

Molecular Diagnosis, Detection and Treatment of Drug Resistant Mycobacterium tuberculosis

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خلاصة:

مرض السل أصاب الكثير من البشر خلال القرون الماضية و عدد الإصابات يزداد عالمياً والسبب الرئيسي هو ازدياد عدد المصابين بمرض الأيدز، و زيادة أعداد المسنين، و زيادة مرض السل المقاوم للعلاج التقليدي. المرض سببه الإصابة بجرثومة "mycobacterium tuberculosis" والاستنشاق هو الطريق الرئيسي للإصابة بهذه الجرثومة، وبالنتيجة يكون الجهاز التنفسي هو الجهاز الرئيسي الذي تظهر عليه أعراض هذا المرض، و مرض السل ينتج عن الإصابة الحديثة بالجرثومة أو نتيجة جرثومة خاملة في الجسم تستعيد حيويتها و نشاطها و بالتالي تسبب المرض.

من الممكن إن يصيب مرض السل أجزاء من جسم الإنسان خارج الجهاز التنفسي والذي تسببه جرثومة خاملة لربما في الرئة، تنشط ثم تنتشر عن طريق الدم أو اللمف من المكان الأولي للإصابة. عادةً ما يكون التشخيص النهائي لمرض السل في الرئة و يعتمد بصورة رئيسية على طرق عدة منها : الفحص النسيجي، زراعة البلغم، التشخيص الجزيئي الوراثي الخلوي للجرثومة المعزولة من غسيل القصبات الهوائية أو من عينة نسيجية.

جرثومة السل يمكن أن يكون لديها مقاومة للأدوية العلاجية فيما إذا كانت مقاومة لنوعين من هذه الأدوية "rifampin and isoniazid" وهي أدوية الخط الأول أو يمكن أن يكون لديها مقاومة عالية إذا كانت مقاومتها إلى أي من مجموعة الـ "fluoroquinolone" بالإضافة لمقاومتها لـ "isoniazid and rifampin" وعلى الأقل مقاومة لواحد من الأدوية الثلاث "amikacin, kanamycin, capreomycin".

ABSTRACT

Tuberculosis has affected humans for centuries and the number of mycobacterium tuberculosis infections have been shown to be increasing worldwide, mainly due the increased number of patients with HIV infection and AIDS disease worldwide, an increasing number of elderly patients and the emergence of multidrug resistant tuberculosis. The disease is caused by a bacterium called Mycobacterium tuberculosis (MT) and is also called tubercle bacilli (TB). Inhalation is the predominant pathway of MT infection, making pulmonary tuberculosis the most common form of tuberculosis. Tuberculosis may arise either from a recent infection with MT, or from the reactivation of dormant bacilli, years or decades after initial infection. Extrapulmonary tuberculosis mainly results from reactivation of a tuberculous focus after hematogenous dissemination or lymphogenous spread from a primary, usually pulmonary focus. Tuberculosis may demonstrate a variety of radiological features depending on the organ site involved and may mimic other pathologies. The final diagnosis of tuberculous

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disease mainly depends on the detection of the causative organism on histopathological examination, culture and molecular diagnostic assay for mycobacterial DNA on material obtained during bronchoscopic washings, fine needle aspiration cytology (FNAC) or biopsy. Tubercle bacilli can be multidrug resistant-TB (MDR-TB), if the tubercle bacilli are resistant to at least isoniazid and rifampin (first-line drugs) or extremely drug resistant-TB (XDR-TB), if the tubercle bacilli are resistant to isoniazid and rifampin, plus resistant to any fluoroquinolone and at least one of the three injectable second-line drugs such as amikacin, kanamycin, or capreomycin.

DEFINITION

Tuberculosis (TB) is a potentially fatal contagious disease that can affect almost any part of the body but is mainly an infection of the lungs. The bacteria can attack the kidney, spine, and brain. It is caused by a bacterium called the tubercle bacillus or *Mycobacterium tuberculosis*. Although TB can be treated, cured, and can be prevented if persons at risk take certain drugs. If not treated properly, TB disease can be fatal.

