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UTILITY OF PCR ASSAY IN DIAGNOSIS OF PULMONARY TUBERCULOSIS AND IDENTIFICATION OF MUTATIONS ASSOCIATED WITH ANTIMYCOBACTERIAL RESISTANCE BY DNA SEQUENCING

Thesis

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ABSTRACT

Tuberculosis describes an infectious disease that has plagued humans since the ancient times. It has been affecting man for at least 5.000 years. (Stark, 2000).

The emergence of anti-tuberculous drug resistance, especially multidrug resistant tuberculosis (MDR-TB), poses a serious threat to the success of TB control programs. [Suresh et al., 2006].

This study aimed to evaluate peripheral blood based PCR technique in the diagnosis of active pulmonary tuberculosis . It also aimed to use manual DNA sequencing technique for sequencing 3 selected genes. To achieve this goal, 40 patients were selected from those who were diagnosed to have pulmonary tuberculosis by their history ,clinical examination , radiological finding and laboratory criteria.

The main laboratory criteria for selection was their +ve smear stained by Z.N for sputum samples showing AFB. All patients had history of treatment failure with RIF and INH for at least 6 months. 20 healthy blood donors were chosen as a control group.

PCR results showed 100% specificity and 100% sensitivity.15 samples from those which were PCR +ve for IS6110 gene were randomly selected where manual DNA sequencing was done for 3 selected genes : rpoB , katG and inh-A gene. Our results revealed that mutant rpoB genes were 93% versus 7% which were non mutant. The sequenced katG genes showed 100% point

87% of manually sequenced inh-A genes were mutant versus only 13% which were non mutant.

المستخلص

يعتبر مرض الدرن من اقدم الامراض الوبائية التي اصابت البشرية منذ قديم الازل فهو يؤثر علي الجنس البشري منذ حوالي خمسة الاف عام.

ولقد أثبتت الدراسات الجزيئية أن ميكروب الدرن موجود من حوالي خمسة عشر ألف عام

أولاً: تقييم استخدام عينات الدم بدلاً من عينات البصاق فى تفاعل البلمرة المتسلسل كتقنية جديدة لتشخيص مرض الدرن الرئوى.

ثانياً: إستخدام تفاعل تتابع قواعد الحمض النووي كتقنية جديدة لتحليل ثلاث عينات وهى (rpoB, katG and inh-A genes) وهى إلجينات المسئولة عن حساسيه ميكروب الدرن للريفامفيسين والأيزونيازيد وحدوث طفرات بها يؤدى إلى ظهور الدرن المقاوم للعقاقير.

- تم عمل هذا البحث على عدد ٤٠ مريضاً من مرضى مستشفى صدر المحله الكبرى.
- جميع المرضى تم إختيارهم على أساس التاريخ المرضى لمرض الدرن الرئوى والفحص الإكلينيكى والأشعة ألسينيه والفحوصات المعمليه.
- وقع الإختيار على أساس النتائج الإيجابية للفحص المجهرى لعينه البصاق المصبوغة بصبغه الزيل نيلسن (ZN).تم إستبعاد جميع المرضى الذين كانت عينات البصاق الخاصة بهم سلبيه.
- تم اختيار ١٥ حاله من الحالات التى أظهرت نتائج إيجابيه فى تحليل تفاعل البلمره المتسلسل لوجود ميكروب الدرن وتم عمل ثلاث تفاعلات (بى س أى) منفصله لتضخيم أجزاء محددة من ثلاث جينات وهى (rpoB, katG and inh- A).
- تم عمل تفاعل تتابع قواعد الحمض النووى وتحليل الجينات الثلاث لمعرفه نسبه الجينات التى تحمل طفرات وأماكن توزيع هذه الطفرات ومعرفه الطفرة إن كانت مؤثرة أو غير مؤثرة.

وجود الحامض النووي الخاص بميكروب الدرن الرئوى فى جميع المرضىوعدم وجودة في المجموعة الضابطة أي أن حساسية وتخصص تفاعل البلمرة المتسلسل كانت ١٠٠٪.

وجد أن نسبه الجينات المطفرة فى جين rpoBمطفرة أو التى أظهرت ١٠٠٪ تطابقاً مع الجين الأصلى فى بنك الجينات كانت ٧٪.

كانت نسبه الجينات المطفرة فى katG هى ١٠٠٪.

وجدت الجينات المطفرة فى ٨٧٪ من inh-A جين بينما كانت ١٣٪ من الجينات غير مطفرة.

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Abbreviations

A	:	Adenine
A	:	Alanine
A.C.	:	After Century.
AFB	:	Acid Fast Bacilli
AIDS	:	Acquired Immune Deficiency Syndrome
APCs	:	Antigen presenting Cells.
ARI	:	Annual Risk of Tuberculosis Infection.
ATS	:	American Thoracic Society.
B.C.	:	Before Century.
BACs	:	Bacterial Artificial Chromosomes.
BCG	:	Bacilli- Calmette –Guerine.
BLAST	:	Basic Local Alignment Search Tool.
bP	:	Base pair
C	:	Cytosine
CDC	:	Centre of Disease Control
CMI	:	Cell Mediated Immunity.
D	:	Glutamine
dATP	:	deoxyadenosine triphosphate
dCTP	:	deoxycytosine triphosphate
ddNTPs	:	dideoxy Nucleotide Triphosphates.
dGTP	:	deoxdyguanosine tirphosphate
DTH	:	Delayed Type of Hypersensitivity.
DNA	:	deoxyribonucleic Acid.
dNTPs	:	deoxy Nucleotide Triphosphates.
DOTS	:	Directed Observed Therapy with short courses.
Ds. DNA	:	double Stranded deoxyribonucleic acid.
dTTP	:	Deoxy thymidine triphosphate
E	:	Isoleucine
EIA	:	Enzyme Immune Assay
EMB	:	ethambutol
ETH	:	ethionamide
G	:	Guanine
HIV	:	Human Immune deficiency virus.
HPLC	:	High Performance liquid chromatography.
INH	:	Isoniazid.
IS	:	Insertion Sequence
KAN	:	Aimikan
KD	:	Kilo Dalton.
LAM	:	Lipoarabinomannan.
M.T.B	:	*Mycobacterium Tuberculosis.*
MDR-TB	:	Multidrug Resistant tuberculosis

MGIT	:	Mycobacteria growth Indicator Tubes.
MHC	:	Major Histocompitability
MOTT	:	*Mycobacteria* Other than Tuberculosis
MTC	:	*Mycobacterium Tuberculosis* Complex.
NAA	:	Nucleic Acid Amplification
NCBI	:	National Centre for Biotechnology Information
NTCG	:	National Tuberculosis Control Guide.
P	:	Phenylalanine
PBMC	:	Peripheral Blood Mononuclear cells
PBS	:	Phosphate Buffered Saline
PCR	:	Polymerase chain reaction.
PPD	:	Purified protein Derivative
PZA	:	Pyrazinamide
R	:	Arginine
RFIP	:	Restriction fragment Length Polymorphism
RIF	:	Rifampicin.
RRDR	:	RIF resistance determining region
S	:	Serine
STR	:	Streptomycin
T	:	Threonine
T	:	Thymidine
TAE	:	Tris Acetate EDTA
Taq	:	Thermus aquaticus
TB	:	Tuberculosis
TCT	:	T cell Receptor
TRC	:	Tuberculosis Research Centre.
V	:	Valine
WHO	:	World Health Organization
Y	:	Tyrosine
ZN	:	Zeihl Nelseen.