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Department Of General Practice and Community Medicine, Faculty of Medicine; UIO

Drug resistance among Tuberculosis Re-Treatment Cases; Study on Drug Resistance / Multi-drug Resistance and associated risk factors for developing MDR among Tuberculosis Re-Treatment Cases in Khartoum State.

Researcher : Imad El Amin Obeidalla

Supervisor: MD. Ph. D. Professor Gunnar Bjune , International Community Health, UIO

Co-Supervisors: MD. Dr. Asma Elsony, Sudan National TB Program

MD. Dr Olga Toungoussova , International Community Health, UIO

Collaborators: Department of General Practice and Community Medicine , Faculty of Medicine ,UIO, Sudan National Tuberculosis Programme , Epidemiological Laboratory (Sudanese Research Center) ,Sudan National lab.\ NTP reference lab. and National Institute of Public Health, reference lab of TB Norway

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Contents

Topic	Page
Abbreviations ……	4
Acknowledgement……	5
Abstract……	7
Introduction……	9
Chapter I……	11
I. Literature Review……	11
Global Re-emergence of TB: ……	11
Global efforts to control TB epidemic: ……	12
Global Achievements: ……	12
Constraints of control ……	13
Drug Resistance and Multi-drug resistance DR / MDR: ……	14
The global burden of MDR: ……	14
The basis of resistance to antibiotics in bacteria ……	16
Mycobacterium Drug Resistance: The basis of resistance to antibiotics……	17
Anti-TB drugs mode of action: ……	18
Mechanisms of drug resistance against individual 1st line drugs: ……	19
Risk factors of developing multi-drug resistance……	22
Re-treatment Cases and Drug resistance: ……	23
MDR impact and control: ……	26
Sudan brief Country profile: ……	27
TB in Sudan ……	28
TB in Khartoum state ……	34
Chapter II……	36
Methodology ……	36
Overall objective: ……	36
Specific objectives: ……	36
Patients Sample: ……	37
Inclusion criteria……	37
Sample size……	37
Collected sample……	39
Representativeness of the sample……	40
Design: ……	41
Methods Data collection: ……	41
(1) TB registers in Khartoum state ……	41
(2) Pre-structured questionnaire	42
(3) Laboratory methods: ……	42
(A)Smear microscopy: Zeil –Nielsen (Z-N) staining ……	42
(B) Cultivation in Lowenstein –Jensen (L-J) media: ……	43
(C) Susceptibility testing: ……	44
Variables definitions ……	47
Data analysis: ……	48
Chapter III……	49
Results……	49

The total collected sputum samples …… 49
Drug susceptibility in strains:…… 51
Patients: …… 55
Factors associated with infection with drug-resistant and multi-drug resistant strain of M. tuberculosis…… 59
Tables and graphs …….. 62
Chapter III 71
Discussion 71
First study in newly established reference laboratory 72
Low percentage of positive cultures: 72
Previously treated patients in Khartoum state: 72
M. tuberculosis drug resistance among previously treated patients in Khartoum state: 73
Risk factors for the development of drug-resistant TB 74
Constraints and limitations: 76
Future perspectives 77
CONCLUSIONS AND RECOMMENDATIONS 78
References 80
Annex 86

Abbreviations

AIDS: Acquired Immunodeficiency Syndrome
DOTS: Direct Observed Treatment Short Course
DR : Drug Resistance
H: Isoniazid
HIV: Human Immunodeficiency Virus
IUATLD: International Union against Tuberculosis and Lung Diseases
MDR: Multiple Drug Resistance
NTP: National Tuberculosis Program
PAS : param-amin salicylic acid
PLWH: People Living With HIV
R : Rifampicin
S: Streptomycin
TB: Tuberculosis
TBMU: Tuberculosis Management Unit
VC: Voluntary counseling
WHA: World Health Assembly
Z: Pyrazinamide
NTRL : National Tuberculosis Reference Laboratory

Abstract

Research title : Drug resistance among Tuberculosis Re-Treatment Cases; Study on Drug Resistance / Multi-drug Resistance and associated risk factors for developing MDR among Tuberculosis Re-Treatment Cases in Khartoum State

Researcher : Imad Elamin Obeidalla , **Supervisor** : MD.Ph.D. Professor Gunnar Bjune **Co-Supervisors**: MD. Dr Asma Elsony , MD. Dr Olga Toungoussova **Collaborators:** Department of General Practice and Community Medicine , Faculty of Medicine ,UIO, Sudan National Tuberculosis Programme , Epidemiological Laboratory (Sudanese Research Center) , Sudan national TB reference laboratory and National Institute of Public Health, reference lab of TB, Norway.

Overall objective: To study the prevalence of drug resistance and the risk factors of MDR among re-treatment cases in Khartoum state, Sudan

Design: This is observational analytical cross sectional study

This is the first study to be performed in the newly established Sudan National Tuberculosis reference laboratory (NTRL). Out of 236 sputum samples collected from previously treated Tuberculosis patients, sixty two were culture positives. Low percentage of positive cultures results was associated with type of decontamination method used. Results for sensitivity were obtained for 51(82, 2%) out of the sixty two strains. Thirty five strains (68.6%) were resistant to at least one anti-TBdrug. Nineteen (37.3%) of the 51 strains were multi-drug resistant. The highest rates of drug resistance were observed for S whereas resistance to H and R were found equal and the lower resistant rate was to E.

The number of previous treatment courses and type of health facility were significantly associated with multi-drug resistance.

CONCLUSION: Sudan TB Reference laboratory need more strengthening in issue of cultures and drug susceptibility testing . Drug-resistant TB is common among previously treated TB

patients. Low Weight of the patients, number of previous treatment courses, duration of previous treatment course and health facility where patient received their treatment are the risk factors for increasing the likelihood of development of drug-resistant TB among previously treated patients in Khartoum state.

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Introduction

M .tuberculosis is an ancient infectious killer that still remains one of the leading causes of death by an infectious agent worldwide. [(1)] At the present time, 1.7 billion people, i.e. one third of the human population, are estimated to be infected with the tubercle bacillus and are at risk of developing tuberculosis (TB) disease. More than eight million people develop active disease, and about 2,9 million die of TB every year. Over 95% of TB new cases and deaths occur in the developing world. [(2)] TB is considered as a disease of young people, where 75% of cases are in the most economically productive age group (15-54 years). [(3)]

Beside poverty and collapsing health infrastructure as underlying causes, the WHO stated that TB reached unrecorded rates in the human history also due to HIV pandemic, inadequate cases detection, diagnosis and cure. [(4)]

Despite the fact that the TB incidence rate was falling or stable in some parts of the world, the global TB incidence is growing at approximately 1, 0% for the year 2003.The African countries showed an increased incidence particularly in countries with higher HIV prevalence rates [(5)].

The TB epidemic is accompanied by rising drug resistance. Drug resistance together with the HIV pandemic are considered as real threats to TB control measures. Drug resistant and multi-drug-resistant tuberculosis (MDR) present a serious threat to public health, since it arises wherever there has been, or currently is, inadequate application of anti-TB drugs. In such conditions subsequent transmission and spread of *M. tuberculosis* strains that are not responding to the first line anti-TBdrugs and result in high mortality and morbidity of tuberculosis disease [(6)]

Cure of smear positive TB cases and prevention of drug resistance emergence are the public health priorities of all National Tuberculosis Control Programs (NTPs). An increased emphasis is put on the use of Directly Observed Short Course Therapy (DOTS) as the most cost effective strategy. Inefficient NTPs fail to treat completely newly diagnosed patients and facilitate in the increase of smear positive cases that receive the treatment for more than one time. Previously treated TB patients (re-treatment cases) include those patients treated for TB as new cases for more than one month who are now smear or culture-positive. They are more likely to have drug resistance, which may have been acquired through inadequate previous chemotherapy. Re-treatment cases include failure, relapse, and return after default and chronic cases. **(7)**

Sudan has a well performing NTP that manages to implement and expand TB services nearly allover the country. There are no recent national drug resistance surveillance data to assess the situation of drug resistance in Sudan. Limited information about the magnitude of the problem can be assessed from scattered researches. Sudan NTP encourages research on drug resistance which represents one of its top research priorities.

Chapter I
Literature review

Global Re-emergence of TB:

Since the mid of 1980s TB re-emerge globally as a major public health problem, the reason behind this re-emergence is multifactorial. Demographic, socioeconomic factors and HIV co-infection are considered as the main factors for TB re-emergence. The global population is increasing constantly and in particular in poor countries. The population growth is accompanied by declining socioeconomic standards which result in poverty and the widening gap between rich and poor between nations and in various populations. Collapse of the health infrastructure in countries experiencing severe economic crisis or civil unrest contribute greatly in the TB epidemic to the extend that TB is considered as the 'disease of poverty'. [8,9]

The HIV pandemic have accelerated the TB epidemic and put it in the fast-forward. HIV fuels the TB epidemic in several ways. HIV promotes progression to active TB in recently infected cases and cases with latent *M. tuberculosis* infection. While the life time of developing TB in non-HIV infected person range between 10-20% , the annual risk of developing TB in people living with HIV (PLWH) who is co-infected with *M. tuberculosis* ranges from 5-15%.[10] HIV increases the rate of recurrent tuberculosis, which can be due to either endogenous reactivation (true relapse) or exogenous re-infection. Increasing numbers of TB cases in PLWH pose an increased risk of TB transmission in the general community, whether or not HIV-infected.The AIDS epidemic has played an important role in the re-emergence of TB. At the end of 2000 there were 36.1 million people living with HIV/AIDS (PLWH) in the world, 95% of them living in developing countries (70% in sub-Sahara African courtiers and 16% in South East Asia). About

one third of the 36.1 million PLWH worldwide were co-infected with *M. tuberculosis*, 68% of those co-infected live in sub-Saharan Africa, and 22% in South East Asia. [10,11]

The emergence of drug resistance especially multi-drug resistance MDR facilitates the spread of TB epidemic. [12]

Global efforts to control TB epidemic:

Since the early 1990's the WHO has paid more attention to the global impact of TB. The WHO has defined global targets for TB control to detect 70% of the infectious TB cases and to cure 85% of the new infectious cases. In 1993 TB was declared as global emergency and this is followed by dissemination of the well know TB control strategy (DOTS) in simple and understandable manner which been known as five elements of the DOTS strategy. These five elements are the political commitment; case detection by sputum smear microscopy, standard short-course chemotherapy administered under proper case management conditions including directly observed therapy; a system to ensure regular drug supplies; and standard recording and reporting system including the evaluation of treatment outcomes. [2, 5, 7]. The 70/85 targets are now embedded in the United Nations Millennium Development Goals (MDG) [13].

Global Achievements:

As results of the international efforts the DOTS strategy has become widely accepted. The number of countries implementing DOTS increased from 10 in 1990 to 182 in 2003. The proportion of the global population covered by DOTS increased from 22% in 1995 to 77% in 2003. A total of 17.1 million TB cases, and 8.6 million smear positive TB cases, were notified by DOTS programmes between 1995 and 2003. It is estimated that there were 8.8 million new cases of TB in 2003 (140 per 100 000), including 3.9 million (62 per 100 000) smear-positive cases. In 2003 the detection rate of the new smear positive cases under DOTS was 50% and

the success in treatment was 82%. Both the detection rate and the success in treatment remained unchanged. [5,13]

In African countries with high HIV prevalence rates, the incidence rate of TB was increasing quickly. TB incidence also showed steady annual increase in Eastern Europe during the 1990s and it reached a peak in 2001. The incidence of TB worldwide was growing at a maximum of around 1.5% per year in 1995, but less than 1% per year by 2003. [5,13]

In the cohort of 2002, both African and European regions showed low treatment success rate. Low treatment success rates in these two regions can be attributed, in part, to the complications of TB/HIV co infection and drug resistance and failure of DOTS programmes to monitor the outcome of treatment for all their patients.[5,13]

Constraints of control

Despite the great progress during the past decade in adoption and implementation of the DOTS strategy, the global targets of detecting at least 70% of all estimated infectious cases and curing at least 85% of those might not be reached by 2005 . Experts identified many constrains facing the progress towards reaching the global goals of TB control. One constrain is poor quality of care delivery and uncovered NTP needs for technical assistance to overcome difficulties like under-recognition of suspected cases of TB and underreporting of cases. A second constrain is non-adoption of DOTS strategy. For example some countries did not adopt the DOTS strategy and those who adopted it did not reach a substantial demographic coverage. Also many health care providers are out of DOTS system e.g. private sector and non-governmental organization and even some governmental health providers are not linked to the DOTS system. A third constrain is low access to DOTS. It is limited due to patients or health system factors. Patients factors include limited TB awareness and cultural constrains to healthcare access. Health system factors include lack of human resources, limited laboratory capacity, inconvenient

opening hours and location of health facilities, and limited awareness of TB among some health workers. [14,15,16]

Drug Resistance and Multi-drug resistance DR / MDR:

Generally *M. tuberculosis* drug resistance can be defined as a *M. tuberculosis* strain resistant to one or more anti-TB drugs. Drug resistance can be 'monoresistance' when a strain is resistant to only one anti-TB drug or can be 'poly-resistance' when resistance to more than one of these drugs. Multi-drug resistance is defined as resistance to both insonizide (H) and rifampicin (R), with or without resistance to other anti-TB drugs. [17,18].

Drug resistance is classified into primary and acquired. Traditionally the primary and acquired drug resistances are differentiated on the basis of a history of previous TB treatment. Primary resistance or resistance among new cases defined as the presence of resistant strains *of M. tuberculosis* in a patient with no history of such prior treatment. Acquired resistance is found among previously treated patients is that which is found in a patient who has received at least 1 month of prior anti-TB drug treatment.[17,18].

Drug resistance among new cases is a result of transmission of drug resistant *M. tuberculosis* strains from patients who have developed the resistance during treatment. Primary drug resistance differs from acquired drug resistance in prevalence. The rate of primary resistance , whether resistance to one or more drug or even MDR, in new patients is lower than the rate of acquired resistance. It is proved that the probability of drug resistance and MDR is directly proportionate with the no of previous cases [17,18].

The global burden of MDR:

Resistance of *M. tuberculosis* to anti-TB drugs is a man-made phenomenon. It has existed since the introduction of anti-TB chemotherapy and it represents a potential threat to the standard international method of TB control.

The global magnitude of drug-resistant TB has not been well studied until recently, when the WHO/IUATLD in collaboration with several partners launched the Global Project on Drug-Resistance Surveillance (DRS) starting from 1994, to assess the magnitude of the problem and monitor its trends [18, 19].

The results of the surveillance are released in 1997, 2000 and 2003. During the years of the surveillance , the participating countries has increased from 35 countries and settings in the 1997 results to 77 in the 2003 released results. The three released reports confirmed that drug resistant TB, including MDR- TB, was found in all regions of the world. Also hot spots are identified and confirmed in each report; in these hot spots the prevalence of MDR-TB was exceptionally high. They are, in particular, part of previous Soviet Union (Estonia, Latvia, Ivanovo Oblast Kazakhstan, Uzbekistan) and some areas in China (the provinces of Henan and Zhejiang).

