


Photo 4. 7 days after injecting urine into the submucosa white New Zealand rabbit ultrastructural changes in the structural elements of the private boards of the bladder mucosa: A- neutrophils on the electrogram (they were shown with flowers), lymphocytes (they were shown by a triangle) and platelets (staining - 2% uranyl acetate and 0, 6% pure lead citrate); B- blood vessel in the private board of the urine bladder (LV – vascular interval), fibroblasts, collagen fibers, edema fluid (unpainted areas), macrophages, lymphocytes, neutrophils, eosinophils (they were shown by an arrow). Staining - 2% uranyl acetate and 0,6% pure lead citrate; C – heterochromatin (it was shown by an arrow), plasma cells (PC), staining - 2% uranyl acetate and 0,6% pure lead citrate; D- cisterns of the granular endoplasmic reticulum, plasma cells (PC); nuclear (N), staining - 2% uranyl acetate and 0,6% pure lead citrate.

In the experimental group beginning from the private board of the epithelial covering, release of the collagen fiber bundles were determined; individual collagen fibers of different orientations close to the muscle layer are surrounded by small granular precipitates of plasma proteins, which are a sign of acute impairment of vascular permeability. Near the collagen fibers are flat, fibrous fibroblasts responsible for the synthesis of collagen and the extracellular matrix. Inactive fibroblasts (fibrocytes) characterized by small cytoplasm and flattened heterochromatic nuclei are also found.

On the surface of urotheliocytes facing the bladder cavity can be seen urothelial plaques and intermediate parts of the asymmetric single membrane of the bladder located between them. Discoid (spindle) foams were found in the cortical cytoplasm.

In the experimental group, the edematous fluid that was located mainly in the peripheral cells of the endothelial cells of the capillaries and postcapillary venules of the mucous membrane, which came mainly from the phenes, disrupted the integrity of the individual parts of the basal plate and spread between the basal and interstitial cell layers of the bladder epithelium.

In this case, narrow slit-shaped cavities were found between the individual superficial urothelial cells extending to the bladder cavity.

In the experimental group in the ultranasic incisions even in the 20.000 growth of the microscope cleft spaces between plasmolemmas of urotelial cells were not observed. In the high growth of the electron microscope (80.000-100.000) both the apical and adhesion points of the outer layers of plasmolemmas in the adjacent parts of the lateral surfaces of superficial urotheliocytes were not determined, and it is meant to be the only morphological sign of the blood-urine barrier. In addition, in the experimental animals, the apical parts of the superficial ureteral cells did not have the ultrastructural features characteristic of an asymmetric single membrane of the bladder.

Beginning from a special plate of the epithelial lining under the mucous membrane of the bladder in animal samples injected into the urine, softening to varying degrees were observed in the arrangement

of sets of collagen fibers. As it approaches the muscle layer, small granular precipitates of plasma proteins are found between the collagen fibers of different orientations, which is a sign of acute impairment of blood vessel permeability in experimental cystitis.

So, in the experimental model of ASKS / IS applied by the option of urinary injection under the mucous membrane of the bladder, morphological changes in the structural elements of the plaque and epithelial lining of the bladder mucosa were observed. The changes were expressed by a clearly defined inflammatory infiltrate of the private plaque of the bladder mucosa, an inflammatory infiltrate of the mucosa expressed by lamina propria edema; in this case, the inflammatory process lasted for a long time.

The mean number of all leukocytes and stem cells in groups I, II, and III was statistically significantly higher than in group VI ($p<0,001$). IV and V groups the number of neutrophils and lymphocytes was observed to be decreasing in the samples that were taken from the experimented animals in the group number VI ($p<0,001$). Unlike the groups I, II and III, both the number of the eosinophils in IV and V groups were observed to be lower relevantly comparing the controlled groups ($p<0,01$).

The maximum number of neutrophils and lymphocytes was found in group III, which was statistically significantly higher than in other groups ($p<0,001$). The minimum number of neutrophils was observed in group IV. According to the results of morphometric analysis, the maximum number of eosinophils in animals was detected after administration of 70% alcohol solution ($p<0,001$).

The fat cells in the I, II, III groups were observed in the rabbits though they were not seen in the animal samples in V and VI groups. Besides, the maximum number of these cells were observed in the animals in the IV group; the minimum number of them were observed in the group number I. During the hystological analysis the fat cells were not seen in the experimented animals in the groups I, II, and III. Though it is not possible to state the same opinion about the experimented animals in group number IV. In this group all samples have been observed to carry the fat cells after after urine was taken from the bladder and inserted into the bladder wall.

As a result, the obtained data show the infiltration of different types of leukocytes and stem cells into the tissue of the bladder wall, in which case the obvious infiltration of stem cells was observed in experimental group IV.

The study of correlation among the cells of the bladder wall with NGF size in the blood revealed different trends. The weak direct relationship was observed to be correlated with NGF lymphocytes ($r=+0,322$, $p<0,05$) and eosinophils ($r=+0,400$, $p<0,05$). The NGF was correlated with all cells in the second group and the opposite relationship was noticed to dominate. At the same time, high correlation was noted to be seen in the lymphocytes ($r=-0,722$, $p<0,01$); contact with the remaining cells was observed to be weak, but statistically significant ($p<0,05$). The high correlation was discovered with neutrophils ($r=-0,864$, $p<0,01$) and lymphocytes ($r=0,749$, $p<0,01$) in the III group. The middle relationship as well as the opposite relationship with fat cells ($r=-0,612$, $p<0,05$) were noticed in the IV group, and direct weak relationship was determined with lymphocytes ($r=0,470$, $p<0,05$). The direct middle correlation was discovered with eosinophils of the NGF in the V group ($r=0,523$, $p<0,05$). The middle opposite relationship with lymphocytes ($r=-0,507$, $p<0,05$) and neutrophils ($r=-0,652$, $p<0,01$) in the intact rabbits.

