



Detection of Latent Tuberculosis Infection in Nursing Students by Combined TST and IGRA Serial Testing

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ABSTRACT ;

Nursing students are occupationally at risk group for tuberculosis. Tuberculosis can either be as Latent tuberculosis (LTBI) or as active tuberculosis infection (TBI). Lack of awareness and not taking appropriate self defence exposes this group to acquire tuberculosis. It is the prime responsibility of governments and the managements of hospitals must take care of the health care workers.

There is no optimal or single specific test for detection of LTBI. Tuberculin skin test (TST) is used for screening to detect LTBI. But TST has many variations, modern immunological tests like detection of interferon gamma release assay are found to be more reliable. In TB high burden countries, persistently elevated interferon gamma level is found in some health care workers, constantly exposed to M.tuberculosis positive clinical samples. Serial testing of combined TST and Quantiferon gold helps in detecting nursing students with LTBI. Methodology ; 85 nursing students on admission were tested for detection of Latent TBI. In TST the induration size was increased to 15 mm. these tests . 25 first year medical students and fifteen microbiology technicians were included as controls. Result : only one nursing student showed 15 mm induration and a very high Gamma interferon level, persistently positive gamma interferon was found in 5 technologists, combined test after three, six and twelve months overcame the false positives faced in single TST or IGRA.

Key Words :

IGRA, Latent Tuberculosis, Nursing students, serial testing.

HIGHLIGHTS ;**Known :**

- Tuberculosis is Nosocomial
- Nursing students are the liaison for TB transmission and acquiring self.
- Lack of awareness is the major contributory factor.
- Tuberculin skin test is still used as the gold standard
- TST has variables

FUTURE

- LTBI in nursing students may be persisting or these students may be reinfected after treatment . this could be due to ongoing intensive nosocomial occupational exposure of HCW's .
- There is very limited data on T cell responses in LTBI nursing students
- There is no reliable test that can detect new exogenous or nosocomial TB infection in nursing students .
- our study offers a different perspective , because it was done among nursing students to detect NOSOCOMIAL INFECTION RATE OF LTBI

NO single test is reliable.

- IGRA is ideal in low endemic countries.
- Persistent positive result poses problems in result interpretation.
- Modification of the test interpretation needed to eliminate these ‘ grey zone’ values.
- 15 mm or more should be the cut off in TST. ,

INTRODUCTION

Last two decades, there has been an intense global concern regarding implementation of teaching the basics of Tuberculosis (TB) ^[1] for medical and nursing students. World Health Organization (WHO) and other national Consortiums are insisting that intensive teaching about TB must be

compulsorily included in curriculum of Nursing and medical courses. A shocking revelation of a survey was that the health care students in some countries are unaware of many facts regarding TB ^[2] Thus these students have placed themselves at occupational TB risk. Nursing students may develop Latent TB infection (LTBI), where the

bug remains silent in some part of the body, activating later to cause Tuberculosis infection (TBI). Adopting Good nursing practices or employing standard work precautions is poorly followed by the nursing students. Lack of awareness about transmission of Mycobacterium tuberculosis (MTB), weak financial status of the hospital, forgetfulness or emulating the senior are the factors contributing to occupational risk of LTBI in nursing students. There is no national or regional data about TBI/ LTBI among nursing students in Indi^[3]. Non availability of a reliable, economical and specific test to diagnose LTBI has hampered this important surveillance. Ten percent of nursing students having LTBI progress to active TB, making such nursing student a liaison for Nosocomial TB spread. The 'Gold Standard' diagnostic tool for LTBI detection is the tuberculin skin test (TST). Recently, tests detecting release of mycobacterium tuberculosis (MTB) specific interferon gamma (IFN- γ) by T cell are developed which are more specific and rapid tests for LTBI detection^[4]. In Germany, section 4 of the regulation on occupational safety and health (OSH) prescribes regular compulsory screening of employees in infectious diseases including TB. Quantiferon gold In tube test (Cellestis, Australia) is widely used for detection of LTBI. Single TST or TB gold test results are less reliable, hence serial testing after 3, 6 and 12 months may be more specific and detect true cases of LTBI. This is the first study in south India employing serial testing for detection of LTBI in nursing students

using both TST and Interferon Gamma release assay (IGRA).

Materials & Methods: This is a target group preliminary surveillance study. 125 health care students comprising of, 85 first year nursing students posted for clinical ward duty (test group), Twenty five first year MBBS (Healthy control) and fifteen technical staff working in microbiology laboratory (Risk group) who gave a written consent to participate voluntarily were included in the study. The students were explained in detail about the study protocol and permission was obtained from all the participants to publish the data, keeping the identity confidential.

