

A high rate of recurrent tuberculosis in western Kenya independent of human immunodeficiency virus infection.

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SUMMARY

Background: Previous studies have shown that recurrent TB develops in about 2-5% of the patients after curative treatment with short-course anti-TB chemotherapy. With the advent of HIV/AIDS, the rate TB recurrence is anticipated to rise.

Objectives: To determine whether HIV infection and TB recurrence are associated with anti-TB drug resistance and the rates of ZN microscopy and culture positivity among the recurrent TB cases in western Kenya.

Design and methods: A cross-sectional study was carried out between 2007 and 2009. Sputa from 872 tuberculosis suspects underwent mycobacteriologic evaluation using Ziehl Neelsen smear microscopy, Lowenstein-Jensen and BACTEC MGIT 960 culturing, and Hain's GenoType[®] *Mycobacterium* CM and GenoType[®] *Mycobacterium* AS molecular identification tests. Consenting participants were screened for HIV infection using Uni-Gold[™] test and positives were confirmed with the enzyme linked immunosorbent assay. **Results:** In total, 361/872 (41%) of the suspects mycobacterial disease (346 TB, 4.2% non-tuberculous mycobacterial disease). HIV testing was accepted by 695 (79.7%) and 39.1% of these (272/695) were found positive. Recurrence of TB constituted 44.8% (155/346) of the TB cases, with 41.9% (65/155) of them co-infected with HIV. There was no significant difference in TB recurrence rates with HIV status [OR = 0.57; 95% CI: 0.29-1.13; P = 0.10].

Conclusions and recommendations: This study reports a much higher (44.8%) rate of recurrent TB, compared to that of National TB control Programme of 5% in 2008 and a combined retreatment rate of 14% in 2009. The HIV co-infection and TB recurrence were not associated with anti-TB drug resistance. The majority of TB recurrent cases were ZN smear negative (67.7%) and culture negative (80%). The high TB recurrence observed in this study calls for studies to determine the proportions of the disease attributable to endogenous re-activation (relapse) and exogenous re-infection.

Key words: Recurrent tuberculosis; HIV co-infection

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Introduction

Even after effective short-course chemotherapy for active tuberculosis (TB), some patients experience a recurrent TB episode. The recurrence of active TB after treatment can be due to relapse (endogenous reactivation) or exogenous re-infection with a new strain

of *Mycobacterium tuberculosis* complex. A relapse is a patient previously treated for TB who was declared cured or treatment completed, but is diagnosed with bacteriologically confirmed TB again [1]. However, the role of exogenous re-infection in recurrent TB episodes

has been debated for decades [2-4], but the proportion of recurrent TB attributable to relapse or re-infection has not been accurately determined [5]. However, a study in South Africa suggested that up to 75% of the recurrences after curative treatment may be caused by exogenous re-infections [6].

Following treatment with short-course chemotherapy, patients are considered to have a small risk for developing recurrent TB as a result of a relapse or exogenous re-infection [5, 7]. However, studies have shown HIV infection to be one of the risk factors for TB recurrence, as this is a major opportunistic infection in persons infected with HIV [8], and TB recurrence attributable to exogenous re-infection is more common in people who are HIV-positive [9].

Elucidating the role of TB recurrence and its underlying factors in Kenya is important because the country has the 13th highest incidence of TB in the world ¹⁰. Understanding the cause for recurrence helps clinicians evaluate the effectiveness of therapeutic regimens. It also facilitates TB prevention and control programmes to assess strategies and interventions. The aim of our study was to determine whether HIV infection and TB recurrence are associated with anti-TB drug resistance. The study also sought to determine rates of ZN microscopy and culture positivity among the recurrent TB cases.

Materials and Methods

Study Design: A cross-sectional study was conducted.

Study site and population: The study was done at chest and paediatric clinics at one provincial, one level 5 hospital and eight district hospitals in western Kenya. These were Busia, Bungoma, Kisumu, Migori, Kisii (Level 5), Narok, Kericho, Uasin Gishu and Lodwar district hospitals, and Nakuru Provincial General Hospital. Western Kenya includes the expansive former Rift Valley, Nyanza and Western Provinces, with a cumulative population of about 19.8 million people, constituting about 52.1% of the Kenyan population.

Sampling frame and patient characteristics: Participants suspected of having TB were included at random if they sought healthcare services at the chest and paediatric clinic between September 2007 and September 2009. They had to be resident in western Kenya for at least six months and consented to participate in the study. Cases that had prior treatment

were carefully screened and those already on anti-TB were excluded. Participants were suspected of having TB if they fulfilled the National TB Programme criteria: had a cough of more than two weeks and were not responding to antibiotic treatment [11].