The statistically significant correlation of the NGF levels was discovered with neutrophils and lymphocytes in the urine in the I group ($p<0,05$), and in the first case the relationship in the opposite direction was noted. The NGF neutrophils, lymphocytes, eosinophils and fat cells were correlated in the opposite relationship in the urine as well as in the blood in the second group. In all cases the relationship was noticed to have been important from the statistically point of view and has been changed from the high level to the weak one. The opposite correlation was noticed among neutrophils and lymphocytes with NGF in the III group, but the relationship with neutrophils was seen to be weak ($r=-0,367$, $p<0,05$), though it was high ($r=-0,805$, $p<0,01$) in the lymphocytes. The growth factor of the nerves was correlated in the direct relationship with fat cells in the same group ($r=0,410$, $p<0,05$). Very weak relationship was

determined among neutrophils, lymphocytes, eosinophils with NGF in the VI group, weak opposite relationship was noted only with fat cells ($r=0,340$, $p<0,05$). The statistically significant relationship was discovered among neutrophils, lymphocytes and eosinophils with NGF in the V group. The statistically middle opposite correlation with eosinophils was discovered in the group that included the intact rabbits.

The positive correlation with EGF levels in lymphocytes and eosinophils in the blood in the I group was discovered though it was negative with neutrophils in the urine. The correlation with fat cells was not noticed. The high direct and statistically significant correlation ($p<0,01$) in the blood was discovered among the numbers of fat cells with EGF concentration in the I group of the research. The statistically significant correlation in this experimental model was discovered among neutrophils with EGF size ($p<0,05$), eosinophils ($p<0,05$), as well as lymphocytes with urine EGF ($p<0,05$) and eosinophils ($p<0,01$). The weak direct correlation only with lymphocytes was discovered in the III group; the EGF level was correlated with lymphocytes both in the blood and in the urine ($p<0,05$). The statistically significant weak opposite correlation was noted with neutrophils in the IV group ($p<0,05$). The EGF in the bllod and in the urine has been correlated with eosinophils directly in the V group. The high relationship with EGF level ($r=0,844$, $p<0,01$) in the blood in the animals of this group was noted though the weak relationship was discovered in the urine ($p<0,05$). The positive weak correlation (but also with an identical correlation coefficient) was discovered with lymphocytes and eosinophils of epidermal growth factor in the contact rabbits. The attention was drawn to the fact that the correlation of the NGF and EGF with fat cells was not observed in the group that includes the intact animals.

So, the models that were created from IC/PBS the option of injecting urine into the bladder cavity was more appropriate for this disease. The information that was got might be useful in the study of the mechanisms of inflammatory processes. Besides, the study of pathogenesis in animal models is also of great importance for applied

science because it also provides an important basis for development and evaluation.

It is necessary to highlight that the option of the animal model may be complex decision that requires scientific and practical opinions. In most cases, it may be better to study different aspects of the pathogenesis or to use several animal models to examine possible treatment or prophylactic interventions.

The results of the clinical studies. 52 women (41,3%) out of 126 area at the reproductive age, 74 women (58,7%) are perimenopause and at menopausal age. During the research all women had the signs of urinary tract infection and the bacteriological analysis of urine was negative.

While investigating the reason of the disease, it was determined that most of the patients who were examined (65) could not coordinate the reason of the signs of IC/PBS; 20 of the people (15,5%) explained the reason of the disease with recurrent bacterial infections of the lower urinary tract and bladder; 19 of them (14,7%) coordinated it with the beginning of the menopause; 17 of them (13,2%) coordinated it with excessive widening of the urine bladder; 8 patient (6,2%) with autoimmune disorders, only three of them (2,3%) with rheumatoid arthritis, two of them (1,5%) with diffuse toxic thyroiditis and respectively autoimmune thyroiditis, one patient (0,8%) coordinated it with psoriasis.

According to the PUF inquiry, 23 (17,8%) patients had light symptoms, 77 (59,7%) had middle and 29 (22,5%) carried hard symptoms. The total average of the points is $8,14 \pm 1,76$.

The results of the survey according to the VAS scale of the patients demonstrated that 25 (19,4%) of the women carried light pain (2-4 points), 84 (65,1%) of them carried some moderate pain (5-6 points) and 20 (15,5%) of the women had severe pain (7-8 points). Average score is $5,45 \pm 0,93$ points.

The analysis of the results according to the scale of the USS scale demonstrated that 5 (3,9%) patients do not have any urgency in this case (1-2 points), 40 (31.0%) of them carries moderate urgency (3 points) and 28 (21,7%) of them had inability to hold urine (4

points). The average point according to the USS scale is 2,63 ± 0,91 points.

According to the results of the scale of O'LearySant Interstitial Cystitis Symptoms Index (ICSI) most of the patients had 14 points - 73 (56,6%), 6 of them had (4,6%) and minimal result is -10 points, 19 of the patients (14,7%) demonstrated maximum 20 points. 75 (58,1%) of the patients had urinary excretion more than 8 times in a day.

Positive potassium test demonstrated 91,5% (n=118), negative 8,5% (n=11). Potassium test sensitivity was 86,5% and its specificity was 84,6%. After injection of potassium solution 100 (77,5%) of the patients demonstrated the aggravation of the pain. In this case, depending on the symptoms, the frequency of the Positive potassium test was different. According to the obtained results, depending on the degree of expression (on the PUF scale) symptoms of the potassium test were positive in the 16 of 23 patients with light pain, in 72 of 77 patients with mild pain (93,5%) and in 27 of 29 patients (93,1%) with severe pain.

