

Isolation and identification of *Mycobacterium tuberculosis* from patients that have relapse infection at Nasseriya province during 2004-2009 year

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Abstract

Mycobacterium tuberculosis that cause disease for inhaled and reach lungs , the disease results from proliferation at organisms and interactions with the host, 3 months or years injected BCG in the normal host, and there the production and development of lesions are caused by healing and multiplication at the lung or progression are determined chiefly by the number of mycobacteria in the inoculum and their subsequent, and the *Mycobacterium* inter the host, tuberculosis is caused by *Mycobacterium tuberculosis* ,persons who have tuberculosis in their lungs can release tiny moulculos contain *Mycobacterium tuberculosis* into the air by coughing ,in 2004 a total of 200 patients (108 male and 92 femal),in 2005 a total of 67 patients (57 male and 10 femal), in 2006 a total of 38 patients (29 male and 9 femal) , in 2007 a total of 28 patients (19 male 9 female),in 2008 a total of 2 patients (2 male and non in female),in 2009 a total of 67 patients (53 male and 14 femal) , the males patints a total of 268 and females patints a total of 134 the total from 2004 to 2009 total of 402 patients , the aim of study isolation and identification of *Mycobacterium tuberculosis* from patients that have relipase infection at Nasseriya province during 2004-2009 year

Keywords: Relipase, Cough fever, phosphatides, Intracellular site.

عزل وتشخيص بكتريا التدرن الرئوي من المرضى الذين حدثت لديهم إنتكاسة في محافظة ذي قار من عام

2009-2004

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الخلاصة

التدرن مرض تسببه بكتريا التدرن نتيجة استنشاق الهواء المحمل بهذه البكتريا وتصيب الرئتين وتؤثر على الجهاز التنفسي واذا لم يتم العلاج بصورة صحيحة ووفق نظام المعالجة يؤدي بعد ذلك الى حدوث أنتكاسة وظهور علامات المرض من جديد وكان عدد المراجعين خلال عام 2004 حوالي 200 مراجع 192 من الذكور و92 من الأناث وعام 2005 حوالي 67 مراجع 57 من الذكور و10 من الأناث وعام 2006 حوالي 38 مراجع 29 من الذكور و9 من الأناث وعام 2007 حوالي 38 مراجع 29 من الذكور و9 من الأناث وعام 2008 حوالي 2 مراجع 2 من الذكور ولا يوجد أي إصابة في الأناث وعام 2009 حوالي 67 مراجع 53 من الذكور و14 من الأناث وان عدد المراجعين الذين حدثت لهم انتكاسة من عام 2004-2009 كان 402 مصاب منهم 268 من الذكور و138 من الأناث من المجموع الكلي 4755 مراجع منهم كان 2842 من الذكور و1913 من الأناث وهدفت الدراسة الى عزل وتشخيص بكتريا التدرن الرئوي من المرضى المصابين والذين حدثت لهم أنتكاسة في محافظة ذي قار من عام 2004-2009.

Introduction

Mycobacterium tuberculosis explained by John Bunyan as the Captain of the men of death tuberculosis was a major threat to human health (Brooks, *et.al.* 2010).

It is a disease of great antiquity thought to have evolved as a human pathogen during the Neolithic period, and its rise has been associated with the change from a hunter-gatherer lifestyle to pastoralism and farming, skeletons from the Neolithic period and Egyptian and Peruvian mummies have evidence of tuberculosis (CDC,2003).

Tuberculosis has been a major cause of death as evidenced by the seventeenth-century bills of mortality in London which indicate that approximately 20% of deaths were caused by tuberculosis. At 1768–1773 pulmonary tuberculosis accounted for 18% of all deaths rising to 25% at twentieth century (Holmberg, 1990). The incidence of tuberculosis in industrialised countries is confounded by a steep rise and after the two world wars, tuberculosis is a disease of overcrowding take place at poor nutrition, HIV has brought about an enormous growth in the incidence of tuberculosis especially in sub-Saharan Africa (Corbett *et al.*, 2003).

Lipids in Mycobacteria, that have include "mycolic acids and waxes, and phosphatides", the lipids in the cell, are largely bound to (proteins and polysaccharides), muramyl dipeptide complexed with mycolic acids can cause granuloma formation; induce caseous necrosis, phospholipids. (Jawetz, *et.al.*2007)

In mycobacterium each type contains several proteins that elicit the tuberculin reaction, proteins bound to a wax fraction can upon injection, induce tuberculin sensitivity, they can also elicit the formation of a variety of antibodies (Kayser, *et.al.* .2005).

