

قال الله تعالى

وَقُلْ اَعْمَلُوا فَسَيَرَى اللّٰهُ عَمَلَكُمْ وَرَسُولُهُ وَالْمُؤْمِنُونَ ^ص وَسَتَرَدُّونَ
إِلَىٰ عَالِمِ الْغَيْبِ وَالشَّهَادَةِ فَيُنَبِّئُكُمْ بِمَا كُنْتُمْ تَعْمَلُونَ

صدق الله العظيم

سورة التوبة الآية ١٠٥

DEDICATION

*To the memory of my brother Ali
May his love, devotion to family, and
his zest for life remain
a constant source of inspiration and
his memory always be for a blessing..*

To

*My father & mother...
Who provide me hopes, happiness, and
successfulness..*

To

*My brothers & sisters..
For their support & kindness*

To

All persons whom I loved

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Abstract

Simple, rapid, and sensitive methods that enhance the detection of *Mycobacterium tuberculosis* (*M. tuberculosis*) from sputum specimens are needed. This study compared the sensitivity of ZN stain and polymerase chain reaction (PCR) in the detection of *M. tuberculosis* from sputum specimens to achieve early and proper diagnosis in order to start accurate regimen.

this is a cross-sectional laboratory-based study in which ١٧١ sputum specimens were collected from patients suspected of having pulmonary tuberculosis attending Abu-Anga Teaching Hospital, El Sha'ab Teaching Hospital and the Tuberculosis Reference Laboratory at the national Health Laboratory in Khartoum, Sudan, during the period from January to March 2010.

Sputum specimens were examined using Ziehl-Neelsen stain; all sputum specimens extracted by Isopropanol method to obtain DNA and subjected to PCR amplified IS6110 insertion sequence in terms of sensitivity. At same time all sputum specimens were inoculated in Lowenstein Jensen (LJ) media and incubated at 37°C.

Only 37(22%) were acid fast bacilli positive, whereas 145(85%) were PCR positive with bands typical to the target sequence of IS6110 as showed by the standard DNA marker, Ziehl-Neelsen technique sensitivity was found to be ٢٦% compared to PCR assay.

Concerning cultivation of all sputum specimens on LJ media there were only 23.4% showed MTC-like colonies in LJ media (dry, rough and pale yellow), whereas 5.8% were considered rapidly growing *Mycobacterium*, and 70.8%

samples revealed contamination or no growth. Further biochemical tests were used beside colonial morphology in order to confirm the existence of *Mycobacterium tuberculosis* complex.

The study concluded that though the sensitivity of ZN stain was quietly decreased, PCR assay provides a significant improvement in diagnosis of pulmonary tuberculosis.

ملخص الاطروحه

للحوجه الماسه لاكتشاف طرق بسيطه ،سريعه، وحساسه تساعد على تشخيص المتفطره السليه المسببه للسل الرئوي هدفت هذه الدراسه لتقارن حساسية صبغة زيل – نلسون وتفاعل البلمره التسلسلي في التعرف على المتفطره السليه من عينات البلغم.

تم جمع ١٧١ عينه بلغم لمرضى اشتبهوا في اصابتهم بالسل الرئوي و أحضروا من مستشفى أبو عنجه التعليمي، مستشفى الشعب التعليمي، والمعمل المرجعي للدرن بالخرطوم، السودان، في الفترة من يناير إلى مارس ٢٠١٠.

تم فحص العينات باستخدام صبغة زيل – نلسون و تم أيضا استخراج الحمض النووي الريبي منقوص الأكسجين من جميع عينات التفاف بطريقة الأيسوبروبانول وتم إختبارها بواسطة تفاعل البلمرة التسلسلي للتحديد حساسيه صبغة زيل – نلسون. وفي نفس الوقت قد تم تزرير كل العينات بوسط ليونيستين جنسن وتحضيئها تحت درجة حراره 37°C .

اظهرت النتائج ٣٧ (٢٢%) عينه موجبة لصبغة العصويات المقاومة للأحماض بينما 145 (٨٥%) عينه كانت موجبه لتفاعل البلمره التسلسلي ذلك أنها أظهرت حزمة مطابقة في القياس للمستهدف *IS 6110* كما هو مشار إليه بواسطة المؤشر القياسي للحامض النووي الرايبوزي منزوع الأوكسجين، وجد ان حساسية صبغة زيل – نلسون ٢٦% مقارنة بتفاعل البلمرة التسلسلي .

وفيما يتعلق بزراعة عينات البلغم في بوسط ليونيستين جنسن فقط ٢٣.٤% اظهرت مستعمرات تشبه عضيات الباكثيريا المتفطرة الدرنية بوسط ليونيستين جنسن، بينما ٥.٨% اعتبرت متفطرة سريعة النمو و ٧٠.٨% من العينات اظهرت تلوث أو لم تنمو . تم استعمال اختبارات بايوكيميائيه اضافيه بجانب شكل المستعمرة للتأكد من وجود المتفطره السليه .

وبهذا اكدت الدراسه أن حساسيه صبغة زيل – نلسون ضعيفه ، وأن إستخدام تفاعل البلمرة التسلسلي يوفر تحسين ذو محتوى ملحوظ في تشخيص فحص السل الرئوي

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LIST OF Abbreviation

AFB	Acid Fast Bacilli
AIDS	Acquired Immuno Deficiency Syndrome
BCG	Bacillus Calmette-Guerin
bp	Base pair
CD	Culster Differentiation
CO ₂	Carbon dioxide
CMI	Cell Mediated Immunity
CSF	Cerebro Spinal Fluid
CR	Complement Receptor
DNA	Deoxyribo Nucleic Acid
dntp	deoxynucleotide triphosphates
DTH	Delayed Type Hypersensitivity
DW	Distilled Water
HIV	Human Immunodeficiency Virus
IFN γ	Interferon gamma
IL	Interleukin

INH	Isoniazid
IUATLD	International Union Against TB and Lung Disease
LAM	Lipoarabinomannan
LJ	Lowenstein-Jensen
MDR	Multy Drug Resistant
Mgcl ₂	Magnesium Chlorite
MTB	Mycobacterium Tuberculosis
MTC	Mycobacterium Tuberculosis Complex
NaOH	Sodium Hydroxide
NTB	Non Mycobacterium Tuberculosis
OT	Old Tuberculin
PCR	Polymerase Chain Reaction
PNB	Para – NitroBenzoic acid
PPD	Purified Protein Derivative
RIF	Refampin
rRNA	Ribosomal Ribo Nuclie Acid
TBE	Tris Bolic Acid
TCH	Thiophene-2-Carboxy Acid Hydrozide
UV	Ultra Violet
WHO	World Health Organization