Previously treated cases, worldwide, are not only more likely to be drug-resistant, but also to have resistance to more drugs than untreated patients. [6, 18]

The surveillance results showed also that resistance to isoniazid (H) and streptomycin (S) were more prevalent than Rifampicin (R) or Ethambutol (E) resistance. The last results released in 2003 showed that the median prevalence of any resistance and MDR among new and previously treated cases of TB were 10.2% 1.1%, 18.4% and 7% respectively.

These medians were almost similar in all reports table (1)

Table (1): Global Prevalence of drug resistance to any drug and MDR-TB,the WHO/IUATLD Global Project on Anti-TBDrug Resistance Surveillance1994-2003

Year of data released	New cases		Previously treated cases		Countries/settings surveyed
	DR	MDR	DR	MDR	
1997 data	9.9 %	1.4 %	36 %	13%	35
	Range 2-41%	Range 0-14%	Range 5-100%	Range 0-54%	
2000 data	10.7 %	1%	23 %	9%	58
	Range 2-36%	Range 0-14%	Range 4-94%	Range 0-48%	
2002 data	10.2 %	1.1%	18.4 %	7%	77
	Range 0-57.1%	Range 0-14.2%	Range - 82.1%	Range 0-58.3%	

DR, Drug resistant (at least to one Drug) MDR: Multi-drug resistance

The basis of resistance to antibiotics in bacteria

Generally bacteria have many strategies to hit back and cancel out the action of antibiotics that appear in their neighbourhoods. For example the bacteria can pump out the antibiotic . This will stop the effect of the antibiotic by preventing the antibiotic to accumulate at the concentration that needed to act. A second strategy of resistance is destruction of the chemical warhead in the antibiotic. A third resistance strategy focuses not on removal or destruction of the antibiotic but on a reprogramming or camouflaging of the target. **(20)**

Most anti-bacterial drug resistance that leads to accomplish these strategies are genetically determined either by chromosomal mutations or acquisition of a plasmid or transposon, but also non-genetic resistance can be found (presence of bacteria in cavities or presence of bacteria in resting state) **(21).** Anti-bacterial drug resistance can be divided broadly into inherent (natural) resistance, in which bacteria may be inherently resistant to an antibiotic or acquired resistance, when the bacteria develop resistance to antibioticsfrom a previously sensitive stage. The later

type of resistance results from changes in the bacterial genome. Acquired resistance is driven by two genetic processes in the bacteria: (1) mutation and selection (sometimes referred to as vertical evolution); (2) exchange of genes between strains and species (sometimes called horizontal evolution).

Mycobacterium Drug Resistance: The basis of resistance to antibiotics

M. tuberculosis has several mechanisms of developing drug resistance, naturally mycobacteria have a very hydrophobic cell wall, that is responsible for their acid-alcohol-fast properties, which makes it impermeable for some antibiotics . *M. tuberculosis* possesses some enzymes such as beta-lactamase able to inactivate penicillin. In addition to this natural resistance *M. tuberculosis* can develop resistance to antibiotics through spontaneous chromosomal mutations but does not perform any gene transfer **(22).** This means that *M. tuberculosis* drug resistance doesn't occur due to acquisition of new resistance genes (not horizontal), but is the result of random genetic (chromosomal) mutations (vertical evolution) in particular genes conferring resistance.**(23)**

Mutations occur spontaneously during bacterial multiplication and are not dependent upon exposure to drugs and it was found that the ratio of resistant bacilli to the total number of bacilli is different according to the different anti-TB drugs. Mutations occur with a defined frequency, for example the ratio frequency of genetic mutations resulting in resistance to R is 1 in 10^8 bacilli, whereas the frequency of mutations resulting in resistance to H, S and E is 1 in 10^6 bacilli for. **(24,25,26)**

A tuberculous cavity harbouring 10^7–10^9 bacilli may contain a few (10–1000) bacilli resistant to H, a few (<- 10) resistant to R, a few (10–1000) resistant to E and a few (10–1000) resistant to S, and so on. This does not imply that when a sample of this population of bacilli is cultured in

the laboratory it will be found to be resistant to these drugs; for resistance to be reported in the laboratory, > 1/100 of the bacilli must be resistant to the drug [(27)]

The presence of anti-TB drugs in the environment of *M. tuberculosis* represent a selective pressure which lead. The selective pressure suppresses the growth of susceptible bacilli but permits the multiplication of drug-resistant organisms which will dominate the population .The treatment of *M. tuberculosis* with an inappropriate regimen kills the majority of the bacilli in the population, but the small number of mutants resistant to the drug continue to multiply. After 2 weeks to several months of treatment with the inappropriate regimen, the resistant bacilli will outgrow the susceptible bacilli, causing clinical drug resistance. Selection of drug resistant mutants occurs when therapy is inadequate. This may take place in situation of monotherapy or can occur during irregular treatment. Here comes the importance of using a combination of anti-TB drugs in treating TB patients to decrease the selection of resistant strain to one drug to be controlled by other drugs in the combination [(28)]

M. tuberculosis drug resistance occurs in a sequential process, i.e. monoresistance, double resistance, triple resistance and quadruple drug resistance. The main pathway suggested for *M. tuberculosis* drug resistance starts with monoresistance to S or H followed by double resistance to HS, triple resistance to HSR and quadruple resistance to HSRE. [(18)] The MDR develops when the sequence of such mutations gives resistance to at least R and H one at same time.

Anti-TB drugs mode of action:

Anti-TBdrugs can kill the *M. tuberculosis* providing bacteriocidal effect or prevent its growth providing bacteriostatic effects. Like all other antibacterial agents, anti-TB drugs have a certain

target, either (1) bacterial cell-wall biosynthesis; (2) bacterial protein synthesis; and (3) bacterial DNA replication.

In current standard short-course chemotherapy, H, R, Z and E are given together in the 2-month initial phase and are followed by 4 months of H and R. This can be expressed in a condensed form as 2EHRZ/ 4RH. All of the drugs, with the exception of Z, act on known sites in the genome of the tubercle bacillus to which they or their derivatives bind. E inhibits cell wall synthesis (EmbA, B and C) and a major target of H action is also inhibition of cell wall synthesis (inhA and kasA), while R inhibits transcription by binding to DNA-dependent RNA polymerase (RpoB) and S inhibits protein formation at the ribosome (RpsL and 16S rRNA), though the site causing the important low level of resistance has not been identified. **(29)**

The sterilizing activity of anti-TB drugs, that is the rate at which *M. tuberculosis* is killed in the lesions, depend upon many factors , for example the presence of the bacilli intracellularly or extracellularly, the most difficult to kill are the extra-cellular bacilli in cavities. Bacilli that are actively dividing are probably killed early, while bacilli that are metabolising at a slower rate and may be occasionally dividing (called persisters) are more difficult to kill. Bacterial growth is slowed by low oxygen tension and age of the bacilli **(29)**

Mechanisms of drug resistance against individual 1st line drugs:

Substantial progress has been made in understanding the molecular basis of *M. tuberculosis* drug resistance in the last decade; the molecular basis of *M. tuberculosis* drug resistant has been well documented and there are many genes discovered to be involved in the development of drug resistance **(24).** The most common mechanisms of resistance to the primary anti-TB drugs such as R, H, Z and S in *M. tuberculosis* are well studied. In these different studies the

genes conferring the resistance to different anti-TBdrugs were isolated and are found in different percentages as shown in table (2) below. **(24-26)**

Table (2) Anti-TB drugs and drug resistance mechanisms

Site of action	Anti-TB drug	Gene Associated with resistance	Approximate frequency of resistance %
Inhibitors of cell wall synthesis	Isoniazid	*katG*	40 – 60 %
		inhA	20 -34%
		ahpC	10 – 15%
		kasA	14%
	Ethambutol	*embCAB*	50 – 65
Inhibitor of nucleic acid synthesis	Rifambicin	*rpoB*	>95
Inhibitors of protein synthesis	Streptomycin	*rpsL*	52-59
		rrs(16S RNA)	8-21
Unknown	Pyrazinamide	*pncA*	70-97

Isoniazid (H) (is nicotinic acid hydrazide) , used as a first line TB drug and together with R constitute the backbone of *M. tuberculosis* chemotherapy globally, is a synthetic, bactericidal agent. H is active against growing tubercle bacilli in the presence of oxygen and under favourable temperature (37^0 c), **(22)** H is a pro-drug that is activated by the *M. tuberculosis* catalase-peroxidase enzyme (KatG) to its active form **(22, 26,30)**. Upon activation reactive radicals are formed damaging multiple targets in the cell. Significant evidence supports the idea that H acts on blocking synthesis of *M. Tuberculosis* cell-wall. Targets of active H are enzymes involved in synthesis of mycolic acids which is of the major components of the cell wall of *M. tuberculosis.* Mutations have been found in the gene encoding H activation enzyme, or less commonly in the genes that encode these proteins of mycolic synthesis enzymes (inhA, acpM, ahpC and kasA) **(22,25,30,)**

.**Ethambutol (E)** is a bactericidal first line TB drug. E is only active against growing bacilli. E inhibits synthesis of arabinogalactan, a major cell wall component of mycobacteria [(22).] Genetic studies indicated that the target of E is EmbB, arabinosyl transferase, involved in synthesis of arabinogalactan . Emb has group of proteins that are involved in the cell wall biosynthesis. These proteins are EmbA, EmbB, and EmbC . Mechanisms of resistance to this anti-TBagent are primarily associated with point mutations in the embCAB operon encoding various arabinosyl transferase enzymes necessary for cell wall biosynthesis [(26)]

Rifampin (R) is a first line TB medication and its highly effective bactericidal action against *M. tuberculosis* has made it a key component of the initial anti-TB regimen. It will rapidly kill *M. tuberculosis* as it is introduced, and it acts both against multiplying and slow metabolizing bacilli which contribute extensively in shortening the duration of treatment [(22,29).] Rifamycin and its derivatives, easily diffuses through the cell membrane due to its lipophilic nature and acts by binding to the bacterial RNA polymerase, thereby inhibiting RNA synthesis. Resistance to R in *M. tuberculosis* is due to a single mutation in the rpoB gene, a gene encoding the DNA-dependent RNA polymerase beta subunit .RIF resistance was found to be in some areas very related to H resistant which make R resistance a useful marker for multidrug resistance [(30)]

Streptomycin (S) is another first line anti-TB drug. It was the first antibiotic used against mycobacteria since mid 40s. S inhibits protein synthesis by binding to the 30S subunit of the bacterial ribosome causing misreading of the mRNA message during translation [(22,26)] S is thought to kill actively growing tubercle bacilli at neutral or alkaline pH conditions but is inactive against non-growing or intracellular bacilli. Mutations associated with streptomycin resistance in tubercle bacilli have been identified in the 16S rRNA gene (*rrs*) and *rpsL* gene. [(26)]

Pyrazinamide (Z) it an important anti-TB drug and like R it is responsible for the shortening of TB therapy from the previous 9 to 12 months to the current 6 months due to its killing of the semi-dormant bacillary populations . Z is a structural analogue of nicotinamide. Z shows unique features like it is only active at acidic conditions and it kills old, non-replicating bacilli with low metabolic activity more effectively than young, growing bacilli with high metabolic activity [(38).] Its full function is poorly understood. Z is a pro-drug that requires activation to its active form, pyrazinoic acid, by the enzyme pyrazinamidase. To define the molecular mechanism of Z resistance the *M. tuberculosis pncA* gene encoding pyrazinamidase has been sequenced. The results have provided evidence that *pncA* mutations conferred Z resistance. **(30)**

Beside the study of the mechanism of drug resistance to each drug, many other studies are trying to explore the assumed potential effects of drug resistance genetic changes on fitness of *M. tuberculosis* resistant bacilli. It is hypothesized that mutations leading to multidrug resistance will affect on the reproductive effectiveness of the organism and that these strains will be less widely transmitted than drug-sensitive strains. **(31,32)**

Risk facros of develping multi-drug resistance

The two main causes of emergence of drug resistance are non-adherence to therapy and the use of inadequate treatment regimens. Both nonoadehenrence and inadequate treatment can be contributed to a variety of health system, health provider and patient-related factors**(33)**.

Although, there has been no clear evidence that HIV infection is a risk factor for developing MDR-TB in proper treatment settings, it was noticed that MDR epidemic is some areas is associated with HIV. On the other hand there are some factors that may contribute to an increased risk of MDR-TB in HIV-infected people. For example, as MDR strain are said to be with lower genetic fitness and virulence, they may appear predominating in immuno-suppressed

people. The second possibility is that MDR is mainly nosocomially spread where HIV patients can get it easily. Also HIV-infected TB patients may be subjected more often to functional monotherapy [34, 35]

Although drug-resistant TB was recognized shortly after the introduction of effective anti-TB chemotherapy, the emergence of MDR-TB in the hospital and HIV positive patients in the United States in the early 1990s led to renewed interest in this topic [36]

Re-treatment Cases and Drug resistance:

> "Previously treated cases, worldwide, are not only more likely to be drug-resistant, but also to have resistance to more drugs than untreated patients" ***WHO/IUATLD global MDR surveillance; 2004***

Previously treated TB patients are defined as patients who are treated as new cases for more than one month who are now smear- or culture-positive. These cases include relapse patients previously treated for TB who have been declared cured or treatment completed, and are diagnosed with bacteriologically positive tuberculosis , or failure cases, patients who are started on a re-treatment regimen after having failed previous treatment , or defaulters patients who return to treatment, bacteriologically positive, following interruption of treatment for 2 months or more , or chronic cases **,** a patients who are sputum-positive at the end of a re-treatment regimen. [17] Although all these categories are pulmonary smear positive cases, pulmonary smear-negative and extra pulmonary cases may also be relapses, failures, defaulters or chronic cases.[37] . Although re-treatment cases are not many in well performing NTPs and usually represent about 10% of the total reported cases, the re-treatment cases proportion, together with cure rate and death rate prevented are usually used to measure the impact of TB control programmes. [17,38]

Review of published literature strongly suggests presence of drug resistance including MDR is much less common among new TB cases than among previously treated TB cases. The most

powerful predictor of the presence of drug resistance and MDR-TB is in fact a history of treatment of TB. In the latest (third) report of the WHO/IUATLD surveillance (2003) demonstrated that new cases had an average of 10.2 % (Range 0-57.1%) prevalence of drug resistance to at least one drug and 1.1% MDR (Range 0-14.2%) , whereas in previously treated patients the prevalence of drug resistance and MDR are 18.4 % (Range -82.1%) and 7% (Range 0-58.3%) respectively. **(18)**

Both incomplete and inadequate treatment including inadequate treatment compliance are considered as major risk factors for development of MDR and are commonly observed among previously treated cases. Incomplete and inadequate treatment can be due to use of single drug to treat TB. This could have occurred because of ignorance and economic constraints which make the direct observation of the patients difficult. Furthermore, inadequate treatment can be due to the problem of using unreliable combinations. The common error in prescription practice is the “addition syndrome”. In cases when the patient appears to deteriorate clinically addition of another drug to an existing regimen. If resistance had developed to the drugs in use, adding another drug effectively amounts to monotherapy with the drug added. Also use of unreliable drugs with poor bioavailability can increase the likelihood of monotherapy. Use of anti-TB drugs by unqualified persons or alternative medicine practitioners in bizarre regimens for inadequate periods is an important problem in some settings. Free availability of anti-TB drugs over the counter may contribute to this.

