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Ashrapov Jamshid Raufovich
Signs and surgical tactics in continued growth of gliomas of supratentorial localization in children
Hygienic assessment planning house of mercy of the Republic of Uzbekistan
Babakulov Sharaf Hamrokulovich, Tangriberganov Murat Reyimberganovich, Babakulova Shahlo Hamidullaevna
Specificity of micro-vascular density in superficial bladder cancer
Musabaev Erkin Isakovich, Bayjanov Allabergan Kadirovich, Mustafaeva Dildora Asadovna,
Mamatkulov Adxam Rustamjonovich, Kazakova Evgenia Ivanovna
Introduction of the control system for the HIV medicinal resistance to antiretroviral preparations
Protective efficiency of "Phosphogliv" at high active antiretroviral
therapy in patients with HIV-infection, associated with chronic viral hepatitis C
Berkinov Ulugbek Bozorbaevich, Khalikov Sarvar Pulatovich
Optimization of the surgical treatment for high cicatricle tracheal stenosis
Bakhritdinova Fazilat Arifovna, Narzikulova Kumri Islamovna,
Mirrakhimova Saidakhon Shukhratovna, Khera Akshey
Biochemical parameters of the effect of laser radiation in the experiment
Karimov Ulugbek Rasulovich, Narzikulova Kumri Islamovna
The results of medicated decreasing of intraocular pressure at neovascular glaucoma
Berkinov Ulugbek Bozorbaevich, Sakhiboev Dilshod Parpijalilovich,
Irnazarov Akmal Abdullaevich
Results simultaneous operations in patients with adrenal tumors
Ashurov Azimjon Mirzajanovich, Boymuradov Shukhrat Abdujalilovich,
Khayruddinova Zulfiya Rafikovna, Ibragimov Davron Dastamovich
Posttraumatic rhinosinusitis in patients with cranio-facial injuries
Gafarova Feruza Murathodzhaevna
Dysfunctional state kidney during postnatal adaptation in the newborn80
Davis Nikolay Aleksandrovich, Toychiev Abdurakhim Khodjiakbarovich,
Islamova Jannat Ikramovna, Parpieva Nargiza Nusratovna, Osipova Svetlana Olegovna
Concomitant intestinal parasitic diseases in pulmonary
tuberculosis patients: influence on some immunological indices
Davis Nikolay Aleksandrovich, Toychiev Abdurakhim Khodjiakbarovich,
Djuraeva Zulfiya Baratovna, Parpieva Nargiza Nusratovna, Osipova Svetlana Olegovna
Influence of intestinal parasites on cytokine profile of patients
with pulmonary tuberculosis, including cases complicated with aspergillosis85
Ermatova Gulnara Ahmadovna, Hozhimatov Khusnidin Odilovich
Influence factors of the environment on the state of health of the population at the regional level
Juraev Rivojiddin
The role of viral etiology in the development of acute gastroenteritis in children in Uzbekistan
Zokirkhonova Shahzoda
Medical and biological assessment of the fluoride content of bottled water91
Zakirova Feruza Akildjanovna, Bekbulatova Indira Rinatovna,
Eliseeva Marietta Rafaelevna
The influence of active inflammation on parameters of central
hemodynamics in pregnant women with rheumatic heart defects
Ismailova Savrinisa Sultanovna
Efficiency expectant management in women with premature rupture of membranes
Israilov Radjab Israilovich, Tursunov Khasan Ziyaevich, Eshbaev Erkin Abdukhalimovich
Morphological changes of newborns coronary vessels in preeclampsia in mothers99
Kayumov Abdurakhman Abdumavlyanovich, Karimov Khamid Yakubovich,
Boboev Kadirzhon Tuhtabaevich
Role of polymorphism RS1800629 gene proinflammatory
cytokine TNF-α in the development and clinical course of leukemia

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Influence of intestinal parasites on cytokine profile of patients with pulmonary tuberculosis, including cases complicated with aspergillosis