Mycobacteria contain a variety of polysaccharides in which their role in the disease is uncertain.(Holset, *et.al.* 2005).

Tubercle bacilli spread in the host by direct extension, through the lymphatic channels and bloodstream, and via the bronchi and gastro intestinal tract in the first infection, tubercle bacilli always spread from the initial site via the lymphatics to the regional lymph nodes, the bacilli may spread farther and reach the bloodstream,

which in turn distributes bacilli to all organs, they are aspirated and distributed to other parts of the lungs are passed into the stomach and intestines or swallowed (Jawetz, *et.al.* .2007).

The positive tuberculin test indicates that an individual has been infected in the past tuberculin positive persons are at risk of developing disease from reactivation of the primary infection, it does not imply that a active disease or immunity to disease is present, tuberculin negative persons (Brooks, *et.al.* 2010)

Patients who are heavily the infection of tuberculosis transmit through the increased coughing when the population migration through war and economic factors has resulted in individuals moving from countries of high endemicity to industrialised countries (Maguire *et al.*,2002).

Materials and Methods

Growth characteristics

Mycobacteria are obligate aerobes and derive energy from the oxidation of many simple carbon compounds Increased CO₂ tension enhances growth biochemical activities are characteristic, and the generation time of most bacteria is slower than others, the pathogenic bacteria was produce pigment and grow more rapidly, to proliferate well (Holt,1994).

Samples collection

The samples collected from persons that come to Nasseriya center for examination chest and respiratory system in 2004 a total of 200 patients (108 male and 92 female), in 2005 a total of 67 patients (57 male and 10 female), in 2006 a total of 38 patients (29 male and 9 female), in 2007 a total of 28 patients (19 male 9 female), in 2008 a total of 2 patients (2 male and non in female), in 2009 a total of 67 patients (53 male and 14 female),

Culture

Selective media contain antibiotics to prevent the overgrowth of contaminating bacteria and fungi, there are three general formulations that can be used for both the nonselective and selective media.

1-Semisynthetic agar media these media (e.g.Middlebrook 7H10 and 7H11) contain defined salts, vitamins, cofactors, oleic acid, albumin, catalase, and glycerol

2. Inspissated egg Media-These media (eg, Lowenstein-Jensen) contain defined salts, glycerol, and complex organic substances ,Malachite green is included to inhibit other bacteria, These media with added antibiotics are used as selective media small inocula in specimens from patients will grow on these media in 3-6 weeks.

3-Broth media- Middlebrook 7H9 and 7H12) support the proliferation of small inoculium, ordinarily, mycobacteria grow in clumps or masses because of the hydrophobic character of the cell surface,if Tweens are added,they wet the surface and thus permit dispersed growth in liquid media, growth is often more rapid than on complex media.

Culture media

The Lowiestien –Jenson medium that use for culturing *Mycobacterium tuberculosis* and incubated for seven days, Mollular-Hinton agar to determinate the activation of antibiotic, (Sensetive or Resistance that using the antibiotic

Drugs

The following antituberculosis drugs are contra indicated in pregnant women

1-Streptomycin, 2-Kanamycin, 3-Amikacin, 4-Capreomcin, 5-Fluoroquinolones

Treatment

1-Isoniazid (INH), 2-Ethambutol, 3-Rifampin (RIF), 4-Pyrazinamide (PZA)

Drug Regimens for Treatment of LTBI (CDC, 2003)

Drug	Duration (month)	Interval	Minimum doses
Isoniazid	9	Daily	270
		Twice weekly	76
Isoniazid	6	Daily	180
			52
Rifampin	4	Daily	120
Rifampin Pyrazinamide	Generally shoud not beofferred for treatment of LTBI		

Data analysis

chi sequire test used for data analysis, groups tests were performed using student (analysis of variance) between year months collect the *Mycobacterium tuberculosis* (Newman,2001).