Methodology: TST and IGRA (Quantiferon TB Gold In tube test, Cellestis, Australia) were used to detect LTBI cases. Serial testing at base line, three months, six months and twelve months was done to rule out false negatives and false positive variables which are common in TST. Blood collection, incubation and testing for IFN gamma was done as per the package protocol. QFT was considered positive if the Interferon-gamma response of TB antigen minus nil tube was ≥ 0.35 IU/mL. for TST microlitre of tuberculin containing 2 units of PPD was injected intradermally on the forearm of all participants; the reaction was read for Induration after 48 hrs. Because of subjective variations in reading and interpretation of TST, a five member expert team read and interpreted TST results.

Result: WHO guideline for TST positivity is an induration of 5 mm or more, but in high burden countries healthy people also may develop an

induration of up to 10 mm. Hence following modification in interpretation of TST was adopted in the present study. Induration of ≥ 15 mm as positive, 11- 14 mm as intermediate or doubtful and ≤ 10 mm as negative. This interpretation reduced the false positive results and improved the specificity of TST.

TEST GROUP- 23 (27%) nursing students developed induration of more than 5 mm, twelve of them developed induration of 11 to 13 mm, one had an induration of 14.5 mm and ten had induration between 5mm to 9mm. (27%)(23 – 29%; 95% C.I – 4 to 16). IGRA level was found increased (more than 18 IU/ml) in the student who had developed 14.5 mm induration. IFN-gamma level in the remaining eighty four, was less than the cut off value (0.37 iu/ml).

Healthy controls: Two medical students developed an induration of 7 mm (13%) (11-15%, 95% C.I. 5-8) QFT GOLD test was negative.

Laboratory technicians, TST showed induration of 9-10 mm in eight volunteers (53%).(51-55%; 95%C.I.7-11mm).IFN γ level in these technicians varied between 0.37 to 1.0 IU / ml. This is one of the limitations in IGRA. The cut off value calculation formula and result interpretation programming needs to be reset for use in TB high burden countries. The 'Greyzone' Values must be altered to suit TB endemic countries. TST result was analyzed and interpreted by five microbiologists.to assess concordance and discordance among t two specialists. This was done to prove the subjective reading and variables among individuals during TST result

reading. One of them interpreted 78 negative, 3 with induration of 9 mm, 2 with 6 mm and 1 with 5 mm induration. The second specialist interpretation was 75 negative, 7 with 10 mm induration and 2 with 6 mm induration. Third person's interpretation was still more confusing, she reported 70 negative and 14 with 6-7 mm induration. 4th and 5th person's interpretation was almost identical, 60 less than 5 mm, Twenty with 6-10 mm induration, 3 having 8-9 mm and one with 12 mm induration. only result in concordance among all the five was that of the student who was identified as positive in the study.

First follow up (after three months): Among the 23 who had shown induration of more than 5 mm during the initial screening, 15 developed 8 mm induration, 6 developed 7 mm and one had no induration. . The small induration could be due to hypersensitivity reaction to the protein fraction belonging to the mycobacterium complex or it could be a booster response seen following previous PPD injection or due to BCG. IFN-gamma level had increased to 32 IU / ml in the student who was TST and QFT Gold Positive, Other volunteers were negative by QFT GOLD test.

Control group - Two medical students developed induration of 6-7 mm, but QFT GOLD level was less than 0.37 IU/ ml. The false 'positive' induration is probably due to non specific hypersensitivity reaction.

Risk group: Two technicians who had TST induration of 9-10 mm showed similar induration.

Two others who were TST negative during initial screening developed induration of 5 mm. QFT GOLD test was negative in all the four, thus the hypersensitivity to some protein or 'booster' reaction theory is confirmed.

2nd serial testing (After 6 months): Entire testing procedure was repeated in all the 125 volunteers. QFT GOLD remained positive in the student who was earlier positive. TST positive nursing students continued to develop 6-10 mm induration confirming the false positivity in TST. Four from risk group volunteers developed induration of 8 – 10 mm , but IFN Gamma level was normal .

Third serial Testing (At the end of 12 months): Serial testing of all three groups by both methods was done. **Test group:** eighty were negative by both methods. (94%). 4 students (6%) developed 8-10 mm induration, but IFN gamma level was in the negative range. One (1.1%) had 18 mm induration and a high IFN- gamma level (36 IU/ml). This confirmed the poor PPV and NPV of TST. The control group two medical students showed 7 mm induration, but QFT GOLD was negative. Similar result was seen in 5 of the microbiology technicians who showed induration of 8 mm, but a negative QFT GOLD Test.