INTRODUCTION

Tuberculosis, although a curable disease, continues to be one of the most important infectious causes of death worldwide. More than a century after the discovery of the tubercle bacillus after sputum samples are digested and decontaminated. By Robert Koch, tuberculosis remains one of the major causes and serious public health problem worldwide. A syndemic is defined as the convergence of two or more diseases that act synergistically to magnify the burden of disease.

The syndemic interaction between the human immunodeficiency virus (HIV) and tuberculosis (TB) epidemics has had deadly consequences around the world (1).

Tuberculosis remains a serious public health problem in Iraq affecting 16,000 people every year. (2, 3). Each year around 3000 people in Iraq die from this respiratory disease and primarily spreads by coughing and sneezing, however; over the last year the prevalence of the disease decreased as a result of a successful Iraq national tuberculosis program supported by WHO and funded By the Global Fund (4).

Iraq is one of the countries in WHO Eastern Mediterranean Region (WHO-EMR) with a relatively high TB incidence rate (56/100,000) and low case detection rate (45%) (5). Despite the adoption of Directly Observed Therapy Short-Course (DOTS) strategy by World Health Organization (WHO), TB prevalence continues to increase worldwide, particularly in developing countries. This has been attributed to the human immunodeficiency virus (HIV) pandemic and emergence of drug resistant strains also inadequate health care generally attributed to poor performance of NTPS (1), the emergence of MDR-TB in addition to the above mentioned factors. Recent study conducted in Dahouk province indicated that drug resistance pattern in new and previously treated tuberculosis (TB) patients, using molecular fingerprinting methods, a 10.7% of the studied cases (56 isolates) were MDR-TB (6). Such study will help characterize the MDR-TB strains and in turn will assist in evaluating the function of TB control program. According to a 2000–2004 survey of International TB Laboratories conducted by the WHO and US Centers for Disease Control (CDC), 20% of *M. tuberculosis* isolates were MDR-TB and 2% were XDR-TB (1).

ETIOLOGY

The main cause of tuberculosis is MT and also called tubercle bacilli. It is a small aerobic non-motile bacilli. High lipid content of this pathogen accounts for many of its unique clinical characteristics (7) It divides every 16 to 20 hours, an extremely slow rate compared with other bacteria, which usually divide in less than an hour (8).

HISTORY

Historically tuberculosis (TB) has been and today it remains the single greatest cause of mortality due to an infectious agent, and with the increasing prevalence of TBs resistance to the drugs of choice (9,10) the problem posed by TB to public Health should not be underestimated. Tuberculosis, has been recorded in history since the Greco-Roman and Egyptian civilizations, with evidence of spinal tuberculosis being recorded as long ago as 3400 BC. Ancient Indian scriptures also mention this disease (11) with the first known description of tuberculous spondylitis being written in Sanskrit sometime between 1500 and 700BC. However, the modern name of the disease has been attributed to Laennec in the 1800s (12). It has been postulated that M. tuberculosis existed as an unimportant pathogen to man until the coming of the industrial revolution (13). With resulting urbanization and propinquity of living, a new epidemic, described as a great white plague, evolved. In the newly industrialized countries, the incidence of tuberculosis probably increased sharply from the mid 1700s with subsequent pandemic spread throughout Western Europe over the next century and a peak incidence around 1800 (14). Migration probably resulted in spread to the United States, central Africa and also to South and South-east Asia. As recently as 1950 tuberculosis has affected previously completely uninfected and, therefore, non-immune populations, such as the Inuit Eskimos of Northern Canada and the natives of the highlands of Papua New Guinea (15,16). It has been stated that as tuberculosis moves through a nonimmune population, natural selection would eventually result in a resistant population and subsequent gradual decline of the disease pandemic. In most persons, infection with M. tuberculosis is initially contained by host defenses, and the infection remains latent. Belgium faces a resurgence of tuberculosis. After declining for more than a century, notification rates began to increase in the mid 1980s and the long-term downward trend in mortality also shows signs of leveling off (17). Several factors may have contributed to these trends, including immigration from countries with a high prevalence and the epidemic of HIV and AIDS. In addition, other underlying diseases (diabetes mellitus, chronic renal failure, chronic obstructive pulmonary disease, liver cirrhosis, leukemias and lymphomas) and numerous sociological factors contributed to the re-emergence of tuberculosis: a growing elderly population; overcrowded prisons; poor living facilities; poor nutrition status, alcohol and drug abuse; persons in long-term care facilities and homelessness (18,19). Among health care workers, the risk of occupational tuberculosis varies among and within institutions, but workers involved in autopsies and cough-inducing procedures seem to be at higher risk (20). Finally, it is known that immigrants visiting their country of origin can bring back tuberculosis on their return (21). Tubercular decay has been found in the spines of Egyptian mummies.

