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Modern Problem of Non-Tuberculosis Mycobacteria in Uzbekistan: What is the True Picture of the Situation?

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Abstract: The incidence of tuberculosis (TB) in the Republic of Uzbekistan, according to the expert commission of the World Health Organization (2018), tends to decrease. So, in 2011, it was 52.4 per 100 thousand population, and already in 2018 - 42.5 per 100 thousand population. Until now, there is no official statistics on the registration of diseases caused by NTM in Uzbekistan and it is not possible to reliably assess the incidence rate. The study involved 15122 case histories who had applied to The Republican Medical Center of Phthisiatry and Pulmonology under the Ministry of the Health of Uzbekistan. The ATS/IDSA diagnostic algorithm was used as the criteria for non-tuberculosis mycobacteria detection.Out of 15122 patients, nontuberculosis mycobacteria were detected in 61 (0.41%) patients; while Mycobacterium avium Complex - in 19 (0.13%), Mycobacterium fortuitum - in 9 (0.06%). According to the results of a retrospective study of the treatment results it was revealed that NTM are detected annually in biological samples of 0.4% patients.

KeywordsNontuberculosis mycobacteria; pulmonary disease;COPD;GenoType Mycobacterium AS/CM; Chest computed tomography

Introduction

The incidence of tuberculosis (TB) in the Republic of Uzbekistan, according to the expert commission of the World Health Organization (2019), tends to decrease. So, in 2012 it were 52.2

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per 100 thousand populations, and already in 2019 - 42.9 per 100 thousand populations[1,2]. Of these countries, six (belonging to the high TB burden, high multidrug-resistant TB [MDR-TB] burden or high TB/HIV burden groups) reported over 1000 contacts started on preventive treatment: Azerbaijan, Democratic People's Republic of Korea, Peru, Republic of Moldova, Ukraine and Uzbekistan[1].In many economically developed countries, the societal impact of mycobacterial infections recently underwent a change due to a decrease in TB and a simultaneous increase in diseases caused by non-tuberculosis mycobacteria (NTM)[3].The International Statistical Classification of Diseases and Related Health Problems, the tenth revision (ICD-10) includes infections caused by NTM under the heading A31 "Infections caused by other mycobacteria" [4]. In Uzbekistan, modern technologies are being introduced in phthisio-pulmonological practice, for many years cases of detection of NTM in the country have been recorded; nevertheless, the problem of NTM has not been sufficiently studied, and there is no record of diseases caused by NTM. NTM are widely distributed in the environment with high isolation rates worldwide. An increase in the incidence of NTM is evidenced by data from studies conducted in the USA, Germany, Russia, Great Britain, Japan, India, Brazil, China and Republic of Korea [3,5–8].For example, the annual incidence rates of NTM in Great Britain from 5.6/100,000 in 2007 to 7.6/100,000 in 2012, whereas in Japan it increased from 5.6/100,000 in 2007 to 14.7/100,000 in 2015[3]. Of the more than 150 NTM types currently recognized, and about 25 have consistently been associated with NTM diseases in humans and/or animals[9,10]. The environment is the source of human infections and human transmission is thought to be exceedingly rare, though possible transmission events have recently been reported from a cystic fibrosis (CF) clinic and chronic obstructive pulmonary disease (COPD) [5,11]. The symptoms of NTM lung disease – cough, sputum, hemoptysis, fatigue, malaise, and weight loss – are nonspecific and similar to symptoms of pulmonary TB[8,12]. The fact is known, skin disease is the third most common presentation and again includes local disease (mostly caused by Mycobacterium marinum, or by Mycobacterium ulcerans in endemic areas)[12]. Lymphadenitis caused by NTM usually affects lymph nodes in children and immunocompromised patients[8,11,12]. The interpretation of NTM in the sputum of HIV-infected patients presents a particular problem[8]. The main pathogenic agent of the Mycobacteria avium Complex lung disease, a slow-growing mycobacterium found in various environments[2,8,13].This is due to the fact that NTM are still considered safe in epidemic terms (transmission of infection from person to person is considered unlikely) and not subject to mandatory registration, and the methodological approaches to studying heterogeneous populations are different[14]. The reasons for the increase in the incidence and prevalence of NTM are still not definitively determined[11].

