STUDII ȘI SINTEZE

STUDY OF THE POSSIBILITY OF IMPROVING THE DIFFERENTIATION OF LATENT AND ACTIVE TUBERCULOSIS INFECTION

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Summary

Latent TB infection (LTI)-asymptomatic nontransmissiv tuberculosis infection with hidden persistence of M.tuberculosis in host organism. The problem of detection of latent tuberculosis infection is very urgent and difficult task. 140 individuals suspected of tuberculosis were examined using conventional clinical and laboratory methods of examination, setting skin immunological reactions to detected delayed hypersensitivity- tuberculin skin test (TST) and Diaskintest (DST). All the patient underwent determination of total antibodies to M.tuberculosis by ELISA. According to the data of examination 97 individuals were found to have latent tuberculosis infection and 43-different forms of pulmonary tuberculosis. Based on the results of the study it can be agued that: 1) The use of DST allows more accurately differentiate latent tuberculosis infection and its activation. 2) Detection of total antibodies to MBT in the range 0,14-2,28 UOD is typical for persons with latent tuberculosis infection. 3) Determination of anti-TB antibodies and their level by ELISA can serve as an additional criterion for the diagnosis of tuberculosis and determine the activity of tuberculosis infection.4) With the exception of the local form of tuberculosis, persons with positive DST, with the level of anti-TB antibodies in the range 0,14-2,28 UOD and with the syndrome of General disorders should be considered as patients with a borderline condition who need preventive anti - tuberculosis chemotherapy.

Key words: latent tuberculosis infection, skin tuberculin tests, Diaskintest, anti-tuberculosis antibodies.

Резюме. Изучение возможности усовершенствовании дифференциации латентной и активной туберкулезной инфекции

Латентная туберкулезная инфекция (ЛТИ) –асимптоматичная нетрасмиссивная туберкулезная инфекция со скрытой персистенцией. M.tuberculosis в организме человека. Дифференциация ЛТИ и активного туберкулеза все еще остается довольно сложно разрешаемой проблемой. 140 лиц с подозрением на туберкулез были комплексно обследованы с применением общепринятых клинико-лабораторных методов обследования, а так же проведения кожного туберкулинового теста (TST) и Диаскинтеста (DST). Качественного и количественного определения суммарных противотуберкулезных антител методом ИФА. По результатам обследования у 97 лиц была выявлена ЛТИ, у 43 - различные формы туберкулеза легких. Основываясь на результатах исследования можно утверждать что:

1) Применение DST позволяет более точно дифференцировать ЛТИ и ее активизацию. 2) У лиц с ЛТИ выявляются суммарные ПТА в диапазоне 0,14-2,28 ед. ОП. 3) Определение уровня ПТА методом ИФА может служить дополнительным критерием активности туберкулезной инфекции. 4) При исключении локальной формы туберкулеза, лица с положительным DST с уровнем ПТА в диапазоне 0, 14- 2,28 ед. ОП и синдромом общих нарушений должны рассматриваться, как пациенты с пограничным состоянием, которым необходимо проводить превентивную противотуберкулезную химиотерапию.

Ключевые слова: латентная туберкулезная инфекция**,** кожный туберкулиновый тест, Диаскинтест, противотуберкулезные антитела.

Introduction. In many contries, regardless of the level of economic development, the incidence of tuberculosis is increasing, so that tuberculosis can be classified as a resurgent infection (1).

Latent tuberculosis infection is a reservoir of future tuberculosis, the problem of detection of latent tuberculosis infection is a very urgent and difficult task (2,3,4,5).

Latent TB infection (LTI)-asimptomatic nontransmissive tuberculosis infection with hidden persistence of Mycobacteria in the host organism [2,6]. The phenomenon of persistence of bacteria causes an increased interest around the world [2,3,4, 5,7,8,9,10]. The problem of differentiation of latent and active TB is very important, but difficult and not yet completely solvable [6,11]. According to the World Health Organization (WHO), one third of all humanity has LTI 5-20% of those infected there is a risk of development of active tuberculosis (TB) during their lifetime and in the majority of cases TB develops in 2-5 years of infection [8,12]. The gap between infection and the development of tuberculosis is unique for contagious disease [9,13]. Thus, the LTI is a tank of the future TB. Infection control