TRANSMISSION

TB is spread from person to person through the air. When a person with infectious TB disease (TB that can be spread) coughs, sneezes, speaks, or sings, tiny particles containing M. tuberculosis may be expelled into the air are called droplet nuclei, are

about 1 to 5 microns in diameter. Droplet nuclei can remain suspended in the air for several hours, depending on the environment (22). This type of transmission means that when a TB patient exhales, coughs, or sneezes, tiny droplets of fluid containing tubercle bacilli are released into the air. This mist, or aerosol as it is often called, can be taken into the nasal passages and lungs of a susceptible person nearby. Tuberculosis is not, however, highly contagious compared to some other infectious diseases. TB is not passed on by contact with a patient's clothing, bed linens, or dishes and cooking utensils. The fetus of an infected mother may contract TB by inhaling or swallowing the bacilli in the amniotic fluid.

PATHOGENESIS

It is the way TB infection and disease develop in the body. Microbial pathogenicity is usually not attributed to a single contributing factor. It is multifactorial and is the culmination of several adaptations by the organism in order to survive within the hostile environment of the host. These are summarized below:

1. Due to its distinctive architecture and composition, of *M. tuberculosis* cell envelope that contains a large hydrophobic layer of mycolic acids and a vast array of lipids and glycolipids confers extreme hydrophobicity to the outer surface of the organism(23,24). They also can survive long exposure to acids, alkalis, detergents, oxidative bursts, lysis by complement, and many antibiotics.
2. Pathogenic mycobacteria also inhibit phagosome-lysosome fusion (25, 26)
3. A selective advantage to *M. tuberculosis* of staying in an early endosome is that there would be less host immunosurveillance by CD4⁺ T cells. a decrease in the expression of major histocompatibility complex class II (MHC-I) proteins and in the MHC-II presentation of bacterial antigens in macrophages after *M. tuberculosis* infection (27).
4. Mycobacteria can colonize their hosts without the hosts showing any adverse signs.
5. They are naturally resistant to a number of antibiotics that disrupt cell-wall biosynthesis, such as penicillin.
6. Surface and secreted proteins of *M. tuberculosis* contribute significantly to the virulence of this organism. There is an increasing list of extracytoplasmic proteins proven to have a function in the virulence of *M. tuberculosis* (28).
7. TB infection begins when the mycobacteria reach the pulmonary alveoli, where they invade and replicate within the endosomes of alveolar macrophages. The primary site of infection in the lungs is called the Ghon focus, and is generally located in either the upper part of the lower lobe, or the lower part of the upper lobe (29). Bacteria are picked up by dendritic cells, which do not allow replication, although these cells can transport the bacilli to local (mediastinal) lymph nodes and is through the bloodstream to other tissues and organs and establish foci of infection and appear as a tiny white tubercles and is called military tuberculosis. People with this disseminated TB have a fatality rate near 100% if untreated and if treated, the fatality rate is rate is reduced to about 10% (30).

