Sudan University of Science and Technology

College of Graduate Studies

Feasibility and Acceptability of Antimycolic Acids Antibodies in the Diagnosis of Pulmonary Tuberculosis in TB/HIV Co-infected Patients

الجدوى والمقبولية لتشخيص السل الرئوى بالأجسام المضادة لحمض المايكوليك في مرضى السل الرئوي المصاحب لمرض نقص المناعة المكتسبة

A dissertation submitted for partial fulfillment for M.Sc. Degree in Medical Laboratory Science (Microbiology)

By

Ossama Abdelrahman Elhassan

(B.Sc in Medical Laboratory Science (Microbiology), 2006)

Supervisor

Dr. Mogahid Mohamed Elhassan

Ph.D in Medical Laboratory Science (Microbiology)

JUNE 2010

الآيـــــة

(رَبِّ أَوْزِعْنِي أَنْ أَشْكُرَ نِعْمَتَكَ الَّتِي أَنْعَمْتَ عَلَيَّ وَعَلَى وَالِدَيَّ وَأَنْ أَعْمَلَ صَالِحاً تَرْضَاهُ وَأَدْخِلْنِي بِرَحْمَتِكَ فِي عِبَادِكَ الصَّالِحِينَ }

صدق الله العظيم

سورة النمل ، الآية (19)

Dedication

I would like to dedicate this research

to all those who always have been there for me.

To my Father and mother

To my wife and kids ..

With much love ….

Osama

Acknowledgment

First of all, thanks for Allah who gave me the power for preparation and completion of this study.

With a great deal of respect I would like to express my thanks to my supervisor Dr. Mugahid M. El hassan, Head of Microbiology Department, College of Medical Laboratory Science, Sudan University of Science and Technology for his advice, enthusiasm, help and endless guide.

My thanks are extended to my colleagues for their kind support and their beautiful friendship.

I would also like to thank my old and little family for their patience and support.

Abstract

This study aimed to dertermine antimycolic acid antibodies [IgG and IgM] among TB/HIV Co-infected patients in Khartoum State.

Sputum and blood specimens were collected from patients attended to Alsha'ab Teaching Hospital, Tubercolosis Reference Laboratory, Ibrahim Malik Hospital and Abu Anga Hospital, patients were all ready informed.

Direct smears from 90 sputum of suspected patients showed that 17 (18.9%) were acid fast bacilli, while 73 (81.1%) were non acid fast. The ninety sputum specimens were subjected to PCR to ampilify IS6110. The result showed that 79 (87.8%) were positive for IS6110 while 11 (12.2%) were negative.

The 90 serum samples were investigated for HIV using dot polt technique,were 9 samples (10%) were found HIV positive and they were all TB positive by PCR.

80 Serum samples were analysed by ELISA, 16 (20%) gave positive result for antimycolic acid IgG while 64(80%) were negative and 55 (68.8%) were positive for antimycolic IgM, while 25(31.3%) were negative. In correlation between HIV and antibodies for antimycolic acids, in 6 HIV positive samples one (31.3%) was positive and 5 (6.3%) negative samples for IgG, and in correlation between HIV and IgM, in the 6 HIV positive

there were 2 (2.5%) IgM positive and 4 (5%) IgM negative samples.

This study concluded that patients with TB/HIV Co-infection have less antimycolic acids antibodies.

ملخص الدراسة

هدفت هذه الدراسة للتعرف علي الاجسام المضادة من فصيلة IgG و IgM الخاصة لحمض المايكوليك لدى المرضى المصابون بالسل الرئوي المصاحب لمرض نقص المناعة المكتسبة بولاية الخرطوم.

جمعت عينات الدم و التفاف من مستشفى الشعب التعليمي ومعمل الدرن المرجعي ، مستشفى ابراهيم مالك ومستشفى أبو عنجة. المرضى تم إعلامهم بهذه الدراسة.

المسحات التي أخذت من 90 عينة تفاف تم صبغها بالذيهل نيلسن 17 (18.9%) وجدت موجبة و 73 (81.1%) سالبة. أما من جملة الـ 90 عينة تفاف التي تم فحصها باختبار البلمرة التسلسلي وجد أن 79 (87.8%) كانت موجبة و 11 (12.2%) سالبة. 90 عينة دم تم فحصها لمرض المناعة المكتسبة بطريقة الاختبار المناعى بتقنية الفصل النوعى ، 9 عينات (10%) كانت موجبة. العينات الموجبة لمرض نقص المناعة المكتسبة كلها كانت موجبة لمرض السل الرئوي عندما تم فحصها عن طريق اختبار البلمرة التسلسلى.

80 عينة دم تم تحليلها بواسطة الاختبار المناعى المرتبط بالانزيم وجد 16 (20%) عينة كانت موجبة للجاما قلوبيولين و 64 (80%) كانت سالبة.اما اختبار الاميونوقلوبيولين ام كانت 55(68,8%) موجبة و 25(31,3%) سالبة. أما عندما أجريت المقارنة مع المرضى المصابين بمرض نقص المناعة المكتسبة من بين 6 مصابين بمرض نقص المناعة المكتسبة وجد شخص واحد موجب الجاماقلوبيلين وذلك يعزى للنقص الشديد في الأجسام المضادة في الدم. بينما وجدت عينتان موجبتان

للقلوبيولين المناعى M مقابل اربعة عينات سالبة من اصل 6 عينات تم إجراء الاختبار المناعي المرتبط بالإنزيم لها.
لقد وضح لنا في هذه الدراسة أن المرضى الذين يعانون من مرض نقص المناعة المكتسبة يقل عندهم أجسام مضادة لحمض المايكوليك الموجودة في العصيات السلبية للسل الرئوي.

