Latent Tuberculosis is Highly Prevalent in Sub-Saharan Africans in Dublin - a Study Intended to Establish Normal CD4 Reference Ranges in this Population

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ABSTRACT
Objectives: To establish the normal reference range for CD4 lymphocyte cells in human immunodeficiency virus (HIV) negative sub-Saharan Africans attending the Genito-Urinary and Infectious Diseases (GUIDE) Outpatient Clinic at St. James's Hospital in Dublin. To correlate CD4 Count with lymphocyte count. **Design:** This was a prospective observational study. **Methods:** Volunteers were recruited among new sub-Saharan African patients attending the GUIDE Outpatient Clinic at St. James's Hospital, Dublin. Recruitment took place over an eight-week period between July and August 2003. The study objectives and methods were explained to volunteers, and informed consent for participation was obtained. History and relevant physical examination, together with measurement of haematological parameters, screening for sexually transmitted infections (STIs) and examination of stool and urine samples were performed to exclude confounding co-morbidities. A chest radiograph and Mantoux skin test was performed to exclude pulmonary tuberculosis (TB). **Results:** Seventeen participants were recruited. Two (12%) were excluded on the basis of HIV infection. Ten men and five women form the CD4 study group. Of the fifteen suitable patients recruited, the range of CD4 count is 532–1537 x 10^6/L. The reference range used by the laboratory at St. James's, based on CD4 counts in largely Caucasian populations, is 380 – 1500 x 10^6/L. Despite a high level of coincidental findings in the cohort, the range of CD4 counts measured falls within the range currently used by the laboratory. Women had significantly higher CD4 cell counts than men (median = 1245 and 899 respectively. $P < 0.01$), as has been previously described. There was a strong linear relationship between CD4 cell count and absolute lymphocyte count (r^2=0.5). Twelve of thirteen patients (92%) screened had evidence of latent pulmonary tuberculosis (TB), on the basis of a positive Mantoux reaction without radiographic evidence of TB. Two of fifteen (13%) HIV-negative patients defaulted before the result of their tuberculin skin test could be read. Only one patient in the entire cohort had a negative reaction to tuberculin challenge. We have referred 92% of this cohort for tuberculosis chemotherapy. **Conclusions:** The range of CD4 lymphocyte counts measured in this cohort falls within that used by the central pathology laboratory at St. James's Hospital, and by clinicians at GUIDE. This is the range that is used to guide clinical care among HIV-positive patients at GUIDE. A high rate of latent TB exists in this cohort.

INTRODUCTION

Tuberculosis (TB)

The World Health Organization estimates that one third of the world's population is infected with *Mycobacterium tuberculosis*, and that there are eight million new cases of active TB annually. Nearly 2 million persons die of TB worldwide each year, including persons infected with the human immunodeficiency virus (HIV).[1] The global incidence rate of TB is growing by approximately 0.4% per year, with a much faster growth rate in sub-Saharan Africa.[2] TB is currently responsible for approximately 11% of deaths occurring due to the acquired immunodeficiency syndrome (AIDS) worldwide. HIV is the single most important precipitant of the increased incidence of TB in Africa in the past 10 years.[1] TB is now the most common AIDS-defining illness in Africans resident in the UK.[3] The crude incidence rate of tuberculosis in Ireland fell in four consecutive years until 2000, where it was recorded at 10.9 cases per 100,000 population.[4] In the year 2000, 11.4% of cases of tuberculosis notified nationally were known to affect persons born outside Ireland.[5]

Background and Original Aims of the Study

International guidelines for the treatment of HIV infection have been drawn up using CD4 lymphocyte cell count reference ranges determined by studies conducted on HIV-negative North American and European subjects.[6,7] Several studies have shown that there is a significant difference in reference ranges between ethnic groups.[8,9,10] The primary aim of this study was to establish the normal reference range for CD4 cells in HIV-negative sub-Saharan Africans attending the Genito-Urinary and Infectious Diseases (GUIDE) Outpatient Clinic at St. James's Hospital, Dublin. This was a prospective observational study.