RISK FACTORS

1. People with silicosis have an approximately *30-fold* greater risk for developing TB (31-33)
2. Persons with chronic renal failure and also on hemodialysis have an increased risk (34)

3. Persons with diabetes mellitus have a risk for developing active TB that is two to four times greater than persons without diabetes mellitus (35).
4. Other clinical conditions that have been associated with active TB include gastrectomy with attendant weight loss and malabsorption, jejunoileal bypass, renal and cardiac transplantation, carcinoma of the head or neck, and other neoplasms (e.g., lung cancer, lymphoma, and leukemia) (37).
5. Low body weight is associated with risk of tuberculosis as well. A body mass index (BMI) below 18.5 increases the risk by 2–3 times. An increase in body weight lowers the risk (35, 36). People with diabetes mellitus are at increased risk of contracting tuberculosis,(37) and they have a poorer response to treatment, possibly due to poorer drug absorption.(38)
6. Sharing of needles among IV drug users
7. prolonged corticosteroid therapy and other immunosuppressive therapy
Immunocompromised people (30–40% of people with AIDS in the world also have TB) hematologic and reticuloendothelial diseases, such as leukemia and Hodgkin's disease; end-stage kidney disease; intestinal bypass; chronic malabsorption syndromes; vitamin D deficiency; (39) . low body weight.
8. Twin studies in the 1940s showed that susceptibility to TB was heritable. If one of identical pair of twins got TB, then the other was more likely to get TB (40). These findings were confirmed by studies in South Africa.(41-42). Specific gene polymorphisms in *IL12B* have been linked to tuberculosis susceptibility(43)
9. Some drugs, including rheumatoid arthritis drugs that work by blocking tumor necrosis factor-alpha (an inflammation-causing cytokine), raise the risk of activating a latent infection due to the importance of this cytokine in the immune defense against TB (44).
10. Elderly people, Babies and young children

SYMPTOMS

In an active TB disease, 75% of the cases involve infection in the lungs (pulmonary TB). Symptoms include chest pain, coughing up blood, and a productive, prolonged cough for more than three weeks. Systemic symptoms include fever, chills, night sweats, appetite loss, weight loss, pallor, and fatigue. In the other 25% of active cases, the infection moves from the lungs, causing other kinds of TB, collectively denoted extrapulmonary tuberculosis (40). This occurs more commonly in immunosuppressed persons, elderly and young children. Extrapulmonary infection sites include the pleura in tuberculous pleurisy, the central nervous system in meningitis, the lymphatic system in scrofula of the neck, the genitourinary system in urogenital tuberculosis, and bones and joints in Pott's disease of the spine. An especially serious form is disseminated TB, more commonly known as miliary tuberculosis. Extrapulmonary TB may co-exist with pulmonary TB (46).

WHO SHOULD GET TESTED FOR TB

Persons should get tested for TB by their doctor or local health department if they

- have spent time with a person known or suspected to have active TB disease
- have HIV infection or another condition that weakens the immune system and puts them at high risk for active TB disease
- have symptoms of active TB disease

- are from a country where active TB disease is very common (most countries in Latin America and the Caribbean, Africa, Asia, Eastern Europe, and Russia)
- live somewhere in the United States where active TB disease is more common such as a homeless shelter, migrant farm camp, prison or jail, or some nursing homes)
- inject illegal drugs.

LATENT TB INFECTION (LTBI): This means that the body of infected individual harbor the tubercle bacilli, but the body's immune system contains the spread of the bacilli and keep it under control and inactive. The immune cells surround the tubercle bacilli and form a fence around it.

TB DISEASE: It develops when the immune system cannot contain the tubercle bacilli and the bacilli begin to multiply rapidly. The major differences between LTBI and TB disease is summarized in table 1.