The nature of the clinic symptoms were determined due to the complaints of the patients, information on the questionnaires they filled out and the frequency of the urination. The patients with the symptoms who had the lower urinary tract complained about the pain in the urine bladder and the frequency of the urinary. Most of the patients (n=125) spoke about the presence of imperative urges to urinate, 91 patients complained about the frequent urination at night and 74 patients stated to have some pain in the urethra. Involuntary urination was observed in only 26 (19,4%) patients, with imperative urges to urinate (5,0 times, $p<0,001$), emergency daytime urination (5,1 times, $p<0,001$) and emergency night urine output (3,6 times, $p<0,01$) is statistically significantly lower.

The imperative urges and urinary incontinence were reported respectively by 54 (41,9%) and 27 (20,9%) patients. 25 (19.4%) women with urinary incontinence had both accelerated urination and imperative urges to urinate.

The classic disease type (type 3C) were examined in 44 (34,1%) out of 129 patients who had IC/PBS, 85 (65,9%) patients had the non-ulcer type of IC/PBS. Clinical manifestations of the

disease were observed in 13,6% of the patients with Hunner's injury under the age of 40, but in the group without Hunner's injury, 25,9% of patients reported symptoms at this age (p = 0, 05). The group of patients with Hunner's injury also had high scales and low average volumes, frequency of urination, and maximum volume of discharge, but the differences were not statistically significant. At the same time, the maximum capacity of the urine bladder during hydration was 42,01% (p<0,001, t-krit. – 3,32) less than in patients without Hunner's injury.

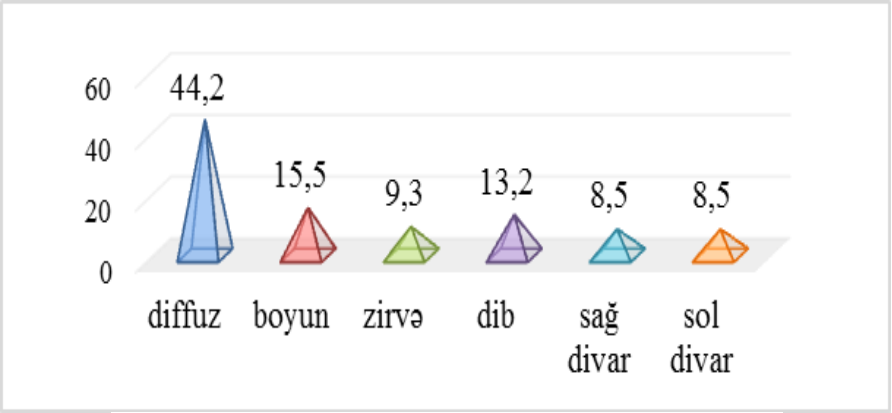
The maximum anatomical capacity of the urine bladder changed between 92 to 410 ml, and it is 308,0±77,5 ml on average. This indicator changed between 300 to 390 ml in the 25 patients (according to the VAS scale) who had light pain, and it is 340,4±88,6 ml on average. The size of maximum anatomical capacity was 120-280 ml in the 84 patients who had moderate pain. In 20 patients with severe pain, this figure was the lowest at an average of 160,0±48,8 ml and it changed between 92-200 ml. A comparative analysis of maximum cystometric capacity measurements in patients with varying degrees of pain intensity revealed statistically significant differences. In patients with mild pain, the average value of maximum cystometric capacity was respectively higher 30, 9% (p <0.05) and 53, 0% (p <0.01) compared to moderate and severe pain.

While examining the outer hole of the urethra, some urethral polyps were discovered among the patients of 20 (15,5%). In other cases no changing were observed in the outer holes of the urethra.

The specifically painful changing has been paid special attention to the mucous membrane of the urine bladder. While examining the cystoscopic view, the evaluation system of 5 indicators has been used: 0 – normal mucous membrane, I- rare, at least two quadrants, glomerulation, II – ecchymoses, diffuse submucosal hemorrhages, III – diffuse complete bleeding of the mucous membrane, IV – Hunner injuries. The results of the research showed no changing in the mucous membrane of 16 women. The diffuse bleeding was observed (III degree) while examining the mucous membrane of the urine bladder; it was examined among 49 patients. The examination of the mucous membrane demonstrated no

changing among 16 (12,4%) patients; the 18 (13,9%), 30 (23,2%) and 16 (12,4%) demonstrated relatively rare glomerulation, diffuse mucous membrane bleeding and the Hunner's injuries.

The changes detected by cystoscopy after urine bladder hydrodistension ranged from the complete absence of ulcerative wounds of the bladder mucosa to their presence. The changing in the mucous membrane has the character of diffuse (n=57), or it is put in two parts of the urine bladder. In rare cases, some changing in the mucous membrane were discovered in the walls of the urine bladder. The results show that such changes are present in the right and left walls respectively among 10 patients (Graph 1).



Graph 1. The patients who had IC/PBS after (%) hydrodistension according to the cystoscopy information the localization of the changing in the mucous membrane of the urine bladder (n=129)

The investigation of the mutual relationship of the correlation was able to demonstrate the maximum cystoscopic capacity of the bladder as well as a statistically significant correlation between the degree of expression of deviations in the mucous membrane of the urine bladder (r=-0,57, p<0,01). The degree of expression of changing in the mucous membrane of the urine bladder was positively correlated with the total points of the PUF questionnaire (r=+0,61, p=0,0003), the total points in the VAS questionnaire

(r=+0,59, p=0,0008) and the total points in the USS questionnaire (r=+0,66, p=0,005). The patients who had obviously changing in the mucous membrane of the urine bladder carried more severe clinical symptoms.