Results

Table (1) The patients that have relipase infection at Nasseriya province during 2004

Month	Male	Female	Total
January	8	6	14
February	8	7	15
March	13	9	22
April	14	14	28
May	13	5	18
June	8	3	11
July	11	4	15
August	8	10	18
September	5	12	17
October	12	7	19
November	4	7	11
December	4	8	12
Total	108	92	200
$X^2= 15.566,$ $df=11$			$P \geq 0.05$
There is no significant difference			

Table (2) The patients that have relipase infection at Nasseriya province during 2005- year

Month	Male	Female	Total
January	2	-	2
February	2	1	3
March	8	-	8
April	5	-	5
May	5	1	6
June	5	2	7
July	5	-	5
August	7	1	8
September	3	2	5
October	4	1	5
November	5	1	6
December	6	1	7
Total	57	10	67
$X^2=7.981$ $df=11$			$P \geq 0.05.$
There is no significant difference			

Table (3) The patients that have relipase infection at Nasseriya province during 2006- year

Month	Male	Female	Total
January	2	1	3
February	2	1	3
March	4	-	4
April	4	2	6
May	3	1	4
June	4	-	4
July	3	-	3
August	2	2	4
September	2	-	2
October	2	1	3
November	1	1	2
December	-	-	-
Total	29	9	38
$X^2= 7.110$ $df=11$ $P\geq 0.05$ There is no significant difference			

Table (4) The patients that have relipase infection at Nasseriya province during 2007- year

Month	Male	Female	Total
January	1	-	10
February	-	2	10
March	2	1	12
April	4	-	4
May	3	2	5
June	1	-	1
July	1	-	19
August	-	1	18
September	-	1	16
October	3	2	16
November	3	-	13
December	1	-	17
Total	19	9	28
$X^2= 13.940$ $df=11$ $P\geq 0.05$ There is no significant difference			

Table (5) The patients that have relipase infection at Nasseriya province during 2008- year

Month	Male	Female	Total
January	1	-	1
February	1	-	1
March	-	-	-
April	-	-	-
May	-	-	-
June	-	-	-
July	-	-	-
August	-	-	-
September	-	-	-
October	-	-	-
November	-	-	-
December	-	-	-
Total	2	-	2
$X^2= 00.000$ $df=11$ $P\geq 0.05$ No statistics are computed.			

Table (6) The patients that have relipase infection at Nasseriya province during 2009- year

Month	Male	Female	Total
January	4	1	5
February	3	-	3
March	4	1	5
April	3	-	3
May	4	2	6
June	6	1	7
July	7	3	10
August	5	2	7
September	5	1	6
October	3	-	3
November	5	2	7
December	4	1	5
Total	53	14	67
$X^2= 4.197$ $df=11$ $P\geq 0.05$ There is no significant difference			

Table (7) The patients that have relipase infection at Nasseriya province during 2004-2009 year

Year	Relipase tuberculosis in male	Relipase tuberculosis in female	Total
2004	108	92	200
2005	57	10	67
2006	29	9	38
2007	19	9	28
2008	2	-	2
2009	53	14	67
Total	268	134	402
$X^2= 31.931$ $df=5$ $P\geq 0.05$ There is a significant difference			

Table (8) The patients that have relipase infection at Nasseriya province during 2004-2009 year

Year	Total patients that come through -year		
	Male	Femal	Total
2004	623	354	977
2005	580	367	947
2006	414	317	731
2007	477	337	814
2008	481	370	851
2009	267	168	453
Total	2842	1913	4755
$X^2= 15.003$, $df=5$, $P\geq 0.05$ There is a significant difference			

Discussion

A person with tuberculosis disease spread by *Mycobacterium tuberculosis*, that person was to inhale droplet nuclei containing the bacteria, tuberculosis is not spread by brief contact, anyone who shares air with tuberculosis disease of the lungs is an infectious stage is at risk, in 2004 a total of 200 patients (108 male and 92 female), in 2005 a total of 67 patients (57 male and 10 female), in 2006 a total of 38 patients (29 male and 9 female), in 2007 a total of 28 patients (19 male 9 female), in 2008 a total of 2 patients (2 male and non in female), in 2009 was a total of 67 patients (53 male and 14 female), the males patients a total of 268 and females patients a total of 134 the total from 2004 to 2009 total of 402 patients, the total patients that come to Nasseriya center for examination chest and respiratory system from 2004-2009 year, the males patients a total of 268 and females patients was 134 the total from patients from 2004 to 2009 was 4755, male was 2842 and female 1913 at 5% significance level there is no significant difference between months in every year and there is a significant difference between years from 2004 to 2009 at probability ($p \geq 0.05$). The results in the table 1 to table 6 there is no significant difference between months every year and there is significant difference between year at table 7 and table 8, the human was excreted the mycobacterium from the respiratory tract, tuberculosis is a function of the risk of acquiring the infection and the rise risk of acquiring tubercle bacilli depends on exposure to sources of infectious bacilli-principally sputum-positive patients, this risk is proportionate in the population rate of active infection, socioeconomic disadvantage, crowding, and inadequacy of medical care, the development of clinical disease after infection may have a genetic component and by status immunologic, coexisting diseases for example silicosis, diabetes, and other individual host resistance factors. In urban the infection occurs at an earlier age at in rural populations, disease occurs only in a small proportion of infected individuals (Jawetz, et al. 2007).

