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**COMPARATIVE STUDY FOR SOME TECHNIQUES USED
FOR DETECTION OF *MYCOBACTERIUM TUBERCULOSIS*
IN AL- MUTHANNA PROVINCE**

A THESIS
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BY
NIHAYA AWAD ARYAN AL-DHALMI
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SUPERVISED BY
ASSIST. PROF. DR. HAIDER HAMEED MITAB

Abstract:

The current study aimed to evaluate the interferon gamma release assay (IGRA) in the diagnosis of TB in comparison to other tests (conventional and molecular tests) and to assess the distribution rate of tuberculosis in Al- Muthanna province during the period of October/2015 to June/2016 in advisory clinic for respiratory diseases and thoracic in Al-Samawa city. A sample of five hundred and forty one were collected from suspected TB individuals and TB patients. Samples were subjected to the conventional tests which include AFB, culture and molecular diagnosis (TB GeneXpert MTB/RIF) assay for the detection of *Mycobacterium tuberculosis* (MTB). The positive results for AFB, culture and TB GeneXpert MTB/RIF assay were 10.5%, 15.2% and 12.9% respectively. The diagnostic sensitivity between acid fast bacilli (AFB) smear test and culture were 65.9% and the diagnostic specificity was 99.4%, as well as, the diagnostic sensitivity between culture and GeneXpert test were 84% while diagnostic specificity was 99.8%. In the current study showed that the difference among age groups of PTB patients according to cases rate and present non-significant differences at the level of ($P > 0.05$) between female and male, the highest rate of infection with female (62%) compared with male (38%).

Sixty sputum and blood specimens were taken as cross sectional study is conducted with randomly selected individuals to evaluate the immunological detection of active and non-active tuberculosis compare with control individuals by using T-Spot.TB test, compare with tuberculin skin test (TST), conventional and molecular tests. A total of 60 individuals, the results of T-Spot.TB test were 29 positive and 27 negative specimens, unclear (indeterminate) results in 1 patients and three of patients had T-Spot test results into borderline zone. The diagnostic sensitivity and specificity that estimated for T-Spot.TB test compare to TST were 51% and 47% respectively and the overall rate agreement (ORA) was 50 %. This study concluded that high incidences rate of tuberculosis for Al-Muthanna province. Also the study confirmed the failure of TST test in evaluation of PTB positive and negative incidences in respect of vaccinated or non-vaccinated status and the study showed capability of T-Spot.TB test in diagnosis of active, under- treatment and latent PTB cases with more benefit to diagnose of extra pulmonary cases.

Introduction:

Tuberculosis (TB) is a contagious and airborne disease caused by various species of mycobacteria usually *Mycobacterium tuberculosis* (MTB) (Nagata and Koide, 2012). In 1882, Robert Koch identified MTB as a causative agent of TB (Sakula, 1983). The main cause of TB is *Mycobacterium tuberculosis* a small, aerobic, non-motile bacillus and the high lipid content of this bacteria accounts for several of its distinctive clinical features (Glickman and Jacobs, 2001). TB disease could be pulmonary or extra pulmonary but the pulmonary TB involvement is more important, for the reason that it is considered as a reservoir of diseases in the population (Charati and Moradi, 2013).

Nearly one third of the world's population are infected by TB but it doesn't definitely progress to an active disease because the human immune system protecting against it and in immune suppression conditions, 5 -10% of infected individuals progress to an active TB disease if left untreated can transmit the disease (Charati and Moradi, 2013). The persons with latent TB considered as the biggest reservoir of the tubercle bacilli and detecting these cases is a part of the global plan of TB control (Druszczynska *et al.*, 2012).

Diagnosis of TB is based on assessment of all the findings derived from epidemiology, TB contacts history and TB symptoms, clinical examination and relevant investigations like chest radiography, acid fast bacilli (AFB) test, culture, molecular tests and immunological tests (Nagata and Koide, 2012). Immunological diagnosis of TB is based on the immune responses against MTB (Beata *et al.*, 2012). The most available tool for the diagnosis of Latent TB infection is the tuberculin skin test (TST), which has a very low specificity, and does not differentiate between MTB sensitization and non-tuberculous mycobacterial (NTM) infections or history of *M. bovis* BCG vaccination (Chesov *et al.*, 2015). Interferon-gamma release assays (IGRAs) more accurately diagnose MTB infection than the TST; two IGRAs are commercially available are Quantiferon-TB gold in-tube assay (QFT-GIT) and T-Spot .TB test (T-SPOT) (Fujiwara *et al.*, 2014). These tests measure the interferon gamma (IFN- γ) release after stimulation of blood cells with MTB specific antigens (Connell *et al.*, 2009).