Collection of demographic data: A questionnaire was administered by the designated Nurse or Clinical Officer in-charge of the chest and paediatric clinics to collect demographic and medical history data of TB suspects. Data collected included age, gender, previous anti-TB treatment, and HIV status.

Collection and transportation of sputum and blood samples: Three sputum specimens (spot, early morning, spot) were collected from 872 participants suspected with TB under the supervision of trained and competent medical staff. The patients were requested to cough so that expectoration would come from deep down the chest as possible, and spit into a sterile 50 ml blue cap tubes. For children less than 5 years of age and those less than 10 years of age unable to expectorate sputum had sputum induction performed at the Nakuru provincial and Kisii level 5 hospitals. The samples were refrigerated at 4°C awaiting transportation in cool boxes to the Mycobacteria Reference Laboratory, Moi University School of Medicine (MRL, MUSOM) weekly for analysis. Samples were processed within 7 days of collection in order to minimize loss of viability of the mycobacteria. Consenting 695 participants also underwent phlebotomy for HIV testing. The blood was delivered into Vacutainer Brand STERILE interior EDTA (K3) tubes and stored at -20°C awaiting processing. The samples were transported in cool boxes to MRL, MUSOM, Eldoret, and processed within two weeks.

HIV testing: Screening for HIV infection was done by screening serum using Trinity Biotech Uni-GoldTM test [12] and confirmed with enzyme linked immunosorbent assay (ELISA) [13].

Microscopic examination of sputum specimens: Sputum smears were examined for acid-fast bacilli (AFB) after staining following the Ziehl-Neelsen (ZN) method [14].

Isolation and identification of mycobacteria: Sputum specimens were processed for isolation of mycobacteria following standard protocols [15]. The mycobacterial isolates were identified as *M. tuberculosis* complex or species of non-tuberculous mycobacteria (NTM) using Hain's GenoType[®] Mycobacterium CM and GenoType[®]

Mycobacterium AS Molecular Genetic Assays, following manufacturer's instructions [16].

Identification of recurrent TB cases: Those who indicated to have been previously treated for TB (during demographic data collection) and declared cured and re-notified at least 12 months from the date of the initial notification were considered recurrent TB cases [17, 18]. This is after verification with their records held at the hospital.

Data analysis: Data was entered in MS Excel 8.0 and analysed using Epi Info version 3.5.1. Descriptive statistics were used to summarize data and proportions compared using Chi-square (χ^2) testing. Univariate odds ratios (OR) with 95% confidence intervals (CI) were calculated to assess risk factors (gender and age-group) with regard to TB infection and recurrence and HIV infection. Logistic regression was used to analyze multivariate data.

Ethical issues: The proposal for this study was approved by ITROMID / KEMRI's Scientific Steering Committee (SSC) and Ethical Review Committee (ERC) [SSC No. 837] and by Moi University School of Medicine (MU-SOM) / Moi Teaching and Referral Hospital (MTRH) Institutional Research and Ethics Committee (IREC) [FAN No.00092]. Clearance was also obtained from respective district health authorities and hospital administrations. Informed consent / assent were obtained from candidates or their guardians before they were enrolled into the study. The purpose of the study was explained to the potential candidates in English, Kiswahili or a local language before consent was sought. Code numbers rather than names were used to identify candidates in order to maintain confidentiality. The study did not expose candidates to any unusual risks as competent hospital staff obtained sputum and blood specimens from candidates using standard procedures.

Results

A total of 872 suspected TB cases were enrolled into the study, 54.9% (477) males and 45.1% (393) females. Their median age was 32 years. The majority of study participants (33.1%) were in the 25-34 age bracket, followed by those in the 35-44 (21.8%) and 15-24 (18.7%) age categories respectively. Children in the 0-14 age-group constituted 4.6%, of the TB suspects, with the under fives (<5 years) contributing 0.6%.

Microscopy and culture: A total 361 participants were diagnosed with mycobacterial disease, 346 (95.8%)

being TB, and 15 (4.2%) being NTM disease cases. Hence, 39.7% (346/872) of the participants had TB, 62.1% (215/346) males and 37.9% females. Children below 15 years constituted 4.9% (17/346) of the TB cases.