Patient’s non-adherence is considered as one of the major risk factor in developing MDR among TB patients. Non-adherence is defined as the patient’s inability or refusal to take TB drugs as prescribed. When medical treatment is complicated or lasts for a long time, as in the treatment for TB disease, patients often do not take their medication as instructed. Patients no longer feel sick, or the lack of knowledge, access to health care or motivation to adhere to a TB

regimen will cause non-adherence. In many studies in different developed country settings demographic factors such as age, sex, marital status, education level and socio-economic status have not been found to correlate with the degree of compliance. Other certain factors such as psychiatric illness, alcoholism, drug addiction and homelessness do predict non-compliance. This may not be entirely true in the developing countries context and the relevance of these factors in these countries merits further study. **(39-41)**

Espinal reviewed of global report of MDR, he noticed that previous treatment was the major risk factor for development of MDR although he argued that the sample was limited in the international survey because it targeted newly diagnosed cases and the absolute number of these cases was low. (7). Djuretic T. et al paper on MDR in UK between 1993-99, found the prevalence of MDR to be 9.4% among re-treatment cases compared to 0.8% in new cases. Beside the fact that previous treatment is a major risk factor other factors were identified to be significantly correlated to development of MDR among these cases like male gender, adulthood, place of birth, residence and non-DOT treatment. **(42)** Liu Z et al in thesis New Jersey survey (1991 -1995), that MDR was 13.4% in re-treatment cases compared to 2.5% in new cases ; HIV positivity and male sex were found to be significantly associated with MDR and ethnicity, homeless, drug use and alcoholism were excluded as risk factors (43) . In Toungoussova et al's study, risk factors for MDR in the Archangelsk oblast, Russia 2001, prevalence of MDR was found to be 60.0% in re-treatment cases compared to 13.5% in new cases. Female gender, physical work and interruption of treatment were proved to be significantly associated with development of MDR. **(44)** Afranio L et al did a study on re-treatment cases in Brazil 1986 -90. They found that failure cases had higher prevalence of MDR than other re-treatment groups, and presence of lung cavitations was the major risk factor. **(45)** in another study in Brazil, poverty and number of previous courses were identified among the risk factors.**(46)** Also there are many others studies from many places in the world, verify the fact that previous anti-TB treatment is a

major risk factor for drug resistance and MDR and that there are associated risk factors among re-treatment cases that increase the likelihood of MDR among them. [47-53] In Obeid et al's study in Khartoum and Gezira states in Sudan in 2001, among strains isolated from 144 cases (only 51 with previous records of treatment) , 31 cases had of MDR (22% of the total cases). Only two cases were from new patients and the rest were from previously treated patients [54]

MDR impact and control:

MDR TB represents a real threat to TB control measures. Short-course chemotherapy (SCC), which is recommended as part of the DOTS strategy, is not appropriate for treatment of MDR TB patients. Although MDR-TB patients are not absolutely untreatable by SCC , even in good and well implemented DOTS strategy the success rate for treatment of new MDR cases is very low (range between 6- 59%) compared to new susceptible cases which can reach 95% success rate with SCC [34,55].

Another impact of MDR is that mortality is significantly higher among persons infected with multi-drug resistant strain than of those infected with sensitive strain. MDR cases also remain infectious for longer time that increases the risk of infection transmission. Management of MDR-TB relies on strong laboratory support and qualified, dedicated personnel for treatment oversight and supervision. Treatment should be individualized for each patient on the basis of in vitro susceptibility data. The treatment of patients with MDR-TB is much more difficult and relies extensively on second-line drugs these agents are generally proved to have poorer activity than the first-line drugs and also they have greater tendency to cause adverse reactions. In addition they are very expensive. WHO and its partners are currently piloting a strategy called "DOTS-Plus" that aims a developing global policy recommendations for the management of MDR-TB with second-line anti-TB drugs [33,34].Treatment of MDR-TB in developing countries is a particular dilemma because the susceptibility testing and second-line agents are usually insufficient [56]

The future trend of MDR control is challenged by many constrains. At the outset is the poorly functioning and poorly funded TB control programs which are considered one of the great challenges since the best preventive strategy available now is to prevent the emergence of new MDR cases, and this is proved to be achieved through good quality application of SCC in well functioning NTP. The other challenge is investing in MDR control to offer the second line drugs, which should be carefully introduced in good DOT adopting programmes. To provide the reliable laboratory test for drug susceptibility and to train the health personnel needed to implement MDR control policy. All these challenges the WHO is trying to solve by the proposed MDR control strategy known as DOTS plus.[33,34, 57, 58]

Sudan brief Country profile:

Sudan is the largest and most colourful country in Africa and is located in the northern part of Africa. The large area of the country expand through three distinct geographic regions starting from the harsh deserts of the Sahara in the north, passing the flat lands of the central region and end at the dense rainforests of the south. The country has a wide ethnic spectrum with over 570 groups which speak over 100 different languages. Sudan is classified as a low-income country. The GDP per capita was US$395 in 2001. The country is suffering a civil war in the south part for more than 20 years that ended in 2005 but with emerging other conflict areas. The estimated population as of 2002 is about 33 million.[58] The administrative division in Sudan follows the federal system where the whole country is divided into nine zones and 26 states; each state is divided into provinces. Malaria, diarrhoea and acute respiratory infections are major diseases, especially in children. [59]

TB in Sudan :

TB has been recognised as a major public health problem since the 1950s [60] but before this time, TB among Sudanese was coming into attention when a pioneer study was done among Egyptian army in 1911. The study showed a relatively higher prevalence of TB among Sudanese soldiers than among Egyptians. The first tuberculin survey was done in Sudan in 1925 in the Blue Nile province. Extensive surveys done between 1929 and 1932 in different regions threw more light on the TB problem in the country. Late in the 40s it was notice that TB cases were slowly rising among Sudanese and in the early 50s chest units were established in hospitals and TB was considered by Sudanese health services as one of the major health problems, it still remains a health priority in the country **[60,].**

Until 1990 Sudan National Tuberculosis Control Programme (SNTP) was mainly hospital based and poorly implemented. Cure rate was 30% and case detection 38%. In 1990 the Manual of the National Tuberculosis Control Programme was published. Up to 1993, regular monitoring of treatment was limited to pilot areas. Since 1995 expansion started and the programme was finding its way among other International Union Against Tuberculosis and Lung Diseases (IUAT-LD) collaborative programmes and has fully adopted the World Health Organization (WHO) policy package of TB control. [61,62]

Sudan is a high burden country in the Eastern Mediterranean Region of WHO. It shoulders 11.6% of the total cases in the region. The annual risk of *M. tuberculosis* infection is estimated as 1.8, which indicates for each 100,000 of Sudanese population we are expected to find 180 tuberculosis cases. It is also estimated that the average incidence of all forms of TB is twice the incidence of pulmonary smear-positive cases i.e. 90 out of the total 180 cases will be smear positive cases **[61,62]**

Since it was revitalised in 1993 the NTP managed to put DOTS components in place. NTP introduced gradually the 8-month TB treatment regimens for different case categories (Table 2). The programme reached its 100% expansion when 'DOTS all over' was achieved at the beginning of 2003. Decentralization of TB control services and integrating them within the PHC services improved significantly the epidemiological picture of TB in the country. This can be shown in the improvement of case finding, in particular, in the PHC facilities, changing the TB patient profile with more benefit for vulnerable and deprived groups like women and great improvement of treatment success rate. [(63)] Case finding was greatly improved. In 1993 when the SNTP was still a pilot project it managed to detect only 897 new smear positive cases. Then the program steady increased its case finding to reach the peak at 1999 with 14085 cases and plateaued at 10,000 -11,000 cases annually since 2001. The programme manage to detect 242,640 cases, 114100 of them were new smear positive cases (table 3). Out of the total detected cases 45-50% are new smear positive , and the cases that receive the treatment for the second time or more (re-treatment category) represent around 6-10%(fig 1) [(61,64).] The treatment success rate reached 81.1% for the 2003 cohort. [(64)]

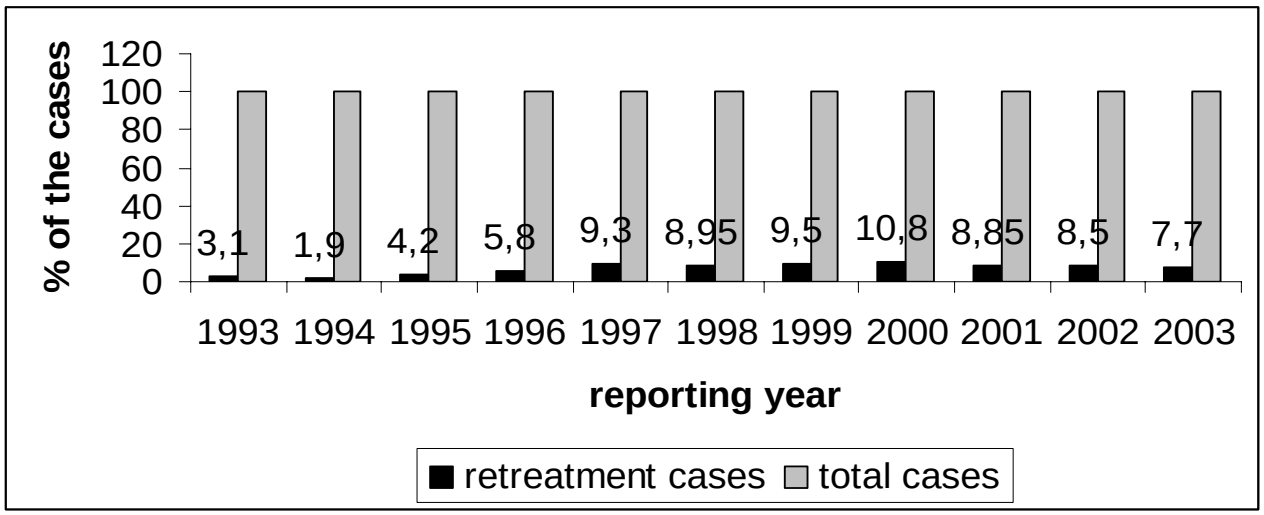
Table (3) Recommended treatment in Sudan TB program

Cat.	**TB patients**	**Intensive phase**	Continuation phase
I	• **New smear-positive pulmonary TB** • **New smear-negative and extra-pulmonary TB that are severely ill**	**2 HRZE (2 HRZS)**	6 TH
II	• **Sputum smear-positive relapses** • **Sputum smear-positive cases requiring treatment after interruption** • **Sputum smear-positive treatment failure cases**	**2 SHRZE/1 HRZE**	5 HRE
III*	New smear-negative and extra-pulmonary TB	2 STH	10 TH

Table (4): Sudan-Case finding summary 1993-2003 [63]

Year	New SP	Relapses	Smear Neg.	EP	Total
1993	897	39	170	167	1273
1994	3728	513	9471	12866	26578
1995	8761	604	3001	1954	14320
1996	8978	1185	7641	2476	20280
1997	10835	1652	5859	2548	20894
1998	10820	1655	6901	3442	22818
1999	14075	1806	6933	4136	26950
2000	12440	2159	6600	3892	25091
2001	11136	1776	7071	4014	23997
2002	10338	1657	7871	4713	24579
2003	11003	1640	7802	4666	25111
2004	11089	1593	7791	3988	24461
total	114,100	16279	74111	38150	242640

Fig (1) Percent of re-treatment cases VS total cases in Sudan



<u>Prevalence of TB drug resistance</u>:

The information concerning the situation of drug resistance throughout the country is limited. *M. tuberculosis* drug resistance has been given attention since the year 1965 when a survey was conducted in Khartoum and Wad Medaini areas. This survey evaluated the resistance to anti-

TB drugs namely S, PAS and H among new and previously treated patients. The survey included limited number of patients for analysis however it showed that *M. tuberculosis* strains resistant to anti-TB drugs were prevalent in Sudan and previously treated patients show higher prevalence of drug resistance than new ones (table 4). Patients aged 20 and above also had more drug resistance (table 5). [61, 65].

TABLE (5) Prevalence of strains resistant to one or more drugs according to source of sputum (patients with doubtful, or unknown chemotherapy omitted)

Source of sputum	Treated			Untreated			Treated			Untreated		
	Total	Resistant		Total	Resistant		Total	Resistant		Total	Resistant	
		No	%		No	%		No	%		No	%
Shaab	87	74	85	65	15	23	48	38	78	28	5	1
Abu Anga	41	37	90	30	15	50	3	3	100	2	1	5
Wad medani	2	1	50	39	10	25	2	2	100	15	4	2

table (6) Prevalence of strains resistant to one or more drugs according to age of treated and untreated patients.

Age (Yrs.)	Treated			Untreated		
	Total tested	Resistant		Total tested	Resistant	
		No	%		No	%
0	17	15	88	28	2	7
20	58	45	78	59	21	36
30	54	49	91	42	14	33
40	23	20	87	28	8	29
50 % over -	29	22	76	24	8	33

The most recent study was carried out in 2001 in collaboration with University of Oslo. It was a study on the drug resistance prevalence among new and re-treatment cases. The main findings

were that drug resistance and MDR were more prevalent among previously treated cases, (table 5). Most of the previously treated cases enrolled in the study were cases selected from chest hospital to which they are referred as difficult cases from other health care centers [(55)].

TABLE (7) Drug resistance to anti-TB drugs among new and previously treated patients in Khartoum and Gezira states, 2001

Resistant to	No of new cases (total 93) (%)	No of previously treated cases (total 51) (%)
H	11 (11,8%)	30 (58,8%)
R	3 (3,2%)	29 (56,6)
S	33 (35,4%)	35(68,6%)
E	0	21(41,1%)
single drug	34(36,5)	38(74,5)
MDR	2(2,1)	29(56,8%)

HIV /TB co-infection: For Sudan, although the neighboring countries Kenya and Uganda are examples of high burden of HIV-associated TB, it was found that the prevalence of HIV among TB cases was 7.9% which is relatively low. This can be explained by the low HIV prevalence in the country which is 1.4% and 5% in northern and southern part of the country respectively. The low HIV prevalence among TB patients and minor effect of HIV co-infection on treatment outcome of TB patients, as shown in El sony et al's study, may make the effect of HIV in development of MDR is less common.[(66)]

Sudan NTP laboratory network:

Sudan NTP expanded a microscopy network. For each 100,000 of the population a microscopic centre (TBMUs) is launched. At each state there is a state quality control laboratory and centrally there is there reference laboratory. The laboratory network is organized in three levels:

1. Level I: Central level, the National TB Reference Laboratory at the central level, it is the leading body in microscopy network and is placed in Khartoum.
2. Level II: state level: The TB quality control laboratory in the state, it is responsible for the strengthening the laboratory network at the state level.
3. Level III: peripheral level: The peripheral laboratory in the diagnostic centres (TBMUS), is responsible for diagnosis of TB through sputum smear microscopy.