DIAGNOSIS OF TB DISEASE

Persons suspected of having TB disease should be referred for a medical evaluation, which should include:

- Medical history,
- Physical examination,
- Test for TB infection (TB skin test or special blood test),
- Chest radiograph (X-ray), and
- Appropriate laboratory tests

TESTING FOR TB INFECTION

There are two kinds of screening tests that can be used to help detect TB infection – the TB skin test (TST) and TB blood tests. A positive TB skin test or TB blood test only tells that a person has been infected with TB bacteria. **It does not tell whether the person has latent TB infection (LTBI) or has progressed to TB disease.** Other tests, such as a chest x-ray and a sample of sputum, are needed to see whether the person has TB disease. Mantoux tuberculin skin tests tuberculin (also called purified protein derivative or PPD) are often used for routine screening of high risk individuals (47). Interferon- γ release assays are blood tests used in the diagnosis of some infectious diseases. There are currently two interferon- γ release assays available for the diagnosis of tuberculosis: QuantiFERON-TB Gold (licensed in US, Europe and Japan); and T-SPOT.TB, a form of ELISPOT (licensed in Europe). Chest photofluorography has been used in the past for mass screening for tuberculosis. There are three methods of testing, the Mantoux test, the Heaf test and the Tine test. Mantoux tuberculin skin tests are often used for routine screening of high risk individuals.(48) Interferon- γ release assays are blood tests used in the diagnosis of some infectious diseases.

γ -INTERFERENCE TESTING

There are currently three commercially available interferon- γ release assays (IGRAs): QuantiFERON-TB Gold, QuantiFERON-TB Gold In-Tube and T-SPOT.TB. These tests are not affected by prior BCG vaccination, and look for the body's response to specific TB antigens not present in other forms of mycobacteria and BCG (ESAT-6). Whilst these tests are new they are now becoming available globally.

MANTOUX TUBERCULIN SKIN TEST

The TB skin test (Mantoux tuberculin skin test) is performed by injecting a small amount of fluid (called tuberculin) into the skin in the lower part of the arm. A person given the tuberculin skin test must return within 48 to 72 hours to have a trained health care worker look for a reaction on the arm. World Health Organization standardized the method of conducting and reading the results of Mantoux test. 0.1 ml of tuberculin (100 units/ml) is given by intradermal injection into the volar surface of the forearm (subcutaneous injection results in false negative results). A waterproof ink mark is drawn around the injection site so as to avoid difficulty finding it later if the level of reaction is small. The test is read two to seven days afterwards. The area of induration (NOT erythema) is measured transversely across the forearm (left to right, not up and down) and recorded to the nearest millimeter

HEAF TEST

The Heaf test was first described in 1951 (Heaf 1951, pp. 151–3) (47). The test is named after F. R. G. Heaf. Until 2005, the test was used in the United Kingdom to determine if the BCG vaccine was needed; the Mantoux test is now used instead. Also known as the **Sterneedle test**,(48) is administered by a Heaf gun (trademarked "Sterneedle") (49) which is a spring-loaded instrument with six disposable needles arranged in a circular formation (50).

TUBERCULIN TINE TEST. The tuberculin tine test is used to determine whether someone has been infected with the bacteria that cause tuberculosis. It is a screening test for tuberculosis in which an instrument having four sharp prongs dipped in tuberculin antigen is pressed into the skin of the forearm. (51)

TB BLOOD TESTS

TB blood tests measure how the immune system reacts to the bacteria that cause TB. If your health care provider or local health department offers TB blood tests, only one visit is required to draw blood for the test. The QuantiFERON®-TB Gold test (QFT-G), QuantiFERON®-TB Gold In-Tube test (GFT-GIT) and T-SPOT®.TB test are three test approved in the United States. Test results are generally available in 24-48 hours.