Recurrences and HIV infection: A total of 155 (44.8%) of the TB cases were recurrences, having previously been treated for TB and declared cured. Questionnaire data and hospital records regarding history of TB treatment of participants were fully in agreement. No treatment failures or default cases were observed in this study. Males constituted 54.2 % (84/155) and females 45.8% (71/155) of the recurrent TB cases. A total of 50 (32.3%) of the TB recurrent cases were ZN smear positive of which 25 were culture positive, and 105 (67.7%) cases were smear negative of which 6 were culture positive. Similarly, a total of 31 (20%) recurrent cases were culture positive and 124 (80%) culture negative. A total of 65 (41.9%) of these cases were HIV sero-positive, 70 (45.2%) cases HIV sero-negative, and the HIV status of 20 (12.9%) cases was unknown.

The majority (35.2%) of the recurrences were in the 25-34 age-group, followed by the 35-44 age-group with 23.9%. Also within age-groups, recurrences were more frequent among males in the 25-54 age-group. There was no statistically significant difference in TB recurrence between the genders [OR = 1.04; 95% CI: 0.73-1.47; P = 0.84] and HIV status [OR = 0.57; 95% CI: 0.29-1.13; P = 0.10]. (Table 1).

Table 1: Tuberculosis recurrence prevalence and the logistic regression output for gender and age-group

Age-group	N (%)	Males (%)	Females (%)	OR	95% CI	P-value
0-14	5(3.2)	1(0.6)	4(2.6)	5.714	0.576-56.675	0.137
15-24	18(11.6)	9(5.8)	9(6.8)	0.960	0.360-2.555	0.934
25-34	57(36.8)	32(20.6)	25(16.1)	1.006	0.561-1.804	0.985
35-44	40(25.8)	23(14.8)	17(11.0)	0.967	0.478-1.957	0.925
45-54	17(11.0)	10(6.5)	7(4.5)	1.038	0.354-3.040	0.946
55-64	12(7.7)	4(2.6)	8(5.2)	2.941	0.763-11.336	0.117
> 64	6(3.9)	5(3.2)	1(0.6)	0.182	0.020-1.690	0.134
Total	155(100)	84(54.2)	71(45.8)	1.037	0.734-1.469	0.839

Discussion

An episode of recurrent TB has been defined as a case re-notified at least 12 months from the date of the initial notification [18]. This is based on the reporting criteria of Enhanced Tuberculosis Surveillance that a year has to elapse before a case in the same patient can be notified again, and that any case reported from the same patient twice within a year is considered a single episode. The assumption is that most cases would be expected to have completed their treatment within 12 months, which is also why 12 months is the standard cut-off time at which treatment outcomes are recorded [18, 19]. Previous studies have shown that recurrent TB develops in about 2-5% of the patients after curative treatment with short-course anti-TB chemotherapy [20]. Among TB patients cured by short-course treatment in trial conditions, up to 7% develop recurrent TB needing retreatment within 1 to 2 years [7]. Also re-infections with new strains of *M. tuberculosis* complex may play a significant role in the recurrence of TB especially in settings with a high prevalence of the disease [21].

The high TB recurrence rate of 44.8% reported in this study compared to national rates by the 2007 and 2008 reported by the Division of Leprosy, TB and Lung Disease (DLTLD) [22, 23] is highly discouraging and deserves major attention. In the official 2007 DLTLD [22] annual report, retreatment cases contributed 9% of the TB cases, of which 3% were classified as PTB relapse category. The rest were recurrent smear negative PTB and EPTB (4%), treatment failures (0.1%), and return after default (1.4%). The 2008 annual report published by the DLTLD [23] shows a

smear positive relapse rate of 3% and a combined recurrent smear negative pulmonary TB and extra-pulmonary TB rate of 5%. However, in the 2009 annual report, the DLTLD [24] reports a combined retreatment rate of 14%. In all these reports however, the DLTLD cautions that due to the high prevalence of HIV in this population it is possible that some of the cases reported as TB could represent undiagnosed HIV related disease. Many National TB Control Programmes in Sub-Saharan Africa countries have no capacity to distinguish between relapse (endogenous reactivation) from (exogenous) reinfection TB, which constitute recurrent TB. It is not clear how the DLTLD identified TB relapse cases given in their annual reports. This is because diagnosis and treatment of new TB cases at majority of health facilities in Kenya is based on clinical symptoms, ZN smear microscopy, and occasionally augmented with chest X-ray in some of the health facilities.