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Perhaps this is due to the improvement of laboratory methods for the isolation and identification of NTM, the growth of immunocompromised patients (Predisposing factors include immunocompromised host, HIV infection, diabetes, malnutrition, alcohol, poverty, low socioeconomic status, and crowded environment with improper ventilation)[15,16]. The CT findings of NTM lung disease in patients today is CT findings: MAC and Mycobacteriumchelonae causing inflammation of the lung parenchyma[17].

Materials and Methods:

Study Design

This was a diagnostic accuracy study using the electronic database of NRL in Uzbekistan.

General setting

Uzbekistan is a lower- middle-income country in Central Asia with a population of 33 million, two-thirds of who live in the rural areas. The country is divided into 12 provinces, an autonomous republic (Karakalpakstan), and a capital city (Tashkent). The national effort in fighting TB is chaired by the Republican Specialized Scientific Practical Medical Centre of Phthisiology and Pulmonology under Ministry of Health of the Republic of Uzbekistan. The laboratory network consists of the National Reference Laboratory, 10 regional bacteriological laboratories and district microscopic laboratories. Each regional laboratory is equipped with modern systems for TB diagnosis: Xpert MTB/RIF (Cepheid Inc, Sunnyvale, CA, USA), LPAs (Hain LifeScience GbH, Nehren, Germany) and mycobacterial culture and phenotypic drugs sensitivity testing using BACTEC MGIT (Becton Dickinson and Company, NJ, USA).

Specific setting

In Uzbekistan, TB services are vertically structured and provided at central, oblast, district, and primary health care levels. Funding for the National TB program comes mainly from on external donors, in particular, The Global Fund to Fight AIDS, Tuberculosis and Malaria (Global Fund), The United States Agency for International Development (USAID) and Médecins Sans Frontières (MSF).

Study Population

In the course of this study, the task was set to study and analyze the medical documentation of patients who applied for qualified assistance to the Republican Specialized Scientific and Practical Medical Center for Phthisiology and Pulmonology in the period from 2009 to 2020, who, when examined in sputum,bronchoalveolar lavage fluid, urine, feces, pleural fluid or surgical material were identified with NTM. In total, 48 366 diagnostic samples isolated from 15122 patients with various diseases of the respiratory system were analyzed, the main

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diagnostic samples are sputum, urine and surgical material, as can be seen (Table 1).The age of the patients varied from 20 to 60 years (mean age 47.1 ± 6.5 years). By gender distribution: there were men 64.6%, women 35.4%.

Data sources and variables

The variables related to the study objectives were extracted from the electronic NRL database. These were demographic characteristics of patients (age, gender, region), date when a specimen was received by the laboratory, type of specimen, and results of XpertMTB/RiF Ultra, MGIT and Löwenstein–Jensen medium culture tests.

Specimen collection, storage and preparations

Specimens entering to microbiological laboratory for further testing are divided into two parts; portions A and B. Portion A is for Xpert MTB/RIF Ultra testing and to microscopy, while portion B is was subjected for examination on liquid medium using the MGIT 960 system (Becton, Dickinson and Co) and LJ medium (Becton, Dickinson and Co). The sample preparation is conducted according to the Global Laboratory Initiative (GLI), NRL SOP’s which are based on the WHO recommendations and guidelines [18].

Isolation procedure

Diagnostic samples were processed with N-acetyl-L-cysteine hydroxide sodium (NALC) according to WHO standards and GLI recommendations in biosafety cabinet class 2 [19].Microscopic examination of sputum smears stained and etc. were by the Ziehl-Nelsen or Auramine-O methods (the method is nonspecific, therefore a positive sample test result (ABC +) may indicate the presence of both TB and NTM in the sample) [8,20].The cultivation was carried out on liquid culture medium Middlebrook 7H9 (base broth 0.47% weight/volume 7 ml) with inoculation of 0.5 ml of decontaminated diagnostic sample (BACTEC™MGIT™960 System, Becton Dickinson, USA) [19,21]. The identification of the culture of NTM isolated during growth on days 4-42 was performed using SDMPT64 (SD Bioline TB Ag MPT64 test, Korea). M. tuberculosis complex produce, that is, release more than 30 different proteins are introduced into the nutrient medium, one is - MPT64. It was found that NTM do not produce this protein, thus, the detection of MPT64 in the sample is evidence about the belonging of the studied strain to the M. tuberculosis complex [22,23]. The species of NTM cultures was carried out using the GenoType Mycobacterium AS/CM version 1.0 DNA*strip hybridization technology (The test procedure consisted of three stages: DNA isolation from cultures grown on a solid or liquid medium, multiplex amplification (PCR) with biotinylated primers, reverse hybridization of the DNA of the strain under study with DNA probes specific for the mycobacteria species