Objectives of the study:

1. Main characteristics of the study population.
2. Screening for tuberculosis by AFB smear, culture and TB GeneXpert tests.
3. Correlating between AFB smear and culture tests.
4. Correlating between culture and TB GeneXpert tests.
5. Comparing between T-Spot.TB with other tuberculosis tests.
6. Correlating between T-Spot.TB and TST tests.

Aims of the study:

1. Evaluating the IGRA in the diagnosis of TB in comparison to other tests (conventional and molecular tests.)
2. Prevalence of tuberculosis in Al- Muthanna province.
3. Prevalence of latent tuberculosis incidences in Al- Muthanna province.

الخلاصة

تهدف الدراسة الحالية لتقييم فحوصات ال (IGRA) في تشخيص مرض السل بالمقارنة مع غيرها من الفحوصات (التقليدية والجزيئية) وكذلك لتقييم معدل انتشار مرض السل في محافظة المثنى خلال الفترة من تشرين الاول/ 2015 وحتى حزيران/ 2016 والتي اجريت في العيادة الاستشارية للأمراض التنفسية والصدرية في مدينة السماوة. تم جمع خمسمائة واحد وأربعون عينة (البلغم) من مرضى السل والافراد المشتبه بهم، وتم اختبار هذه العينات للاختبارات التقليدية التي تشمل AFB، والزرع والتشخيص الجزيئي (TB GeneXpert MTB/RIF) لتحديد بكتريا السل وكانت النتائج ايجابية ل AFB والزرع و TB GeneXpert MTB/RIF (10.5٪، 15.2٪ و 12.9٪ على التوالي). في حين كانت الحساسية التشخيصية بين اختبارات AFB و الزرع (65.9٪) مع خصوصية التشخيصية (99.4٪)، وكذلك، كانت الحساسية التشخيصية بين اختبارات الزرع و TB GeneXpert هي (84٪) مع خصوصية تشخيصية 99.8٪. أظهرت الدراسة الحالية أن هناك اختلاف بين الفئات العمرية من مرضى السل الرئوي وفقا لمعدل الحالات وقدمت الدراسة اختلافات غير ملحوظة عند مستوى ($p > 0.05$) بين الذكور والإناث، وان أعلى معدل للإصابة في الإناث (62٪) مقارنة مع الذكور (38٪). تم سحب ستين عينة دم وبلغم كدراسة عرضية من اشخاص تم اختيارهم عشوئيا من الدراسة الحالية لتقييم الكشف المناعي لمرض السل النشط وغير نشط مقارنة مع اشخاص اعتبروا كسيطرة باستخدام اختبار T-Spot.TB، مقارنة مع اختبار التيوبركولين (TST) tuberculin skin test والاختبارات التقليدية والجزيئية الاخرى. من مجموع ستين شخص كان 29 مريض موجب لاختبار T-Spot.TB و 27 سالب مع نتائج غير واضحة (غير محددة) لمريض واحد وثلاثة من المرضى لديهم نتائج ضمن منطقة borderline zone (كانت الحساسية والخصوصية التشخيصية ل T-Spot.TB مقارنة مع TST هي 51٪ و 47٪ على التوالي، وكان معدل الموافقة (ORA) هو 50٪. تستنتج هذه الدراسة أن هناك ارتفاع في معدل حالات السل في محافظة المثنى. كما أكدت الدراسة فشل اختبار TST في تقييم حالات PTB الإيجابية والسلبية فيما يتعلق بوضعهم محصنين بلقاح السل من عدمه كما وأظهرت الدراسة قدرة اختبار T-Spot.TB في تشخيص حالت السل النشط او مرضى السل تحت العلاج بالاضافة الى تشخيص الحالات الكامنة من السل مع الاستفادة من الاختبار في تشخيص الحالات الخارج رئوية extra pulmonary



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دراسة مقارنة لبعض التقنيات المستخدمة في تشخيص
Mycobacterium tuberculosis في محافظة المثنى

رسالة مقدمة إلى مجلس كلية العلوم/ جامعة المثنى كجزء من متطلبات
نيل درجة الماجستير علوم/ علوم حياة/ أحياء مجهرية

من قبل

نهاية عواد عريان الظالمي

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بإشراف

أ.م.د. حيدر حميد متعب