In the current study males dominated females with 54.2% of the recurrent TB cases, just like in TB and TB-HIV co-infection. The majority (36.8%) of the recurrences were in the 25-34 year age-group, one of the most productive segments of society. Even if there was no significant difference in recurrence rate between genders and HIV status, the relatively high (44.8%) recurrence rate observed in the current study require further investigation to distinguish re-activations from re-infection as the predominant cause. This may lead to consideration of further intensification of the initial regimen or use of secondary prophylaxis [7]. Results of

the current study are also indicative of the necessity for evaluation of the performance of the short course (SCC) TB chemotherapy in Kenya. Kenya has been fully under directly observed therapy short course (DOTS) programme since 1998 [24]. However, it cannot be concluded categorically that DOTS functions as may be expected on basis of its naming. In the current study for instance, patients in western Kenya were provided with drugs to take at home for at least two weeks and adherence is not routinely checked. Therefore, it is unknown what the degree of compliance is. The degree of compliance can only be determined if differentiation between relapse and re-infection is established. From results of the current study, it becomes clear that this major issue should be addressed appropriately in order to pin point the underlying reasons for such a high rate of recurrence. However, acquired resistance does not seem to play a major role in this problem, since the 27 *M. tuberculosis* complex isolates obtained from the recurrent TB cases and subjected to first-line anti-TB drug susceptibility testing were all susceptible to streptomycin (STR), ethambutol (EMB) and rifampicin (RIF). Only 3 isolates from cases co-infected with HIV were resistant to isoniazid (INH).

However, the present study could not determine whether the recurrences were due to endogenous re-activations or exogenous re-infection, or even multiple/super infections since there were no *M. tuberculosis* complex isolates from previous disease episodes of the study candidates available. However, DNA fingerprinting studies in South Africa have reported a higher rate of reactivation (74.8%) versus recent transmission or re-infection (25.2%) [6]. The DNA fingerprinting of *M. tuberculosis* complex is an excellent tool to address this important issue and it is clear this type of molecular epidemiology becomes highly important in determining whether a recurrent TB episode is due to endogenous reactivation, meaning treatment failure, or exogenous re-infection with a different strain [25, 2]. Different *M. tuberculosis* complex strains can be differentiated using information on genetic markers and their distribution in the genome. Among persons with recurrent TB, if the isolates from two TB episodes have the same genotype, the second episode is a relapse, defined as an endogenous reactivation; otherwise, it is defined as exogenous re-infection [26].

Other studies have reported proportions of re-infection among recurrent TB cases ranging from 1.2-7% in low-incidence areas [27], 12-75% in medium-incidence areas [28], and 23-75% in high-incidence areas which include India, China, Indonesia, Nigeria, South Africa

and former Soviet states [29]. However, re-infection is an uncommon cause of recurrent TB in low TB prevalence settings [28, 30]. For instance, Jasmer *et al.* [28] evaluated cases of recurrent TB in two prospective clinical trials in Canada and United States by genotyping using IS6110. They reported 96% of the recurrences to have been caused by the same genotype; only 4% had a different genotype and were categorized as re-infection, indicating that recurrent TB in the USA and Canada, countries with low rates of TB, is rarely due to re-infection with a new strain of *M. tuberculosis* [28].

The significantly high rate of TB (39.7%) observed in the current study compared to the national standards to 20-25% reported by the DLTLDD is worthy noting. The difference could be attributed to regional variations of TB disease rate since the national reports cover the whole country with some regions have low disease rate. It may also be attributed to challenges faced by the DLTLDD in data collection countrywide which may lead to inaccurate data collection. The present study also observed a high TB-HIV co-infection prevalence of 41.8% even though this was slightly lower than those reported by the DLTLDD. The 2007, 2008 and 2009 DLTLDD annual reports give TB-HIV co-infection prevalence of 48%, 45% and 44%, respectively. The discrepancy could be attributed to some TB cases declining to undergo HIV testing, or decreasing HIV infection rate. However, the TB-HIV co-infection rate in the present study was higher than the global prevalence of 14.8% during the same period [10].

Conclusions and Recommendations

The HIV co-infection and TB recurrence were not associated with anti-TB drug resistance. The majority of TB recurrent cases were ZN smear negative (67.7%) and culture negative (80%). The high TB recurrence rate reported in the current study requires further investigation to distinguish relapse (endogenous reactivation) from exogenous re-infection as the predominant cause. In order to know whether the *M. tuberculosis* population structure is changing toward genotypes with selective advantage to circumvent BCG induced immunity and to become resistant against tuberculostatics / tuberculocidals, it would be wise to compare the genotypes found in young people (recent infections) and older people (more frequently due to endogenous re-infections of remote infections). What is found in old people is a reflection of the past (reactivation), while what is found in young people tells what strains are currently circulating and will be found in the future.

Competing interests

The authors declare that they have no competing interests.

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