DISCUSSION

Nurses and nursing students are at the highest risk of not only TB infection but also active TB disease. Screening health care students at admission helps in knowing the possibility of LTBI OR TBI in these students [5] Early detection helps to initiate appropriate treatment

and prevent progression to active disease. 10 % LTBI cases are known to progress to become active. Initial negative TST indicates the serological status and helps in knowing any future seroconverted student. A study done as early as 1935 found that TB disease and infection risk was 2-3 times more in nursing students than medical students, 3 times that of physicians and 3-20 times comparing with non HCW's [6] [7]. }As early as 1950's health care industry agreed that care of TB patients is an occupational hazard and nursing students are at increased risk of acquiring TB by their prolonged contact with TB patient [8]. Occupational exposure to MTB can occur in different modes. These modes include contact (direct and indirect) transmission, droplet transmission, airborne transmission, percutaneous exposure and mucus membranes exposure [9] Transmission of MTB can be via any of these routes [10]. Co morbid factors like not practicing standard precautions are the predictors of LTBI in nursing students. In India only a few studies have attempted detecting LTBI rate in nursing and medical students. A Survey at a Medical school in North India showed a very high LTBI rate among medical students [11]

Non availability of a reliable and economic tool has resulted in underestimation of LTBI in nursing students. TST is still the gold standard method employed in many countries. Non specific nature of antigen, cross reactivity with non tuberculous mycobacteria, false positive result seen in BCG vaccinated population and false negativity seen in immunosuppressed persons makes it a unreliable

diagnostic aide for screening nursing, medical students and other HCW's ^[12]. Newer tests employing methods to detect MTB specific cellular products/ cytokines in the blood are better methods with a higher specificity. ^[13]

Interferon-gamma release assays (IGRAs) are increasingly used in many European countries for the tuberculosis (TB) screening of health care workers (HCWs) and also as pre employment screening. But increased rates of TB conversions and variable interferon gamma production in the same person has placed doubts regarding the reliability of this test as a better marker for early detection of LTBI in nursing student ^([14])

Detection of specific markers specific to TB pose problems by showing persistently elevated titre in some percentage of population, especially in countries with a high TB burden. This limitation needs to be looked in to by these commercial companies and reset the calculation of IGRA cut off level in such countries. In the present study also similar situation arose, while testing microbiology technical staff who were included as 'risk group', because of their constant contact with sputum and other clinical samples containing mycobacterium tuberculosis. Prevention of Nosocomial TB transmission in health care workers is the first step in reaching the WHO goal. TB control requires continuous learning and proper implementation of strict measures by the public health department and sincere involvement of national health ministry.

There is paucity of data regarding LTBI rate in nursing students in India, A study in south India found a high rate of ARTI in nursing students¹. TB is Nosocomial in origin, and is proved by Phylogenetic study of MTB isolates from patients admitted to the same health care centres at different time ^[15]

In India Annual MTB rate of infection (ARI) in the general population is about 1.5%, but the rate in Nursing fraternity is about 5 to 6%. This data is self explanatory for increased risk of LTBI in nursing students ^[16] In the present study LTBI rate in nursing students is 1.1% , comparing to other reports ,LTBI and TBI rate is low in this region A larger study and for extended follow up may help in detecting the correct rate of prevalence. In the study only one nursing student was detected LTBI positive by both methods and in all three serial testing. The surveillance was extended to find the source for this nursing student, one of the family members was the 'contact' source for MTB.

It is depressing to note that the billion dollar mega 'global control' programme is still depending on TST to find whether the skin positivity in PPD injected individuals is either due to 'anergic' or 'allergic' reaction to tuberculin .The present study is the first of its kind in this southern state of India and was undertaken to find the rate of LTBI among Nursing students in a multi specialty teaching hospital and also to evaluate the specificity and sensitivity of TST and Interferon gamma release assay. IGRAs are modified in vitro tests detecting a in vivo response

against TB antigen. These are not affected either by 'booster effect' of ppd or BCG. IGRAs have a high specificity and correlate well to the occupational risk of TB exposure. They also possess certain logistical advantages over the TST in that a second appointment to read the result is no longer required [17), (18),(19).] LTBI / TBI in HCW'S must be detected at the time of admission professionally , so that transmission nosocomially can be prevented. Limitations of the present study are small number of nursing students tested, low cut off level of IGRA which may have to be altered so as to include accurately detecting LTBI/TBI in TB endemic nations and to avoid 'grey zone' results

CONCLUSION

LTBI in nursing students is a global issue. Early detection and appropriate treatment prevents occurrence of HCW mediated Nosocomial TB transmission. TST has several limitations and variables. QFT GOLD test has a good PPV and is to be adopted for screening. There is NO single definite laboratory test to accurately diagnose LTBI . Serial testing at an interval of 6 months and combined usage of conventional TST and IGRA is more reliable than single test. Proper infection control policy and implementation of strict bio safety measures in health care centres is practiced by nursing students and all other HCW,s to prevent Nosocomial TB transmission. One of the Bio safety measure is detection of TBI or LTBI.

