Original Article

Comparison of Ziehl-Neelsen staining microscopy and immunochromatographic tuberculosis test for diagnosis of pulmonary tuberculosis

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Background: Tuberculosis continues to be a great public health problem in Nepal. The evaluation of suitable diagnostic method to diagnose tuberculosis is urged.

Methods: Three consecutive early morning sputum collected from 413 patients were subjected to Z-N staining and serum from 224 patients to ICT-TB test.

Results: Overall positive results of Z-N staining and ICT-TB test were 13.1% and 14.3%, respectively. In ICT-TB test, males were found more positive (14.7%) than females (13.9%)($P>0.05$) but in microscopy, it was vice-versa ($P>0.05$). Age group 41-60 was significantly more seropositive compare to younger, however, in combination it wasn't significant ($P>0.05$). Alcoholics were significantly high (25.5%)($P<0.05$) and smokers were marginally high (17.3%) ($P>0.05$) AFB positive compared to non-alcoholics (11.5%) and non-smokers (11.6%). Students and service men were remarkably lower AFB positive compare to farmers and workers but interestingly nobody was found positive among business personnel ($P>0.05$).

Conclusion: Z-N staining microscopy is cheaper and equally sensitive hence it is more useful diagnostic tool than ICT-TB test for pulmonary tuberculosis.

Key words: Pulmonary Tuberculosis, Ziehl-Neelsen staining, ICT-TB test, Nepal

Introduction

Tuberculosis is a disease of significant public health importance worldwide.[1] WHO (1998) estimated that the infection of one-third of world population and 2 million death each year.[2] Majority (95%) of these cases and 98% of deaths condensed only in developing countries[3,4] is the most serious point. By 2000, annual death of 3.5 million[5] and by 2003, 8.8 million new cases of disease were estimated.[6] South Asia is the worst affected region[4,7] and India alone bears major burden.[4] About 45% of population of Nepal is supposed to be suffering from this disease, out of which 60% are in reproductive age group. Every year, 44,000 people develop active tuberculosis, of whom 20,000 are infectious pulmonary disease and 8-11 thousands death range in Nepal are believed.[8] National health policy has been made in Nepal to achieve the global target which hoped to save 60,000 lives over 5 years.[8]

In spite of various socio-cultural, environmental and economic factors; habit of smoking, consumption of alcohol, drug addiction, hereditary diseases and immune dysfunctions are known to increase a person's vulnerability to the disease. The increasing incidence of infection is also associated with infection by resistant strain[9,10,11] and the growing problem of HIV infection.[1,12] For laboratory diagnosis, direct microscopy is simple and rapid,[13] however, it needs a huge numbers of bacilli, from 50,000 to 1,00,000/ml of sputum. Now, as specific antigens have been identified and recombinant antigens are readily obtainable, serological test is alternate realistic proposition. We carried out this study to compare the efficient of 2 different diagnostic

methods and some risk factors of pulmonary tuberculosis among patients attending at National Medical College Teaching Hospital, Birgunj, Nepal.

Material and Methods

The study was carried out among patients attending at National Medical College Teaching Hospital (NMCTH), Birgunj, Nepal, presumptively diagnosed as a case of pulmonary tuberculosis during July 2004 to June 2005. Presumptive diagnosis was based on following criteria: cough more than 2 weeks, hemoptysis at any time, account of illness and radiological examinations. Three consecutive early morning sputum samples were collected and questionnaire regarding various predisposing factors were filled up. Microscopy was carried out from smear of sputum stained by Ziehl-Neelsen (Z-N) method for direct detection of acid fast bacilli (AFB)- *Mycobacterium tuberculosis*. Serological diagnosis was also carried out by immunochromatographic tuberculosis (ICT-TB) kit (Zydus Pathline, Cadila Healthcare Ltd, Ahembdabad, India) using serum from patients. Significant difference was found out by chi-square (c^2) test.

Results

Among 413 suspected cases of pulmonary tuberculosis, 54 (13.1%) were found to be AFB positive. Among 224 patients subjected to ICT-TB test, 32 (14.3%) were seropositive against TB *(Table1).*

Table 1: Result by different diagnostic methods

Test performed	Total no.	Positive no.	(Percentage)
AFB Microscopy	413	54	13.1
ICT-TB test	224	32	14.3

In ICT-TB test, males were found to be more positive than females but in microscopy it was vice-versa (P>0.05) *(Table 2, 3).*

Table 2: Microscopic positive cases between genders

Sex	Total no.	Positive no.	(Percentage)	p-value
Male	234	27	11.5	P>0.05
Female	179	27	15.1	
Total	**413**	**54**	**13.1**	