LABORATORY TESTS

The WHO reported that 16 countries achieved by the end of 2009, the recommended target of having at least one laboratory with capacity to perform culture per 5 million population, and one laboratory with capacity to perform drug susceptibility testing per 10 million population. Eleven countries are introducing the rapid MDR-TB Xpert diagnostic test (2). There are two types of tests:

- a. Phenotypic methods
- b. Molecular Diagnostic tests

DIAGNOSIS AND TARGETED TESTING

Major advances in molecular biology and the availability of new information generated after sequencing the complete genome of *M. tuberculosis* (52, 53) stimulated the development of new tools for the rapid diagnosis of tuberculosis, differentiation of *M. tuberculosis* from nontuberculous mycobacteria, and For the rapid detection of drug resistance (54). In this review, a summary of the phenotypic and molecular diagnostic tests are discussed.

A. Phenotypic tests

1. Drug susceptibility testing

The Laboratory Branch performs drug susceptibility testing for selected *Mycobacterium* species referred from state or other authorized health facilities. Cultures of mycobacteria are tested by the indirect proportion method with anti-tuberculosis drugs incorporated into 7H10 agar plates.

2. Other Phenotypic Methods:

E Test: The Epsilometer test (usually abbreviated **E test**) is a laboratory test used by microbiologists to determine whether or not a specific strain of bacterium or fungus is susceptible to the action of a specific antibiotic. This is most commonly used in the setting of medicine, where a particular organism has been found to infect a patient, and the doctor treating the patient is seeking guidance on what concentration of antibiotic is suitable.

MODS: Microscopic Observation Drug Susceptibility [MODS] assay is a simultaneous detection and direct drug susceptibility test [DST] method which relies on the characteristic growth of *Mycobacterium tuberculosis* (MTB) in a liquid medium.

Pha B Assay: the phage amplified biologically (PhaB) assay, has been described previously and is based on the inability of susceptible isolates of *M. tuberculosis* to support the replication of bacteriophage D29 in the presence of inhibitory doses of rifampin

Microcalorimeter. Faster methods have been developed, but these tend to be very expensive and are, therefore often unavailable in developing countries. Dr Braissant and his colleagues used a microcalorimeter to detect the growth of *Mycobacterium tuberculosis*. This method proved to be faster than growing the bacteria in the lab and as fast as other more expensive methods (between 5.5 and 12.5 days) (55).

B. Molecular Detection of Drug Resistance (MDDR)

Molecular diagnostic test is utilized DNA sequencing for the detection of drug resistance and rapidly identify multidrug-resistant MT, by detecting mutations most frequently associated with rifampin and isoniazid drug resistance. Additional testing will be conducted to identify mutations associated with resistance to the most effective second-line drugs; fluoroquinolones, amikacin, kanamycin, and capreomycin.

Three genotyping methods to identify MT strains:

1. **Spoligotyping:** Identifies the *M. tuberculosis* genotype based on presence or absence of spacer sequences found in a direct-repeat region of the *M. tuberculosis* genome where 43 identical sequences and 36 base pairs are interspersed by spacer sequences. This highly reproducible method gives results in a standardized 15-digit code that can be easily analyzed and communicated between laboratories and TB programs

2. Variable number of Tandem Repeat- Mycobacterium Interspersed Repetitive Units (VNTR-MIRU):

Distinguishes the *M. tuberculosis* strains by the difference in the number of copies of tandem repeats at specific regions, or loci, of the *M. tuberculosis* genome. Like spoligotyping, this typing method yields results in a standardized code that can be easily analyzed and communicated between laboratories

and TB programs. A total of 41 MIRU loci have been reported; however, most laboratories target only 12 loci. Newer versions of the method include 24 loci, which may increase discriminatory power.