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immobilized on a nitrocellulose strip; then the results were taken into account by comparing the ones that appeared on the strip) [24,25].

CT imaging technique

The computed tomography scan (CT)is a medical imaging technique that uses computer processed combinations of multiple X-ray measurements taken from different angles to produce tomographic images of a body, allowing the user to see inside the body without cutting. A CT scan can be used for detecting both acute and chronic changes in the lung parenchyma, the tissue of the lungs. It is particularly relevant here because normal two-dimensional X-rays do not show such defects. The examinations were performed on two scanners: Helical technique included 10-mm collimation for individual scans that were then reconstructed using a standard algorithm for mediastinal-windowed images and a lung or bone algorithm for lung-windowed images. High-resolution CT technique consisted of 1-mm collimated images obtained at 20-mm intervals that were constructed using a high-spatial-frequency algorithm and retrospectively retargeted to each lung with a smaller field of view [26].

Diagnostic criteria

The American Thoracic Society in conjunction with the American Society on Infectious Diseases published a guidelines in 2007/2020 dedicated to diseases caused by NTM, and developed the main diagnostic criteria for mycobacteriosis of the lungs:

Data management and analysis

Data were collected during February 2009 – December 2020, selected variables from the NRL database, which is in MS Exce. We entered selected variables from paper health records into a structured EpiData database created for the purpose (version 3.5.2 Copyright (C) 2019 for entry EpiData Association, Odense, Denmark).

Results

Microbiological data

As a result of identification of NTM cultures using GenoType Mycobacterium AS/CM, 9 types ofNTM were isolated. The total number of NTM for the period from 2009 to 2020 of the total number of 15 122 patients was 61 (0.4%) cases, which are presented in Table 2. Among the slowgrowing, the most common were the NTM species belonging to the Mycobacterium aviumComplex(MAC) - 28 (0.18%), represented by similar microorganisms: non-photochromogenic species Mycobacterium avium–19(0.12%) and Mycobacteriumintracellulare–9 (0.06%) (M. avium complex or MAC includes at least two mycobacterial species, Mycobacteriumavium and Mycobacterium intracellulare. These two species cannot be differentiated on the basis of traditional physical and biochemical tests. There are specific DNA

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probes for identification of and differentiation between Mycobacterium avium and Mycobacterium intracelluare).Mycobacterium gordonae were lesscommon - in 11 (0.07%) cases. M. kansasii wasdetected in 3 (0.01%).,Mycobacterium fortuitum - 11 (0.07%) andMycobacterium chelonae - 4 (0.02%) prevailed (Mycobacterium chelonae is a less common cause of pulmonary disease than Mycobacterium abscessus. The symptoms and radiographic presentation are similar to Mycobacterium abscessus and Mycobacteriumfortuitum). Mycobacteriumabscessus was detected 1 (0.006%).Mycobacterium marinum was detected 1 (0. 006%),Mycobacterium septicumwas detected 1 (0. 006%), and Mycobacterium immunogenumwas detected 1 (0. 006%).