Abstract: influence of intestinal parasites on immune imbalance in pulmonary tuberculosis (PT), including cases complicated by pulmonary aspergillosis (PA) was studied. 300 and 111 patients with active PT were examined for intestinal parasites. Group of comparison: 200 residents of Tashkent and Tashkent region. The group of 111 patients was examined for PA (clinical, X-ray, mycological and serological data). Serum IFN-γ and IL-4 were assessed by ELISA. Ascariasis was found in patients with PT and PT with invasive PA respectively 5 and 12 times as frequent as in population. Decrease of IFN-γ and increase of IL-4 level (P < 0.05) was observed in these groups in comparison with healthy individuals and patients with PT free of intestinal parasites and PA. Conclusion: Ascariasis as a concomitant disease enhances immune imbalance, typical for PT and PT + invasive PA. Keywords: Ascariasis, pulmonary tuberculosis, intestinal parasites, aspergillosis, cytokines.

Control of Mycobacterium tuberculosis replication is mainly mediated by production of Th1-cytokines IFN- γ and TNF- α and cytotoxicity of CD8*-lymphocytes, directed against infected macrophages. Control efficiency is specified by the balance of Th1-and Th2-response (IL4, IL-10, IL-13) [7, 694–701]. Protective immu-

nity in helminthiasis is mediated by Th2-response [6, 459–466]. Protective mechanisms in pulmonary tuberculosis (PT) and aspergillosis are similar and are based on elevated IFN- γ production [5, 403–413]. Our previous study showed that pulmonary aspergillosis (PA) complicates course of the disease in 40% of the

patients with PT and the majority of Aspergillus spp. strains were resistant to widely applied antimycotics. Maximal susceptibility was detected to voriconazole [2, 113–118]. It was of interest to study influence of intestinal parasites on immune imbalance specific for active PT, including cases complicated by PA under conditions of the region endemic on intestinal parasitic diseases.

The purpose of the communication is to determine level of serum cytokines IFN- γ and IL-4 in patients with PT including cases with concomitant intestinal parasitic diseases and complicated with PA.

Materials and methods. Two groups of patients with active PT, hospitalized at Republican specialized scientific research medical center of phthisiology and pulmonology of the Ministry of Public Health of the Republic of Uzbekistan (respectively included 300 and 111 patients) were examined, the last group was additionally examined for PA. Patients with infiltrative PT dominated in both groups. Diagnosis of PT was based on clinical, X-ray and bacteriological data. HIV-infected and individuals with viral hepatitis were excluded. All the patients were at the age of 18–64 and received standard antituberculosis therapy: isoniazid, rifampicin, pyrazinamide, ethambutol, streptomycin.

Group of comparison included 200 inhabitants of Tashkent city and Tashkent region. Family where patients with PT, HIV-infected individuals and patients with chronic viral hepatitis were excluded. Control groups for IgG to Aspergillus spp. and cytokines detection consisted of 30 and 20 healthy persons respectively. Sexual and age structure of groups of comparison were analogous to the patients with PT and PA.

Intestinal parasites were diagnosed by the triple coproscopy, stool samples were collected with 2-3 days interval.

The cause for examination for PA was sudden aggravation of patients condition against the background of antituberculosis therapy or torpidly current tuberculosis.

Diagnosis of PA was based on clinical, radiological (chest computerized tomography (CT) scan), and laboratory findings: isolation of Aspergillus spp. of sputum, bronchoalveolar lavage and blood samples, detection of IgG antibodies to Aspergillus spp. by ELISA (kits from LLC Vector-Best, Novosibirsk, Russian Federation).

We used as differential diagnostic sign a rapid improvement of patient's condition on the 3rd – 5th day of empiric therapy: decrease of temperature, relief of dyspnea, significant diminution of weakness in the cases without mycological confirmation (absence of sputum and bronchoalveolar lavage refusal) and nonspecific results of CT (progression of infiltrative process) against the background of clinical impairment and positive serology.

Blood samples for mycological study were taken at fever period once a day (5–7 ml.) for 3 days. For prevention of contamination we used flasks of the system HiSafe, Hi-Media, India, with diphase system Hi-Combi for fungi with CC addition). In 2, 5 and 10 days blood samples from flasks were inoculated on Petri dishes with Sabouraud agar which were incubated at 37 °C up to 3 days, Sabouraud agar with glucose (pH 5.0), (Hi-Media, India) was used for inoculation of sputum and bronchoalveolar lavage samples.

