Dedication

To my mother . . .

To my father . . .

To my sisters and brothers . . .

To my teachers . . .

To my friends . . .

To all whom I love in ALLAH . . .

I

dedicate this effort . . .

Acknowledgment

First of all my thank to Almighty Allah for giving me the courage, ability and strength to accomplish this work, I'm deeply indebted to my supervisor Dr. Mogahid Mohamed Elhassan, Head Department of Microbiology, Sudan University of Science and Technology, for facilitating the completion of my research by providing support, advices and encouragement through out the course of the study. Thanks are also extended to the members of the Tuberculosis Reference Laboratory, National Health Laboratory, especially Miss Eman Osman, Ahmed Hassan, Ramia Mohamed and Miss Nuha Yousif for providing facilities, help and integration.

Abstract

This study aimed to identify and characterize Non-Tuberculous Mycobacteria (NTM) among suspected pulmonary tuberculosis / HIV co-infected patients in Khartoum state by using conventional methods and PCR technique.

171 sputum and blood samples were collected from patients attending Abu Anga Hospital, The Reference Tuberculosis Laboratory, ELshaab Teaching Hospital, Ibrahem Malek Hospital, Omdurman Teaching Hospital, and the Academy Charity Hospital during the period from January to March 2010.

HIV screening tests has been conducted to all patients, 25 (14.6%) were found positive while 146 (85.4%) were negative.

All the 171 samples were tested for the presence of acid fast bacilli by using direct Ziehl-Neelsen stain, 37 (21.6%) were positive while 134 (78.4%) were negative. All sputum samples were cultured in Lowenstein Jensen media. 40 (23.4%) of the isolates gave a characteristic growth of *Mycobacterium tuberculosis* and six out of 10 (5.8%) rapidly grower isolates were confirmed by the indirect Z.N stain, culture characteristics and biochemical properties as NTM.

In this study, 112 (69.6%) males were found to be affected compared to 52 (30.4%) females. Age groups between 21-49 years were more affected with both *M. tuberculosis* complex and NTM.

21 (84%) out of 25 patients with positive HIV were found infected with MTB while 4 (16%) were infected with NTM.

Ten rapidly grower isolates were subjected to PCR. Six isolates showed a band typical in size (136 bp) to target gene (*rpoB*) as indicated by the standard DNA marker. Four isolates were found negative.

From this study, it is obvious that NTM infections are frequent in HIV infected patients.

The results obtained from this study revealed clearly the importance of conventional methods in the diagnosis of TB and NTM as well as the effectiveness of PCR as confirmatory diagnostic tool.

الهدف من هذه الدراسة هو عزل المتفطرة اللا سلية بين المرضي الذين يشتبه فى اصابتهم بمرض السل الرئوى بالاضافة لاصابتهم بفيروس نقص المناعة البشرية في ولاية الخرطوم باستخدام الطرق التقليدية واختبار البلمرة التسلسي .

تم جمع 171عينة قشع ودم من مرضى حضروا لمستشفي أبو عنجة ، المعمل القومي المرجعي للدرن, مستشفى الشعب التعليمي, مستشفى إبراهيم مالك, مستشفى الأكاديمية الخيري ومستشفى أم درمان التعليمي خلال الفترة من يناير إلى مارس 2010. أظهر فحص فيروس نقص المناعة البشرية 25 (14.6٪) من المرضى نتائج ايجابية في حين 115 (83.6٪) اظهروا نتائج سلبية.

كل العينات خضعت لفحص وجود العصيات المقاومة للاحماض باستخدام صبغة زيل نلسون ، 37 (21.6٪) عينه كانت موجبه, بينما 134 (78.4٪) عينة كانت سالبة. جميع 171عينه تم تزريعها في وسط ليونيستين جنسن. 40 (23.4٪) من العينات المعزولة أظهرت خصائص عصيات الباكتيريا المتفطرة الدرنية. ستة من أصل 10 عزلات سريعة النمو تم التعرف عيها كمتفطرة اللا سلية اعتمادا علي خصائصها المجهرية غير المباشرة, المزرعية و الاختبارات البيوكيميائية.

في هذه الدراسة وجد أن 112 (69.6 ٪) من الذكور كانوا مصابين مقارنه مع الإناث 52 (30.4٪). بينما وجد أن الفئة العمرية بين 21-49 سنة هم الأكثر عرضة للإصابة بالمتفطرة السلية والمتفطرة اللا سلية.

من أصل 25 مريض مصابين بفيروس نقص المناعة البشرية ، 21 (84٪) وجدوا مصابين بالمتفطرة السلية و 4 (16٪) مصابين بالمتفطرة الغير سلية .

عشر عزلات من المتفطرة سريعة النمو, تم إختبارها بواسطة تفاعل البلمرة التسلسلي. ستة عزلات أظهرت حزمة مطابقة في القياس (**bp136**) للجين المستهدف **(rpoB)** كما هو مشار إليه بواسطة المؤشر القياسي للحامض النووي الرايبوزي منزوع الأوكسجين.

النتائج التي تم الحصول عليها من هذه الدراسة كشفت بوضوح أهمية الأساليب التقليدية في تشخيص المتفطرة السلية والغير سلية, وأيضا فعالية تفاعل البلمرة التسلسلي كاختبار تشخيصي **تأكيدي.**

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List of abbreviations

AFB	Acid Fast Bacilli
AIDS	Acquired immunodeficiency syndrome
BC	Before Century
BCG	Bacillus Calmette-Guerin
DNA	Deoxy Nuleic Acid

HIV	Human immunodeficiency virus
IUATID	International union against tuberculosis and lung disease
LJ	Lowenstein Jensen
MOTTS	Mycobacteria other than tuberculosis
MTB	Mycobacterium tuberculosis
NTM	Non tuberculous mycobacteria
PCR	Polymerase chain reaction
PNB	Para-nitrobenzoic acid
PPEM	Potentially pathogenic environmental mycobacteria
RGM	Rapid growing mycobacteria
rRNA	Ribosomal Ribo nucleic acid
TB	Tubercles bacillus
TBE	Tris base EDTA
TCH	Thiophene-2-carboxylic acid hydrozide
Bp	Base pair
dNTP	deoxy nucleotide triphosphates
dw	deionized water
NAOH	Sodium hydroxide
NAP	nitro-alpha-acetylamino-beta-hydroxy propiophenone
UK	United kingdom
USA	United states American
UV	Ultra violet
WHO	World health organization
ZN	Ziehl Neelsen

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