STUDY METHODS

General Considerations

Measurement of CD4 count at St. James's Hospital is undertaken as part of measurement of lymphocyte subsets. This is carried out by the department of immunology at the hospital's central pathology laboratory. Candidates for the study were drawn from new sub-Saharan African patients who registered with the GUIDE outpatient service over an eight week period between July and August 2003.

Consent, History-taking and Examination

Prior to participation, a detailed consent form was discussed with the patient, and informed consent was obtained for participation in the study. Demographic details and past medical and infection history were recorded, as were details pertaining to smoking preference. All patients underwent infectious disease screening in order to exclude ongoing infection. Participants had a general physical examination. Chest radiograph and tuberculin skin test (Mantoux method) were performed to identify tuberculosis. Stool samples were collected for culture and examination for ova, cysts and parasites. Optimal™ monoclonal antibody kits were used to screen for malaria. Urinalysis was performed to exclude pathological proteinuria, haematuria, glycosuria, and bacteruria (evidenced by the presence of nitrite in the urine), and of leucocyte esterase (indicative of the presence of white cells in the sample). Female patients underwent a pregnancy test based on urinary qualitative human chorionic gonadotrophin (βHCG) measurement.

A screen for sexually transmitted infection was performed. This included genital swabs for chlamydia, gonorrhoea, trichomoniasis and non-specific urethritis, and blood tests for HIV, hepatitis A, B and C, and syphilis. Absolute lymphocyte count was measured in each volunteer, together with lymphocyte subsets (including CD4 count).

Statistical Analysis

Statistical analysis of CD4 lymphocyte counts was undertaken using the Statistical Program for the Social Sciences (SPSS). The range of a group of CD4 cell counts is positively skewed. "Skew" or "bias" is used to describe a distribution that is not normal. This reflects the fact that although the upper limit of a range of CD4 cell counts may be very high, the lower limit cannot be less than zero.

Because of this positive skew, standard statistical tests based on normal distributions such as the Student's T-test are weakened. Non-parametric analysis does not require that variables under examination must be distributed normally about the mean of the sample. This is the reason that non-parametric analysis is more appropriate to a sample consisting of CD4 cell counts than a simple parametric test such as the t-test. The Kruskal-Wallis rank sum test used in this instance is a non-parametric statistical test.

The coefficient of determination r^2 was calculated as a test of the strength of the correlation between CD4 Count and absolute lymphocyte count. For two given variables *x* and *y* measured in a sample, r^2 represents the proportion of the variability of *y* that can be attributed to its linear relationship with *x*, where r is the Pearson product moment correlation coefficient "correlation coefficient". In this study, r^2 is quoted as a measure of the strength of the relationship between CD4 count and absolute lymphocyte count.

RESULTS

Seventeen new patients of sub-Saharan origin were recruited over an eight week period between July and August, 2003. Eleven men and six women were enrolled during this time. The original intention of the study was to establish a range for CD4 lymphocytes in sub-Saharan African patients.

Recruitment was hampered by a sharp decline in the number of new patients from sub-Saharan Africa attending GUIDE outpatient clinics during the study period. Most new patients from sub-Saharan Africa at GUIDE are asylum seekers who are in the process of applying for refugee status in this country. This curtailment of numbers may reflect a national trend in the rate at which people sought asylum in Ireland during 2003 (figure 1).