The incidence of tuberculosis in HIV infections is high minority persons with Primary infection, patients who have had tuberculosis can be infected exogenously

a second time. Endogenous reactivation tuberculosis occurs at persons that have AIDS (Kayser, et al. 2005). The lesion communicates with a bronchus, drains leading to the formation of a cavity, the cavity destroyed the alveoli, cavities are especially difficult to manage conditions for the multiplication, the antibiotic therapy may not be effective, the draining cavities are a source of bacteria that spread to other parts of the lung. Histopathologically the cavity is surrounded by a fibrous the bacteria are found in this outer zone, the lack of T-cell help means that the macrophages are unable to control the growth of bacteria (Kaplan, et al., 2003).

Tuberculosis is spread by the respiratory patients who are sputum smear positive are considered to be infectious and those who have extrapulmonary disease or who are smear negative are not, several factors can influence transmission rates, explain the infective dose and the energy with which they are expelled into the air, bronchoscopy and autopsy exposure is result in infections, transmission is occur in overcrowded conditions (Beggs et al., 2003).

Throughout the twentieth century the average age of tuberculosis patients increased and a similar process was occurring in developing countries until the appearance of the HIV epidemic which reversed this trend, the epidemiology of tuberculosis is quite different from most other infectious diseases, this is due to the relatively long incubation period and the presence of both primary and reactivation forms of disease, Mathematical modeling of the epidemiology suggests that epidemics of tuberculosis are slow to develop peaking between 50 and 200 years after introduction of the disease (Blower et al., 1995). The interaction between HIV and tuberculosis was facilitating the spread of the disease (Corbett et al., 2003).

The directly observed therapy short course (DOTS) strategy was devised, in countries where patients are required to pay for their drugs, the poorest member of the community, most likely to suffer tuberculosis, are likely to stop treatment before bacteriological cure can be achieved. As the patient feels well long before bacteriological cure, this is a major problem in tuberculosis therapy. The homeless and those addicted to drugs a 6-month course of therapy often lack social structures or self-discipline to complete, the DOTS idea depends on the development of effective tuberculosis treatment and control program to deliver directly observed therapy. However, DOTS is not a

panacea. It can be difficult to organise and in some countries the results of such programs have been disappointing (Walley *et al.*, 2001).

The chest X-ray has a vital role in diagnosing pulmonary disease, in addition to identifying patients with changes suspicious of tuberculosis, it can demonstrate the presence of cavities, pleural effusions, empyema and pericardial effusions, CT scanning is also gaining a place in diagnosis of *Mycobacterium tuberculosis* and depended at Bergey's manual (Holt, 1994).

Conclusions

The patients with relapse tuberculosis can use tuberculin tests, x-rays, treatment are not complete if the relapse takes place, use the drug treatment of asymptomatic tuberculin-positive persons in the age groups, these organisms can not. Vaccination with is a substitute for primary infection and BCG is given to children in many countries, the use of BCG is suggested only for tuberculin-negative persons, the eradication of tuberculosis in cattle and the pasteurization of milk have greatly reduced *M. bovis* infections.

Recommendations

Treatment of patients with relapse infection with tuberculosis drug treatment of asymptomatic tuberculin-positive persons in the age groups, HIV infection is a major risk factor for tuberculosis, BCG (bacillus Calmette-Guérin, an attenuated bovine organism), Vaccination with these organisms is a substitute for primary infection with virulent tubercle bacilli, the eradication of tuberculosis in cattle and the pasteurization of milk have greatly reduced *M. bovis* infections.

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