So, with the Hunner injuri the following has been discovered: the cystoscopic examination of the mucous membrane investigation of the glomerulations, the diffuse mucus bleeding and diffuse bleeding. The results of the investigation demonstrate the correlation of the changing of the mucous membrane of the bladder with the expression of the clinic symptoms of IC/PBS.

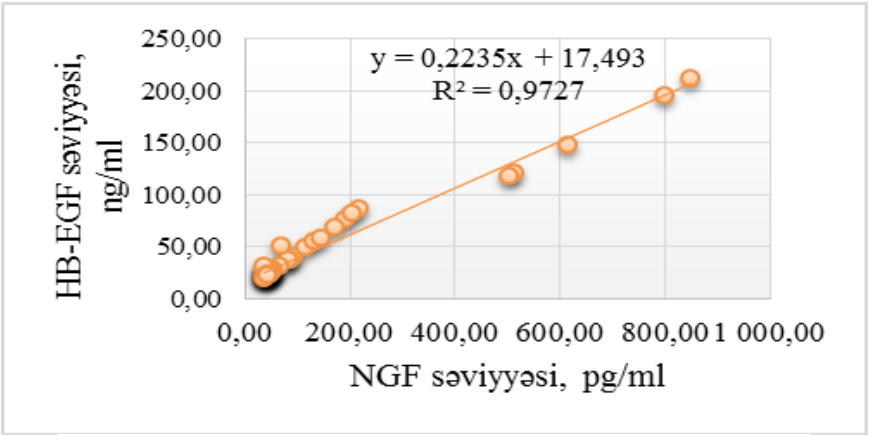
The patients who have IC/PBS in the blood the level of NGF is 1,70-113,70 pg/ml, in the control group it was changed in the interval of 2,60-45,40 pg/ml, relevantly the changing in HB-EGF indicator is 19,60-68,60 ng/ml and 19,10-74,20 ng/ml. The middle level of NGF became higher: 8,82% (t=0,10, p=0,920). In this case the patients who had IS/ASKS in the blood the HB-EGF concentration 30,84% got decreased (t=0,62, p=0,537).

The determination of the identification indicators of NGF in the patients who had IC/PBS in the urine could demonstrate the changing of it widely. The concentration of this biomarker (a measurable substance in an organism whose presence is indicative of some phenomenon such as disease, infection, or environmental exposure) changed between 35,7-848,80 pg/ml. The level of NGF in the control group changed between 0 -146,10 pg/ml. The level of HB-EGF in the main and control groups were discovered relevantly between 19,80-211,90 ng/ml and 4,0-54,8 ng/ml. The EGF indicator in which the IS/ASKS was observed was 2,80-60,70 ng/ml, in the control group it was 0,60-7,7 ng/ml.

The middle level of NGF and HB-EGF was high statistically significant from the control indicator – respectively 50,44% (t=2,06, p=0,043) and 56,44% (t=2,01, p=0,049). Though the EGF concentration in the patients who had IC/PBS was higher than the control indicator it was not important from the statistic point of view (t=1,37, p=0,175).

The levels of NGF in the blood and the urine of the main group patients were correlated directly with one another with weak

unimportant relationship (r=+0,102, p>0,05). The HB-EGF correlation of measurements in the blood and the urine in the main group and in the controlled group have been differentiated practically (relevantly r=-0,322, p>0,05 and r=-0,358, p>0,05), and in the opposite direction, it has been unimportant from mild and statistic point of view. It becomes clear that the measurement of NGF in the urine directly with HB-EGF with a statistically significant relationship (r=+0,922, p<0,05) has been correlated (Graph 2).



Graph 2. The patients who had IC/PBS in the urine the correlation between the indicators of NGF and HB-EGF

The patients who did not have the IS/ASKS, the intermediate level of relationship has been discovered (r=+0,449, p<0,05) between these indicators there was some direct, statistically significant relationship that unlike the main group. The correlation between these indicators in the blood during IC/PBS is direct, weak, statistically insignificant in the main group (r=+0,104, p>0,05) and direct, noticeable and statistically significant in the control group (r=+0,642, p<0,05).

The concentration of the NGF in the urine in the main group was correlated with the weak and insignificant correlation with EGF in the opposite direction (r=-0,151, p>0,05). On the contrary, direct, noticeable and important relationship has been discovered between the indicators studied in the control group(r=+0,511, p<0,05). The

weak, unimportant relationship was observed between the indicators of the HB-EGF in the urine in both of the groups.

The level of CRP changed between 0,55-16,5 mg/l in the blood serum of the main group patients, and on average it is 3,56±1,66 mg/l (control – 1,02±0,18 (0,30-1,80 mg/l). The CRP concentration exceeded the level of control 71,3% (p=0,015) in the blood of the patients who had IS/ASKS.

According to the obtained information, the patients who carried the proportion with high (<3, 5 mg / l) CRP in the serum in the main group was 63,6%, in the control group – 35,0%, i.e. in the patients with IS / ASKS, the high level of CRP was 45,0% compared to the control group was more (p <0,05).

We have analyzed the correlation between NGF, HB-EGF, EGF and CRP. The analyze proved some positive weak correlation of CRP with the level of NGF in the blood in the main group (r=+0,179, p=0,42), in this case the determination coefficient was R^2=0,032, it means that the factor sign of NGF may determine the 3,2% dispersion of CRPP. The middle approximation error that characterizes the regression model consisted of 81,3%. The correlation coefficient between NGF and CRP in the control group was r=-0,187 (p=0,43), the determination coefficient was R^2=0,035. The NGF factor sign determines 3,5% the dispersion of CRP. The average approximation error characterizing the adequacy of the regression model was 70,3%.

