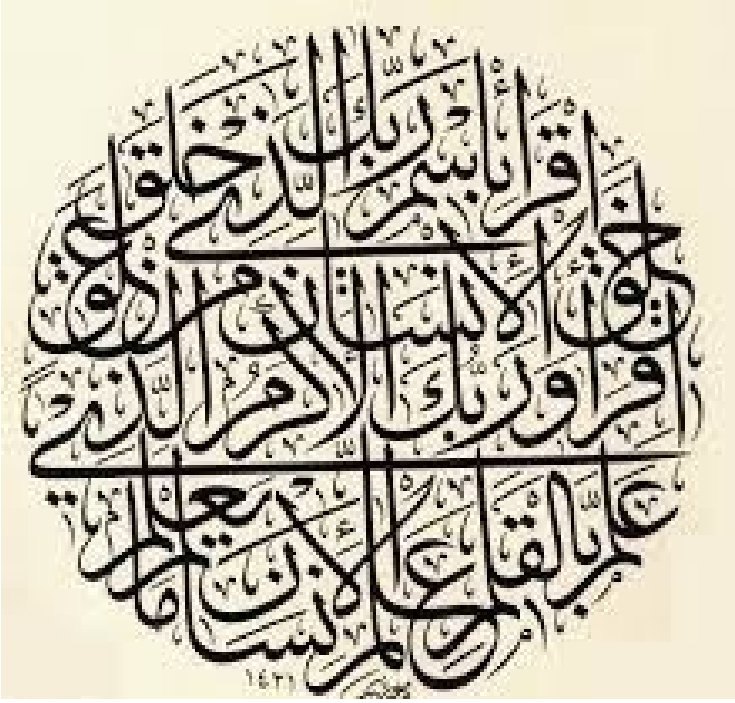
بسم الله الرحمن الرحيم



صدق الله العظيم

Dedication

This study is dedicated to my parents and my lovely family for their unconditional support, and for all Sudanese children.

Acknowledgements

All thanks to Allah for giving me courage and the determination, as well as guidance in conducting this research, despite all difficulties.

Special thanks to my great father for his moral and financial support.

Many people have contributed to my success in completing this study. I was fortunate to have an outstanding supervisor. Dr. NageebSuliman and Co-supervisor Dr. Mogahid Mohammed El Hassan who is a wonderful teacher and mentor, and I want to thank him for all of his help from the first time we talked about a study topic to my final discussion where he provided the leadership for success.

Special thanks to my colleagueHiba Abdul Azim for her assistance, encouragement and support during this research, I also must acknowledge staff of Tuberculosis Department in National Reference Laboratory and staff of research laboratory in Sudan University of Science and Technology for their help by opinions and advices out ofoutstanding experience in this aspect. Finally, thanks to my family, my wife and mother, thanks for listening and understanding when I responded to your many requests with “I need to be working on my paper.”