Sudan NTP reference laboratory (NRTL):

A reference laboratory has been planned for many years. Establishment of the reference laboratory was supported by the Norwegian Lung and heart association LHL, WHO and IUATLD. The main tasks for the Sudan NTRL are:

1- Maintaining high proficiency in routine smears microscopy carried out peripheral health facilities.

2- Training of laboratory staff involved in the national laboratory network for tuberculosis.

3- Surveillance of anti-TB drug resistance.

4- Collaboration in research activities.

5- Implement external quality assurance by blind rechecking of the slides. [67,68]

The laboratory has been integrated in the premises of the national health laboratory in Khartoum. The necessary infrastructure in terms of facilities, equipment, and manpower were prepared for starting the culture and susceptibility testing since 2001.The reference laboratory opened in 2004 and started by performing the quality assurance of the smear microscopy performed in the NTP microscopy network. With the help of external technical advisor the laboratory started culture and susceptibility testing since September 2004.

TB in Khartoum state :

Khartoum State is located in the centre of Sudan at the junction of White Nile and Blue Nile. According to the 1993 census, Khartoum's population was 3,512,000, and estimated at 5,352,000 in 2003. Around 1.8 million IDPs are estimated to be living in Khartoum. All ethnic groups of Sudan are represented in Khartoum state population. Administratively Khartoum state is divided into seven localities namely, Khartoum, Jebel Awlia, Sharag el Neal, Khartoum Bahri, Omdurman, Karrarrie and Umm Bada. Relatively Khartoum state had the best health services in Sudan. There are 39 public hospitals, 131 health centers, 177 dispensaries, 29 dressing stations, 30 primary health care units.[(69)]

NTP started in Khartoum since 1993 , when the NTP was a pilot project , now it is 100% covered by NTP centres (either TBMUs (diagnostic centres) or DOT (treatment centres) , NTP in Khartoum state is a well functioning program compared to other states. It has good political commitment in the state, 100% coverage of TBMUs and a good reporting system. NTP in Khartoum state is implemented in three categories of health facilities:

1. Federal level, consisting of federal hospitals. Usually they are specialized chest hospitals or specialized chest units in big hospitals. There are nine such centres in total.
2. State hospitals and health centres and PHC units that belong to Khartoum state Ministry of health. There are forty four such centres in total.
3. Internally Displaced People (IDP) health units. These are centres run by the Sudan Council of Churches and offer health services to the IDP. There are five such centres.

Through steady decentralization NTP in Khartoum manage to move all the cases from the big hospital. In the year 2004, Khartoum sate centres reported the majority of cases, followed by

the federal centres and the IDP health units. The number of re-treatment cases reported by the federal hospitals was very near to those reported by state TB centres (table 8).

Table (8): Cases finding in the different categories in Khartoum state 2004: **(64)**

Category of centres	New Smear Positive cases	re-treatment cases	Smear Negative cases	Extra pulmonary cases	Total
Khartoum State Centres (43 centres)	1852	261	1445	498	4056
Federal Ministry of health Centres (9 centres)	1259	237	495	357	2348
Internally Displaced Centres (5 Centres)	600	57	294	221	11172
Total	3711	555	2234	1076	17576

Because of the relatively well functioning health services, Khartoum state represents a referral site for re-treatment cases from other states as they are considered among the complicated TB cases. Khartoum state reports the majority of re-treatment cases in Sudan, it reports a range of 30 -46% of the total reported cases in the country (table 9).

Table (9): Re-treatment cases in Khartoum State: (64)

Year	Total reported	No of re-treatment cases	% of re-treatment	% of the total re-treatment reported cases in Sudan
1997	7525	701	9,3	42,4
1998	8156	730	8,95	44,1
1999	8749	835	9,5	46,2
2000	8523	922	10,8	42,7
2001	7485	663	8,85	37,3
2002	6244	533	8,5	32,2
2003	7563	632	8,3	38,5
2004	7576	555	7,3	34,8

Chapter II

Methodology Section:

Overall objective:

To study the prevalence of drug resistance and the risk factors of MDR among re-treatment cases in Khartoum state, Sudan.

Specific objectives:

This study was conducted to fulfill the following specific objectives

1. To assess the drug resistance and MDR among re-treatment cases and distribution of drug resistance /MDR among the different re-treatment categories in Khartoum state
2. To identify the risk factors associated with the development of MDR-TB among these cases in Khartoum state
3. To assess the quality of the susceptibility tests performed at the reference laboratory of NTP in Sudan.

Patients Sample:

The study was performed in Khartoum state by using previously treated TB patients. Sudan NTP uses definition and classifications of re-treatment categories according to the WHO/IUATLD recommendations. Patients diagnosed with TB are treated according to NTP guidelines in different centers of Sudan NTP in Khartoum state [(61).]

In Sudan according to annual risk of infection an estimated 180 TB new cases per each 100,000 Sudanese population. Half (50%) of them could be expected new smear positive cases and around 9% are previously treated cases and the rest are smear negative and extra-pulmonary cases. Therefore for the total population of 5,352,000 (2003 estimation) in Khartoum state , a total of 9633 TB cases is expected annually, of which 4817 are expected to be new pulmonary smear positive cases and around 967 are expected to be re-treatment cases and the remaining are smear negative and extra-pulmonary cases.

Inclusion criteria

All re-treatment cases registered in Khartoum state NTP centers, during the period of September /2004 – February /2005, were included in the study. The following inclusion ccriteria were used:

- Patients who received the re-treatment regimen (registered as failure, relapse, return after default and chronic case during the period of September 2004 –February 2005.
- Patients were enrolled in the study after informed consent was revealed.
- Patients aged 15 and more were enrolled.
- Only smear positive sputum pulmonary TB cases were enrolled.

Sample size

The sample size for the study was calculated according to the WHO and IUATLD guidelines for sample size calculation for a survey on the prevalence of anti-TB drug resistance. [(70)] It was

recommended that sample calculation for drug resistance prevalence should be based on the expected prevalence of drug resistance or the minimum prevalence of resistance to any of the anti-TB drugs that would affect program performance, logistically feasible sample and a recommended confidence of 95%.

In Sudan there are no available valid data for the prevalence of *M. tuberculosis* drug resistance. For this study we lack observations to base all assumptions for sample calculation. Therefore the international prevalence of MDR among re-treatment cases was taken as base for the calculation and a determined intake period was identified.

Using the student formula

$N= Z^2pq / d^2$ where

N: number of observations needed,

p: estimated prevalence ,

Z= 1.96,

d=allowable error (0.05 – 0.08),

q=1 – p

In the latest data, released from the WHO/IUATLD drug resistance surveillance the median prevalence of acquired (among patient with previous treatment records) MDR TB was found to be 7%, therefore *p*=0.07 was assumed as prevalence for MDR among retreatment cases and *a*=0.05 was chosen as allowable error.

The total no of observations required then $= (1.96)^2 (0.07x\ 0.93) / (0.05)^2 = 100$ cases . the sample size was increased by 20% to account for unexpected losses. The final sample consisted of **120 cases.**

An alternative way to calculate the sample size might have been to use the results of the study carried out by Dr. Obeid.[55] The results of the mentioned studyare , however, not valid

because all the re-treatment cases in the Obeid's study were selected from specialized chest units and represented only the most complicated cases. In addition the total number of the cases was small and some re-treatment categories were not well represented. The prevalence of MDR among re-treatment cases was found to be very high.

Collected sample

A total of 238 sputum sample were obtained from previously treated TB patients in Khartoum state during the period of 6th September 2004 to 20th of February 2005. Samples were cultivated on Lowenstein-Jensen media.

The 238 sputum samples were collected in two phases. First,120 cases were collected from 6th of September – 1st of December 2004 and 118 sputum sample were collected in the second period from 15th of December 2004 – 20th of February 2005. The TB reference laboratory in Khartoum did not have centrifuge machine during the first phase of sample collection. Therefore the modified Petroff's Method without centrifugation (known as neutralization method) was used for decontamination of sputum. (71) The number of the samples that showed growth and produced colonies was very small. The centrifuge was available during the second phase of collection sample and therefore modified Petroff's Method with centrifugation was used in decontamination of sputum. (71) The growth improved greatly.

Sixty two (26.1%) of the 238 cultivated sample showed growth and produced colonies.

Sixty two positive cultures were tested for their susceptibility to anti-TB drugs using the proportion method and results were obtained from fifty one (51) strains out of sixty two. Cultured Samples were forwarded to the Norwegian Public Health Institute (NIPH) Reference Laboratory for Tuberculosis, 10% of the strains will be checked for quality assurance.

Representativeness of the sample

Any person living in Khartoum state suspected to have TB was either self presented or routinely referred for diagnosis or treatment to a NTP centre. TB suspects were diagnosed and classified as new or previously treated cases in diagnostic centres. TB centres personnel were trained to treat TB patients according to National guidelines. TBMUS are distributed all-around the seven provinces in Khartoum state. The study sample included patients from the different types of health facilities (table 6).

To ensure representativeness, all TB patients with previous records of TB treatment were included into the study according to the inclusion criteria.

Because of the relatively well functioning health services, Khartoum state represent a referral site for these retreatment cases from other states as they are considered among the complicated TB cases. The study sample included patients from the seven Khartoum state provinces in addition to cases resided outside Khartoum state (table 10).

Table (10) Number of patients according to patient's residence inside Khartoum state provinces and outside the state.

Patient' residence	No of patients	Percent
Ouside Khartoum	15	24,2 %
Khartoum Province	22	35,5%
Ondueman Province	10	16,1%
Ombada Province	5	8,1%
Gabawlia Province	4	6,5%
Sharqnile Province	3	4,8%
Karary Province	2	3,2%
Bahery Province	1	1,6%
Total	62	100,0

The cases included in the study were collected from the different Khartoum state health centres that report to NTP (table 11)

Table (11) Number of patients according to different types of TB centres

Type of health centre	No. of patients	Percent
Chest Hospitals	22	35,5
Health Centres	20	32,3
General Hospitals	15	24,2
IDP Centres	5	8,0
Total	62	100,0

Design:

A quantitative non-interventional study design was selected. The design of the study chosen was observational analytical cross sectional. Retrospective data were collected from the study population, through person –to- person questionnaire, to identify risk factors associated with development of MDR among these cases, at the same time laboratory tests were carried out to assess the drug resistance status of the selected cases. The relation between the risk factor and resistance status was analyzed.

Methods Data collection:

Different information sources were used for data collection. TB registers, pre-structured questionnaire and results of different laboratory tests were used.

(1) TB registers in Khartoum state (from the different centers in Khartoum state, federal hospitals, state centers and displaced population, are TB registers found in all centers, laboratory registers and TB patients treatment cards were also used for data collection. All

these documents give detailed information about a patient including disease classification, identification information (age, residence, weight, ..etc), laboratory result and date of start of treatment. The documents were used for patients selection and validation of the information given by the patient.

(2) A pre-structured questionnaire to study the association of drug resistance / MDR and different independt variables was used in order to identify risk factors. The questionnaire was constructed to include different demographic data (age, gender,), socioeconomic status data (marital status, place of origin, residence ...etc) and data about the previous TB chemotherapy. The questionnaire was filled during friendly person to person interview. The different data collected through the questionnaire were analyzed. See annexed questionnaire (annex 1)

(3) Laboratory methods:

For all sputum samples collected from patients the following laboratory tests were performed:

(A) Smear microscopy: Zeil –Nielsen (Z-N) staining

Smear microscopy using Z-N staining is routinely used for identification of acid fast bacilli in the sputum of the patients. It is the most important tool in the diagnosis of TB. The technique is simple and inexpensive, and detects the most infectious cases of TB. It has been shown that at least 5000 bacilli per ml of sputum are required for direct microscopy to be positive, three samples are examined for diagnosis.**(72)** Smear microscopy is routinely done in all Sudan NTP diagnostic centers.

The sputa of all included cases were examined by smear microscopy using Z-N staining in order to diagnose the disease. Patients with positive samples were included in the study.

(B) Cultivation in Lowenstein –Jensen (L-J) media:

Löwenstein–Jensen (LJ) media is the most widely used solid medium for cultivation of *M. tuberculosis*, it takes approximately 6-8 weeks for M. tuberculosis to grow and form cultures. Incubation was performed at 35-37oC until growth was observed or sample was discarded as negative after eight weeks of incubation. **(72).**

Cultivation is not routinely done in Sudan NTP centers. Sputum of smear positive re-treatment cases were transferred to NTP reference laboratory for cultivation. All included cases were cultured in L-J media.

Sputum samples were first decontaminated. Decontamination was carried out very carefully so as not to kill the tubercle bacilli. For decontamination and homogenization of the collected samples **modified Petroff's Method was used (71,73).** Two methods of **modified Petroff's Method** were used. One of them was with centrifugation and the other was without centrifugation.

I. Modified Petroff's Method : With centrifugation :

Each sputum sample was transferred into a 50 ml centrifuge tube. A double volume of 4% sodium hydroxide was added and the tube was tightly closed, and mixed thoroughly by shaking or vortex briefly. The tube was allowed to stand for 15 minutes at room temperature with occasional shaking or placed in an incubator at 37 ºc for 30 minutes, but removed briefly for further mixing by shaking at intervals. Then the tube was centrifuged at 3000xg for 15 minutes. The supernatant fluid was removed and discarded into strong disinfectant. 15-20 ml sterile saline or distilled water was added and the sediment was resuspended. Another centrifugation

was performed at 3000xg for 15 minutes. Again the supernatant was discarded and two or three drops of the deposit were inoculated onto L-J culture media.

II. Without centrifugation : Neutralization method :

The sputum was transferred into a 50 ml centrifuge tube. An equal volume of 1N sodium hydroxide was added and the tube was tightly closed and mixed thoroughly by shaking or vortex briefly. The tube was kept at room temperature for 20 minutes. A neutralizer 1N Hydrochloric acid was added with phenol red until the solution became yellow to clear
(usually about 5 or 6 ml fast, then drop by drop), with shaking. 4 drops were inoculated on slopes of LJ media.

(C) Susceptibility testing:

All strains with positive culture on L-J media were tested for susceptibility to anti-TB drugs (H, R, E and S) by the proportion method. BACTEC (Becton Dickinson Diagnostic Systems, Towson, MD) method was used to ensure the quality of the proportion method.