The NGF level was correlated with serum in CRP direct, noticeable and statistically significant relationship in the urine-r=+0,529 (p<0,05), the correlation coefficient in the control group was r=+0,028 (p>0,05), determination coefficient is R^2=0,295, NGF factor sign determines 29,5% the dispersion of CRP. The average approximation error that characterizing the adequacy of the regression model consists of 71,5% of it. The HB-EGF level was correlated with CRP in a reverse, weak, statistically insignificant relationship (r=-0,231 (p>0,05), in the urine - with a direct, mild and statistically insignificant contact (r=+0.352 (p>0,05). The relationship between indicators that were investigated in the control group relevantly was r=+0,256 (p>0,05) and r=-0,006 (p>0,05).

While calculating the correlation of EGF with CRP in the patients who were in the main group, direct, weak, insignificant contact was found out r=+0,003 (p>0,05), in the control group the indicators were correlated with one another in a direct, weak and insignificant relationship r=+0,153 (p>0,05).

The frequency of fat cells in the bioptats changed among the cells of 0 – 237, the frequency of plasma cells changed among the cells of -221. The number of fat cells in the patients who have or do not have the Hunner's injury on average, respectively was 114,8±14,8 (from 35 till 237) cells and 68,1±3,44 (from 0 till 168) cells (p=0,003, t-krit. – 3,07). The number of the plasma cells in samples of patients with Hunner's injury changed on average, respectively 0- 221, and it was on average 68,7±16,04 cells. The range of the change in the patients who did not have the Hunner's injury were not different from the patients who had the Hunner's injury, but the average number of the cells were little, and they form 56,4±16,72 cells (p=0,596, t-krit. – 0,53). The fat cells had a reverse, weak, statistically insignificant relationship with plasma cells (r = -0,208 (p> 0,05)), neutrophils (r = -0,227 (p> 0,05)), and lymphocytes (r = -0,156 (p > 0,05) was correlated. The analyses of the correlation demonstrated the weak unimportant relationship of cells with NGF and HB-EGF as well as with EGF. Only neutrophils were correlated with a statistically significant association with EGF (r = + 0.460, p <0.05).

In the blood serum of the examined patients in the main group the middle level of the inflammatory cytokines such as IL-1β, IL-6, IL-8 and TNF-α compared with the control group relevantly was high like this 63,34% (p=0,020), 65,99% (p=0,001), 67,14% (p=0,006) and 70,16% (p=0,042).

The level of IL-1β, IL-6, IL-8 and TNF-α was high on average from the indicators in the control group: 2,44 (p=0,000), 2,12 (p=0,000), 2,35 (p=0,049) and 1,97 times (p=0,000). The correlation of IL-1β/IL-6 in the group of IS/ASKS patients was 1,10, and it was 0,95, and TNF-α/IL-6 correlation respectively was 0,95 and 0,99.

In the urine of the patients, who had or did not have any Hunner's injury, the concentration of the cytokines studied was

statistically significant from the control measures was high. The level of IL-1β was 2.4 higher ($p<0,05$) than the indicators in the control group. The levels of IL-6, IL-8 and TNF-α was higher than the control indicators relevantly 2,0 times ($p<0,05$), 2,5 times ($p<0,05$) and 2,0 dəfə ($p<0,05$). The women patients who did not have the Hunner's injury the contents of IL-1β, IL-6, IL-8 and TNF-α was relatively higher 2,4 times ($p<0,05$), 2.0 times ($p<0,05$), 2,0 times ($p<0,05$) and 1,9 times ($p<0,05$). According to the comparatve analysis between groups, the important significant difference in practice in the IL-1β, IL-6 and TNF-α levels did not exist. The concentration of IL-8 in the group of patients who had the Hunner's injury was 20,3% higher than in the group of patients who did not have the Hunner's injury.

While investigating the mutual relationship between the levels of cytokines in the blood and urine of the patients who had IC/PBS, a direct, moderately statistically significant correlation ($r=0,531$, $p<0,05$) was discovered between IL-8 concentrations in the blood and urine. In this case, a weak correlation ($r=0,144$, $p>0,05$) was noted between the levels of IL-6 in the blood and urine.

The levels of IL-8 were correlated ($r=-0,256$, $p>0,05$) in a weak, reverse contact with one another in the blood and urine in the control group, the same was observed with IL-6 as well ($r=-0,116$, $p>0,05$).

Analysis of serum inflammatory cytokines in patients who had IS / ASKS demonstrated a significant relationship between IL-1β and IL-6 ($r=+0,584$, $p<0,05$), while in the control group the relationship between these cytokines was weak and reversible ($r=-0,241$, $p>0,05$).

The patients who had IC/PBS the attention was drawn to the fact that there was not any relationship between IL-6 and TNF-α. In this case, in the control group a direct, noticeable, significant connection was noted between these cytokines ($r=+0,714$, $p<0,05$).

The serum levels of IL-1β, IL-6, and IL-8 in patients who had IC / PBS were not correlated with a number of fat cells in biopsy specimens. So, the correlation coefficient of the number of fat cells in the biopt with the level of IL-1β in the blood was 0, IL-6 with – $r = -0.024$ ($p = 0.1$). A moderately negative relationship ($r = -0,418$, $p =$

0,02) was defined between the number of the fat cells in the biopsy samples and serum TNFα levels.

The analysis of the correlation between inflammatory cycotins and proinflammatory cytokines and CRP in the blood discovered a weak multifaceted relationship. IL-8 was correlated with CRP compared to other cytokines (r=-0,152, p>0,05).

While investigating the relationship of the correlation between the cytokines and the studied growth factors in the patients who had IS/ASKS, weak correlations were identified (Table 3).