IS6110-based RFLP: This method detects variations in a specific section of the *M. tuberculosis* genome called insertion element IS6110. The first step of RFLP is purification of DNA from an *M. tuberculosis* isolate. A restriction enzyme is added that cuts the DNA into hundreds of different fragments at specific sequences. The fragments are separated by size on an agarose gel and transferred to a membrane. A probe is then used to detect fragments containing IS6110, and the image is captured on film. Each copy of IS6110 produces one band. IS6110-based RFLP patterns containing 7 or more bands provide more specificity in discriminating between isolates than do patterns with 6 or fewer bands. The performance of molecular detection of drug resistance is detailed in table 2.

TREATMENT

TB disease can be treated by taking several drugs for 6 to 12 months. It is very important that people take the drugs exactly as prescribed. If they stop taking the drugs too soon, they can become sick again; if they do not take the drugs correctly, the germs that are still alive may become resistant to those drugs. TB that is resistant to drugs is harder and more expensive to treat. It is recommended that staff of the local health department meet regularly with patients who have tuberculosis to make sure that the patients take their medications. This is called directly observed therapy (DOT). DOT helps the patients complete treatment in the least amount of time.

PRINCIPLES OF TB TREATMENT

The principles of TB treatment are the same for adults and children. The combination regimens used to treat active disease aim to eliminate actively replicating and dormant or near-dormant mycobacteria using a combination of drugs with different actions while preventing the emergence of drug-resistant organisms, and all being achieved with a minimum of toxicity. Bactericidal drugs That kill actively metabolizing and replicating organisms are important to achieve a rapid reduction in microbial load which leads to clinical improvement, contains disease progression and terminates transmission. Isoniazid (H) and rifampicin (R) are Important first-line bactericidal drugs with isoniazid having the Most potent early bactericidal activity. Sterilizing drugs aim to eradicate those organisms that are less active metabolically and those that are in an acidic environment in order to prevent relapse. Rifampicin and pyrazinamide (Z) are important first-line sterilizing drugs. Protection against emergence of drug-resistant organisms is achieved by the combination of effective early bactericidal activity to reduce microbial load combined with effective sterilizing activity of more slowly replicating organisms, and strengthened by the addition of a fourth drug such as ethambutol (E) or streptomycin (S). The recommended treatment regimens for each TB diagnostic Category are also generally the same for children as for adults. The recommended regimens by disease category are reported by WHO (56, 57, 58) as in table 3.

There are several treatment regimens currently in use:

9H — Isoniazid for 9 months is the gold standard (93% effective).

6H — Isoniazid for 6 months might be adopted by a local TB program based on cost-effectiveness and patient compliance. This is the regimen currently recommended in the UK for routine use. The U.S. guidance excludes this regimen from use in children or persons with radiographic evidence of prior tuberculosis (old fibrotic lesions) (69% effective).

6 to 9H- An intermittent twice-weekly regimen for the above 2 treatment regimens is an alternative if administered under Directly observed therapy (DOT).

4R — Rifampin for 4-months is an alternative for those who are unable to take isoniazid or who have had known exposure to isoniazid-resistant TB.

3HR — Isoniazid and rifampin may be given daily for three months.

2RZ — The two month regimen of rifampin and pyrazinamide is no longer recommended for treatment of LTBI because of the greatly increased risk of drug-induced hepatitis and death.

3RPT/INH - three-month (12-dose) regimen of weekly rifapentine and isoniazid.