IFN- γ and IL-4 in serum samples were assessed using commercially available enzyme-linked immunosorbent assay (ELISA) kits from LLC Vector-Best, Novosibirsk, Russian Federation.

Statistics. Comparison between indices under investigation were made using Student's t-test. The significance was determined at P < 0.05.

Results. Results of intestinal parasites diagnosis in patients with PT are represented in table 1. Ascariasis was diagnosed in

 $10.0\pm1.7\%$ of patients with PT but in $2.0\pm0.9\%$ of population (P < 0.001). This index was even higher in patients with infiltrative PT: in 23 (12.3 \pm 2.4 %) of 187 patients.

Table 1. – Prevalence of intestinal parasites in patients with PT (n/M±m)

Parasites	Patients with PT (n = 300)	Population of Tashkent city and Tashkent region (n = 200)
Ascaris lumbricoides	30/10.0±1.7*	4/2.0 ± 0.9
Enterobius vermicularis	14/4.7 ± 1.2	9/4.5 ± 1.4
Giardia lamblia	14/4.7±1.2*	32/16.0 ± 2.6
Blastocystis hominis	161/53.7 ± 2.8*	36/18.0 ± 2.7

Note: * — significant difference between patients with PT and population (P < 0.05).

Prevalence of Enterobius vermicularis in patients with PT and population was the same in spite of more efficient mode of transmission in enterobiasis, belonging to contact parasitic diseases.

Morbidity with giardiasis in patients with PT was 4 fold lower, than in population. This phenomenon appears to be stipulated by the influence of antituberculosis therapy. Aminoglycosides are known to possess a limited antigiardial activity (paromomycin) [9, 8–10], probably other antituberculosis preparations exert analogous effect.

Prevalence of Blastocystis hominis was 3 fold higher than in population. It seems to can be connected with an expressed immune imbalance and decrease of activity of local immunity to control the B. hominis number. It is confirmed by analogous tendency in HIV-infected individuals, a high intensity of B. hominis infection ($\geq 5-6$ parasites in a field of view, oc \times 10, ob \times 40) being detected only in patients with PT and HIV-infected individuals, not in healthy persons and patients with various pathology of gastrointestinal tract (gastritis, enterocolitis, cholecystitis) [3, 8–11].

Table 2. – Level of serum IFN-γ and IL-4 in patients with PT, invasive PA and intestinal parasites (pg/ml)

Cohort under study	IFN-γ	IL-4
Healthy individuals (n = 20)	125.7 ± 6.7	2.6 ± 0.7
Patients with PT without intestinal parasites and aspergillosis (n = 15)	70.0 ± 6.1*	8.0 ± 2.1*
Patients with PT and ascariasis (n = 17)	54.6 ± 2.8* **	18.2 ± 3.0* **
Patients with PT and enterobiasis (n = 14)	69.3 ± 3.8*	9.3 ± 1.8*
Patients with PT and invasive PA (n = 12)	41.6±3.1* **	27.6 ± 4.7* **
Patients with PT, ascariasis and invasive PA (n = 4)	22.8 ± 4.7* **	31.7±4.9* **
Patients with PT and giardiasis (n = 12)	58.7 ± 3.8*	15.4 ± 2.4*
Patients with PT and blastocytosis (n = 12)	62.8 ± 3.8*	22.4 ± 5.7* **

Note: * — significant difference with healthy individuals (P < 0.05); ** — significant difference in comparison with patients with PT without intestinal parasites and aspergillosis (P < 0.05).

PA was diagnosed in 44 (39.6 \pm 4.6%) from 111 patients with PT, 16 of them suffered from invasive PA. Aspergillus fumigatus were isolated from blood samples of 2 patients and from sputum and bronchoalveolar lavage samples of 10 patients. Invasive PA was diagnosed in 4 patients on the base of significant impairment of condition and progression infiltrative process against back-

ground of antituberculosis therapy (increase of temperature up to 38.5–39.9 °C, appearance/intensification of dyspnea and appearance of blood streaked sputum, progressing of infiltrative process (CT), high level of IgG to Aspergillus spp. and rapid positive effect of voriconazole therapy. Other cases were presented by aspergilloma. We consider positive serology as a test orienting to the disease, because In total positive serology was observed in 4 (20%) from healthy individuals of control group. Positive results were obtained in 1 (5%) from 20 nonsmokers and 3 (15%) from 20 smokers, all three smokers have A. fumigatus colonization of upper part of respiratory tract. Diagnostic level of IgG to A. fumigatus was determined in 31 (70.4%) patients with PA.