It was noticeable that very important correlation relationship was observed in the control group very much. Some significant correlation was noticed between IL-and NGF patients who had IS/ASKS.

Table 3.

The correlation analysis of the secretion levels of cytokines and NGF, HB-EGF and EGF biomarkers in the patients of research groups

Indicators		**A group of patients with IS / ASKS**		**Control group**	
		r	p=	r	p=
IL-1β	NGF	-0,026	0,866	+0,069	0,810
	HB-EGF	+ 0,032	0,711	+0,050	0,787
	EGF	+ 0,101	0,477	-0,665**	0,002
IL-6	NGF	-0,613*	0,003	-0,242	0,121
	HB-EGF	+ 0,038	0,842	+ 0,052	0,723
	EGF	+ 0,217	0,114	- 0,349*	0,029
IL-8	NGF	-0,137	0,370	-0,048	0,748
	HB-EGF	- 0,149	0,284	+ 0,072	0,618
	EGF	+ 0,073	0,601	- 0,135	0,116
TNF-α	NGF	-0,086	0,588	+0,314	0,054
	HB-EGF	- 0,069	0,658	- 0,274	0,063
	EGF	+ 0,005	0,958	- 0,604**	0,002

Note. The statistic importance of the differences: * -p<0,05; ** p<0,01

Considering high levels of NGF and CRP in the women patients who had IC/PBS and who were examined, it was meant to be

possible that chronic inflammation was involved in this urinary bladder disease.

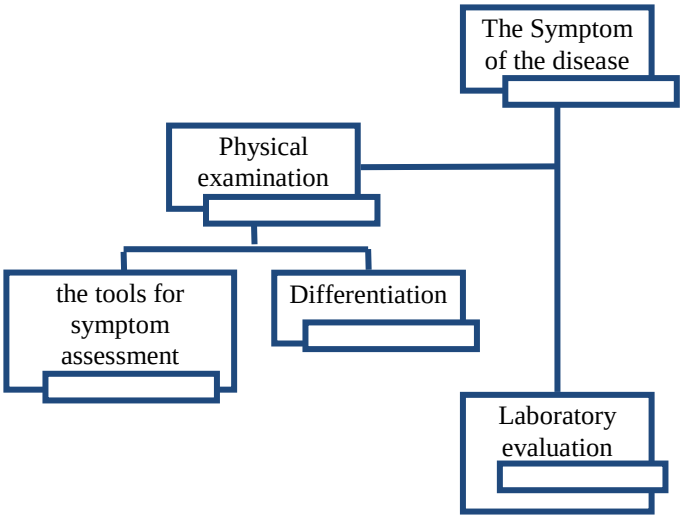
Inflammatory factors have a very crucial role in the etiology of IC/ PBS. The inflammation affects directly the function of the urinary bladder. In female patients who had IC / PBS, an increase in serum levels of inflammatory cytokines such as IL-1β, IL-6, IL-8, TNF-α, CRP and NGF was observed. Significantly positive correlations were recorded between IL-1β and IL-6, and negative correlations between IL-6 and NGF. The inflammatory mediators may be possible to be able to cause damage to the urothelium when they are released; the inflammatory changes are the results of clinical manifestations of IC/PBS. The results obtained help to understand the etiology of IC/PBS and can be taken into account when developing a treatment scheme.

Some sensitivity, specificity and effectiveness have been defined in order to determine the importance of the NGF, HB-EGF and EGF tests in the IC/PBS diagnostics. According to the obtained information, the sensitivity and specificity of the NGF test in the blood relevantly was 84,44% and 12,50%. The sensitivity and specificity of the NGF test in the urine relevantly was 91,18% and 14,78%. The sensitivity and specificity of the HB-EGF test in the blood relevantly was 56,52% and 7,94%; the sensitivity and specificity of the HB-EGF test in the urine relevantly was 97,58% and 68,00%. The sensitivity and specificity of the EGF test in the urine relevantly was 95,76% and 48,39%. The level of HB-EGF had high exactness in comparison with other biomarkers was 92,62% in the urine. The level of EGF (85,91%) in the urine was compared by the exactness. The exactness of these markers such as HB-EGF and EGF was 2,9 times ($p<0,05$) and 2,7 times ($p<0,05$) higher than the exactness of NGF in the urine. The level of NGF in the blood 29,46% (26,54%-32,56% of 95% EI) demonstrated its positive prognostic value; through 65.0% (95% EI 44.26% -81.29%) negative prognostic value was shown. The positive and negative values of the levels of HB-EGF in the blood was 10,08% (7,24%-13,86% of 95% EI) and 50,0% (31,96%-68,04% of 95% EI) relevantly. As it is seen, the positive and the negative prognostic values of the NGF was

higher than HB-EGF in the blood.

High levels of prognostic value were shown by the level of HB-EGF in the urine, which is comparable to the prognostic value of EGF. The difference between the positive and negative prognostic values of HB-EGF levels was 6, 61% and 11, 76% higher compared to EGF respectively. It means that the high levels of HB-EGF detected indicate a 94% probability of the presence of IC / PBS.

According to the research and obtained information, for the diagnostics of IC/PBS the following algorithm can be suggested (Graph. 3.).