Table of Contents

List of Tables

List of Figures

List of Abbreviations

AFB Acid Fast Bacilli

ATSAmerican Thoracic Society

BCG Bacillus Calmette-Guerin

CPCCetylpyridinium chloride

CDCCenters for Disease Control

DNA deoxyribonucleic acid

DOTSdirectly observed treatment, short-course

EtBrEthidiumBromide

HIV human immunodeficiency virus

IFN-γ interferon-gama

LJLöwenstein-Jensen

LMlipomannan

LAMlipoarabinomannan

MDR-TB multiple drug resistance tuberculosis

MTB*Mycobacterium tuberculosis*

mAGPmycolylarabinogalactan–peptidoglycan

NAAT Nucleic Acid Amplification Techniques

NGOsnon-governmental organizations

NTM non-tuberculous mycobacteria

PCR polymerase chain reaction

PIMsphosphatidylinositol mannosides

PZApyrazinamide

TB tuberculosis

TST tuberculin skin test

WHOWorld Health Organization

ZNZiehl-Neelsen

ABSTRACT

Tuberculosis (TB) is a chronic contagious disease which has a major impact on global public health problem. This study was carried out in Khartoum state during the period from January 2011 to December 2013 to improve detection of*Mycobacterium tuberculosis*in children with symptoms of tuberculosis infection using different conventional and advanced diagnostic techniques. One hundred ninety seven specimens of gastric lavage and sputum were collected from different hospitals in Khartoum State including ElbolokHospital, Jafar Ibn Owf Hospital, Elasha'ab Teaching Hospital, Soba University Hospital and Academiy Charity Hospital.

All childrenparticipating in the study were subjected to mantoux test after obtaining appropriate consent injected by 5 tuberculin units of Tuberculin purified protein derivative and the results were taken after three days.Specimens were decontaminated and inoculated on Lowenstein Jensen mediaaccording to modified Pettrof's method, Two smears were prepared and stained by Ziehl-Neelsen stain and Auramin fluorescent dye, bacterial DNA was extracted from each specimen by using phenol chloroform method, and then the Polymerase Chain Reaction technique was adopted to detect Insertion Sequence IS6110 gene of *M. tuberculosis*inthese specimens. This study showed that the positive results for TST, ZN, Auramin, Culture and PCR were 86 (43.7%), 16 (8.1%), 22 (11.2%), 32

(16.2%) and 35(17.8%) respectively. The study concluded that the PCR technique is a most sensitive and specific technique for a fast identification of *M. tuberculosis*in gastric lavage and sputumfrom children who are unable to expectorate good quality sputum sample or diagnosed as negative using conventional diagnostic methods.

الخلاصة

مرض الدرن مزمن ومعدي وله تأثير علي كل المجتمع الطبي في العالم. أجريت هذه الدراسة بولاية الخرطوم في الفترة من يناير 2011 الى ديسمبر 2013 للكشف عن المتفطرة السلية في الاطفال الذين يعانون من اعراض مرض الدرن بأستخدام وسائل تشخيص تقليدية ومتطورة مختلفة. مائة وسبعة وتسعين عينة من غسيل المعدة والقشع تم جمعها من مستشفيات مختلفة بولاية الخرطوم وتتضمن مستشفى البلك للاطفال ، مستشفى جعفر ابن عوف للاطفال، مستشفي الشعب التعليمي، مستشفي سوبا الجامعي و مستشفي الاكاديمي الخيري.

تم حقن جميع الاطفال المشاركين بالبحث بخمس وحدات من التيوبركلين وتم اخذ النتائج بعد ثلاثة ايام من الحقن. تمت عملية ازالة التلوث من جميع العينات ومن ثم تم تزريعها بوسط لونستن جونسن بناء على طريقة بتروف المعدلة ، تم اعداد مسحتين من كل عينة وتم صبغهما بصبغة زيل نلسون و صبغة الاورامين المتألقة، تم استخلاص الحمض النووي للجرثومة بواسطة طريقة الفينول كلوروفورم ومن ثم تم اجراء تقنية تفاعل البلمرة المتسلسل للكشف عن جين المتفطرة السلية.

أظهرت الدراسة نتائج ايجابية لفحص التيوبركلين الجلدي، صبغة زيل نيلسون، صبغة الاورامين، طريقة التزريع و تفاعل البلمرة المتسلسلة بنسب كالاتي 86 (43.7%) , 16 (8.1%) , 22 (11.2%) , 32 (16.2%) و 35 (17.8%) علي التوالي. خلصت الدراسة الي ان تقنية تفاعل البلمرة المتسلسل هو الاكثر حساسية وتخصصية للكشف السريع عن المتفطرة السلية بعينات غسيل المعدة والقشع من الاطفال غير القادرين علي اعطاء عينة جيدة للقشع او المشخصين بنتائج سلبية بواسطة استخدام طرق التشخيص التقليدية.