(measures to reduce the incidence, early diagnosis and treatment) is the most important strategy to combat TB. The decrease in the number of people infected with M.tuberculosis and preventing new cases of disease is achieved by preventive therapy of persons with LTI [10, 11, 12,14,15,16]. Therefore for infection control should be more clearly delineate a the range of persons with LTI in the need of preventive therapy. The main methods of determination LTI are tuberculin tests revealing delayed type hypersensitivity. It is believed that the only convincing evidence of the presence of MBT in the body before the development of local changes is the reaction of delayed hypersensitivity, determined by skin test using tuberculin (13,14,17,18). For carrying out the reaction using PPD (purified protein derivative). However, the PPD contains more than 200 antigens derived from M. bovis and M.humanus, a part of these antigens locate in other non-tubercular mikobacteria. For this reason, a positive test can be registered not only in case of MBT infection, but also in, nontuberculous mycobacteria infection as well as a certain period after BCG vaccination [15,16,17,19,20,21].Decording of M. tuberculosis genome [18] permit to use separate specific MBT proteins for diagnosis of tuberculosis. In the genome of the MBT around 4000 proteins coded and genes profile expressed at different stages of infection can vary [19, 20, 21, 22, 23,24,25,26,27]. The two most widely used for diagnostic purposes antigens ESAT-6 (Early Secreted Antigenic Target 6) and CFP-10 (Culture Filtrate Protein 10) encoded in the zone of RD1 M. tuberculosis genome and, they express when reproduction of Mycobacteria and absent in m. bovis BCG and most non-tuberculosis Mycobacteria. They are related to the virulence of M. tuberculosis [28,29,30,31,32]. These antigens have been used to create diaskintest (tuberculous recombinant allergen) that represents a complex of recombinant protein CFP-10 and ESAT-6 produced genetically modified culture of Escherichia Coli BL-21 (DE3)/p CFP-ESAT and destined for intradermal test with the purpose of revealing delayed-type hypersensitivity [32,33,34,35]. The active stage of tuberculosis is characterized by intensive production of anti- TB antibodies, which can be detected by enzyme immunoassay in a month from the beginning the of activation of mycobacteria. The high specificity and sensitivity of the test is achieved by the use of specific antigens for M.tuberculosis, which excludes false positive results in vaccinated and infected with other bacteria.

The purpose of the study. The aim of the study is finding the objective indicators (other than tuberculin test and history data)to identify LTI and to compare the diagnostic possibilities of tuberculin intradermal tests (TST) and allergen tuberculous recombinant-diaskintest (DST) to identify activation of LTI and determination of the titer of antibodies to MBT antigens in persons with latent tuberculosis infection and in patients with tuberculosis, their comparative analysis.

Materials and methods. We examined 140 individuals suspected of tuberculosis of these, among 140 study were 81 males and 59 females aged from 2 to 17 years old, the average age of 8.7 ± 0.4 years old. All have carefully assembled histories, clinical and x-ray examination, explored the peripheral blood (complete blood count) and sputum examination on the presence of AFB. 97 individuals suspected according to the clinical, radiological, laboratory data and results of skin immunological test reactions were to found to have latent tuberculosis infection, and 43-different forms of pulmonary tuberculosis. Detection of cellular immune response carried out using diaskintest based on an evaluation of delayed-type hypersensitivity. We used the intradermal injection of diaskintest at a dose of 2mkg in 0.1 ml, containing ESAT6-CFP-10 (Lecco, Russia) present in virulent strains of Mycobacterium tuberculosis. The reaction was visually evaluated after 72 h and was measured the size of induration in millimetres. The result was considered negative in the absence of infiltration, doubtful if hyperemia without infiltration, positive if there is infiltration (papules) of any size, hyperergic-when the diameter of infiltration 15 mm and more, formation vesicle and necrosis and (or) the presence of lymphangitis, lymphadenitis. For the evaluation of delayed-type hypersensitivity also conducted intradermal tuberculin test (routine method).TST and DST was carried out in parallel at 100 persons and at 40-only DST.

All the patients underwent qualitative and quantitative determination of total antibodies to M.tuberculosis in serum by ELISA. Tuberculosis antibodies were detected using a set of “АТ-Туб-Бест” on the enzyme immunoassay “Bioscreen-500” (USA).

All the observed persons showed some signs of the syndrome of General disorders (intoxication), which was characterized by pale skin decreased tissue turgor, periorbital cyanosis, body weight deficiency, decreased appetite, General malaise. In individuals with positive DST (62- 44,3%) they were more numerous and pronounced.