Graph 3. The suggested algorithm of the diagnostics of IC/PBS

I. A comprehensive general anamnesis is important in identifying the typical diagnostic symptoms of IC /PBS and other potentially recurrent causes. However, it was the event that widely spread on average from 3 years to the seven one when the delay was offered during the diagnosis. The anamnesis of the disease must cover the signs of the symptoms. In order to determine of IC /PBS to detect chronic and symptomatic infection should be present for at least six weeks with documented negative urine cultures to detect infection. The sense of urgency, as well as the location, nature, and

severity of the pain, pressure, and discomfort should be documented for urinary excretion. The dyspareunia, dysuria, ejaculatory pain in men and the relationship of the pain with menstruation in women should be noted. The typical presentation of IC/PBS consists of a combination of pain, frequency, nocturia, and urgency. The beginning of the symptoms may be gradual and / or only urinary excretion of symptoms though the main pain was pelvic one. The patients may not imagine the obvious pain during the early or less period of IC/PBS, on the contrary, they may feel some feeling of 'blood pressure', 'nausea', 'sharp' or 'unpleasant urination'. This feeling may be felt in the supraorbital area as well as it can also affect the parts of the pelvis, including the urethra, uterus, labia, groin area, intercostal space, and / or the lower abdomen or back. Unlike the symptoms of the endometriosis that were worsening during menstruation became harder some days before menstruation. The patients might feel the symptoms that became worse or 'arose' because of stress, due to sexual intercourse, menstruation or diet. The most widely spread symptom is pain. Symptoms of IC/PBS are usually intermittent and often include pain in areas other than the urinary bladder.

II. The physical review must consist of the view of abdominal cavity and small pelvic organs indicating the existance of mass, pain and hernias. The examination of small pelvic organs must consist of external genitalias, palpation of the base of the urinary bladder in women and the urethra in both sexes with emphasis on subtle areas. It is necessary to conduct purposeful assessment in order to exclude the urethritis, sensitive prostate, urethral diverticulum or other potential source of pain or infection. It is possible to confuse the IS/ASKS and hyperactive urinary bladder with each other as each of the cases is usually seen in the frequency of urinary excretion. The main differential symptom is the pain that is felt in the pelvic area; if such a pain is felt then the hyperactive urinary bladder can be excluded. The patients who have hyperactive urinary bladder, as a rule, should it leak, they should prefer to go to urinate though patients who had IC/ PBS often urinate in small amounts, regardless of day or night, to relieve or alleviate pain. The IC/PBS symptoms of

pelvic pain must be differentiated from endometriosis and dyspareunia. If the endometriosis related pain happens during the period of menstruation, it usually becomes more severe in that period. Women with endometriosis may have a urge to urinate and frequent urination, but these symptoms are less common than in women with IC / PBS. IC / PBS is also distinguished by non-infectious cystitis, vulvar disease, seizures, pelvic bottom disease, and prostate-related pain.

There are different kinds of methods in order to evaluate symptoms. Clinical scales are meant to be useful so that to evaluate and characterize the symptoms of patients. Here belongs not only the pains that demonstrate dyspareunia or other urinary bladder, but also the O'Leary-Sant symptoms that evaluate the frequency of urination and discomfort in the urinary bladder and problem indexes as well as a questionnaire on the scale of symptoms. The survey of O'Leary-Sant is useful so that to get some comprehensive information about the symptoms as well as including some additional symptoms to pain or discomfort. The Urgency / Frequency Scale (PUF) for pelvic pain and patient symptoms is a simple tool for later characterizing patient complaints and narrowing the differential diagnosis.

The IC/PBS patients might have glomerulation (pinpoint-sized red marks on the bladder) during cystoscopy, but these injuries might be usually met with other cases that had IC/PBS. For example: undifferentiated chronic pelvic pain and endometriosis.

The glomerulations may be observed in patients who do not have any symptoms of cystoscopy. It is necessary to note that cystoscopy of the urinary bladder is included in the NIDDK criteria for inclusion in the clinical examination of IC / PBS. The urodynamic diagnostic criteria does not exist having been agreed about IC/PBS. Urodynamic may give some information about symptoms of the lower urinary tract. The potassium test can be used as well.

III. The main laboratory tests cover the urine analysis and its plantation. The general analysis of the urine is meant to be the first step in the diagnosis and the realization of the urine plantation must be fulfilled. Cytological analysis of the urine may be considered in

order to take into account the risk of urinary bladder cancer if the patient has an anamnesis of smoking and / or the presence of undiagnosed microhematuria (It may also be called *microscopic hematuria).*

The plantation of the urine may be indicated in the patients with a negative urine test even for the detection of low levels of bacteria that are clinically important but not easily identified by microscopic examination.

The combination of negative urine analysis and the plantation of the urine, symptoms of chronic urinary incontinence, chronic pelvic pain, and positive results on physical examination is sufficient evidence for the diagnosis of IC / PBS. It gives some confidence if a small amount on the PUF scale is added and the frequently confirmed high urine output is able to ensure the correctness of diagnosis.

The process of diagnosis begins with comprehensive collection of anamnesis and physical examination. It is necessary to get some information about patients' previous diagnoses as well as urinary excretion. The following may be interesting: the succession of symptoms in the patients, nutrition triggers, allergic anamnesis and sexual dysfunction. It is necessary to patients to keep the diary for everyday symptoms. The following needs to be advisable: general urine analysis, the plantation of the urine, C-reactive protein, the level of creatine and the glomerular filtration speed as well as it is necessary to determine the levels of EGF, HB-EGF and NGF in the blood and in the urine.

RESULTS

1. Experimental studies in laboratory animal modeling demonstrated the most effective translational model created by injecting IC / PBS under the mucous membrane of the urinary bladder wall. The model was characterized by persistent symptoms, an increase in the main features of the disease, and autoimmune inflammation [1, 2, 5].

2. In the chemical models of experimental IC/PBS may consist of some typical morphological changing infiltration in the urinary bladder and edema. The following has been determined in the autoimmune model such as mixed inflammatory infiltration, necrosis, accumulation of hemosiderin, multinucleated histiocytes, the infiltration of fat cells [6, 7, 21, 23].