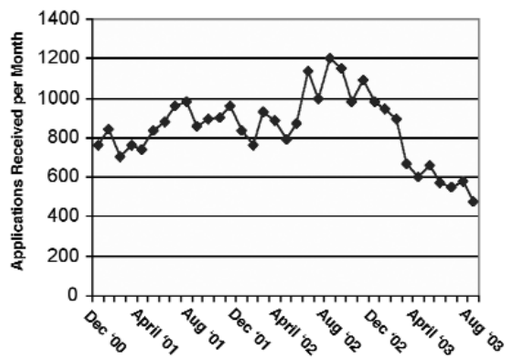

Figure 1: Applications for asylum received by the Refugee Applications Commissioner's Office between December 2000 and October 2003.[11]

Seventeen volunteers from eight countries in sub-Saharan Africa were recruited. The majority of our volunteers were born in

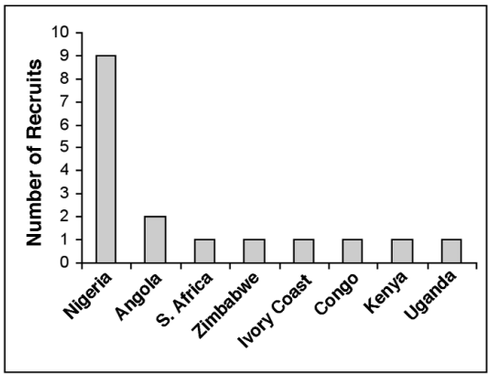

Figure 2: Country of origin of study recruits (N=17).

Nigeria (N=9). Two were born in Angola, and one in each of the other countries listed in figure 2.

Two of our seventeen original volunteers (12%) were diagnosed HIV-positive, a Nigerian woman and a man from the Ivory Coast. These patients were excluded from the CD4 study calculations. The range of CD4 lymphocyte counts in the fifteen suitable patients recruited is 532 – 1537 x 10^6/L. The reference range used by our laboratory, based on CD4 counts in largely Caucasian populations, is 380 – 1500 x 10^6/L.

The range of CD4 lymphocyte cell counts measured in our cohort falls within the range

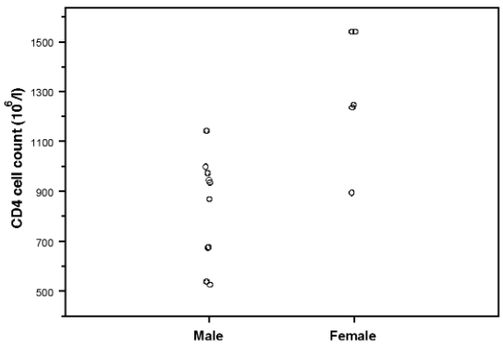

Figure 3: Sex variation in CD4 count.

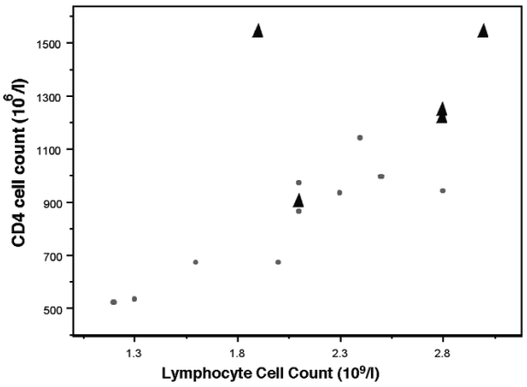

Figure 4: CD4 and lymphocyte counts in men (●) and women (▲).

currently used by our laboratory. Women had significantly higher CD4 cell counts than men (median = 1245 and 899 respectively. $P < 0.01$), as has been previously described (figure 3).[4] This is based on a Kruskal-Wallis rank sum test.

Smoking is known to affect CD4 count. Smokers are known to have significantly higher CD4 counts than non smokers.[12] Three (20%) of the patients in this cohort were smokers. No difference was found between the CD4 counts of smokers and non-smokers in this small group. There was a strong linear relationship between CD4 cell count and absolute lymphocyte count (r^2=0.5) (See figure 4). This value indicates that at least 50% of the variation in CD4 is explained by the variation in lymphocyte count.

In order to establish that the range of CD4 lymphocyte counts in our cohort was valid as a reference range in the wider population, it was important for us to exclude infective causes of altered lymphocyte count in the study cohort. Table 1 details potentially confounding pathologies identified in members of the study group. Despite the wide range of incidental pathologies identified in the study group, measurement of CD4 count lies within the range currently used by the hospital.