I. The proportion method

In Sudan NTP reference laboratory only the proportion method is available. This method enables precise estimation of the proportion of isolated *M. tuberculosis* resistant to a given drug. In principle, the number of colonies growing on drug free media is compared with the number of colonies on drug containing medium and the proportion of resistant organisms is calculated. Several dilutions of *M. tuberculosis* inoculum are planted on to both control and drug – containing media. After bacteria are grown on the media, the total number of viable colonies is observed on the control medium, and compared to the number of colonies resistant to the drug concentrations tested. The proportion of bacilli resistant to a given drug is then determined by expressing the resistant portion as a percentage of the total population tested. Disadvantages of this method are high cost, time consuming, and the need quality control. **(70,73-76)**

Procedure of Proportion method:

1. **Bacterial suspension:** was prepared from *M. tuberculosis* culture. The suspension was prepared by adding approximately 4 mg moist weight of a representative sample of the bacterial mass (isolated mycobacteria), into 0.2 ml of sterile distilled water. To this 3.8 ml of sterile distilled water was added to give a suspension containing approximately 1mg/ml (S1). From this suspension a 10-fold dilution was made by adding 0.2 ml to 1.8 ml sterile distilled water (S2, 10-1). Two further serial dilutions 10-2 (S3) and 10-3 (S4) were prepared in a similar manner. One standard loopful could be inoculated on to drug-free as well as drug- containing LJ slopes as indicated below:

Suspension	Control drug-free	S 4 mg/l	H 0.2 mg/l	R 40 mg/l	E 2 mg/l
S_1 (1 mg/ml)					
S_2 (10^{-1})					
S_3 (10^{-2})					
S_4 (10^{-3})					

The recommended drug concentrations are 4 mg/l for S, 0.2 mg/l for H, 40 mg/l for R and 2 mg/l for E.

2. The slopes were incubated at 37^0C. Proportion tests were read at 28 days and again at 42 days.
3. Interpretation of all tests was based on the 42-day readings. For each strain the number of organisms resistant to each drug concentration was expressed as a percentage of the number of organisms growing on the drug -free slope.
4. **Definitions of drug resistance by proportion method ,** were based on the calculation of number of colonies in drug free and drug containing media. The proportions of colonies in drug containing media to the colonies in the drug free media were calculated, and the strain was considered resistant if the proportion was found to be as follows:

Drug	Concentration (mg/l)	Proportion
Streptomycin	4	1% or more
Isoniazid	0.2	1% or more
Rifampicin	40	1% or more
Ethambutol	2	1% or more

(2) BACTEC 460 radiometric method

This method is not available in Sudan NTP reference laboratory. BACTEC method was used to ensure the quality of the susceptibility testing carried out in Sudan NTP reference laboratory. 50% of the MDR cases and 10% of sensitive strains of the total tested strains were randomly selected and sensitivity using BACTEC method was performed in NIPH.

BACTEC test is a rapid test; results are available in about 10 days. . It is an indirect test in liquid medium. Unlike proportion method liquid median is used instead of solid media. It is like the proportion method based on using the growth indices obtained by inculcation of the *M. tuberculosis* isolates in drug containing media versus drug free media. The test uses Middle brook 7H12 broth. Growth of *M. tuberculosis* releases $^{14}CO_2$ which is measured radiometrically. The amount of $^{14}CO_2$ is expressed as a growth index, the growth indices of *M. tuberculosis* is calculated and measured in both control and drug containing tubes. **(70, 74-76)**

Quality control of cultivation and drug susceptibility testing

To ensure the accuracy and reliability of culture and drug susceptibility testing the following quality control measures were assessed:

- Laboratory arrangement and administration: The laboratory has a drafted manual describes the steps for cultures and susceptibility testing

- Laboratory equipment: all the equipments were newly installed. With exception of the centrifuge which was not available for the first samples.
- Specimens: The entire specimen were tested by spear microscopy and proved to be smear positive. Specimen was kept in refrigerators if they were not cultured in the same day of arrival.
- Digestion and decontamination: two methods were used according to the availability of the centrifuge machine
- Culture media : fresh eggs Löwenstein-Jensen media was used.
- Culture procedures: cross-contamination of cultures was avoided by using individual pipettes or loops and strict aseptic techniques was ensured
- Quality control of drug susceptibility tests the standard strain H37Rv of M. tuberculosis was used for each newly produced batch of drug susceptibility testing media [73,77]

Variables definitions

Dependent variables:

Drug-resistant TB : this is a case of TB excreting bacilli resistant to one or more anti-TBdrugs. MDR-TB is defined as TB caused by an isolate resistant to at least R and H [17].

Presence of MDR: was scaled as, MDR– Non-MDR, and resistance to different types of drugs was scaled as sensitive- resistant.

Independent variables: Risk factors for MDR among TB re-treatment factors

The following variables were checked for association with presence of drug resistance and MDR among study population:

1. Categorization of re-treatment patients:

- **Relapse**. A patient previously treated for TB who has been declared cured or treatment completed, and is diagnosed with bacteriologically positive (smear) TB.
- **Treatment after failure:** A patient who is started on a re-treatment regimen after having failed previous treatment.
- **Treatment after default:** A patient who returns to treatment, positive bacteriologically, following interruption of treatment for 2 months or more.
- **Other**: All cases that do not fit the above definitions. This group includes **chronic case,** a patient who is sputum-positive at the end of a re-treatment regimen.

2. Demographic variables: Age, sex, place of origin, residence, education, marital status, employment, income, housing conditions, smoking, TB contacts.
3. Duration of present illness symptoms and patients weight.
4. No. of previous treatment courses.
5. Duration of previous treatment
6. Mode of drug delivery (directly observed or not)
7. Site of drug delivery (Big Hospitals (chest hospitals , general hospital), Health center , PHC unit , Displaced Camp health unit) See annex (1&2)

Data analysis:

Data was sorted and patients were classified after results of *M. tuberculosis* susceptibility testing were ready into those who proved to be MDR cases and those who were not MDR cases. Presence of drug resistance was also identified. SPSS was used in the analysis and association between independent variables and the MDR status. Associations between categorical variables were assessed by the χ^2 test. Differences between groups were expressed with 95% confidence intervals (95% CI) and P value of < 0.05 was chosen as the level of significance.

CHAPTER III

RESULTS

The total collected sputum samples:

During the period between September 2004 and February 2005, a total of 263 sputum samples were collected from previously treated pulmonary TB patients in the Khartoum state. All samples were cultivated in L-J media and only 62 strains were found to be culture positives. The 63 obtained strains represented (26, 7%) of the collected cases. All the 62 strains were identified as *M. tuberculosis* and tested by the proportion method for susceptibility to the first line anti-TB drugs: E, H, R and S.

Of the total 236 collected strains 159 (66, 8%) were collected from male patients and 79 (33,2%) were from females patients. The 236 strains were collected from different health facilities , 74 (31,1%) were from chest hospitals, 72 (30,3%) were from health centres, 68 (28,6%) from general hospitals and 24 (10,1%) were from IDP camps health units. 91 (38,25%) were found to be relapse cases , 83 (34,9%) were return after default cases , 43 (18,1%) were failure cases and 21 (8,8%) were chronic cases table (13).

Two methods were used for the decontamination of the sputum samples. For the first 120 samples modified Petroff's without centrifugation was used. This method gave growth in only 20 (16.7 %.) of the total 120 cultivated sputum samples. For the rest of the 116 cultivated sputum samples another modified Petroff's with centrifugation was used and growth and formulation of colonies was observed for 43 (36.4%) strains of the 116 strains table (13).

To identify the factors associated with low percent of the positive cultures, the culture results of the total 236 patients were tested for relation to: (a) decontamination method used (b) gender of the patients (c) type of patients and (d) type of health facility. Only type of decontamination method was found to be significantly associated to the positive culture results table (14)

Table (13) Decontamination method, sex of patients, type of patients and type of centre for the total 236 patients.

Variable	Description	No of patients	Percent of the total (%)
Cultivation result	Negative	176	73,9
	Positive	62	26,1
	Total	238	100,0
Decontamination method used	Without centerfugation	127	53,4
	With centerfugation	111	46,6
	Total	238	100,0
Type of center	Chest hospital	74	31,1
	General hospital	68	28,6
	Health center	72	30,3
	IDP center	24	10,1
	Total	238	100,0
Type patient	Relapse	91	38,2
	Default	83	34,9
	Failure	43	18,1
	Chronic excretor	21	8,8
	Total	238	100,0
Sex	Male	159	66,8
	Femle	79	33,2
	Total	238	100,0

Table (14) Decontamination method, sex of patients, type of patients and type of centre in relation to cultivation results for the total 236 patients

Variable	Descreption	Culture result		Total	P value
		Negative	Postitive		
Lab. method	No centrifuge	115	12	127	0,000
	With centrifuge	61	50	111	
	Total	176	62	238	
Sex	Male	118	41	159	0,507
	Femle	58	21	79	
	Total	176	62	238	
Type of patient	Relapse	67	24	91	0,194
	Default	55	28	83	
	Failure	37	6	43	
	Chronc Excretor	17	4	21	
	Total	176	62	238	
Type of center	Chest hospital	52	22	74	0,591
	General hospital	53	15	68	
	Health center	52	20	72	
	IDP center	19	5	24	
	Total	176	62	238	

Drug susceptibility in strains:

The sixty two strains were tested for susceptibility for the first line anti-TB drugs namely for R, H, S and E. Results for susceptibility were obtained for 51 (82,2%) out of the sixty two strains. 6 (9,7%) strains revealed week growth as the amount of inoculums taken was very little or they were dry. 5(8,1%) were repeated because the growth appear in one or two controls and no growth in the others controls media and even drugs containing media.

Out of the 51 strains, resistance to S, R, H and E was detected in 28 (54.9%), 24 (47.1%) 24 (47.1%) and 15 (29.4%) strains, respectively. The highest rate of drug resistance were observed for S, whereas resistance to H and R were found equal and the lower resistant rate was to E (table 16). Sixteen (31.4%) of the 51 strains were susceptible to all tested anti-TB drugs. Consequently the resistance to any of the tested anti-TB among the study population was detected in 35 (68.6%) strains. Nineteen of the 51 (37.3%) strains were multi-drug resistant (table 17). Resistance to one drug was detected in 9 (17.6%) cases. Resistance to two drugs was detected in 6 (11.8%) strains, to three drugs in 9 (17.6%) strains, and to four drugs was detected in 11 (22.6%) strains (table 10). The susceptibility pattern to R, H, S and E of the 51 *M. tuberculosis* strains are represented in (table 18).

The presence of drug resistance varied according to the different re-treatment categories. For the relapse cases 16 (76.2%) out of the 21 cases were drug resistant and 9 (42.9%) were MDR. Among the default cases the drug resistant cases were 11 (50%) cases out of the total 22 cases. All the chronic cases were MDR i.e. the 4 cases. All the four failure cases were drug resistant and 3 (75%) were MDR cases (table 19).

In the study population the drug resistance and MDR cases among the relapse cases represented 76.2% and 42.9% of the total drug resistance and MDR cases. The return after

default drug resistance and MDR cases represented 31.4% and 15.8%, each of the failure and chronic DR cases represented 11.4%. The failure and chronic MDR cases represented 15.8% and 21.1% of the total MDR cases respectively (table 20).

Table 16 Resistance of *M. tuberculosis* strains isolated from 51 patients with previously treated pulmonary TB in Khartoum state, Sudan, to the first line anti-TBdrugs, determined using the proportion method

	Number of cases	Percent (%)
Resistance to streptomycin	28	54.9
Resistance to isoniazid	24	47.1
Resistance to rifampicin	24	47.1
Resistance to ethambutol	15	29.4
Resistance to any one drug	35	68.6
MDR	19	37.3

MDR = multidrug resistance

Table 17 Resistance of *M. tuberculosis* strains isolated from 51 patients with previously treated pulmonary TB in Khartoum state, Sudan, to the first line anti-TBdrugs, determined using the proportion method

	Number of cases	Percent (%)
Not Resistance to any drug	16	31.4
Resistance to one drug	9	17.6
Resistance to two drugs	6	11.8
Resistance to three drugs	9	17.6
Resistance to all drugs	11	21.6
Total	51	100

Table 18 Susceptibility pattern of *M. tuberculosis* strains isolated from 51 previously treated TB patients with pulmonary TB in Khartoum state, Sudan, determined using the Proportion method

S	R	E	H	No	Percent %
S	S	S	S	16	31,37
R	R	R	R	11	21,57
R	R	S	R	5	9,80
S	S	S	R	4	7,84
R	S	R	S	3	5,88
R	R	S	R	2	3,9
R	R	R	S	2	3,96
S	R	S	S	2	3,92
R	S	S	S	2	3,92
R	S	S	S	1	1,96
S	R	S	R	1	1,96
R	R	S	S	1	1,96
R	S	S	R	1	1,96
TOTAL				51	100

S: streptomycin, R: Rifampicin ,E: ethambutol and H: Insoniazid

R = resistant; S = susceptible

Table (19) Percent of drug resistance and MDR cases according to total number of different TB re-treatment cases in Khartoum State

Category of patient	Total no. of patients	Drug resistance DR		MDR	
		Infected with DR strain No (%)	Infected with non-DR strain No (%)	Infected with MDR strain No (%)	Infected with non-MDR strain No (%)
Relapse	21 100,0%	16 76,2%	5 23,8%	9 42,9%	12 57,1%
Return after Default	22 100,0%	11 50,0%	11 50,0%	3 13,6%	19 86,4%
Treatment after Failure	4 100,0%	4 100,0%	0 ,0%	3 75,0%	1 25,0%
Chronic Excretor	4 100,0%	4 100,0%	0 ,0%	4 100,0%	0 ,0%
Total	51 100,0%	35 68,6%	16 31,4%	19 37,3%	32 62,7%

Table (20) Percent of drug resistance and MDR among different TB re-treatment cases in Khartoum State according to the total number of DR and MDR cases.

Category of patient	Total no. of patients	Drug resistance N=35		MDR N=19	
		Infected with DR strain No (%)	Infected with non-DR strain No (%)	Infected with MDR strain No (%)	Infected with non-MDR strain No (%)
Relapse	21	16 45.7%	5 31.3%	9 47.4%	12 37.5%
Treatment after Default	22	11 31.4%	11 68.8%	3 15.8%	19 59.4%
Treatment after Failure	4	4 11.4%	0 0,0%	3 15.8%	1 3.1%
Chronic Excretor	4	4 11.4%	0 ,0%	4 21.1%	0 0,0%
Total	51	35 100 %	16 100%	19 100%	32 100%

Patients:

Among the 51 strains, different categories of previously treated TB cases were represented, 21 (41.2%), 22(43.1%), 4(7.8%) and 4 (7.8%) were found to be relapse, return after default, failure and chronic cases respectively (fig.2).

In Khartoum state the NTP receives reports from chest hospital, general hospitals, PHC or from IDP camp health units, the four different type of health facilities were represented in the 51 cases. 18 (35.3%) were from chest hospitals, 12 (23.5%) were from general hospitals, 16 (31.4%) were from PHC and 5 (9.8%) were from health units of IDPs camp (fig. 3).

Among the 51 cases 33 (64.7%) were found to be male and 18 (35.3%) were female (fig 4). The patient's age varied from 17 to 65 years, with a mean age of 31.5 years. Patients' weight varied from 30 kg to 70 kg, with a mean weight of 45.3 kg. 28 (54.9%) of the 51 patients were below the mean weight and 23 (45.1%) were above the mean weight.

All the patients were originally from outside Khartoum. Darfor zone was the number one source of patients and smallest amount of patients are coming from Eastern zone. 15 (29.4%) of cases were originally from Darfor region and 5 (9.8%) were from eastern zone (table 20).