CONCLUSIONS

Tuberculosis is being considered as one of the main causes of mortality worldwide, its diagnosis in many countries with low-resource and TB-endemic countries, still depends on microscopical examination of sputum smears that lacks sensitivity and specificity. The gold standard for diagnosis of tuberculosis by culture of MT takes several weeks to become positive with additional tests required to confirm its identification as MDR-TB or XDR-TB followed by the proper treatment. For these reasons several rapid molecular tests have been proposed for the rapid diagnosis of tuberculosis. Both in-house and commercial assays are available and they have been evaluated in numerous studies performed in different settings. Real-time PCR-based methods and the LAMP test have also been proposed. LAMP avoids the use of a thermocycler and relies on visual detection of the amplified product appearing as an interesting alternative for implementation in laboratories with limited resources and equipment. Other two tests, the LiPA and the GenoType MTBDRPlus, aimed at MT-drug resistance detection are licensed for use in Europe and are labelled with the 'CE' (Conformit'e Europ'eenne) mark. In a recent policy statement by the WHO on the use of line-probe assays for the rapid screening of patients at risk of MDR-TB. They remain to be used only in smear-positive samples. Current recommendations advice that, in general, the use of molecular tests for the diagnosis of tuberculosis should always be interpreted together with patient clinical information. In Iraq, because of the requirement of equipment, as well as more skilled personnel, and the costs involved, molecular tests have not yet been implemented as routine in tuberculosis diagnostic laboratories, where the lack of proper laboratory infrastructures may hamper a wider implementation of these techniques.

For rapid molecular tests to have a real impact in the diagnosis and better control of tuberculosis:

1. They have to be affordable for low-resource countries, where the burden of tuberculosis is more dramatic.
2. Many molecular tests require dedicated equipment and skilled personnel not always easily available in tuberculosis-endemic settings. More improvements and

simplifications must be explored to make these equipment and techniques simpler and friendlier in order to be adopted by the laboratory networks of the tuberculosis control programs. Additionally, using these equipment stressing the need for high accuracy and strict quality control in all procedures to

be implemented in tuberculosis diagnostic laboratories.

3. Due to the persistence and the increasing rates of drug-resistant tuberculosis around the world and the emergence of new categories of drug resistance, such as XDR-TB, molecular methods for the diagnosis of tuberculosis should not only be directed to the rapid detection of MT in clinical samples but also to the simultaneous detection of the drug of choice for the treatment of these MT strains.

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Table 1. Major differences between LTBI and TB disease

	LTBI	TB disease
Tubercle bacilli	Inactive in the body	Active
Chest x-ray	Usually normal	Abnormal
Sputum smear and cultures	Negative	positive
Symptoms	No symptom	With symptom, such as cough, fever, and weight loss
Infectivity	Not infectious	Infectious
	Not considered a case of TB	Considered a case of TB
Tuberculin skin test	Positive	positive
QuantiFERON TB Gold test	Positive	Positive

Table 2. Performance of Molecular Detection of Drug resistance

	Drug	Gene	Sensitivity (%)	Specificity (%)
First-line MDDR to detect MDR-TB	RIF	<i>rpoB</i>	96.1	97
	INH	<i>inhA + katG</i>	88.6	98.7
Second-line MDDR to detect XDR-TB	FQ	<i>gyrA</i>	82.2	97
	KAN	<i>rrs + eis</i>	86.8	96.9
	AMK	<i>rrs</i>	87.9	99
	CAP	<i>rrs + tlyA</i>	44.6	85.9

Abbreviations: Isoniazid (INH) and rifampicin (RIF) fluoroquinolones (FQ), amikacin (AMK), kanamycin (KAN), and capreomycin (CAP), Molecular Detection of Drug Resistance-tubercle bacilli (MDDR-TB), and extensively drug resistant-tubercle bacilli (XDR-TB)

Table3. Recommended first line drug dosages for children as currently recommended and as previously recommended by WHO

Drug	Currently recommended (56,57) Daily dosage (dose range) in mg/kg	Previously recommended (58) Daily dosage(dose range) in mg/kg	Thrice weekly dosage (dose range)in mg/kg
Isoniazid	10(10-15)	5(4-6)	10(8-12)
Rifampicin	15(10-20)	10(8-12)	10(8-12)
Pyrazinamide	35(30-40)	25(20-30)*	35(30-40)
Ethambutol	20(15-25)	15(15-20)	30(20-35)
Streptomycin	15(12-18)	15(12-18)	15(12-18)
Thioacetazone	No longer recommended	2.5	Notapplicable

*Was not recommended for children of less than 5years of age in 2003