Table 1: A range of pathologies identified in the cohort.

Pathology detected	Number of patients
Cysts of *Endolimax nana**	2
Cysts of *Entamoeba histolytica/dispar***	1
Chronic anemia of unknown origin	1
Active Hepatitis B infection	1
Latent syphilis	1
Chlamydia trachomatis, Trichomonas vaginalis , Bacterial vaginosis	1
Non-specific urethritis	1

* Evidence of inactive disease ** Morphologically identical cysts

Latent TB in the Study Group

No member of this study group had radiographic evidence of infection with TB (N=15). Twelve of thirteen patients successfully screened (92%) had evidence of latent TB, on the basis of positive Mantoux reactions of between 12 and 30 mm induration. Two patients defaulted before the result of their tuberculin test could be read. Only one patient had a negative reaction to tuberculin challenge (see figure 5).

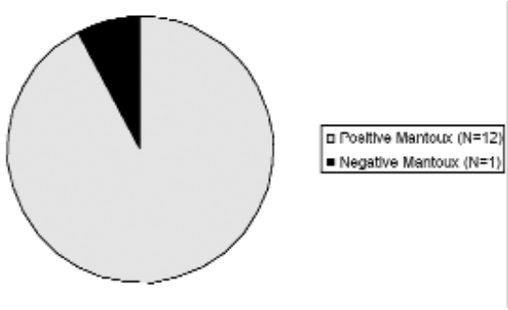

Figure 5: Latent TB infection in screened members of study group (N=13).

DISCUSSION

CD4 Count and the Establishment of a Reference Range

Attempts to establish a reference range for CD4 lymphocyte counts in sub-Saharan Africans were hampered by a low rate of recruitment, as outlined previously (see figure 1). CD4 lymphocyte counts are used to monitor the progression of HIV infection, and to determine intervention thresholds at which highly active antiretroviral therapy (HAART), and antibiotic therapeutic prophylaxis are initiated. In this study, CD4 counts measured fell within the reference range used at GUIDE.

A strong linear relationship was found between CD4 count and absolute lymphocyte count in this study group. Were it to be carried on into a larger group, this would support the use of absolute lymphocyte count as a surrogate marker for CD4 count. Such a finding has implications for the provision of health services in the resource-limited setting of the developing world. It is very much less expensive to measure absolute lymphocyte count than CD4 count. The ability to monitor HIV progression by measuring absolute lymphocyte count would reduce the cost of providing care for HIV-positive patients. More studies are needed in this area.

Other Pathologies in the Cohort

We identified a range of other pathologies in our cohort, including a range of sexually transmitted infections. An untreated sexually transmitted infection increases the likelihood of transmission or acquisition of HIV infection by six- to tenfold. A genital ulcer is thought to increase the risk of becoming infected with HIV during a single exposure by up to 300-fold.[13]

During the initial history taking phase of the study, each member of the cohort was explicitly questioned about relevant infections in their past. No member of this cohort volunteered a history of any of the illnesses with which they were subsequently diagnosed. One must therefore surmise either that existing screening provisions failed to diagnose their conditions at point of entry, or that their conditions were contracted in the interval between their arrival in Ireland, and presentation at GUIDE.

Latent TB in the Study Cohort

According to the current TB statistics published by World Health Organization (WHO), all members of our cohort come from countries that the British Thoracic Society (BTS) define as "high risk" for TB (incidence of 40 per 100,000 population or greater).[2,14]

Thirteen of fifteen members (87%) of the HIV-negative cohort were successfully screened by Mantoux skin testing. In this cohort, twelve of thirteen (92%) of the HIV-negative sub-Saharan African volunteers who were successfully screened had latent tuberculosis. Only one of the thirteen candidates who were successfully screened tested "negative" for TB in this instance.

We have referred 92% of the screened group for prophylactic tuberculosis chemotherapy. Two of fifteen members (13%) of the original cohort defaulted before the results of their Mantoux test could be read. The two-stage process required for Mantoux testing reduced its efficacy as a screening intervention in this case.