The residence of the patients in the different provinces of Khartoum state was assessed in term of the recent habitation of the patient during the treatment. 18 (35.3%) of the patients were resident in Khartoum province. 11 (21.6%) of the patients were resident outside Khartoum state and only came for treatment. Only one patient (2.0%) of the patients was found to be from Bahry province (table 21).

Marital status was assessed in terms of legal status. Marital status was classified as married, single, divorced and widow. A total of 27 of the 51 (52.9%) patients were married, 21 (41.2%) were single, 2 (3.9%) were divorced and only one (2.0%) was widowed (fig 5).

The level of education was found to be very low among the study population. Illiteracy and lower levels of education were frequently demonstrated. The level of education was estimated as the highest last grade attained .13 (25.5%) were illiterate and 8 (15,7%) were able to read and write and did not finished their primary school. Eleven (21.6%) had their primary education, whereas 9 (17,6%) and 8 (15,7%) had their intermediate and secondary education. only two (3,9%) out of the 51 patients were graduated from universities (fig.6).

The monthly income of patients was classified as no monthly income, irregular monthly income and regular monthly income. 12 (23.5%) out of the 51 patients had regular monthly income. 26 (51.0%) and 13 (25.5%) were either had no monthly income or had irregular income respectively.

Unemployment was relatively common among previously treated TB patients. 20 (39, 2%)) out of the 51 patients were not working 7 (13,7%), house wives were 7 (13,7%) of the patients and students were 6 (11,8%) of them. The majority of the employed were working in manual works they were18 patients and represented 35,3% of the total 51 patients and 58.1% of the 31 employed patients (table 22).

Regarding the housing condition, 29 (56.9%) patients were found to own their houses and 22 (43.1%) were not. The number of rooms per each patient’s house was found to be ranging between 1-5 rooms with mean number of rooms of 2.1. 19 (37.3%) were living in a house with one room and only 1 (2.0%) was living in a house of five rooms. The family size (persons living in same house) varied from 2-12 persons and the mean was 6.9. Number of persons per room varied between 1-11, and the mean of persons per room was 4 (3,8). 11 (21.6%) of the patients were living in houses with four persons per one room. Only one patient was living in a house with one room with other 10 family members. Crowded accommodation was very common among the patients as 38 (74.5%) were living in houses with more than three persons per room.

Both smoking and alcohol were not common among the TB re-treatment patients. As show in table (23) habit of smoking and alcohol intake were found in 19 (37.3%) and 8(15.7%) among the patients respectively.

Fourteen (27.5%) were found to have BCG scar. Contact with another TB patient was determined as living in the same house or working together with TB patients. It was relevant for 20 of the 51 (39.2%) patients who had known TB contact and 31(60.8%) had no known TB contact. Cough duration was varied between two weeks and eight months with mean of 2.4 months.

Among the 51 patients the number of previous chemotherapy was varied. The majority of them 38 (74.5%) received one previous TB treatment, 10 (19.6%) received it twice and only three (5.9%) of the patients received it three times before (fig 7).

Among the 38 patients who had one previous treatment course, 22(57.9%) didn't finished their first course and 20 (90.9%) of the 22 stopped it due to patients related factors, 37 (97.4%) received regimen containing R, 21 (55.3%) received their treatment inside Khartoum state 17 (44.7%) received it in general hospitals (table 24). The duration of first treatment varied between 2 – 8 months with mean 4.9 months. 17 (44.7%) out of the 38 patients had their first course for less than three months and 21 (55.3%) had it for more than three months.

The 10 patients who had two previous treatment courses, all of them didn't finish their first course, 3 (30.0%) had stopped treatment because they failed their first course. 7 (70.0%) of them didn't finished their second course. All of the ten patients received regimen containing R, 5 (50.0%) and 8 (80.0%) received their first and second treatment inside Khartoum state respectively. 6 (60.0%) and 5 (50.0%) received their first and second treatment in general hospitals (table 24). Four (40%) out of the ten had their second treatment course for less than four months and 6 (60%) had it for more than four months.

The mode of supervision during the first course was classified as no supervision, daily supervision, weekly supervision and monthly supervision. Out of the 38 who had one treatment course 18 (47.4%) had daily supervision, 8 (21.1%) had monthly supervision and 6 (15.8%) had no supervision and the same number had it on weekly basis (table 24)..

Among the 10 who had two previous treatment courses, 5 (50.0%) had daily supervision in their first treatment course and 8 (80%) had weekly during their second course (table 24).

In general the majority of the cases didn't not finished their first course of treatment as 33 (64.7%) out of the 51 were interrupted their first course of treatment.

Three patients received anti-TB treatment for three times. They were all chronic cases. Two of them completed their first course and one completed the second the other two failed the second course. But all of them completed the third treatment course and their sputum results were still positive. Two of them received their first and second treatment courses outside Khartoum state (table 24).

Since Khartoum state is considered as a referral site for these cases and the state reports a high percent of these cases [(63)]. 24 (47.1%) out of the 51 cases had received their first treatment outside Khartoum state. 69.2 % of those who had more than one treatment course received their second treatment inside Khartoum state. 27 (84.4%) of the 33 who interrupted their first course of treatment were due to patient related factors. 27 (52.9%) out of the 51 cases were not supervised on daily basis during their first course of treatment (table) . twenty four (49.0%) of the 51 cases received their first treatment at general hospital.

Factors associated with infection with drug-resistant and multi-drug resistant strain of M. tuberculosis

To identify factors associated with infection with drug resistant strain among previously treated TB patients in Khartoum state demographical, social and medical factors collected from the patients were studied in relation to susceptibility patterns of *M. tuberculosis* strains.

The majority of patients infected with drug resistant and MDR *M. tuberculosis* strains were males. Male were 60.0% and 75.0% of the drug resistance and MDR cases respectively. Females represented 40.0% and 25.0% of drug resistance and MDR cases respectively (table 25). There was no significant difference in risk of infection with drug resistant strain (P =0.358) and MDR (P = 0.547) according to patients gender in the study (table 26&27)

Age of participants was grouped, according to the mean age, into two groups less than 32 and more than 32 years old. 62.9% of the drug resistant cases were less than 32 years old and 57.9% of the MDR cases were from the same age group (table 25). No significant association between age group and drug resistant (P= 0.162) and MDR (P=0.228) (table 26&27)

Marital status of participants was grouped as married at time of study or living alone which included singles, widowed and divorced. 51.4% of the drug resistant cases and 52.6% of the MDR cases were married (table 25). There is no significance difference found between marital status and infection with drug resistant strains (P= 0.384) and MDR -TB (P=0.575) (table 26&27)

Twenty four of the patients who were below the mean weight were infected with drug resistant strains which represented (68.6%) of the total drug resistance Cases. 16 of these under mean weight were also MDR cases (84.2% of the MDR cases) (table 25). Strong association was found between low weight and presence of drug resistance (P=0.006) and MDR (P=0.001) (table 26&27).

68.6% of drug resistant cases and 57.9% of MDR cases were living inside Khartoum state (table 25). Significance difference was found between residence inside Khartoum and infection with MDR TB. 0.037. But was not significantly associated with presence of drug resistance. The P values was 0.074 and (table 26&27)

The majority of cases with drug resistance and susceptible strains received one previous treatment course in their past medical history. 62.9% of drug resistance strains received one anti-TB treatment course. Among MDR cases patients who receive anti-TB treatment course for two times represented 52.6% (table 25). Significant association was found between presence of drug resistance and MDR and number of previous treatment courses. For drug resistance the P value was 0.009 and for MDR the P value was <0.001 (table 26&27)

The duration of previous treatment was assessed to explore the association between the duration of previous treatment and presence of MDR and drug resistance (table 25). All patients who received two or three previous treatment courses were MDR cases. The duration of the first treatment course was significantly associated with presence of drug resistance (P= 0.003) but not associated with the presence of MDR (P=0.250) (table 26&27)

25 (71.4%) of drug resistant cases and 13 (68.4%) of MDR cases received anti-TB drugs for more than three months in the first treatment course.

The majority of drug resistant and MDR cases were from hospitals, they represented 65.7% and 94.7% of the drug resistant and MDR cases respectively (table 25).. Significant association between type of center and MDR (P< 0.001) but no association was found between type of center and infection with drug resistant strains (P=0.121) (table 26&27)

54.3% and 57.9% of drug resistant and MDR cases were not working (table 25). No significant association between working and DR and MDR (table 26&27)

Cough duration was used to explore the possible association between symptoms duration and presence of drug resistance and MDR. The mean of cough duration among the study population was 2.4 month. 45.7% of drug resistant cases had cough duration more than the mean (2.4 months) (table 25). Cough duration was strongly associated with the presence of DR (P=0.001). 63.2% of the MDR cases had more than mean duration of cough. cough duration was also strongly associated with the presence of MDR (P<0.001) (table 26&27)

No significant difference was observed between patients infected with drug resistant and susceptible *M. tuberculosis* strains in relation to place of origin, smoking, alcohol abuse, crowded accommodation (person per room more than the mean), unemployment, and contact with another TB patient (table 26&27)

Fig (2) Category of previously treated TB patients

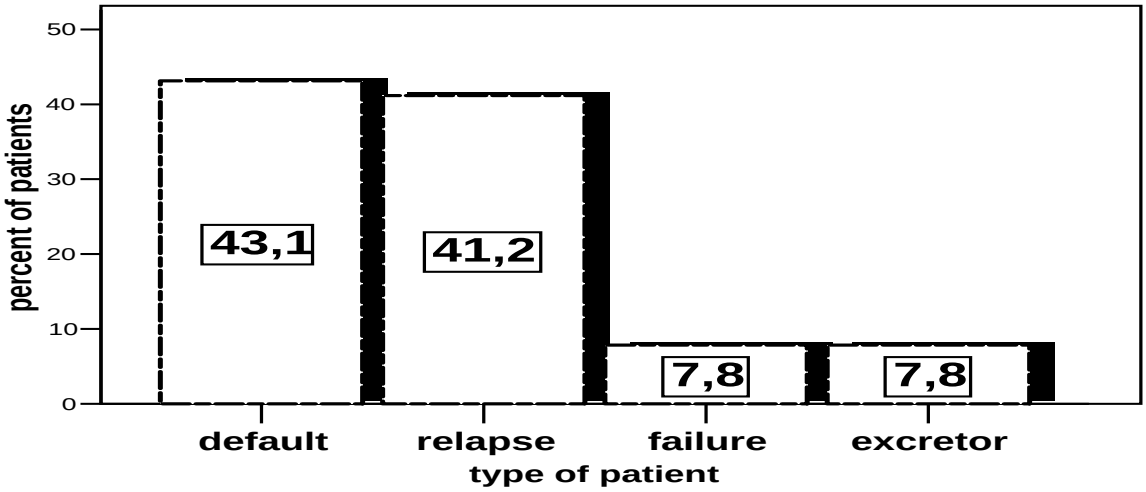


Fig. (3) No of previously treated TB patients according to different health facilities in Khartoum state.

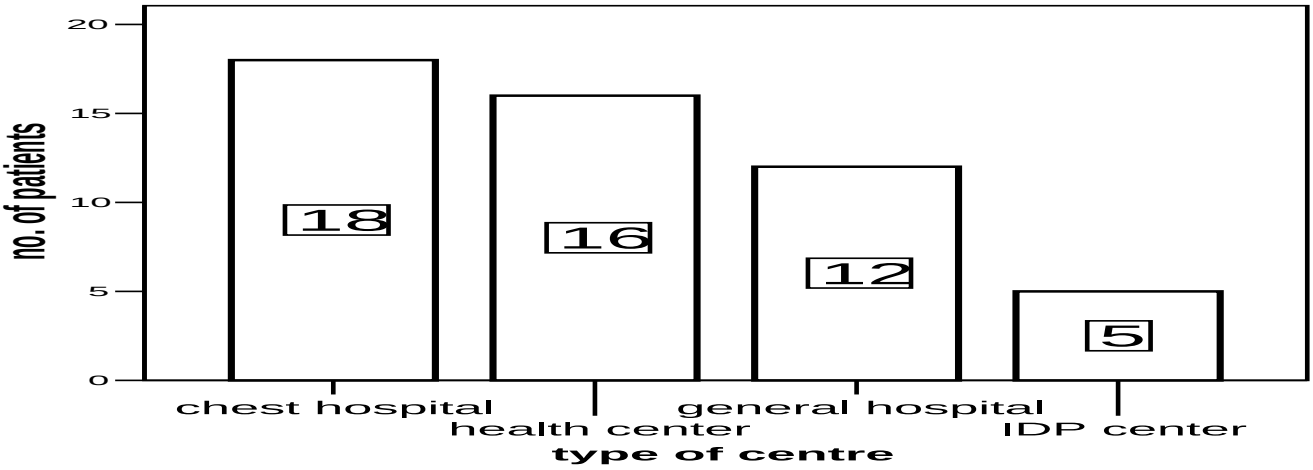


Fig (4) Sex of previously treated TB patients

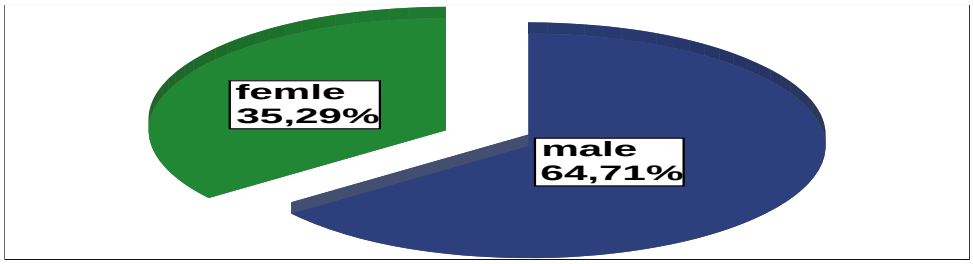


Table (20) Place of origin of previously treated TB patients

Zone of origin	No. of patients	Percent	Cumulative Percent
Darfor zone	15	29,4	29,4
South zone	9	17,6	47,1
North zone	8	15,7	62,7
Centre zone	8	15,7	78,4
Kordofan zone	6	11,8	90,2
East zone	5	9,8	100,0
Total	51	100,0	

Drafor zone include 3 states, South zone include 10 states, Centre zone include 4 states, Kordofan zone includes 3 states, East Zone includes 3 States and North zone includes 2 states.