Statistic calculating of data performed using computer programs Microsoft Excel for Windows, Statistica.

Results and discussion. During the research of skin immune tests (TST and DST) carried out in par-

allel it was found that 99.0 ± 1.0% of examined persons may be considered infected. Only one TST reaction was doubtful (4 mm diameter papule formation). Hyperergic reaction to tuberculin intradermal injection has evolved from 19.0 ± 3.9% surveyed, papule with the formation of vesicles from 5.0 ± 2.2%. At the same time, positive reaction to the DST was detected only in 25.0 ± 1.0% of the surveyed hyperergic reaction in 16.0 ± 3.7%. În 75.0 ± 4.3% surveyed reaction to DST was negative.

Table 1.

Results of skin immune tests (TST and DST) carried out in parallel.

The severity of reaction	TST n = 100	DST n = 100
Positive	99.0 ± 1.0%	25.0 ± 1.0%
Hyperergic	19.0 ± 3.9%	16.0 ± 3.7%
With the formation of vesicles	5.0 ± 2.2%	3.0 ± 1.7%
Doubtful	1.0 ± 1.0%	0.0%
Negative	0.0%	75.0 ± 4.3%

As a result, a comprehensive clinical-radiological and laboratory study among surveyed revealed 3 patients with tuberculosis. In 17 years old patients,TST-doubtful (papule with diameter of 4 mm) DST- hperergic reaction (papule with a diameter of 17 mm), complaining of weakness, loss of appetite, productive cough, radiographically changes were identified, allowing diagnosis "infiltrative tuberculosis in the phase of destruction", in the sputum AFB+.In 14 years old patient DST positive with the formation of papule (diameter of 10 mm) and a negative TST tuberculouse mesenteric adenitis was identified. And finally, 16 years old patient, with hyperergic DST(-papule-18 mm with vezikule) and hyperergic TST (17 mm papule) identified tuberculosis of cervical lymph nodes. Significantly less positive reaction to the DST (25.0 ± 4.3%) than the TST (99.0 ± 1.0%) have surveyed possible because, as shown above, the DST identifies the intensification of latent TB infection as antigens presented in DST expressed when reproduction of Mycobacteria and associated with virulence of the MBT. Match results of TST and DST was determined largely by the hyperergic reactions (9 people). Interest results of TST (papule with a diameter of 4 mm) and with a diameter of 14 mm papules formation on DST from patient with infiltrative pulmonary tuberculosis in the phase of destruction. Thus, we have got several contradictory data necessitate further investigation with a view to a comparative analysis of the effectiveness of two skin immunologic tests in detecting latent TB infection and its activation is necessary.

It was found that in persons without clinical and radiological signs of active tuberculosis, the level of antibodies determined by the optical density was greater than 0, 14 units (negative) and less than 2, 28 units (positive), in the range from 0, 27 to 1, 36 units. In patients with tuberculosis, the level of optical density varied between 2,5 units (positive) and 2,83 units (positive). Thus, at the detection of latent tuberculosis infection, along with convention research methods, it is possible to use the detection of total antibodies to M.tuberculosis in the range of 0,14-2,28 UOD (units of optical density). Taking in account the above mentioned high specificity and sensitivity of the test it can be assumed that this methods of examination can be successfully used in distinguishing the active and latent course of tuberculosis infection. The syndrome of General disorders revealed in the examined persons, taking into account the level of antituberculosis antibodies, can be interpreted as a set of symptoms of tuberculosis intoxication, with the consequents need for preventive measure, one of which is the appointment of preventive chemotherapy to these persons.

Conclusion. So, detection of total antibodies to MBT in the range 0,14-2,28 UOD is typical for persons with latent tuberculosis infection.

Determination of anti-TB antibodies and their level by ELISA can serve as an additional criterion for the diagnosis of tuberculosis and determine the activity of tuberculosis infection.

The results of ELISA on the determination of anti-TB antibodies can be used to confirm the tuberculosis etiology of the syndrome of General disorders.

In all patients with tuberculosis the results of DST was positive, so diaskintest can be used as one of the indicators of latent tuberculosis infection and its activation.

With the exception of the local form of tuberculosis, persons with positive DST, with the level of anti-TB antibodies in the range 0,14-2,28 UOD and the syndrome of General disorders should be considered as patients with a borderline condition who need preventive anti tuberculosis chemotherapy.

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