Two of the seventeen original recruits were diagnosed HIV-positive. One HIV-positive candidate had an anergic reaction to tuberculin. The other HIV-positive patient had a positive Mantoux reaction, and has received TB chemotherapy at GUIDE on this basis.

Current recommendations for chemoprophylactic treatment of latent tuberculosis in Ireland are that the patient should receive isoniazid for 6 months, and for at least 9 months if they are HIV-positive. Severe hepatotoxicity and death have been reported with protracted isoniazid treatment regimes.[15] Because of the risk of peripheral neuropathy in patients receiving isoniazid, it is prudent to prescribe pyridoxine (vitamin B_6) at a dose of 10mg daily from the start of treatment.[16]

Although it is administered only once daily, rates of non-compliance of between 24%

and 28.5% have been reported in patients receiving isoniazid monotherapy for latent TB.[17,18] Ireland has not yet implemented the directly observed treatment short-course (DOTS) endorsed by the WHO at a national level.[2] DOTS allows the documentation of all doses received by a patient during chemotherapy, and requires the direct observation of the patient as they ingest their medicine.[19] The DOTS strategy was first devised to address the resurgence of TB in the context of the emergence of drug resistant TB.

Since the beginning of the new century, drug resistant tuberculosis has emerged in Ireland. Between 2000 and 2001, the number of cases of multi-drug resistant TB notified in Ireland almost trebled. In 2001, in the Eastern Reional Health Authority area alone, eight reported cases of tuberculosis were resistant to one or more antibiotic. Five of eight cases occurred in non-national persons.[4]

TB is a notifiable disease in Ireland, under the provision of the 1947 Health Act. Provision under the act is made for an individual who refuses to cooperate with therapy to be confined to a hospital in order to receive therapy. It is unclear whether this provision has ever been implemented. All aspects of the management of TB are entirely free to patients. This includes hospital care and all medications, which are provided through the local Health Board.

International TB Screening Recommendations

The diagnosis of latent TB is made on the basis of a tuberculin skin test and a normal chest radiograph.[15] The American Thoracic Society and the Centers for Disease Control and Prevention (CDC) classify persons with a positive tuberculin skin reaction, but no clinical, bacteriological or radiographic evidence of active tuberculosis, as having latent tuberculosis.[20] No studies have been carried out on the treatment of latent TB in Ireland. Prophylactic treatment of latent TB has a definite but variable benefit. Almost all clinical studies have used the Mantoux method of tuberculin skin testing. This is the only technique recommended by the World Health Organization and by the International Union against Tuberculosis and Lung Disease.[15]

Mantoux testing involves the intra-dermal injection of 0.1ml of purified protein derivative in the anterior forearm. The result, read 48 – 72 hours later, is recorded as the diameter in millimetres measured transversely to the long axis of the forearm. Only induration or thickening in the skin is recorded; redness or localised oedema is ignored. In Ireland, a reaction of 10mm is commonly taken as evidence of sensitization to the infection.[15]

Where close contact has occurred with individuals who have infectious tuberculosis, there is a 25 – 50% chance of being infected with *Mycobacterium tuberculosis*. Infection rates are similarly high among adults in countries where tuberculosis is very prevalent. In such cases, the specificity of the tuberculin test is very high, and a positive tuberculin skin test indicates a high likelihood of tuberculosis infection.[20] BTS guidelines recommend the following in patients with strongly positive reactions to tuberculin: "chemoprophylaxis is recommended for those with a history of contact with infectious tuberculosis or residence in a high prevalence area within the preceding two years".[14]

The CDC recommend that prophylactic TB therapy is considered for HIV-positive individuals who have a Mantoux reaction with induration greater than 5mm, or in those who fail to react to tuberculin. These guidelines are offered regardless of whether the individual has received Bacillus Calmette Guerin vaccination in the past.[21] It is important to note that American Mantoux testing is based on a preparation of tuberculin that is more biologically potent than the preparation used in Irish testing (5TU as opposed to 2TU tuberculin). This is one reason for the lower threshold of diagnosis (5mm compared to 10mm induration).