Table (21) Residence of previously treated patients

Patient residence	No. of cases	Percent	Cumulative Percent
Khartoum province	18	35,3	35,3
Outside Khartoum	11	21,6	56,9
Omdorman province	9	17,6	74,5
Gabalwlia province	4	7,8	82,4
Sharqelnile province	3	5,9	88,2
Ombada province	3	5,9	94,1
Karary province	2	3,9	98,0
Bahery province	1	2,0	100,0
Total	51	100,0	

Fig (5) Marital status of previously treated TB patients in Khartoum state

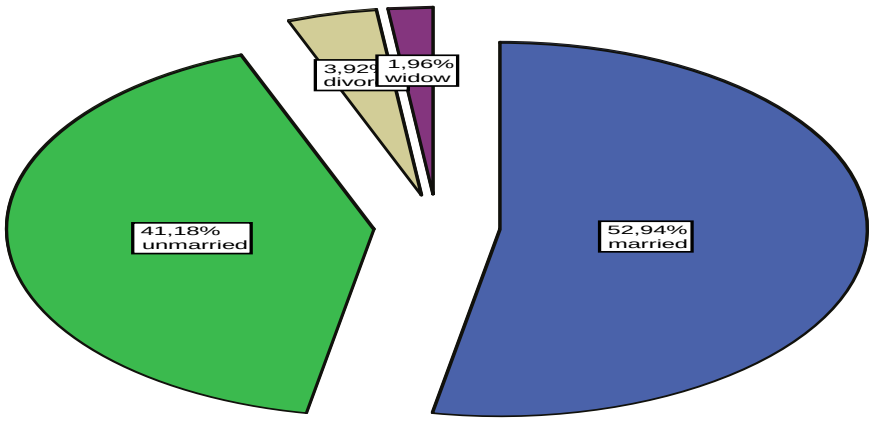


Fig (6) Educational level of previously treated TB patients in Khartoum stat

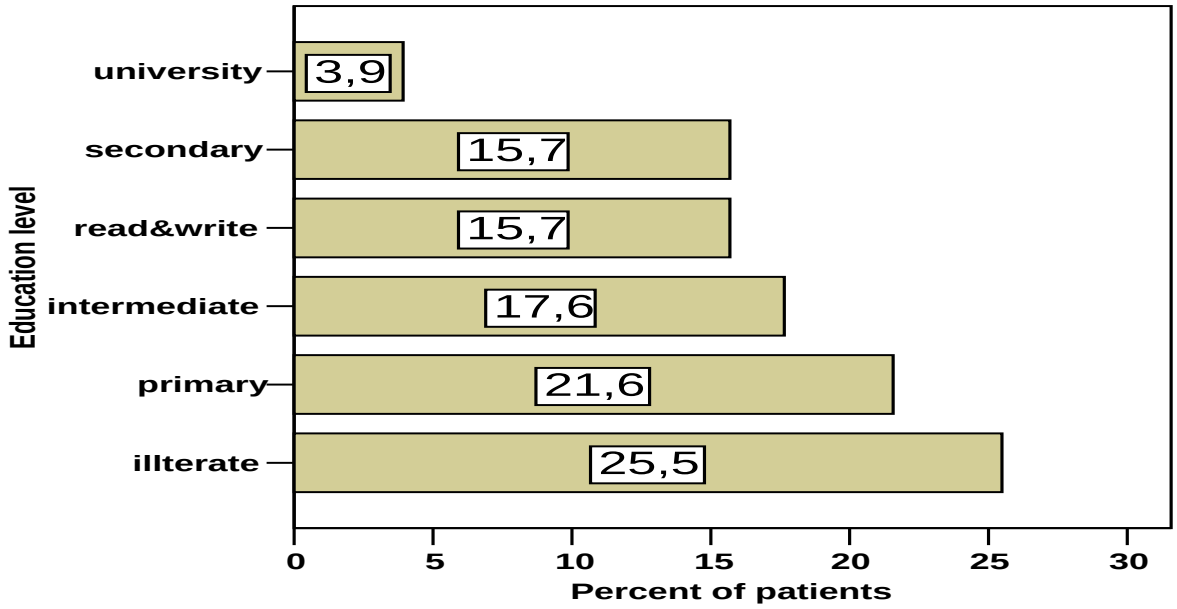


Table (22) employment among previously treated TB patients in Khartoum state

Employment status	No. of patients	Percent
Manual	18	35,3
No work	7	13,7
Housewife	7	13,7
Student	6	11,8
Technical	5	9,8
Trading	5	9,8
Office work	3	5,9
Total	51	100,0

Table (23) Social habit among previously treated TB cases, smoking and alcohol intake.

Habit	Patient response	No, of patients	Percent of the total (%)
smoking	Smoking	19	37,3
	Not smoking	32	62,7
	Total	51	100,0
alcohol	Alcohol user	8	15,7
	Not alcohol user	43	84,3
	Total	51	100,0

Fig (7) No of previous treatment

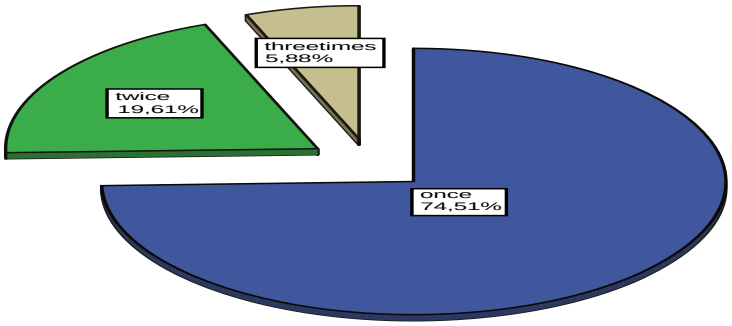


Table (24) Details of previous treatment courses of 51 patients.

Variable	Pateint response	No of patient in group 1 and (%) N=38	No of patient in group 2 and (%) N=10	No of patient in group 3 and (%) N=3
Completion of first treatment course	First course Completed	16 (42.1%)	0	2 (66.7%)
	First course not completed	22 (57.9%)	10 (100%)	1 (33.3%)
Site of first course	Inside Khartoum state	21(55.3%)	5 (50%)	1 (33.3%)
	Outside Khartoum state	17(44.7%)	5 (50%)	2 (66.7%)
Type of centre for first treatment	Chest hospital	4 (10,5%)	0	0
	General hospital	17 (44,7%)	6 (60%)	2 (66.7%)
	PHC	13 (34,2%)	2 (20%)	1 (33.3%)
	IDP camp	4 (10,5%)	2 (20%)	0
Duration of first course	Less than three months	17 (44.7%)	4 (40%)	1 (33.3%)
	More than three months	21 (55.3%)	6 (60%)	2 (66.7%)
Mode of supervision during first course	No supervision	6 (15,8%)	1 (10%)	0
	Daily supervision	18 (47,4%)	5 (50%)	1 (33.3%)
	Weekly supervision	6 (15,8%)	3 (30%)	1 (33.3%)
	Monthly supervision	8 (21,1%)	1 (10%)	1 (33.3%)
Causes of stop first course of treatment	Patient related	20 (90.9%)*	6 (60%)*	1 (100%)*
	Programme related	2 (9.1%)*	4 (40%)*	0
Completion of second treatment course	Second course Completed		3(30%)	1 (33.3%)
	Second course not completed		7 (70%)	2 (66.7%)
Site of second treatment course	Inside Khartoum state		8 (80%)	1 (33.3%)
	Outside Khartoum state		2(20%)	2 (66.7%)
Type of centre for second treatment	Chest hospital		3 (30%)	0
	General hospital		5 (50%)	3(100%)
	PHC		1(10%)	0
	IDP camp		1 (10%)	0

Variable	Pateint response	No of patient in group 1 and (%) N=38	No of patient in group 2 and (%) N=10	No of patient in group 3 and (%) N=3
Duration of second course	Less than four months		4 (40%)	1 (33.3%)
	More than four months		6 (60%)	2 (66.7%)
Mode of supervision during second treatment course	No supervision		0	0
	Daily supervision		2(20%)	0
	Weekly supervision		8(80%)	3(100%)
	Monthly supervision		0	0
Causes of stop second course of treatment	Patient related		3 (30%)	1 (33.3%)
	Programme related		7(70%)	1 (33.3%)
Completion of third course	Third course completed			3(100%)
	Third course not completed			0
Site of third course	Inside Khartoum state			3(100%)
	Outside Khartoum state			0
				0
Type of centre for the third treatment	Chest hospital			3(100%)
	General hospital			0
	PHC			0
	IDP camp			0
Duration of third course	Less than four months			0
	More than four months			3 (100%)
Mode of supervision during third course	No supervision			0
	Daily supervision			3(100%)
	Weekly supervision			0
	Monthly supervision			0
Causes of stop treatment	Patient related			0
	Programme related			0

- (*) % of patient is calculated out of those stopped the treatment
- Group 1 : patient who received one antituberculosis course Group 2 : patients who received two antituberculosis courses
- Group 3 : patients who received three antituberculosis course

Table 25 Distribution of resistant and susceptible *M. tuberculosis in relation to d*demographic and medical profile of 51 previously treated pulmonary TB patients in Khartoum state , Sudan 09/2004 -02/ 2005

Patients characteristic	Descreption	Total N=51	Drugresis N=35 (%)		MDR N=19 (%)	
			yes	no	postive	negative
Sex	Male	33 (64,7%)	21 (60,0%)	127 (5,0%)	11 (57,9%)	22 (68,8%)
	Female	18 (35,3%)	14 (40,0%)	4 (25,0%)	8 (42,1%)	10 (31,3%)
Marital status	Married	27 (52,9%)	17 (48,6%)	10 (62,5%)	9 (47,4%)	18 (56,3%)
	Unmarried	24 (47,1%)	18 (51,4%)	6 (37,5%)	10 (52,6%)	14 (43,8%)
Patient resident	Outside Khartoum	12 (23,5%)	11 (31,4%)	1 (6,3%)	8 (42,1%)	4 (12,5%)
	Inside Khartoum	39 (76,5%)	24 (68,6%)	15 (93,8%)	11 (57,9%)	28 (87,5%)
Education	Illiterate	13 (25,5%)	11 (31,4%)	2 (12,5%)	4 (21,1%)	9 (28,1%)
	Educated	38 (74,5%)	24 (68,6%)	14 (87,5%)	15 (78,9%)	23 (71,9%)
Working	Working	30 (58,8%)	19 (54,3%)	11 (68,8%)	11 (57,9%)	19 (59,4%)
	Not working	21 (41,2%)	16 (45,7%)	5 (31,3%)	8 (42,1%)	13 (40,6%)
Weight of the patient	Below mean wt	28 (54,9%)	24 (68,6%)	4 (25,0%)	16 (84,2%)	12 (37,5%)
	Above mean weight	23 (45,1%)	11 (31,4%)	12 (75,0%)	3 (15,8%)	20 (62,5%)
health facility	Hospital	30 (58.8%)	23 (65,7%)	7 (43,8%)	18 (94,7%)	12 (37,5%)
	Health centre	21 (41,2%)	12 (34,3%)	9 (56,3%)	1 (5,3%)	20 (62,5%)
No. Of previous treatment	One previous course	38 (74,5%)	22 (62,9%)	16 (100,0%)	6 (31,6%)	32 (100,0%)
	Two previous courses	10 (19,6%)	10 (28,6%)	0 (0,0%)	10 (52,6%)	0 (0,0%)
	Three previous courses	3 (5,9%)	3 (8,6%)	0 (0,0%)	3 (15,8%)	0 (0,0%)
Duration of first course	Less than 3 month	22 (43,1%)	10 (28,6%)	12 (75,0%)	6 (31,6%)	16 (50,0%)
	More than three month	29 (56,9%)	25 (71,4%)	4 (25,0%)	13 (68,4%)	16 (50,0%)
TB contact	Tb contact	20 (39,2%)	17 (48,6%)	3 (18,8%)	9 (47,4%)	11 (34,4%
	No TB contact	31 (60,8%)	18 (51,4%)	13 (81,3%)	10 (52,6%)	21 (65,6%)
BCG	Present	14 (27,5%)	7 (20,0%)	7 (43,8%)	2 (10,5%)	12 (37,5%)
	Absent	37 (72,5%)	28 (80,0%)	9 (56,3%)	17 (89,5%)	20 (62,5%)
Age group	Less than 32	35 (68,6%)	22 (62,9%)	13 (81,3%)	11 (57,9%)	24 (75,0%)
	More than 32	16 (31,4%)	13 (37,1%)	3 (18,8%)	8 (42,1%)	8 (25,0%)
cough duration 2gp	Less than 4 months	35 (68,6%)	19 (54,3%)	16 (100,0%)	7 (36,8%)	28 (87,5%)
	More than 4 month	16 (31,4%)	16 (45,7%)	0(,0%)	12(63,2%)	4 (12,5%)

Table 26 Demographic and medical profile of 51 patients with pulmonary TB in selection to infection with resistant and susceptible *M. tuberculosis*

Patients characteristic	Total N=51	Infection with drug-resistant strain N=	Infection with drug-susceptible strain N=35	*P*
Male	33 64,7%	21 (60,0%)	12 75,0%	0.358
Age more than 32 years	35 68,6%	22 62,9%	13 81,3%	0.162
Married	27 52,9%	17 48,6%	10 62,5%	0.384
Rsident inside Khartoum	39 76,5%	24 68,6%	15 93,8%	0.075
Patient Educated	38 74,5%	24 68,6%	14 87,5%	0.185
Working	30 58,8%	19 54,3%	11 68,8%	0.375
Patient weighed below mean wt	28 54,9%	24 68,6%	4 25,0%	0.006
Patient treated at Hospital	30 58.8%	23 65,7%	7 43,8%	0.121
Patient received one previous course	38 74,5%	22 62,9%	16 100,0%	O.009
Duration of first previous course more than three month	29 56,9%	25 71,4%	4 25,0%	0.003
No TB contact	31 60,8%	18 51,4%	13 81,3%	0.065
BCG Absent	37 72,5%	28 80,0%	9 56,3%	0.099

CI = 95% confidence interval;

Table 27 Demographic and medical profile of 51patients with previously treated pulmonary TB in selection to infection with MDR and non-MDR *M. tuberculosis*

Patients characteristic	Total N=51	Infection with MDR strain N=35	Infection with non-MDR strain N=35	*P*
Male	33 64,7%	11 57,9%	22 68,8%	0.547
Age more than 32 years	16 31,4%	8 42,1%	8 25,0%	0.228
Married	27 52,9%	9 47,4%	18 56,3%	0.575
Rsident inside Khartoum	39 76,5%	11 57,9%	28 87,5%	0.037
Patient Educated	38 74,5%	15 78,9%	23 71,9%	0.743
Working	30 58,8%	11 57,9%	19 59,4%	1.000
Patient weighed below mean wt	28 54,9%	16 84,2%	12 37,5%	0.001
Patient treated at Hospital	30 58.8%	18 94,7%	12 37,5%	0.000
Patient received one previous course	38 74,5%	6 31,6%	32 100,0%	0.000
Duration of first previous course more than three month	29 56,9%	13 68,4%	16 50,0%	0.250
No TB contact	31 60,8%	10 52,6%	21 65,6%	0.389
BCG Absent	37 72,5%	17 89,5%	20 62,5%	0.053

CI = 95% confidence interval;

CHAPTER IV

DISCUSSION

First study in a newly established TB reference laboratory:

In the thesis we attempted to describe the problem of resistance to anti-TBdrugs among previously treated patients in Khartoum state and we identified some of the risk factors associated with the development of drug-resistant TB. Cultivation of collected sputum samples and drug susceptibility testing were performed in Sudan NTP reference laboratory (NRTL).

The reference laboratory was newly established and this study is the first to be performed in the laboratory. Since it started its work the laboratory is involved in the quality assurance of the smear microscopy carried out in all TBMUs and culture and susceptibility testing were not routinely performed in the lab. Before our research work started there was a problem of high percent of the contamination among the cultures performed in the NRTL. With the help of the external technical advisor of the NTRL the contamination problem was solved [(67)]. Therefore our research work was delayed and started in September rather than July 2004. The contamination rate was 6% in our study sample.