LIST OF CONTENTS

Topic	Page
آية من الـ قرآن الكريم	I
Dedication	II
Acknowledgment	III
Abstract (English)	IV
Abstract (Arabic)	VI
List of content	VIII
List of Figures	XI
List of Plates	XI
CHAPTER ONE INTRODUCTION AND OBJECTIVES	
1.1 Introduction	1
1.2 Rationale	3
1.3 Objectives	3
1.3.1 General objective	3
1.3.2 Specific Objectives	3
CHAPTER TWO LITERATURE REVIEW	
2.1 Mycobacterium	4
2.1.1 Taxonomy	4
2.1.2 Cell Wall Structure	5
2.1.3 Mycolic Acids	6
2.2.1 Nutritional and Environmental Requirements for Growth	7
2.2.2 Generation time	7
2.2 Epidemiology	8
2.2.1 Epidemiology of TB among HIV patients	9
2.3 Pathogenesis	9
2.3.1 Clinical Features	9
2.3.1.1 Miliary Tuberculosis	10
2.3.1.2 Adult Post – Primary Pulmonary Tuberculosis	11
2.4 Tuberculosis and the interaction with the HIV epidemic	12
2.5 Diagnosis	14
2.5.1 Conventional Diagnostic Methods	14
2.5.1.1 Microscopic techniques	14
2.5.1.2 Traditional Culture Techniques and Colony Morphology	16
2.5.1.3 Biochemical Tests	17
2.5.2 New Diagnostic methods	18
2.5.2.1 Polymerase chain reaction	18
2.6 Treatment	18
2.7 Prevention and control	19

CHAPTER THREE
MATERIAL AND METHODS

3.1 Study Design	21
3.1.1. Type of The Study	21
3.1.2. Study Area	21
3.1.3. Study Population	21
3.1.4. Data Collection	21
3.2 Asepsis and sterilization	21
3.2.1 Hot air oven	21
3.2.2 Disinfection	21
3.3 Method of samples collection	21
3.3.1 Sputum Sample	21
3.3.2 Blood Sample	22
3.4 Methods Identify *M. tuberculosis*	22
3.4.1 Zeihl Neelsen Stain	22
3.4.2 Molecular Identification (PCR)	22
3.4.2.1 DNA Extraction From Sputum	22
3.4.2.2 Primers of Insertion Sequence *IS6110*	23
3.4.2.3 Preparation of PCR Mixture	23
3.4.2.4 PCR Amplification	23
3.4.2.5 Preparation of Agarose Gel	24
3.4.2.6 Visualization of PCR Product	24
3.4.3 Searching for Antimycolic Acid Surrogate	24
3.4.3.1. Material	24
3.4.1.1 PBS buffer	24
3.4.3.1.2 Diluting buffer	24
3.4.3.1.3 Blocking buffer	24
3.4.3.1.4 Washing buffer	25
3.4.3.1.5 Coating antigens	25
3.4.3.1.6 Conjugates	25
3.4.3.1.7 Substrate	25
3.4.3.1.8 Substrate buffer	25
3.4.3.1.9 Human Sera	25
3.4.3.1.10 Plastic ware	25
3.4.3.2 Method for ELISA Assay	25
3.4.3.2.1 Antigen Coating of ELISA Plates	25
3.4.3.2.2 Blocking of ELISA Plates	25
3.4.3.2.3 Binding of Human Antibodies	26
3.4.3.2.4 Detection of the Bound Antibodies	26
3.5 Methods for HIV detection	28
3.5.1 Materials for HIV ½ screening test (ICT)	28
3.5.2 Method of ICT	28

CHAPTER FOUR RESULT	
4.1 Demographic Data	27
4.1.1 Gender	27
4.1.2 Age	28
4.2 Bacteriological Findings	29
4.2.1 Ziehl-Neelsen Staining	29
4.3 Polymerase Chain Reaction	30
4.4 Correlation between the ZN Stain and PCR	31
4.5 Detection of HIV among tuberculosis patients	32
4.6 Correlation between HIV and antimycolic acid IgG	33
4.7 Correlation between HIV and antimycolic acid IgM	34
CHAPTER FIVE DISCUSSION	
Discussion	38
CHAPTER SIX CONCLUSION AND RECOMMENDATIONS	
6.1 Conclusion	40
6.2 Recommendations	40
References	41
Appendices	45

List of Figures

Figure No.	Figure Name	Page
1	Distribution of the study population according to gender	27
2	Distribution of the study population according to age	28
3	Result of ZN stain among target population	29
4	Polymerase chain reaction test	30
5	The amplicon of *M. Tuberculosis* after first run of PCR on 1% agarose gel as follow: Ladder = DNA marker; 2 negative control; 3 = control positive; 4-11 = patients samples = 123 bp bands of M. Tuberculosis.	31
6	Correlation between ZN Stain and PCR	32
7	The result of HIV among target population by using ICT.	33
8	Antimycolic acid IgG for HIV positive and HIV negative individuals	34
9	Antimycolic acid IgM for HIV positive and HIV negative individuals	35
10	Plate of ELISA for Anti-IgG anti-mycolic acid	36
11	Result of ICT for HIV patient Left: positive result, Right: negative result.	37