Efficacy of BCG Vaccination in Preventing TB

The current recommendations of the CDC conclude that a positive reaction to tuberculin challenge subsequent to BCG vaccination cannot predict whether any protection against the disease has been acquired.[22] The protective efficacy of the vaccine is known to diminish over time. The largest study of its kind demonstrated no protection from BCG vaccination against infection by *Mycobacterium tuberculosis* in adults or children 5 years after initial vaccination.[23] Also, no evidence exists of the protective value of the vaccine in patients subsequently infected with HIV.[22]

Four of the thirteen successfully screened members of our cohort (30%) had evidence of BCG vaccination, while 92% had latent TB. This serves to reiterate that vaccination with BCG does not confer universal immunity to TB.

The Ethics and Legalities of Screening Immigrants

The International Health Regulations endorsed by the WHO state that it is illegal to for a nation to require proof of health, or to order the compulsory screening of immigrants on health grounds prior to their arrival in the country.[24]

The United States requires screening for Tuberculosis as a prerequisite for visa applicants.[21] The BTS recommend screening of new entrants to the United Kingdom from high risk areas of the world (TB incidence of more than 40 per 100,000 population per year), and of all refugees.[14] This is a statutory regulation in the UK.

CONCLUSIONS

The range of CD4 lymphocyte counts measured in this cohort falls within that used by the central pathology laboratory at St. James's Hospital, and by clinicians at GUIDE. This is the range that is used to guide clinical care among HIV-positive patients at GUIDE. A high rate of latent TB exists in this cohort.

The findings of this study raise questions about the efficacy of screening for TB in this cohort of foreign born persons. It is important that strategies be found to increase the uptake rate and successful completion of voluntary screening for TB in vulnerable groups from high-risk areas of the world.

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CONFLICT OF INTEREST STATEMENT

No conflicting interests are declared.

REFERENCES

1. World Health Organization Tuberculosis Fact Sheet No 104 Revised August 2002 Available from: URL: http://www.who.int/mediacentre/factsheets/who104/en/

2. WHO Report 2003: Global Tuberculosis Control - Surveillance, Planning, Financing 7th Annual WHO Report on Global TB Control Available from: URL: http://www.who.int/gtb/publications/globrep/download.html

3. Sinka K, Mortimer J, Evans B, Morgan D. Impact of the HIV epidemic in Sub-Saharan Africa on the pattern of HIV in the UK. *AIDS* 2003; 17: 1683 – 169.

4. ERHA Service Plan 2003 Eastern Regional Health Authority Available from: URL: http://www.erha.ie/news/uploads/560-Service%20Plan%202003.pdf

5. Feely E, O'Sullivan P, O'Flanagan D. Report on the epidemiology of TB in Ireland 2000 National Disease Surveillance Centre. Available from: URL:http://www.ndsc.ie/Publications/Tuberculosis/d461.PDF

6. Yeni P, Hammer S, Carpenter C, Cooper D, et al. Antiretroviral Treatment for Adult HIV Infection in 2002. Updated Recommendations of the International AIDS Society-USA Panel. *JAMA*. 2002;288:222-235.

7. Collins S, Fisher M, Johnson M, Khoo S, et al. British HIV Association guidelines for the treatment of HIV-infected adults with antiretroviral therapy July 2003 Available from: URL: http://www.bhiva.org

8. Messele T, Abdulkadir M, Fontanet AL, Petros B, et al. Reduced naïve and increased activated CD4 and CD8 cells in healthy adult Ethiopians compared with their Dutch counterparts. *Clin Exp Immunol*. 1999 Mar; 115(3): 443 – 50.

9. Howard RR, Fasano CS, Frey L, Miller CH. Reference intervals of CD3, CD4, CD8, CD4 / CD8, and absolute CD4 values in asian and non-asian populations. *Cytometry.* 1996 Sept 15; 26(3): 231-2.