To accomplish the research we totally depended on the available equipments, supplies and personnel. Most of equipments are installed recently with the exception of the centrifuge which was not available at start of the research. All reagents and supplies needed for culture and susceptibility testing of *M. tuberculosis* has been purchased from LHL which is the prinicipal donor for the establishment of the laboratory. WHO and IUATLD also assisted. Personnel had been trained externally and internally and most of the susceptibility testing

was carried out after a training course facilitated by an external expert from 6 April, 2005 to 26 April, 2005 [67].

Low percentage of positive cultures:

This study was challenged by the low percentage of positive cultures from the sputum samples cultivated. In our study different methods were used for decontamination of the collected sputum samples. Sputum samples usually contain many other micro-organisms (contaminants). The contaminants are more rapidly growing than *M. tuberculosis*. Decontamination is very crucial in killing these contaminants. Decontamination affects *M. tuberculosis* bacilli. Under mildest and optimal conditions decontamination procedures kill 10 - 60% of the mycobacteria in the specimen. Decontamination methods that yield high percentage of positive cultures depend upon well trained staff and availability of equipments (e.g. centrifuge) and supplies (reagents). Methods involving the use of a centrifuge are more efficient than simple decontamination and culture of sputum directly onto medium [72.73.76]. For the first 120 sputum samples the centrifuge was not available. Therefore, as shown in the results, failure to obtained high percentage of positive cultures was significantly associated with the method of decontamination used and in particular method with centrifugation was responsible for the good results obtained.

Previously treated patients in Khartoum state:

In our study relapse and return after default were the majority of previously treated patients. A remarkable portion of these patients were coming from outside Khartoum. Darfor zone is one of the main sources of these patients; this can be explained by the insecurity in that region due to the civil conflict which started since 2002. Khartoum state represents a referral site for these patients. Eleven (21.6%) of the patients were resident outside

Khartoum and came for treatment. Unemployment, low level of education crowded housing condition were relatively common among previously treated patients.

M. tuberculosis drug resistance among previously treated patients in Khartoum state:

The real situation of *M. tuberculosis* drug resistance and MDR in Sudan is not fully determined. The NTP intended to undertake surveillance for assessing prevalence of primary and multi-drug resistance in TB patients. Based on the surveillance results further intervention and careful introduction of the second line anti-TB drugs ,using standard guidelines, will be ensured. Many steps are taking place to achieve the surveillance. Establishment of the reference lab is on of these steps.

According to recent study conducted by Dr. Obeid et al, drug resistance and MDR among new cases were 36.5% and 2.1% respectively. Among previously treated cases resistant to one drug were 74.55% and MDR was 56.1%. High resistance to S was found among both types of patients. The study was conducted in Khartoum and Gezira state, the majority of cases were from Khartoum (82.6%) collected from big hospitals and IDP camp. The MDR rate in Khartoum state patients was (22.7%). [(55)]

In Obeid's study the prevalence of DR and MDR among previously treated TB cases was apparently high. In our study we targeted to elaborate *M. tuberculosis* drug resistance among previously treated TB cases. In order to overcome the shortages of Obeid's study we targeted to represent the different categories of previously treated TB patients and to include other health facilities.

In our study the majority of the previously treated cases were relapse cases (41.2%) and return after default cases 22 (43.1%). Most of the cases are coming from hospitals. 35.3%

were from chest hospitals, 23.5% from general hospitals and 31.4% were recruited from PHC.

Our study showed that resistance to at least one drug among previously treated cases was 68.6%. MDR among previously treated cases was found to be 37.3%. Both drug resistance and MDR rates were less than the previous study rates.

In our study, monoresistance to S was the found to be at higher rate compare to resistance to R, H and E. resistance to H and R was found to be equal and the resistance to E was found to be the least resistance. The resistance rates to the different drugs are slightly different from Obeid's study rates. The small sample of our study put remarkable limitations on the final results.

Resistance to two and more than two drugs was higher than resistance to one drug among previously treated TB patients. Resistance to three and four drugs was detected (39.2%). Resistance to one drug was observed in (31.4%) of the strains.

Risk factors for the development of drug-resistant TB

TB drug resistance develops as a result of spontaneous genetic mutations in *M. tuberculosis.* Drug resistance occurs in a sequential process, i.e. monoresistance, double resistance, triple resistance and quadruple drug resistance. Clinical TB drug resistance emerges when resistant strains are selected during inadequate use of anti-TB drugs, e.g. if monotherapy is applied. Monotherapy may take place if TB patients are receiving regimens include drugs they are already resistant to, or if additional drug is added to failing regimen. In this respect previous treatment with anti-TB drugs is considered as the major risk factor for development of *M. tuberculosis* drug resistance and MDR. All surveys and researches revealed that the prevalence of drug resistance is significantly higher among previously

treated patients than among new patients [78, 79]. Other associated risk factors, which increase the likelihood for development of MDR among these cases, were identified. [80,81]

Identification of factors associated with the development of drug resistance is very important and helpful in planning anti-tuberculosis activities. In case of limited resources NTPs (like Sudan NTP) the routine use of drug susceptibility testing is beyond the capacity of their health-care resources. So the clinical and epidemiological background of a re-treatment patient can be used to assess the likelihood of development of MDR-TB and appropriate control measures may be constructed and priorities can be settled to prevent development and spread of MDR among these cases and the entire community.

In our study significant association was found between infection with DR and MDR and number of previous treatment courses. All patients who received more than one previous treatment were MDR cases. Prevalence of DR and MDR varies with the number of courses of chemotherapy received by the patients and it may reach (up to 80%) in patients who receive two or more courses of chemotherapy [17]

Previously treated cases are considered among the complicated TB cases. Therefore they are treated in hospitals, which can be a cause for the nosocomial spread of DR and MDR in hospitals. In our study the majority of previously treated patients are collected from hospitals (65.5%). Significant association was found between type of hospital and MDR and no association with DR (table 24)

Residence inside Khartoum was also associated with MDR but not associated with drug resistance.

Long duration of TB symptoms indicates long duration of TB diseases and also it indicates the long infectiousness of TB patient. Cough is the major TB symptom. In our study cough duration was significantly associated with development of both DR and MDR.

Low weight was strongly associated with infection with both drug resistant and MDR strains table 26,27.

Male patients were the majority of re-treatment cases, but no association was found between the patient's gender and development of DR and MDR. This finding is also going with Obeid's study results. The small size of re-treatment cases in the two studies made it difficult to conclude about the association of gender and infection with DR and MDR.

Certain social factors such as smoking habit, alcohol abuse and unemployment were not associated with the development of drug resistance in the study population.

Tables 26 and 27 summarize the risk factors which were significantly associated and those who were not associated with development of DR and MDR.

Constraints and limitations:

Our study is the first study performed in the Sudan NTP National Reference Laboratory (NRTL). It faced many constrains and challenges.

Firstly it was challenged by the problem of contamination of the cultures in the reference NRTL. Our work was delayed and started after the problem of the contamination was solved. After this we faced the lack of centrifuge machine for the decontamination of the collected sputum samples, we alternatively used the neutralization method (decontamination method without centrifugation). This method was recently introduced to the laboratory staff. They were not very familiar or rather experienced to it and the results we had were very low. We decided to continue the collection of the sputum sample and the centrifuge was available for the rest of the collected samples and the culture results were improved.

A training course was conducted by an external expert to strengthen and improve the quality of cultures in the NRTL. The susceptibility testing was postponed, for most of the culture strains, to be carried out after the training course.

For quality assurance of the susceptibility tests of Sudan NRTL, all the positive 62 culture positive strains were forwarded to Norway. Again we challenged with difficulties of shipping the strains. The transfer of the strains took almost more than 2 months.

Our study showed many limitations. One limitation of our study was the small number of the total previously treated TB cases who had susceptibility testing results. Out of the total 236 collected sputum sample the susceptibility results were available for only 51 patients. Inability to verify the drug susceptibility status for all enrolled previously treated TB patients, challenge any statistical conclusion about the prevalence of DR and MDR among these patients. Another limitation is failure to get the quality assurance results for the tested samples. it was proposed to check the quality assurance for 50% of the MDR cases and 105 of the susceptible strains or alternatively we check 10% of the total strains.

Future perspectives

Despite these limitations, our study can serve to initiate a discussion about the prevalence of DR and MDR among previously treated patients. Before that the study can be a helpful tool to improve the quality of work performed in the reference laboratory. The study showed the urgent need for training and standardization of laboratory methods used in the NRTL. The results of present study can serve as a helpful tool for planning further strengthening of the reference laboratory functioning. The research and the network created during the data collection should continue collecting more data. Susceptibility tests will be carried and quality assurance will be carried out.

CONCLUSIONS AND RECOMMENDATIONS

Conclusions

1. Sudan TB Reference laboratory need more strengthening on the issue of cultures and drug susceptibility testing.
2. The reference laboratory is not following a standardized method for cultivating TB patients sample and different methods are used. The different methods had different results. Laboratory personnel are much experienced with the decontamination methods with centrifugation.
3. Khartoum state still represent a referral site for previously treated TB patients. They are still accumulated in chest hospitals and big referral hospitals.
4. Drug-resistant TB is common among previously treated TB patients, and resistance to more than one anti-TB drug is also common among these patients.
5. The highest rates of resistance are observed for S.
6. Resistance to R and to H are present in equal proportions.
7. Number of previous treatment courses and duration of previous treatment course and health facility where patient received their previous treatment are the risk factors for increasing the likelihood of development of drug-resistant TB among previously treated patients in Khartoum state.

Recommendations

1. Methods of culture used in the reference laboratory should be standardized. Methods to be used are the methods which the laboratory personnel competent on it.
2. It is important to monitor and control resistance to anti-TBdrugs among previously treated patients.
3. More concentration should be paid for accumulation of previously treated patients in big hospital. Big hospital can be used for their diagnosis and to ensure more patients compliance they should be referred to the nearest TB health facility.
4. Treatment of TB based on different case categories administered under direct supervision is useful to prevent non-compliance and development of drug resistance.
5. TB control programme should use all efforts to ensure proper treatment of previously treated TB patients.
6. Drug resistance surveillance should be conducted in its due time (end of this year).
7. The study work should continue. More patients should be recruited and drug susceptibility tests should be carried out and quality of susceptibility should be assured.

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Drug resistance among Tuberculosis Re-Treatment Cases; Study on DR/MDR and associated determinants factors for MDR in TB Re-Treatment Cases in Khartoum State.

Questionnaire

NO of case:

1. Date of interview …… Name of the centre ……
2. Type of the centre
    1. Chest hospitals
    2. General hospital
    3. Health centre
    4. PHC unit
    5. Displaced Camp health unit
3. Type of patient
    1. Relapse
    2. Return after default
    3. Failure
    4. Chronic case
4. Patient card no: ……
5. Sex: …….
6. Age: …….
7. Place of origin …….
8. Residence …….

9. Marital status : 1. Married 2. Unmarried 3. Divorced 4. Widowed

Socioeconomic status:

9. Education (edu)
    1 = illiterate 2 = read & write 3= primary
    4 = preparatory 5 = secondary 6 = university
    7 = postgraduate
10. Monthly Income (Minc)
    1. Regular
    2. Irregular
    3. without income generating
11. Approximate monthly income aminc:…….
12. Occupation (occ)
    1 = manual labour 2 = technical labour
    3 = office work 4 = tradesman 5 =no work
    6 = house wife 7 = student
13. Spouse occupation (socc)
    1 = manual labour 2 = technical labour
    3 = office work 4 = tradesman 5 =no work
    6 = house wife 7 = student
    8 = not applicable

14. House ownership (hous)
    1 = yes 2 = no

15. Room number (room) numerical

16. Family size (fsiz) numerical

16] Person / room (p/r)

17. Habits,
    1. Smoking, yes no duration ……
    2. Alcohol users, yes no duration …….

Current illness:

18. Main complaints of suspect

- Cough present absent duration …….
- Weight loss present absent duration …….
- Chest pain present absent duration …….
- Fever present absent duration …….
- Night sweat present absent duration …….
- Tiredness present absent duration ……
- Haemoptysis present absent duration ……

19. BCG present absent
20. Weight of patient …….
21. TB among relatives or other persons living together with the patient:
    No, Yes

Previous treatment:

22. No of previous treatment ……

23. First course
    1. Date of start of first treatment ……..
    2. Did patient complete first course: Yes no
    3. Duration of first treatment course ……..
    4. Type of drugs used Same as this time not the same
    5. Site where patient receive first treatment
       Inside Khartoum outside Khartoum
       Chest hospitals, general hospital, Health centre
       PHC unit, Displaced Camp health unit)
    6. Type of supervision during first course no supervision
       Daily, weekly, monthly
    7. Treatment outcome ……
    8. Did the patient interrupt treatment Yes no
    9. How many times …….
    10. For how log …….
    11. Reasons for stopping treatment
        - system related (irregular drug supply)
        - patient's related (non-compliance with hospital rules, patient's decision)

24. Second course

    1. Date of start of second treatment
    2. Did patient complete second course Yes no
    3. Duration of second treatment course ……..
    4. Type of drugs used Same as this time not the same
    5. Site where patient receive second treatment
       Inside Khartoum outside Khartoum
       Chest hospitals, general hospital, Health centre
       PHC unit, Displaced Camp health unit)
    6. Type of supervision during second course no supervision
       Daily , weekly , monthly
    7. Treatment outcome ……..
    8. Did the patient interrupt treatment Yes no
    9. How many times ……
    10. For how log ……
    11. Reasons for stopping treatment
        - system related (irregular drug supply)

- patient's related (non-compliance with hospital rules, patient's decision)

25 . Third course

1. Date of start of third treatment ……..
2. Did patient complete third course Yes no
3. Duration of third treatment course ……
4. Type of drugs used Same as this time not the same
5. Site where patient receive third treatment
   Inside Khartoum outside Khartoum
   Chest hospitals, general hospital, Health center ,
   PHC unit, Displaced Camp health unit)
6. Type of supervision during third course no supervision
   Daily , weekly , monthly
7. Treatment outcome ……..
8. Did the patient interrupt treatment Yes no
9. How many times …….
10. For how log ……
11. Reasons for stopping treatment
    - system related (irregular drug supply)
    - patient's related (o non-compliance with hospital rules, o patient's decision)

26. Patients non-compliance: causes

1. Patient don't know the duration of treatment
2. Patient a way from centre
3. Patient not agree with diagnosis as TB
4. Patient feels well and no need for treatment
5. Due to centre errors (shortage of drugs , bad attitudes , poor health education …etc)
6. Others identify …….

27. Lab data

1.	Positivity grading	scanty	+1	+2	+3
2.	Culture	positive	negative		
3.	Result of susceptibility	Susceptible	resistant		
4.	R	Susceptible	resistant		
5.	S	Susceptible	resistant		
6.	E	Susceptible	resistant		
7.	H resistant to any drug	Susceptible	resistant		
8.	MDR	positive	negative		