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CONFLICT OF INTEREST

The authors have read the manuscript and have no conflict of Interest

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REFERENCES

- [1] Jensen PA. 2005. Where should infection control programs for tuberculosis begin? Int J Tuberc Lung Dis. 2005;9:825
- [2] Teixeira EG, Menzies D, Comstock GW.et.al 2005. Latent tuberculosis infection among undergraduate medical students in Rio de Janeiro State, Brazil. Int J Tuberc Lung Dis. ;9:841–84
- [3] Joshi R; Reingold AL; Menzies D; Pai M. 2006 ; Tuberculosis among health-care workers in low- and middle-income countries: a systematic review. PLoS Med. 3(12):e494 (ISSN: 1549-1676
- [4] Pai M, Joshi R, Dogra S, Mendiratta DK, Narang P, et al. (2006) Serial testing of health care workers for tuberculosis using interferon-gamma assay. Am J Respir Crit Care Med 174: 349–355
- [5] Hohmuth BA; Yamanija JC; Dayal AS; Nardell E; Salazar JJ; Smith Fawzi MC; Latent 2006 tuberculosis infection: risks to health care students at a hospital in Lima, Peru. Int J Tuberc Lung Dis. 2006; 10(10):1146-51 (ISSN: 1027-3719).]
- [6] Daniels M, Ridehalgh F, Springett VH 1935-34, Hall IM. Tuberculosis in Young Adults: Report on the Prophit Tuberculosis Survey, 1935-1944. London: HK Lewis and Co., Ltd. 1948).

- [7] Sepkowitz KA. 1994 Tuberculosis and the health care worker: a historical perspective. *Ann Intern Med* ; 120:71-9.)
- [8] Pai M, Kalantri S, Aggarwal AN, Menzies D, Blumberg HM ,2006] . Nosocomial tuberculosis in India. *Emerg Infect Dis* [serial on the Internet]. 2006 Sep]
- [9] Jackson M; Harrity S; Hoffman H; Catanzaro A;2007 . A survey of health professions students for knowledge, attitudes, and confidence about tuberculosis, 2005 *BMC Public Health* . ; 7:219 (ISSN:1471-2458
- [10] K A. Sepkowitz 1995 . AIDS, Tuberculosis, and the Health Care Worker, *Clinical Infectious Diseases*, Vol. 20, No. 2 e(F. b, 1995), pp. 232-4
- [11] Rao KG, Aggarwal AN, Behera D 2004. Tuberculosis among physicians in training. *Int J Tuberc Lung Dis* ;8:1392-1394).
- [12]Swaminathan.S, Subbaraman.R,Venkatesan.P, Subramanyam.S, Kumar.SR Mayer.KH, Narayanan PR.; 2008 Tuberculin skin test results in HIV-infected patients in India: implications for latent tuberculosis treatment; *Int J Tuberc Lung Dis*. Feb;12(2):168-73.)
- [13] Pai M, Gokhale K, Joshi R, Dogra S, Kalantri S, et al. (2005) Mycobacterium tuberculosis infection in health care workers in rural India: comparison of a whole-blood interferon gamma assay with tuberculin skin testing. *JAMA* 293: 2746–2755
- [14] Felix C Ringshausen, Anja Schablon, and Albert Nienhaus;2012. Interferon-gamma release assays for the tuberculosis serial testing of health care workers: a systematic review. *J Occup Med Toxicol*; 7: 6
- [15] Christopher DJ; Daley P; Armstrong L; et.al Tuberculosis Infection among Young Nursing Trainees in South India. *PLoS One*. 2010; 5(4):e10408
- [16]Chadha VK, Kumar P, Jagannatha PS, Vaidyanathan PS, Unnikrishnan KP (2005) Average annual risk of tuberculous infection in India. *Int J Tuberc Lung Dis* 9: 116–118).
- [17] Mazurek GH, Jereb J, Lobue P, Iademarco MF, Metchock B, Vernon A. Guidelines for using the QuantiFERON-TB Gold test for detecting Mycobacterium tuberculosis infection, United States. *MMWR Recomm Rep*. 2005;54(RR-15):49–55.] [PubMed
- [18] Diel R, Goletti D, Ferrara G, Bothamley G, Cirillo D, Kampmann B, Lange C, Losi M, Markova R, Migliori GB. et al. Interferon-gamma release assays for the diagnosis of latent Mycobacterium tuberculosis infection: a systematic review and meta analysis. *Eur Respir J*. 2011;37(1):88–99.
- [19] Bhanu NV, Banavalikar JN, Kapoor SK, Seth P. Suspected small-scale interpersonal transmission of Mycobacterium tuberculosis in wards of an urban hospital in Delhi, India. *Am J Trop Med Hyg* 2004;70:527-531)