10. Shahabuddin S. Quantitative differences in CD8+ Lymphocytes, CD4/CD8 Ratio, NK Cells and HLA-DR(+)-activated T cells of racially different male populations. *Clin Immunol Immunopathol*. 1995 May; 75(2): 168–70.

11. Office of the Refugee Applications Commissioner, 79 – 83 Lower Mount Street, Dublin 2, Ireland Monthly Statistics Available from: URL: http://www.orac.ie/Pages/Statistics.htm

12. Maini MK, Gilson RJC, Chavda N, Gill S, et al. Reference ranges and sources of variability of CD4 counts in HIV-seronegative women and men. *Genitourin Med*. 1996; 72:27-31.

13. AIDS Strategy 2000: Report of the National AIDS Strategy Committee Department of Health and Children of Ireland. Available from Government publications statistics office Sun Alliance House Molesworth Street Dublin 2. URL: http://www.doh.ie/pdfdocs/aids_strategy.pdf

14. Joint Tuberculosis Committee of the British Thoracic Society: Control and prevention of tuberculosis in the United Kingdom: Code of Practice 2000. *Thorax* 2000; 55:887-901.

15. Clancy J, Kelly P. Tuberculosis and Mycobacterial Diseases: Proceedings of the 6th Tuberculosis Conference, 2002 Peamount Hospital, Newcastle, Co. Dublin (Unpublished)

16. British Medical Association *British National Formulary* 45th edition March 2003 section 5.1.9 pg 284 Available from: URL:http://www.bnf.org

17. Pang SC, Harrison RH, Brearley J, Jegathesan V, et al. Preventive therapy for tuberculosis in Western Australia. *Int J Tuberc Lung Dis*. 2000; 12:984–988.

18. Eidlitz-Markus T, Zeharia A, Baum G, Mimouni M, et al. Use of the Urine Color Test to Monitor Compliance With Isoniazid Treatment of Latent Tuberculosis Infection. *Chest*. Mar 2003; 123: 736 - 739.

19. Frieden TR, Sterling TR, Munsiff SS, Watt CJ, et al. Seminar: Tuberculosis. *Lancet*. September 13, 2003; 362:887-899.

20. Diagnostic Standards and Classification of Tuberculosis in Adults and Children. This Official Statement of the American Thoracic Society and the Centers for Disease Control and Prevention was adopted by the ATS Board of Directors, July 1999. This Statement was endorsed by the Council of the Infectious Disease Society of America, September 1999. *Am. J. Respir. Crit. Care Med*. 2000; (161): 1376-1395.

21. Centers for Disease Control and Prevention. The role of BCG vaccine in the prevention and control of tuberculosis in the United States: a joint statement by the Advisory Council for the Elimination of Tuberculosis and the Advisory Committee on Immunization Practices. *MMWR Morb Mortal Wkly Rep*. 1996; 45(No. RR-4):[13 – 14] Available from: URL: http://www.cdc.gov/mmwr/PDF/rr/rr4504.pdf

22. Centers for Disease Control and Prevention. Screening for tuberculosis and tuberculosis infection in high-risk populations: recommendations of the Advisory Council for Elimination of Tuberculosis. *MMWR Morb Mortal Wkly Rep*. 1995; 44(RR-11):18-34.

23. Tripathy SP. Fifteen-year follow-up of the Indian BCG prevention trial. In: International Union Against Tuberculosis - Proceedings of the XXVIth IUAT World Conference on Tuberculosis and Respiratory Diseases. Singapore: Professional Postgraduate Services International, 1987:69–72.

24. International Health Regulations (1969) adopted by the Twenty-second world health assembly in 1969 and amended by the Twenty-sixth world health assembly in 1973 and the Thirty-fourth World Health Assembly in 1981. 3rd Annotated Edition WHO Geneva 1983 reprinted 1995 Available from: URL: http://www.who.int/csr/ihr/current/en/