Table 3: ICT-TB positive cases between genders

Sex	Total no.	Positive no.	(Percentage)	p-value
Male	116	17	14.7	P>0.05
Female	108	15	13.9	
Total	**224**	**32**	**14.3**	

Age group 41-60 was significantly more seropositive compare to younger one (Table 5) but in combination there were no significant difference among different age groups (P>0.05) *(Table 4, 5).*

Table 4: Microscopic positive cases among different age groups

Age group	Total no.	Positive no.	(Percentage)	p-value
d"20	42	4	9.5	P>0.05
21-40	134	18	13.4	
41-60	181	25	13.8	
61-80	56	7	12.5	
Total	**413**	**54**	**13.1**	

Table 5: ICT-TB positive cases among different age groups

Age group	Total no.	Positive no.	(Percentage)	p-value
d"20	31	3	9.6	P>0.05
21-40	81	9	11.1	
41-60	76	14	18.4	
61-80	36	6	16.7	
Total	**224**	**32**	**13.4**	

AFB positive rate (17.3%) was found relatively more in smokers than non-smokers (11.6%) (P>0.05) *(Table 6)*

Table 6: Microscopic positive finding between smokers and non-smokers

Smoking	Total no.	Positive no.	(Percentage)	p-value
Yes	104	18	17.3	P>0.05
No	309	36	11.6	
Total	**413**	**54**	**13.1**	

and significantly more (25.5%) among alcoholics than non alcoholics (11.5%) (P<0.05) *(Table7).*

Table 7: Microscopic positive finding between alcoholics and non-alcoholics

Alcohol	Total no.	Positive no.	(Percentage)	p-value
Yes	47	12	25.5	P<0.05
No	366	42	11.5	
Total	**413**	**54**	**13.1**	

Remarkably lower AFB incidence rates were found among young students and service men compare to farmers and workers but no one was found to be positive among businessmen (P>0.05) *(Fig. 1).*

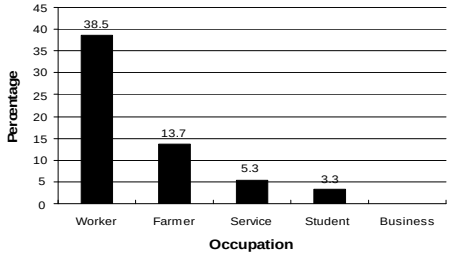

Fig. 1: Positive cases in different occupation by microscopy

Discussion

Overall positive findings by microscopy and ICT-TB kit were 13.1% and 14.3%, respectively. Nearly equal findings by both test methods could be because of microscopy detects all kinds of acid fast bacteria and 3 consecutive days samples and ICT-TB test detects only antibody to *M. tuberculosis* and because of less sensitive in the first 3 months of disease.[14] The report of ICT-TB test as a poor aid in the diagnosis of pulmonary and extra-pulmonary tuberculosis[15] also supports the result, however, our explanation is not corresponding to the report of cross reaction[14] and independent seropositivity with duration of disease,[16] so reason has to be further investigated. Our finding is nearly comparable to nation's tuberculosis detection rate (10%),[8] slightly high percentage in this study could be due to more endemic area and easily accessible health care facilities compared to other remote Nepal, however, AFB prevalence rates vary (1.8 per 1000,[17] 8.4 per 100,000[18] and 76 per 100,000[19] population to 26% in a study conducted in a hospital of Abuja, Nigeria with high HIV prevalence area[20]) with places and types of studies conducted and it is reported that the identification of pulmonary tuberculosis had increased by approx 85% when best of the personal and best of the techniques were employed.[21,22]

In most of the world, males are more infected and dying from tuberculosis than females.[23,24] Our serological study also showed same results as previous findings,[23,24] however, in microscopy it was vice versa supporting Annual Report of Department of Health Services, HMG Nepal in old age group.[8] The observed prevalence of disease among different age groups shows similar trend with finding of Murhekar *et al*.[25] In compare to younger one, age group 41-60 years were significantly more seropositive which could be due to impaired immune system in elderly people leading increase susceptibility to infectious diseases.[26] AFB incidence rate was significantly high among alcoholics than non-alcoholics and remarkably high among smoker than non-smokers. This suggests that smoking and alcoholism are main risk factors for pulmonary tuberculosis. And more than 95% of AFB incidence was found among farmers and workers which could be due to poor living condition, lack of health education and overcrowding.

Finally, it is concluded that ICT-TB is highly specific,[15] rapid, easy to perform and doesn't need sophisticated laboratory so it could be used in field survey or mass screening for research purpose but it is expensive and less sensitive.[14,15] On the other hand, Z-N staining microscopy is cheaper than ICT-TB test and hence more useful for laboratory diagnosis of disease in poor and developing countries like Nepal.

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