Respiratory tract infections data

Most of the patients were patients with various forms of TB and chronic nonspecific inflammatory diseases of the respiratory system including COPD and CF. The overwhelming majority of patients have respiratory symptoms: cough - in 11 432 (75.6%), dyspnea of varying severity - in 3 825 (25.3%), weakness - in11 870 (78.5%), loss of appetite – in 10 025 (66.3%), weight loss - in 3 402 (22.5%)of cases. The main respiratory complaints in patients with NTM included (n=61): prolonged productive cough with a small amount of sputum –36 (0.59%); shortness of breath - 2 (0.03%); chest pain – 18 (0.29%), weakness – 50 (0.81%), fever – 52 (0.85%), weight loss –44 (0.72%), loss of appetite –58 (0.95%), night sweats –39 (0.63%). At the same time, weakened breathing was heard in patients: rales of various sizes in 23 (0.37%), dry scattered rales in 18 (0.29%), crackling rales in 8 (0.13%) of cases.

CT imaging data

CT imagination of the 61 NTM patients showed the following results of identifying the lung parenchyma with destructive manifestations: focal changes in the lungs, in small and medium focal, disseminations in 27 (44.2%) cases, infiltrative changes in 4 (6.5%) cases, parenchymal interstitial infiltration in 5 (8.1%) cases, CF in 14 (22.9%) cases, lymphadenopathy in 23 (37.7%) cases and a combination of various radiological symptoms in 4 (6.5%) cases.

Discussions

Infection with NTM can rarely lead to symptomatic disease: cervical adenitis in children, pulmonary disease in adults with and without underlying lung pathology, and disseminated disease in patients with HIV infection [27]. Chronic pulmonary infections are among the most common clinical manifestations of NTM disease [28]. Our study highlights the superiority of molecular methods over conventional culture for the diagnosis of NTM disease [29] .

Conclusion

According to the results of a retrospective study of the treatment results of 15 122 patients who

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underwent inpatient therapy at The National Specialized Medical Science Center for Phthisiology and Pulmonology for the period from 2009 to 2020, with suspected respiratory TB, it was revealed that NTM are detected annually in biological samples of 0.4% patients. NTM in the lungs rarely occur extensive spread of the lesion to the surrounding tissue; there are gross pleural changes, respectively, local of the lesion, while the main pleura do not have specific foci. Difficulties in diagnosing NTM of the lungs are included in the fact that the clinical, radiological and histological slimes are similar to those in TB. Isolation of NTM culture most often occurs due to the development of mycobacteriosis of the lungs, however, it can also be caused by the carriage of NTM, which can colonize individual organs and systems of a person (including the respiratory tract) and persist there without causing clinical manifestations of the disease. In terms of gender composition, NTM did not show much difference in the frequency of occurrence in male samples from female ones. Mycobacterium avium Complex is the most commonly found NTM.

Recommendations

Further integration into the work on the creation of national protocols and the creation of a national algorithm for diagnostics in Uzbekistan, the introduction and treatment of patients with NTM and their analysis is necessary. Provide as much NTM inoculum with clear instructions to the microbiological laboratory for the cultivation of mycobacteria. All cultures for NTM should include both broth (liquid) medium for rapid detection and solid media such as LJ. For materials grown from lesions of skin, joints and bones, supplemented culture media and special culture conditions (lower incubation temperatures) should be used in accordance with ATS/IDSA recommendations. Evaluation of a patient with suspected NTM lung disease should include a chest X-ray or chest CT.

Data Availability Statement: The data that support the findings of this study are available from the corresponding author.

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Table 1.Shows the diagnostic samples isolated from patients and sent for microbiological research to the National Reference Laboratory (NRL) (n= 48 366)

Sputum	bronchoalveolr lavage fluid	Stool	Pleuralfluid	Surgicalmaterial	Punctate	Urine
31713 (65.5%)	861 (1.78%)	790 (1.63%)	696 (1.43%)	2267 (4.6%)	706 (1.45%)	11313 (23.3%)

Table 2.Types of NTM identified the period from 2009 to 2020 (n=61):

Title	
M.abscessus	1 (0.016%)
M.avium	19 (0.31%)
M.chelonae	4 (0.06%)
M.fortuitum	11 (0.18%)
M.gordonae	11 (0.18%)

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M.immunogenum	1 (0.016%)
M.intracellulare	9 (0.14%)
M.kansassii	3 (0.04%)
M. marinum	1 (0.016%)
M.septicum	1 (0.016%)

M.- Mycobacterium is a genus of Actinobacteria, given its own family, the Mycobacteriaceae. Over 190 species are recognized in this genus.