Results of serum cytokines detection are represented in table 2. Level of serum IFN- γ in patients with PT without intestinal parasites and PA was significantly lower, than in healthy individuals (P < 0.05). This index in patients with PT and ascariasis was even lower, and is significantly different from control values as well as values, obtained in patients with PT and without parasites and PA (P < 0.05). Enterobiasis didn't induce significant changes in IFN- γ concentration in patients with PT. Invasive PA, diagnosed in 12 patients with PT, was developed against a background of low level of serum IFN- γ : 41.6 ± 3.1pg/ml; ascariasis diagnosed in 4 patients with PT and invasive PA induced even more expressed decrease of IFN- γ : 22.8 ± 4.7pg/ml.

Level of serum IL-4 was significantly higher in all groups under study in comparison with control value. The highest IL-4 level was detected in patients with PT+invasive PA+ascariasis and PT+invasive PA and PT+ ascariasis, respectively 31.7 ± 4.9 pg/ml; 27.6 ± 4.7 pg/ml μ 18.2 ± 3.0 pg/ml. In patients with PT without parasites and PT with enterobiasis IL-4 level was similar and significantly higher than in control group.

Significantly higher prevalence of A. lumbricoides in patients with PT can be considered as a factor provoking manifestation or exacerbation of tuberculosis process due to augmentation of IFN- γ and IL-4 imbalance. Larvae migration stage through respiratory tree is of importance due to direct affection of lung tissue and probably activation and spreading of mycobacterial infection. Data of J. Potian et al. [8, 1863–1874] indicate to such possibility.

They showed in experiment with mice, infected with Nippostrongylus brasiliensis (model equivalent ascariasis in human) and M. tuberculosis that Th2 response can enhance the intracellular persistence of M. tuberculosis, in part by mediating the alternative activation of macrophages via the IL-4Ra signaling pathway unlike classic one, mediating by IFN-7. May be ascariasis triggers other mechanisms. Diniz L. M. et al. (2010) [4, 142-150] found A. lumbricoides in 12% of patients with tuberculoid lepra and in 2% of individuals who were in contact with patients but were not sick. These numbers are completely in agreement with our data. Although tuberculosis and lepra characterized by different target organs the infection gate (respiratory tract) are similar at these infections. Diniz et al. (2010) received analogous dynamics of IFN-y and IL-4 in patients with lepra and concomitant ascariasis and without it [4, 142-150]. Thus we can conclude with good grounds that ascariasis influences susceptibility to Mycobacterium and course of mycobacterial infections. Our data are consistent with results of Abate et al. (2012), showed that one third of bacteriological positive PT patients in Ethiopia are infected by helminths and this level is significant higher than in population [1].

Ascariasis seems to influence development of invasive PA in patients with PT. Absence of significant shifts in the level of IFN- γ and IL-4 in patients with PT and concomitant enterobiasis and similar prevalence of E. vermicularis in patients with PT and population point to lack of enterobiasis effect on susceptibility to M. tuberculosis and course of PT. Influence of concomitant giardiasis and blastocystosis (patients only with high intensity of infection were considered) on the level of serum IFN- γ was essentially weaker: in significant decrease in comparison with control value, differences with patients with PT without parasites and PA were negligible (P > 0.05). Some other regularities were elucidated in IL-4 detection: if its values in patients with PT + giardiasis and PT without parasites and PA were similar, blastocystosis resulted in a significant increase of IL-4 level (table 2). Thus influence of protozoan infections on cytokine profile of patients with PT is insignificant.

Conclusion

Ascariasis as a concomitant disease influences development of PA due to worsening immune imbalance typical for PT.

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