3. In the autoimmune model of IC/PBS the ultrastructural changes along with neutrophils and macrophages in inflammatory infiltrate are expressed by the presence of lymphocytes and plasma cells. The toxic damage of a special layer of the mucous membrane of the urinary bladder may cause the apoptosis of fibroblasts of a special layer, the release of collagen fibers and the reduction of protective factors of the mucous membrane [11, 15, 22].

4. In the IC/PBS initiation after 1 and 14 days in the NGF levels increased in dynamics in a disease model obtained by injecting urine into the urinary bladder wall. Significantly tight correlation ($r=+0,715$, $p=0,05$) between the levels of NGF in the blood and the urine was discovered in the animal models created by protamine sulfate injection. The indicators of NGF in the blood and the urine during IC/PBS was conditioned by inflammatory components; an obvious increase in biomarker levels in animals with a urinary bladder wall urinary injection model is associated with chronic inflammation and toxicity of urinary components. The relationship of NGF with fat cells confirms neuroimmune inflammation during the IC/PBS. After 14 days the level of EGF the dynamic increase with 70% alcohol fluid in the experimental model with innerbladder instillation, a slight decrease in the blood (31,4%, $p <0,05$) and the urine was observed compared with the first day; during innerbladder instillation of protamine sulfate (10 mg), EGF levels in the blood decreased by 19,4% and in the urine by 55,0% ($p <0,05$) on the day of 14; innerbladder instillation of 0,5% HCl (0,2 ml) demonstrated a tendency to be increased in the blood and 59,9% ($p <0,05$) in the urine; in the autoimmune model, the levels of EGF in the blood and the urine were increased respectively by 61,1% ($p <0,01$) and 22,3%; when 0,9% NaCl fluid was injected into the urinary bladder wall, the EGF concentration in the blood decreased by 85,8% ($p <0,001$) and

in the urine by 12,3%; NGF was correlated with EGF in the chemical model (protamine sulfate) on the first day of the experiment in the blood ($r = -0,942$, $p < 0,001$) and after 14 days in the 70% alcohol model ($r = +0,936$, $p < 0,001$) [3, 4, 9, 13, 14, 20, 24, 27, 28].

5. The mucous membrane changed in the 12,4% of patients, the following existed relevantly in 13,9%, 23,2% and 12,4% of patients such as rare glomerulations, diffuse mucus hemorrhage and the Hunner's injuries. The diffuse changes during cystoscopy after hydrotherapy in 44,21% of patients in the mucous membrane of the urinary bladder, in the neck - in 15,5%, in the upper part of the urinary bladder - in 9,3%, in the bottom - in 13,2% , on the right and left wall – 8,5% [9, 10, 17, 18, 26].

6. The levels of NGF and HB-EGF in the urine exceeded the controlled values respectively by 50,44% ($t = 2,06$, $p = 0,043$) and 56,44% ($t = 2,01$, $p = 0,049$). The level of NGF was directly correlated with urinary HB-EGF ($r = + 0,922$, $p < 0,05$). The level of NGF in the urine was correlated with a direct, significant relationship with CRP serum ($r = + 0,529$, $p < 0,05$). The urinary bladder cells of neutrophils were mildly correlated with EGF ($r = + 0,460$, $p < 0,05$) [4, 8, 16, 19].

7. High levels of inflammatory cytokines in the blood and urine have been reported in the patients who had IC / PBS. A high coefficient of IL-1β / IL-6 in the group of patients who had IC / PBS (1,10) indicates an imbalance between the cytokines Th1 and Th2 compared with the control group in the group of patients compared with the control group (0,95). IL-6 excretion was significantly correlated with the levels of NGF ($r = 0,613$, $p = 0,003$) [12, 17, 25].

8. The recommended algorithm for the diagnosis of IC/ PBS requires a comprehensive anamnesis, physical examination and laboratory examination to document the main symptoms that characterise the clinical diagnosis of the disorder and to rule out infections and other disorders [19].

PRACTICAL RECOMMENDATIONS

1. It is useful that an experimental IC / PBS model with the instillation of animal urine into the urinary bladder wall of that animal for studying the pathophysiology of the disease and the effects of potential treatments.

2. It is recommended that the method of injecting animal urine into the urinary bladder wall of that animal should be used in the experimental medicine to model autoimmune cystitis.

3. It is advisable to use cysticcopy and hydrodistension during the examination of the patients who had IC/PBS.

4. It is recommended to determine the concentration of NGF, HB-EGF and EGF in the blood and the urine in the diagnostics of IC/PBS.

5. Expression of cytokines in combination with growth factors (HB-EGF and EGF) can be used to explain the pathophysiology of the clinical features of IC / PBS.

6. The results of cytokine profile analysis are proposed for the differential diagnosis of IC / PBS and other urinary bladder disorders.

7. The NGF and CRP with clinical symptoms in the blood serum may be used in order to define the diagnostics of IC/PBS with them.

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LIST OF ABBREVIATIONS

CRP	–	C-reactive protein;
EGF	–	Epidermal growth factor,;
ESSIC BPS	–	Interstitial cystitis / International Society for the Study of Painful Bladder Syndrome (European Society for the Study of IC/PBS);
HB-EGF	–	Heparin binding EGF-like grow factor;
İL	–	Interleukins;
İS/ASKS	–	Interstitial cystitis / painful bladder syndrome;
NGF	–	Nerve growth factor, NGF;
PUF Scale	–	Pelvic Pain and Urgency/Frequency; Patient Symptom Scale, PUF Scale;
TNF- α	–	tumor necrosis factor-alpha;
USS	–	urgency severity scale;
VAS	–	visual analog scale;

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